Research Article Life Conflux

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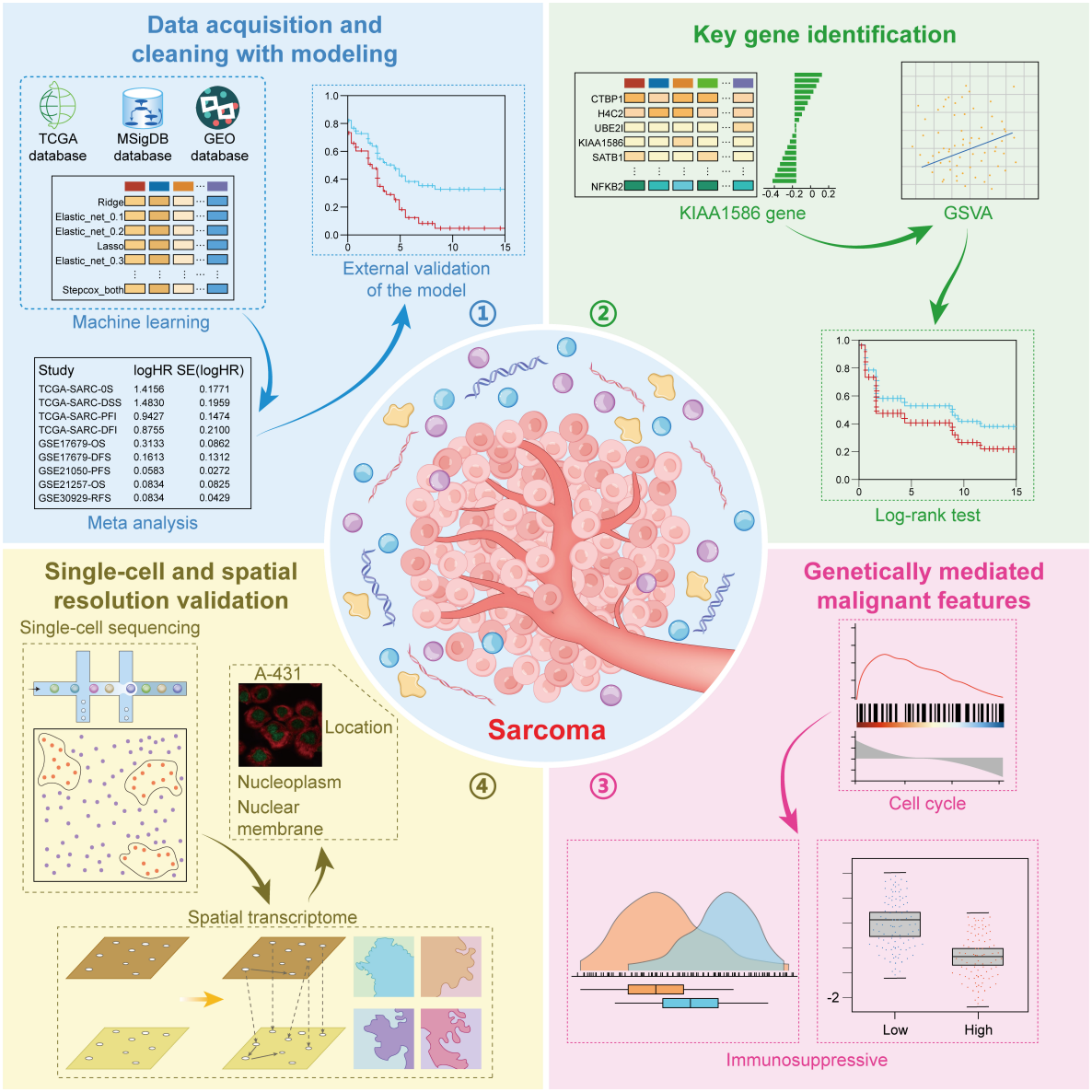
Graphical Abstract



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Research Article

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Integrating machine learning and multi-omics to identify key SUMOylation molecular signature in sarcoma

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Background: Sarcoma (SARC) is a rare and heterogeneous cancer originating from mesenchymal tissue. Due to its complex molecular mechanisms and limited treatment options, patients often have poor prognoses. Protein SUMOylation is an important post-translational modification process that plays a key role in regulating cellular functions and is closely related to the onset and progression of various cancers. However, the specific mechanisms by which SUMOylation affects SARC progression are not fully understood.

Methods: In this study, comprehensive bioinformatics approaches were utilized to analyze multiple datasets of SARC samples. By screening and identifying SUMOylation-related genes, we further explored the expression patterns of these genes in SARC and their association with prognosis and then constructed a consensus prognostic model. In particular, we focused on the KIAA1586 gene, which has attracted increasing attention in cancer biology, and conducted an in-depth study of its role in SARC.

Results: The study revealed that 19 SUMOylation-related genes were significantly correlated with the prognosis of SARC. Subsequently, the consensus prognostic model constructed by ridge regression could accurately predict the survival of patients in multiple data sets. Afterward, we identified KIAA1586 as the key gene, and its expression level was closely related to the prognosis of patients. GSEA enrichment analysis demonstrated that KIAA1586 might affect the progression of SARC by regulating the cell cycle and immune-related pathways, providing new insights into the molecular mechanism of SARC.

Conclusion: We have constructed a SUMOylation signature model that can accurately predict the prognosis of SARC patients, and identified KIAA1586 as a key SUMOylation gene that plays a crucial role in the onset and development of tumors by participating in cell cycle regulation and immune suppression.

Keywords: SUMOylation, Sarcoma, KIAA1586, prognosis, bioinformatics.

Introduction

As a type of malignant tumor originating from mesenchymal tissue, sarcoma is highly heterogeneous and complex [1]. Epidemiologically, sarcomas are relatively rare. Current treatments mainly include surgery, (neo)adjuvant chemotherapy, and/or radiotherapy. However, the mortality rate cannot be ignored [2]. Due to the diverse pathological types, varied clinical manifestations, and significant differences in the responsiveness to conventional treatments of sarcomas, the prognosis of patients is often poor, with a survival rate of approximately 12 to 18 months [3]. Although certain progress has been made in the diagnosis and treatment of sarcomas in recent years, the overall survival rate still needs to be improved [4-6]. An in-depth understanding of the molecular mechanisms of sarcomas, and the search for new therapeutic targets and prognostic markers, are of great significance for improving the prognosis of sarcoma patients.

Post-translational protein modification (PTM) is an important regulatory mechanism that occurs in cellular proteins during or after translation. These modifications can alter the conformation, stability, hydrophobicity, and charge state of proteins, thus influencing their functions in various biological processes [7]. To date, more than 450 types of PTMs have been identified, including ubiquitination, acetylation, phosphorylation, and SUMOylation [8]. SUMOylation is a post-translational modification process that depends on the conjugation of the small ubiquitin-like modifier (SUMO) to the target protein. As a member of the ubiquitin-like protein family, SUMO weighs approximately 11 kDa and can conjugate to the target protein in the form of a single monomer, multiple monomers, or different types of polymers [9]. SUMOylation plays a crucial role in cellular life activities and is involved in regulating multiple biological processes such as transcription, DNA repair, cell cycle progression, and signal transduction [9]. Abnormal SUMOylation processes are closely related to the occurrence and development of various diseases, including cancer. In cancer, abnormal SUMOylation may lead to the activation of oncogenes, the inactivation of tumor suppressor genes, and cell cycle disorders, thus promoting the progression of cancer [10].

Although the role of protein SUMOylation in cancer has been extensively studied [11-15], the specific mechanism of its action in sarcoma remains unclear. As a highly heterogeneous type of cancer, the occurrence and development of sarcoma

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may involve a variety of complex molecular mechanisms, including SUMOylation. Therefore, an in-depth exploration of the role of protein SUMOylation in sarcoma is of great significance for understanding the molecular mechanisms of sarcoma and identifying new therapeutic targets, and prognostic markers. In addition, developing personalized therapeutic signatures using SUMOylation-related genes also has potential application value for achieving individualized treatment of sarcoma.

This study aims to deeply explore the role of protein SUMOylation in the progression of sarcoma, with particular attention paid to the KIAA1586 gene, which has been attracting increasing attention in cancer biology. Through bioinformatics methods, we analyzed a comprehensive data set containing sarcoma samples to identify key SUMOylation-related genes and evaluate the expression pattern of KIAA1586 in sarcoma and its relationship with the prognosis of patients. Our study found that KIAA1586 plays an important role in sarcoma, and its expression level is closely related to the prognosis of patients. This novel finding not only provides a new perspective for understanding the molecular mechanism of sarcoma but also offers new ideas and potential intervention targets for the treatment of sarcoma. We believe that the results of this study will provide strong support for the individualized treatment of sarcoma.

Methods

Data acquisition

The TCGA data we utilized underwent rigorous standardization, normalization, batch correction, and platform correction as part of The Pan-Cancer Atlas, accessible via https://gdc.cancer.gov/about-data/publications/pancanatlas. We meticulously extracted samples from the SARC (Sarcoma) dataset and retrieved corresponding survival data from the UCSC Xena database (https://xena.ucsc.edu/). To validate survival outcomes, we incorporated external datasets including overall survival (OS) data from GSE17679 (88 tumor samples), GSE21257 (53 tumor samples), E-TABM-1202 (101 tumor samples), GSE59455 (122 tumor samples), and GSE119041 (50 tumor samples); progression-free survival (PFS) data from GSE21050 (310 tumor samples), GSE71118 (312 tumor samples), GSE71119 (132 tumor samples), and GSE71120 (41 tumor samples); disease-free survival (DFS) data from GSE17679 (also included in the OS datasets); and relapse-free survival (RFS) data from GSE39055 (37 tumor samples) and GSE30929 (140 tumor samples). By harmonizing these data with the expression files from the SARC dataset, we curated a comprehensive cohort for downstream analysis. The 17 SUMOYLATION gene sets sourced from the MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb) encompass a comprehensive range of processes related to protein SUMOylation. We have obtained a total of 227 genes that are related to SUMOylation.

Data cleaning and preprocessing

Data cleaning steps included removing missing values and non-tumor samples to ensure data integrity and accuracy. Additionally, survival times were converted from days to years to standardize the time units. All validation datasets underwent z-score normalization to transform the data into a normal distribution with a mean of 0 and a variance of 1, eliminating dimensional differences between variables. To further optimize the data distribution characteristics, the exponential function exp was used to convert z-scores into their exponential form.

Identifying key genes involved in SUMOylation

We conducted univariate Cox survival analysis on overall survival, disease-specific survival, and progression-free interval using the Coxph function, and screened for intersecting genes that showed significant correlation across all three survival outcomes through a Venn diagram.

Constructing a consensus prognostic model

We chose a linear model to model the input genes due to its simplicity, intuitiveness, and interpretability, which clearly reveals the specific contribution of each gene to prognosis. Multiple modeling algorithms were employed to construct prognostic models, including Lasso regression, Elastic Net, Ridge regression, stepwise Cox regression, and CoxBoost. Lasso regression was implemented using the glmnet package, with the family parameter specified as Cox and the alpha parameter set to 1. The cv.glmnet function was used to perform ten-fold cross-validation to select the optimal λ value. Model coefficients and feature names corresponding to the optimal λ value were extracted from the training results, and non-zero coefficients and their corresponding gene names were filtered out. Elastic Net and Ridge regression were also implemented using the glmnet package, with the alpha parameter for Elastic Net ranging from 0 to 1 (i.e., 0.1 to 0.9) and set to 0 for Ridge regression. Stepwise Cox regression involved first constructing a multivariate Cox regression model using the Coxph function, followed by stepwise regression analysis using the stepAIC function, with direction parameters including both, forward, and backward. For the CoxBoost model, we first optimized the penalty parameter using the optimCoxBoostPenalty function, then performed cross-validation using the cv.CoxBoost function to select the optimal number of steps. Finally, the CoxBoost function was used to construct the final CoxBoost model with the optimal number of steps and penalty parameter. Model coefficients and corresponding genes could be extracted using the coef function or obtained from the regression coefficient slot of the corresponding model. The linear combination of gene expression data and model coefficients was calculated to generate a risk score for each sample.

Evaluating the predictive performance of the prognostic model

The area under the receiver operating characteristic curve (ROC) and the area under the curve (AUC) were used as evaluation metrics, and the timeROC package was used to calculate AUC values at different time points to assess the performance of multiple prognostic models. Univariate Cox analysis was performed using the Coxph function to calculate the hazard ratio (HR) of the risk score calculated by the top-ranked algorithm across different datasets, followed by Meta-analysis and Kaplan-Meier survival analysis of the risk score.

Assessment of KIAA1586 as a key risk gene for SARC

We employed a multi-step approach for identification and validation. Firstly, we calculated the coefficients for each

gene using various algorithms and presented the results in a heatmap, where a coefficient of 0 indicates that the gene was not included in the model for a particular algorithm. Next, we conducted a univariate Cox survival analysis for KIAA1586 and performed a meta-analysis using the inverse variance method, with the log-hazard ratio (HR) as the primary measure. The HR indicates the tendency of the gene's effect, with values less than 1 suggesting a tumor-suppressive effect and values greater than 1 indicating a pro-oncogenic effect. Statistical analyses and visualizations were performed in the R environment using the "Meta" package. Additionally, we reflected the activity of given pathways by integrating characteristic gene expression, calculating combined z-scores for 14 different functional states of tumor cells based on the z-score algorithm. These scores were standardized and defined as gene set scores, followed by the calculation of Pearson correlations between KIAA1586 and each gene set score. Finally, to further explore the relationship between KIAA1586 expression levels and patient survival rates, we performed a Kaplan-Meier survival analysis using the survival package. The optimal cutoff value for distinguishing between high and low-expression cohorts was determined using the R package "survminer," ensuring that the proportion of high and low-expression groups was no less than 30% of the total sample. The log-rank test was used to assess the significance of survival differences between the two groups. DEPMAP (Cancer Dependency Map) is a research project aimed at creating a detailed map of cancer cell dependencies. CERES stands for CRISPR Essentiality Screen, a method used to quantify gene essentiality in cancer cells. The CERES score is an indicator derived from this method, reflecting the impact of gene knockout on cell survival or proliferation. A negative CERES score indicates that knockout of the gene leads to growth arrest or death of cancer cells, typically implying that the gene is crucial for the survival or proliferation of cancer cells. We have identified the top 200 cancer cell lines with negative CERES scores.

Exploring the carcinogenic pathway of KIAA1586.

This study initially classified samples into high and low-expression groups based on the expression level of the KIAA1586 gene, with the top 30% of expressers designated as the high-expression group and the bottom 30% as the low-expression group. Subsequently, the limma package was utilized to perform differential analysis, calculating the log2 fold change (log2FC) for each gene and ranking all genes according to their log2FC values. Following this, the clusterProfiler package was employed to conduct gene set enrichment analysis based on KEGG gene sets, GO-BP gene sets, GO-MF gene sets, GO-CC gene sets, Reactome gene sets, and WikiPathways gene sets, computing the enrichment score (ES) for each gene set and performing significance testing and multiple hypothesis testing on the ES values. To validate whether the cell cycle was enriched in the KIAA1586 high expression group (using the median value as the cutoff), we downloaded the cell cycle gene set from the MsigDB database (https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/WP_CELL_CYCLE) and conducted gene set enrichment analysis, calculating the ES and performing significance testing and multiple hypothesis testing. Additionally, the PROGENy method from the easier package was used to compute scores for four immune-related pathways: JAK-STAT, NF-κB, TNF-α, and Trail. The specific features of these pathways were derived from studying gene expression changes during pathway perturbation experiments, and a linear regression model was employed for fitting. Finally, across multiple cohorts, various algorithms (including CIBERSORT, CIBERSORT ABS, EPIC, ESTIMATE, MCP-counter, Quantiseq, TIMER, and xCell) were applied to calculate the Spearman correlation between the KIAA1586 gene and different immune infiltrating cells.

Verifying the immunosuppressive potential of KIAA1586

To delve into the intrinsic relationship between KIAA1586 and immune-related expression signatures, we meticulously obtained a valuable dataset encompassing 68 published immune-related expression features from the USCS Xena database. To precisely assess the correlation between KIAA1586 gene expression and these immune features, we skillfully employed the cor.test function in R to conduct detailed, pairwise correlation calculations between KIAA1586 expression levels and each immune-related feature and carefully extracted the Spearman correlation coefficients along with their corresponding p-values. To visually present the results of this correlation analysis, we cleverly utilized the hplot1 function from the fromto package to craft a vivid heatmap. Immune regulatory molecules play a pivotal role in cancer immunotherapy, and numerous agonists and antagonists of these molecules are undergoing rigorous evaluation in clinical oncology. To advance this research, there is an urgent need to understand the expression patterns and regulatory mechanisms of these molecules under different KIAA1586 states. To this end, we comprehensively investigated the expression of immune regulatory molecules, somatic copy number alterations (SCNA), and expression regulation through epigenetic pathways. To accurately assess MeTIL signatures, we adopted a principal component analysis (PCA) approach, skillfully converting individual methylation values of MeTIL markers into unitless MeTIL scores [16]. We standardized the data into Z-scores using the formula $(x-\mu)/\sigma$ and divided the samples into high and low-expression groups based on the median value of KIAA1586. Subsequently, we employed the Wilcoxon Rank Sum Tests to rigorously compare the statistical differences in MeTIL scores between the two groups. Furthermore, we adopted Spearman correlation analysis to meticulously calculate the correlation between genes and TIP scores, as well as the autocorrelation between TIP scores and utilized the linkET package for intuitive visualization [17].

Visualization and correlation analysis of gene expression in pan-cancer single-cell data

We obtained gene expression files at pan-cancer single-cell resolution from the TISCH database and constructed heatmaps using the pheatmap package to visualize the gene expression landscape at the pan-cancer single-cell level. Hierarchical clustering was performed using Euclidean distance as the metric and Ward's minimum variance method, which helped us to more clearly identify patterns and trends in the data and assess the conservation of KIAA1586 expression sources. For visualization of the gene expression data, we employed the Uniform Manifold Approximation and

Projection (UMAP) dimensionality reduction technique to display the distribution of cell types. For the visualization of single-cell transcriptome genes, we used the Nebulosa package. Due to the presence of numerous dropout events in single-cell transcriptome data, some genes have expression levels of zero or near-zero, even for marker genes. The Nebulosa package estimates weighted kernel densities, incorporates similarities between cells, and allows for the convolution of cellular features, thereby recovering lost gene signals and better presenting single-cell data. In addition, we calculated the average expression levels of KIAA1586, MKI67, CENPF, and PCNA in each single-cell dataset from the TISCH2 database, and used Spearman correlation analysis to assess the correlations between KIAA1586 and these three genes (MKI67, CENPF, PCNA).

Pan-cancer spatial transcriptome analysis revealed KIAA1586 expression landscape

Using the Sparkle database (https://grswsci.top/) and SpatialTME (https://www.spatialtme.yelab.site/), we conducted a pan-cancer spatial transcriptomics analysis [18]. The SpatialTME database utilized the Cottrazm package to deconvolute the cellular composition of the tumor microenvironment (TME) [19]. The Sparkle database integrated 10xVisium sequencing data from the SpatialTME database to construct a pan-cancer spatial transcriptomics atlas. Based on the proportion of cell types in each microregion, we named or characterized the microregions according to the dominant cell type and used a heat map to display the expression landscape of KIAA1586 across different cellular microregions. Subsequently, we selected sections from five tumors for further analysis. We presented the tissue sections, localized the dominant cell types after deconvolution, and identified the tumor boundaries. Additionally, we calculated the Spearman correlation between KIAA1586 expression and the proportion of different cell types in the microregions, as well as the difference in KIAA1586 expression among tumor, tumor boundary, and normal groups.

KIAA1586 expression and subcellular localization analysis

We systematically analyzed the immunohistochemistry staining results of KIAA1586 in various tumor tissues from the HPA database. HPA categorizes the staining intensity into four levels: High, Medium, Low, and Not detected, and we calculated the proportion of each level in detail. To more intuitively demonstrate the expression of KIAA1586 in specific tumors, we specifically selected immunohistochemistry staining sections of breast cancer and ovarian cancer for display. In addition, to further investigate the localization of KIAA1586 within cells, we conducted KIAA1586 immunofluorescence experiments using three cell lines (A-431, U-251MG, and U2OS) from the HPA database. By observing the immunofluorescence staining results, we visualized the subcellular localization of KIAA1586 within the cells.

Delve into the transcription and regulation of KIAA1586

We made use of Cistrome DB, also referred to as the Cistrome Data Browser, an interactive database that facilitates the visualization of public ChIP-seq, DNase-seq, and ATAC-seq data [20-21]. With the robust support of the extensive Cistrome DB database, the Toolkit enables users to swiftly test their hypotheses regarding gene regulation using publicly available ChIP-seq data (for protein factors and histone marks) and chromatin accessibility data (DNase-seq and ATAC-seq). The Cistrome DB Toolkit website provides three core functionalities. It leverages the BETA algorithm [22] to compute a regulatory potential (RP) score, which gauges the probability of a factor regulating a gene. For ChIP-seq, DNase-seq, or ATAC-seq samples, BETA adopts a distance-weighted method to assess the regulatory potential of all binding sites of the factor within a specified distance from the target gene. Factors with high RP scores are likely candidates for regulating the given gene. We downloaded the potential regulatory scores for specific transcription factors from http://dbtoolkit.cistrome.org/ and utilized the ggplot2 package for visualization. Pearson correlation analysis was applied to evaluate the correlation between transcription factors and KIAA1586. To examine the correlation between copy number variation scores and gene expression levels, we employed scatter plot analysis in conjunction with the Spearman rank correlation coefficient. A scatter plot serves as a graphical depiction that intuitively illustrates the relationship between two variables, enabling us to discern whether a linear or nonlinear relationship exists between them. Meanwhile, the Spearman rank correlation coefficient constitutes a non-parametric statistical method used to measure the monotonic relationship between two variables, irrespective of the data distribution. The Spearman rank correlation coefficient spans from -1 to 1, with values nearer to 1 or -1 indicating a more pronounced correlation, and the p-value is utilized to ascertain the significance of this correlation.

Results

Nineteen key SUMOylation Genes were identified and a robust SUMOylation signature was constructed

The Venn diagram reveals that there are 19 genes, specifically UBE2I, NDC1, SEC13, RELA, TOLLIP, UHRF2, AURKA, AURKB, BLM, CDCA8, CTBP1, DNMT3B, H4C2, MDC1, MRTFA, NFKB2, NR3C2, SATB1, and KIAA1586, that exhibit significant associations across multiple survival metrics (Figure 1A). Supplementary Figure 1 presents a forest plot displaying the results of univariate Cox analyses for SUMOylation-related genes in terms of overall survival, disease-specific survival, and progression-free survival. The heatmap presents the average AUC values at 1, 3, and 5 years for prognostic models constructed using different algorithms, with the algorithms ranked from highest to lowest average AUC values. The top-performing algorithm, Ridge Regression, exhibits the best performance across multiple datasets (Figure 1B). The meta-analysis results synthesize the findings of the Ridge Regression model across different survival outcomes in multiple datasets, enhancing statistical power and the reliability of the conclusions. The results indicate that the Ridge Regression model is a significant risk factor for various survival outcomes across multiple datasets (Figure 1C). Figure 1D-F present the Kaplan-Meier (KM) survival analysis results of the Ridge Regression model across different datasets, demonstrating that patients in the high-score or high-risk group have poorer prognoses ($p < 0.05$).

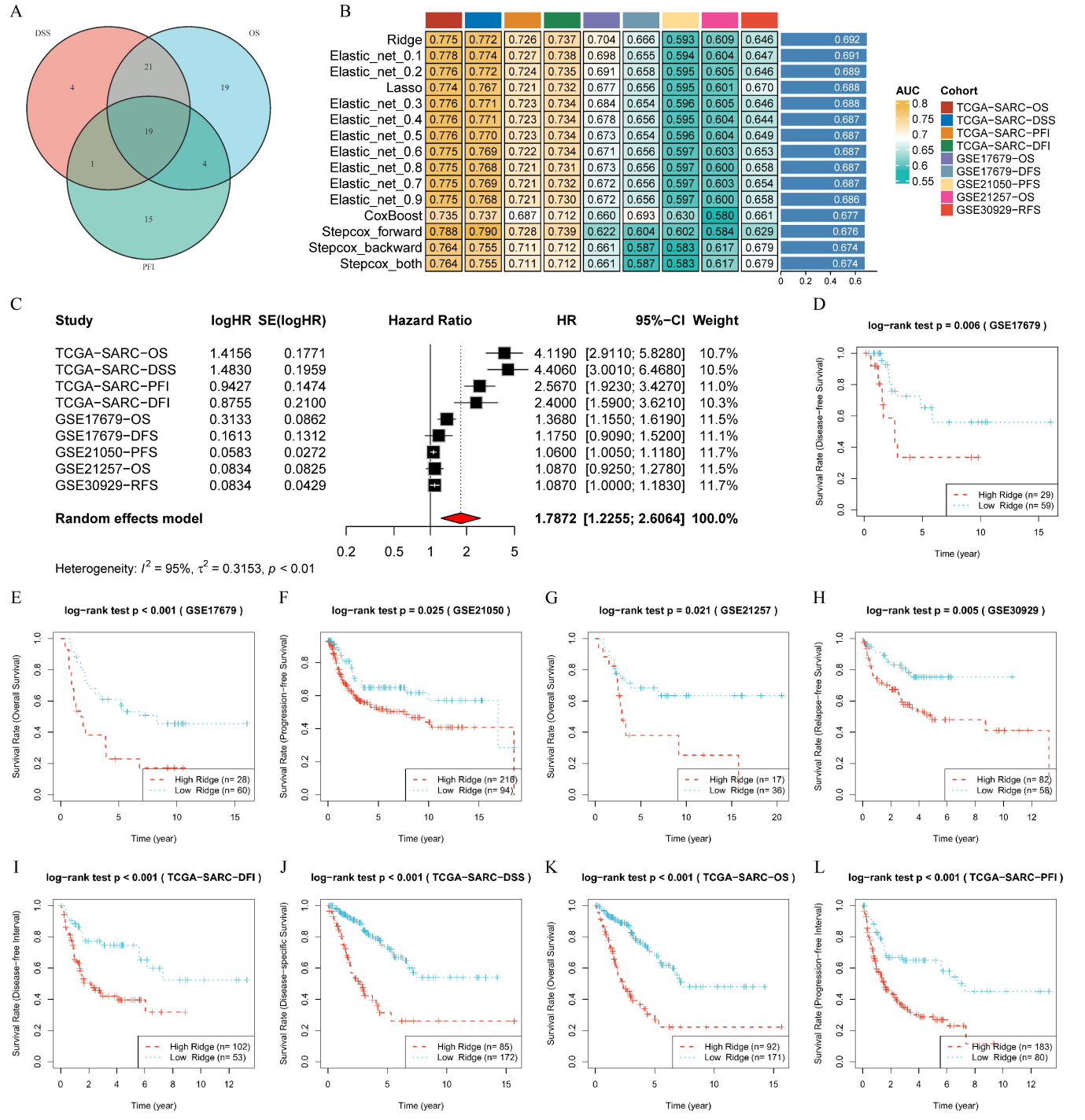

Figure 1. Identification of Key SUMOylation Genes and Construction of a Prognostic Prediction Model. (A) Presents 19 intersecting genes that exhibited significant correlation across overall survival, disease-specific survival, and progression-free interval, screened through univariate Cox survival analysis. (B) Comparison of the average AUC values at 1, 3, and 5 years for prognostic models constructed using different algorithms: This heatmap displays the average AUC (Area Under the Curve) values at 1, 3, and 5 years for prognostic models built with various algorithms. The algorithms are ranked from top to bottom based on their average AUC values, with the top-performing algorithm exhibiting the highest average AUC across multiple datasets. This ranking aids in identifying the most stable prognostic model algorithm over time. (C) Meta-analysis results of the hazard ratios for the prognostic model are presented, which helps synthesize findings from multiple independent studies, enhancing statistical power and the reliability of conclusions. (D) Kaplan-Meier survival analysis results for the final prognostic model, namely the Ridge Regression model, are shown, revealing a significant difference between the high-risk and low-risk groups.

KIAA1586 is a risk gene for SARC

Across all models, KIAA1586 exhibited remarkable consistency with positive coefficients, indicating that higher expression levels contribute to increased risk scores, suggesting that KIAA1586 is a risk gene for SARC (Figure 2A). The results of the meta-analysis revealed that the combined HR for KIAA1586 was 1.23, with a 95% confidence interval ranging from 1.11 to 1.37, confirming KIAA1586 as a risk gene for SARC, despite moderate heterogeneity observed across different survival outcomes (Figure 2B). Pearson correlation analysis uncovered associations between KIAA1586 expression levels and multiple cancer-related gene set scores, particularly showing significant positive correlations with cell cycle, DNA damage, and repair pathways (Figure 2C). Kaplan-Meier survival analysis further substantiated the significant link between KIAA1586 expression levels and the prognosis of SARC patients, demonstrating a consistent pattern across 10 survival cohorts where higher expression was associated with poorer outcomes, reinforcing the evidence that KIAA1586 is a risk gene. Supplementary Figure 2 suggests that KIAA1586 has a negative CERES score in a large number of cell lines, implying that knockout of KIAA1586 leads to growth arrest or death of these cell lines.

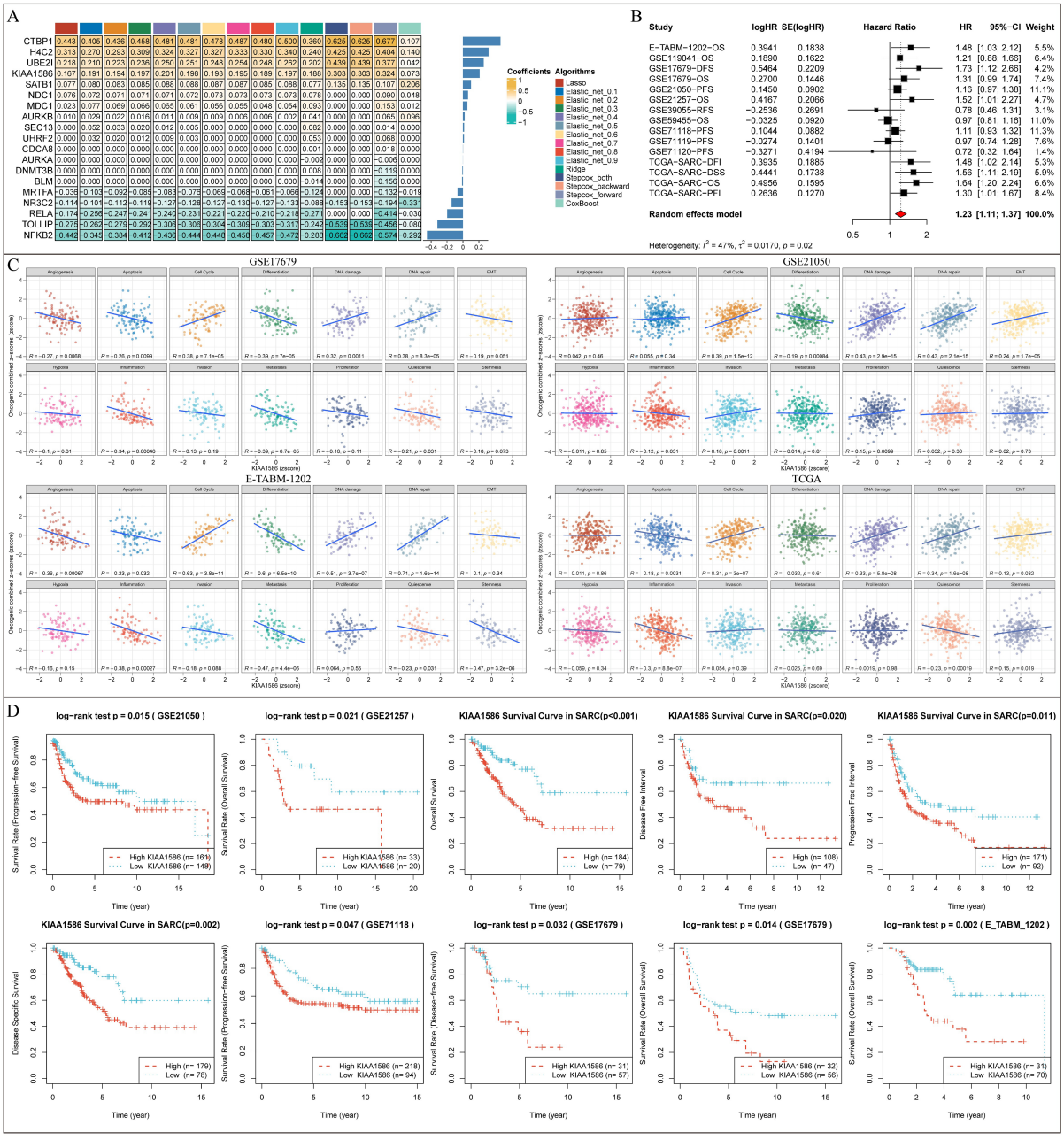

Figure 2. Identification and Validation of KIAA1586 as a Key Risk Gene for SARC. (A) The heatmap presents the coefficients of each gene across multiple algorithms, with a coefficient of 0 indicating that the gene was not involved in the model construction. (B) The results of the meta-analysis reveal the combined log-HR value from the univariate Cox survival analysis of KIAA1586, suggesting its role as a risk gene for SARC. (C) Dataset from the CancerSEA database, combined with the z-score algorithm, were used to calculate the combined z-scores reflecting 14 functional states of tumor cells, and their correlation with KIAA1586 expression. (D) The Kaplan-Meier curves illustrate the survival distribution of SARC patients grouped by KIAA1586 expression levels, with the log-rank test assessing the significance of survival differences between the two groups.

KIAA1586 is involved in tumor progression by participating in cell cycle and inhibiting immunity

Gene Set enrichment analysis revealed significant differences in biological processes and pathways between the high and low-expression groups of KIAA1586 (Figure 3A, B). Specifically, cell cycle-related pathways were significantly enriched in the high-expression group of KIAA1586, whereas immune-related pathways were more prominently enriched in the low-expression group. These findings suggest that the expression level of KIAA1586 may play a regulatory role in cellular proliferation and immune responses. To further validate these observations, we conducted an independent enrichment analysis focusing on the cell cycle gene set, which was corroborated by external datasets (Figure 3C). This analysis reaffirmed the association between high KIAA1586 expression and increased cell cycle activity. Moreover, the scoring of four key immune signaling pathways—JAK-STAT, NF-κB, TNF-α, and Trail—indicated higher activity levels in the low-expression group of KIAA1586 (Figure 3D). This suggests that reduced expression of KIAA1586 may enhance immune system activation. Finally, Spearman correlation analysis provided additional support, demonstrating a negative correlation between KIAA1586 and components of the immune cells with tumor-killing functions (Figure 3E). This not only underscores the potential immunosuppressive capability of KIAA1586 but also highlights its importance as a potential target for cancer therapy. Collectively, our analyses offer new insights into the role of KIAA1586 in tumorigenesis and development, suggesting it could be a critical factor influencing immune reactions within the tumor microenvironment.

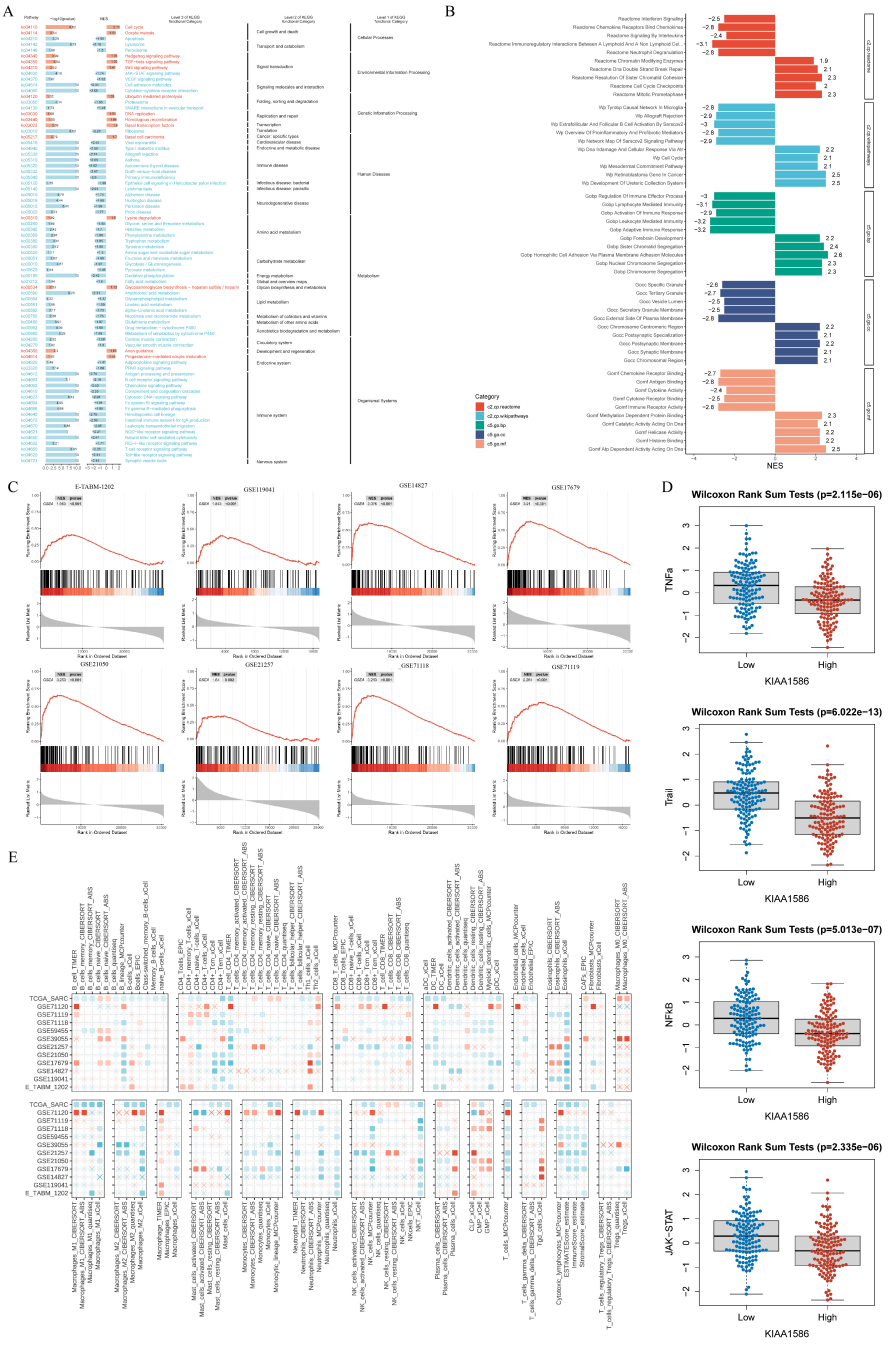

Figure 3. Impact of KIAA1586 Expression Levels on Gene Expression Patterns and Pathway Activity. (A) The pathways significantly enriched in the KIAA1586 high-expression group are represented in red, while those enriched in the low-expression group are shown in blue. A more significant p-value indicates a greater number of enriched genes, resulting in a longer bar graph. Additionally, a semantic summary of each pathway is provided. (B) The distribution of the top five significantly enriched pathways from the GO and Reactome/Wikipathways databases in the KIAA1586 high/low-expression groups is illustrated. Different colors represent distinct gene sets. Bars pointing to the left indicate significant enrichment in the low-expression group, whereas bars pointing to the right signify enrichment in the high-expression group. (C) The enrichment of gene sets at the top indicates that the core molecules within the custom gene set are primarily concentrated in the high-expression group on the left side of the KIAA1586 spectrum. This suggests that the target pathways are significantly enriched, and thus activated, in the high-expression group, while they are inhibited in the low-expression group. (D) A box plot compares the Z-Score values of four key immune pathways in the KIAA1586 high/low-expression groups, reflecting the relative activity of these pathways in different expression groups. (E) A heatmap presents the Spearman correlation between KIAA1586 and various types of immune infiltrating cells, highlighting its negative correlation with tumor-killing immune cell components. Red represents a positive correlation, blue indicates a negative correlation, and squares denote significant correlations ($p < 0.05$); otherwise, the correlation is considered insignificant.

KIAA1586 expression correlates with immune regulation and suggests potential as an immunotherapy target

Through in-depth exploration using Spearman correlation analysis, we revealed a widespread and significant negative correlation between KIAA1586 expression and various immune-related features, strongly suggesting that KIAA1586 may play a crucial role in the immune regulation process (Figure 4A). To provide a more comprehensive picture, we meticulously mapped the expression profile of immune regulatory molecules, somatic copy number alterations (SCNA), and the expression landscape regulated by epigenetic mechanisms (Figure 4B). The results indicated that the expression levels of immune molecules were significantly elevated in samples with KIAA1586 expression levels in the top 25% and between 25% and 50%. Based on previous research findings, we know that the median MeTIL score is significantly higher in tumors enriched with functional cytotoxic T lymphocytes (CTLs), establishing MeTIL score as a valid indicator for assessing CTL function. Further analysis showed that the MeTIL score was significantly lower in the KIAA1586 high-expression group, implying a close association between high KIAA1586 expression and diminished CTL function (Figure 4C). Additionally, we found that multiple immune scores, including CYT, also exhibited a decreasing trend in the KIAA1586 high-expression group (Figure 4D-G). More notably, there was a significant correlation between KIAA1586 expression and the scores of cancer immune steps (Figure 4H). These findings not only provide strong support for our in-depth exploration of KIAA1586 as a potential target for immunotherapy but also open up new research avenues and therapeutic strategies in the field of cancer treatment.

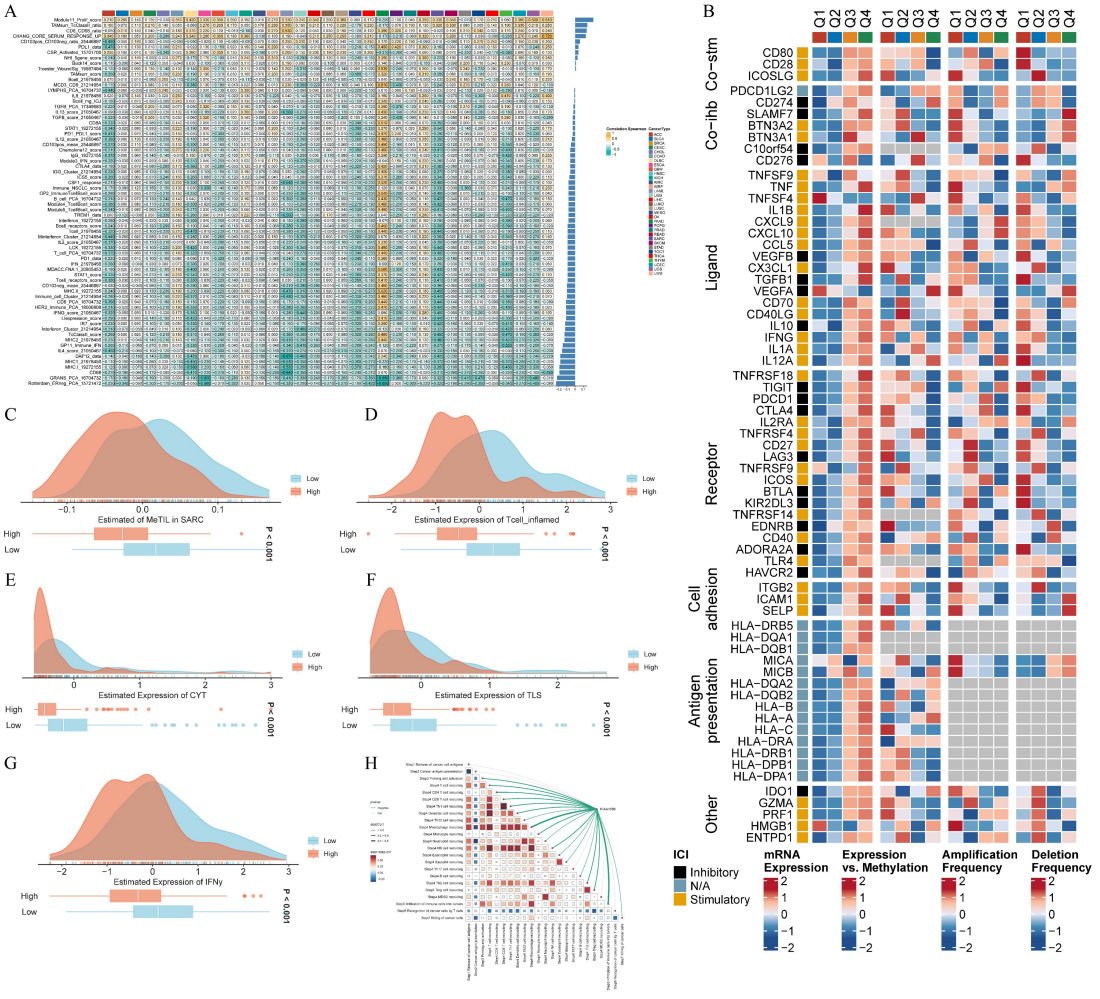

Figure 4. Validation of KIAA1586's Immunosuppressive Characteristics. (A) The heatmap depicts the Spearman correlation coefficients between specific genes and immune features. A larger absolute value of the correlation coefficient is represented by a deeper color, with shades closer to blue indicating negative correlation and otherwise indicating positive correlation. (B) Regulation of immune modulators. KIAA1586 expression is divided into quartiles: Q1, Q2, Q3, and Q4. Each component of the heatmap is arranged from left to right. mRNA expression levels are represented by the median of standardized expression values. The "Expression vs. Methylation" section shows the correlation between gene expression and DNA methylation β-values. Amplification frequency represents the difference between the proportion of samples with amplification of the immune modulator in a specific subtype and the proportion across all samples. Deletion frequency indicates the difference between the proportion of samples with deletion of the immune modulator in a specific subtype and the proportion across all samples. (C-G) Comparison of immune scores, including MeTIL, Tcell_inflamed, CYT, and TLS levels, between KIAA1586 high- and low-expression groups. The distribution of immune score levels for individual samples in the high- and low-expression groups is provided above each plot. The ends of the boxes below each plot represent the interquartile range of the values. The line within each box indicates the median value. Wilcoxon Rank Sum Tests were performed to compare the statistical differences in expression levels between the two groups.

KIAA1586 was expressed in malignant cells and co-expressed with proliferation genes

Our analysis revealed that KIAA1586 is predominantly expressed in malignant cells, demonstrating a significant advantage over immune cells (Figure 5A). To further explore the distribution of cell types and gene expression patterns, we employed the Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction technique for visualizing the gene expression data. UMAP illustrated the distribution of cells and genes, clearly showing higher expression of KIAA1586 in clusters associated with malignant cells (Figure 5B-C). Additionally, we calculated the average expression levels of KIAA1586, MKI67, CENPF, and PCNA in each single-cell dataset from the TISCH2 database, and used Spearman correlation analysis to assess the correlation between KIAA1586 and these three proliferation-related genes. The results indicated a significant positive correlation between KIAA1586 and the three proliferation genes at the pseudo-bulk level, further confirming our previous findings that KIAA1586 is associated with proliferation (Figure 5D-F).

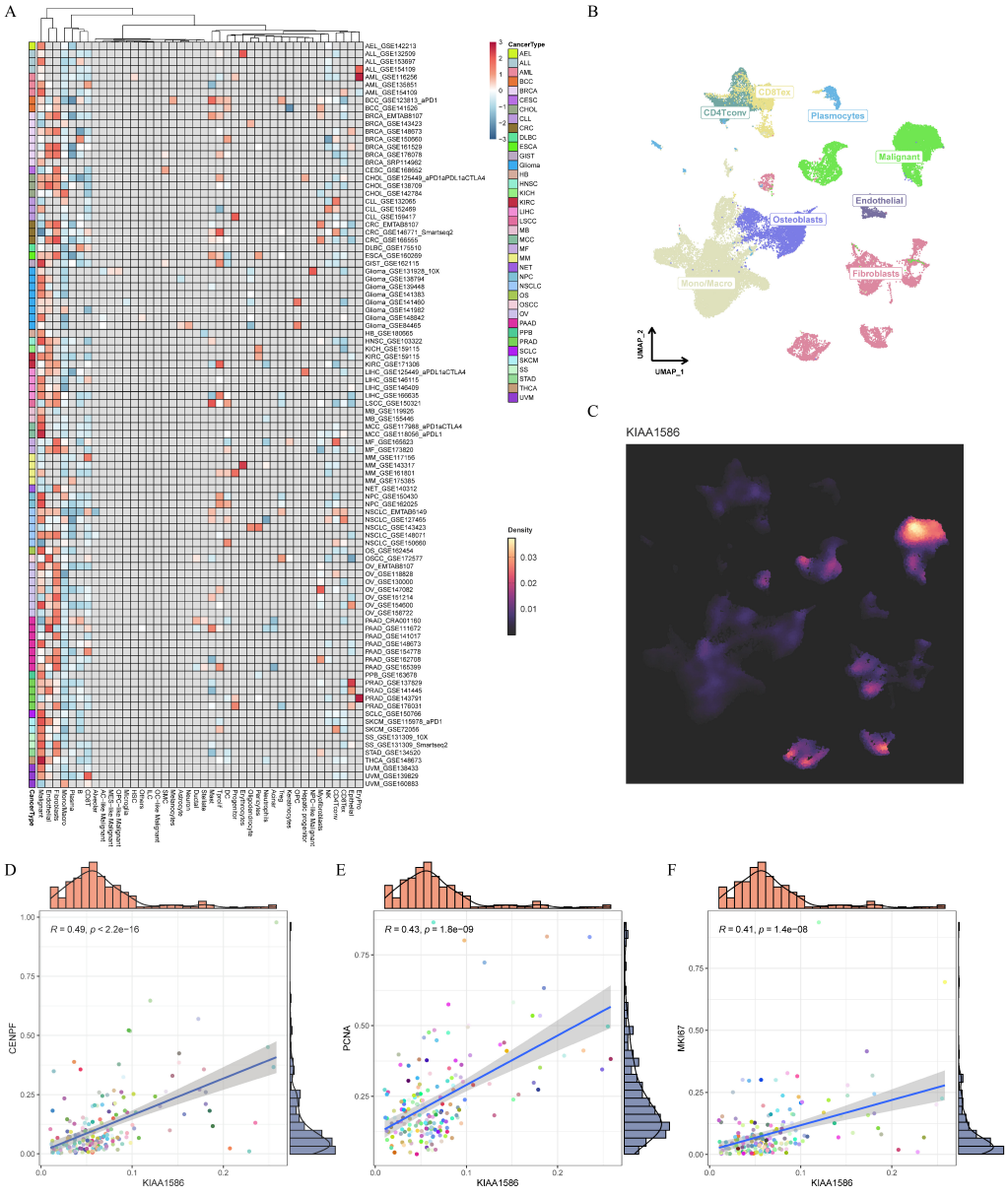

Figure 5. Expression of KIAA1586 in Malignant Cells and Its Co-expression with Proliferation Genes. (A) The heatmap visually presents the relative expression of KIAA1586 across different datasets and cell types. We observe a striking consistency in the expression pattern of KIAA1586 across different tumor types or datasets: it is predominantly highly expressed in malignant cells, demonstrating a significant advantage over immune cells. (B-C) In the osteosarcoma dataset, we employed UMAP dimensionality reduction technique to vividly illustrate the distribution of cells and genes. The results show that regions with higher expression of KIAA1586 highly overlap with malignant cell clusters, further confirming the specific expression of KIAA1586 in malignant cells. (D-F) The scatter plots clearly display the Spearman correlation between the average expression levels of KIAA1586 and the three proliferation genes, MKI67, CENPF, and PCNA, in each single-cell dataset. This result once again demonstrates the close association between KIAA1586 and proliferation genes.

KIAA1586 expression predominant in malignant regions of tumors

KIAA1586 Predominantly Expressed in Malignant Regions (Supplementary Figure 3). In spatial transcriptomics sections of breast cancer, clear cell renal carcinoma, hepatocellular carcinoma, ovarian cancer, and cutaneous melanoma, we observed a significant positive correlation between KIAA1586 expression and the proportion of malignant cells in the microregions, while a negative correlation was noted with other components, particularly immune cell content. Furthermore, we found that KIAA1586 expression exhibited a gradient decrease from tumor regions to tumor boundaries, and finally to normal regions (Figure 6A-E). These consistent results indicate that the deregulated expression of KIAA1586 and its resulting biological effects in the tumor microenvironment can be attributed to malignant cells.

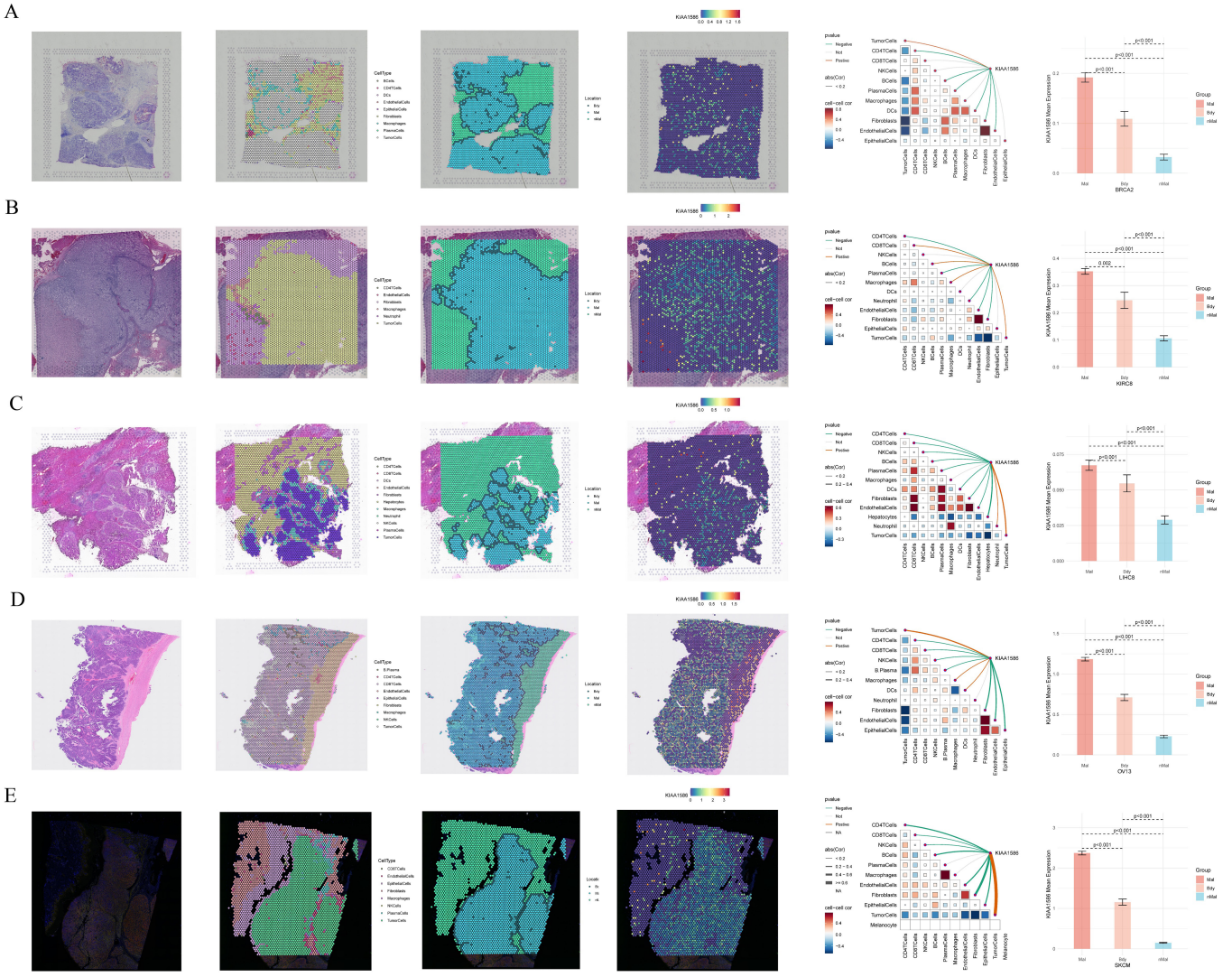

Figure 6. Predominant Expression of KIAA1586 in Malignant Regions of Tumors. (A-E) Each row of images is derived from breast cancer, clear cell renal carcinoma, hepatocellular carcinoma, ovarian cancer, and cutaneous melanoma, respectively. Each row contains six images, from left to right: 1) Tissue section serving as a blank control; 2) Each scatter point represents a microregion, named after the most abundant cell type, with different cell types represented by different colors; 3) Tumor boundary analysis, with different microregion types represented by different colors; 4) Each dot represents a spot from spatial transcriptomics sequencing, with deeper red indicating higher expression of the gene in that spot; 5) Correlation analysis, where red lines indicate positive correlation, green lines indicate negative correlation, gray lines indicate no significant correlation, and line thickness represents the absolute value of the correlation coefficient; the correlation in the triangular region is represented by the color intensity and size of the squares, with red indicating positive correlation, blue indicating negative correlation, and more significant p-values resulting in darker colors, larger absolute correlation coefficients, and larger squares; 6) Microregion differential analysis, with different groups represented by different colors, and the height of the bars representing the average expression level of each group, with differences analyzed using the Wilcoxon rank-sum test.

Expression and localization analysis of KIAA1586 in different tumors and cell lines.

As illustrated in Figure 7A, the expression levels of KIAA1586 vary across different tumor tissues. In tissues such as breast cancer, colorectal cancer, and ovarian cancer(Figure 7B-C), the expression distribution of KIAA1586 ranges from high to not detected. For instance, in breast cancer, 27% of the cases exhibit high expression, while 58% show medium expression, and the rest fall into other categories. Furthermore, in the A-431, U-251MG, and U2OS cell lines, KIAA1586 is primarily localized to the nucleoplasm and nuclear membrane(Figure 7D-F).

HDAC2 is a potential regulator of KIAA1586 expression in Sarcomas

The Cistrome DB database suggests that HDAC2 serves as a potential transcriptional regulator of KIAA1586. Additionally, there exists a correlation between the mRNA levels of these two genes, with a correlation coefficient of 0.31. In sarcomas (SARC), the Spearman's rank correlation coefficient between the copy number score of KIAA1586, as calculated by Gistic2, and its mRNA expression level is 0.45. This indicates that the expression of KIAA1586 in sarcomas is influenced by copy number variations(Supplementary Figure 4A-C).

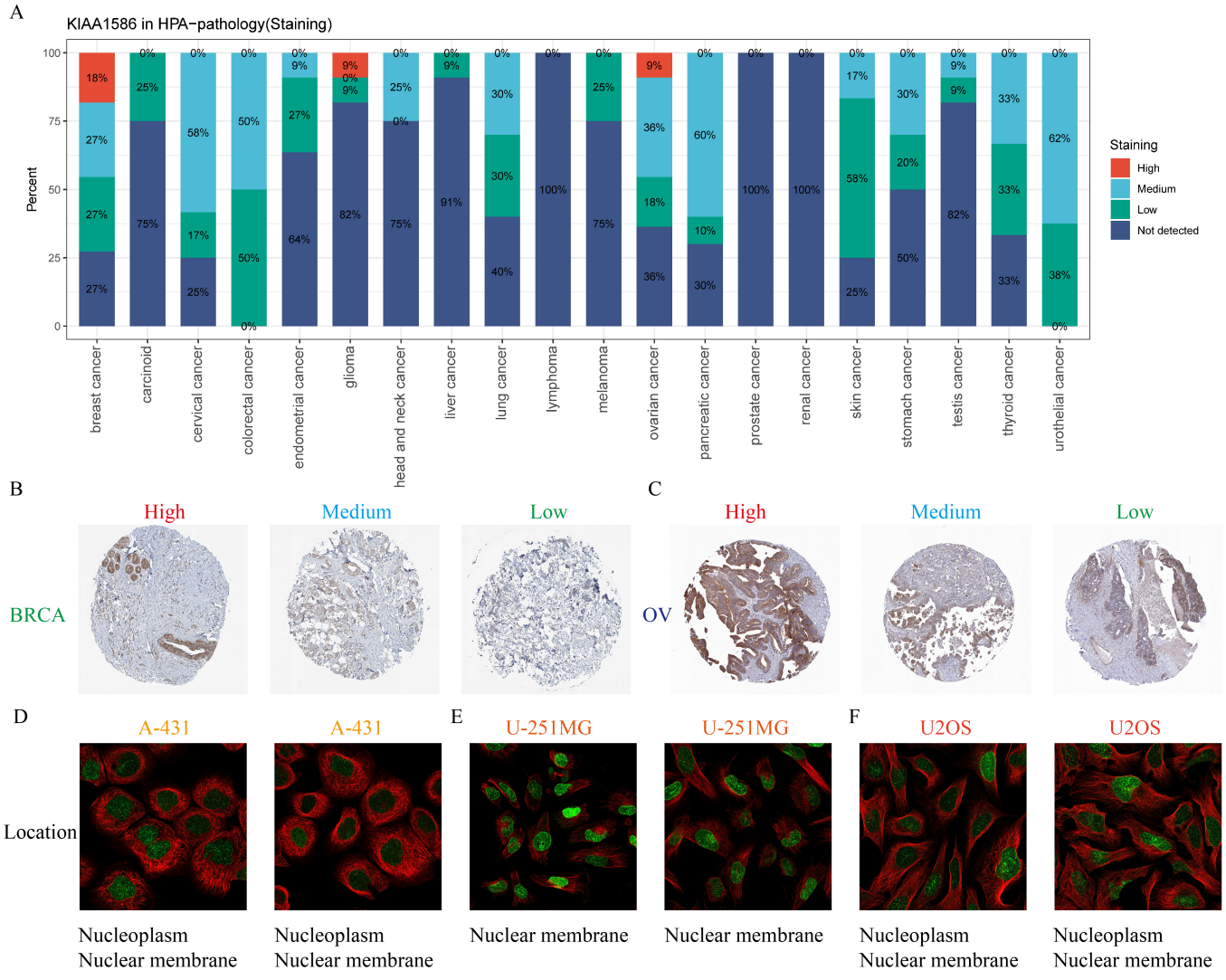

Figure 7. Comprehensive Analysis of KIAA1586 Expression and Localization in Different Tumor Tissues and Cell Lines. (A) The x-axis represents different tumor types, and the y-axis indicates the proportion of different staining intensities within a specific tumor. (B-C) Immunohistochemistry staining sections are presented, with darker brown indicating higher expression levels of KIAA1586. (D-E) Immunofluorescence staining sections are shown, with green fluorescence representing the target protein KIAA1586 and red fluorescence representing the microtubule structure.

Discussion

In this study, through comprehensive bioinformatics analysis, we deeply explored the role of protein SUMOylation in cancer progression, with a particular focus on SARC. Using the TCGA dataset and an external validation cohort, after careful data extraction and preprocessing, we identified 19 key SUMOylation-related genes. These genes showed significant correlations with multiple survival indicators, highlighting their importance in prognosis. Among these genes, KIAA1586 stood out as an important risk gene for SARC. This was confirmed by the consistent positive coefficients of KIAA1586 in various prognostic models and the results of meta-analysis.

We established a consensus prognostic model that includes 19 genes, namely AURKA, BLM, MRTFA, NR3C2, RELA, TOLLIP, NFKB2, UBE2I, NDC1, SEC13, UHRF2, AURKB, CDCA8, CTBP1, DNMT3B, H4C2, MDC1, SATB1, and KIAA1586. It is noteworthy that the risk coefficients of AURKA, BLM, MRTFA, NR3C2, RELA, TOLLIP, and NFKB2 are negative, indicating that these are protective genes. The risk coefficients of the remaining genes are positive, meaning that these are risk genes. Most of these genes have been found to be risk factors for multiple tumors or to be associated with tumor progression. UBE2I is upregulated in various cancers and is associated with cancer progression and poor prognosis. In hepatocellular carcinoma, the high expression of UBE2I is related to the enhanced migration and invasion ability of tumors. The deletion of UBE2I can significantly inhibit the migration and invasion of hepatocellular carcinoma cells [23]. AURKB plays an important role in the process of cell mitosis. Its abnormal expression is closely related to the occurrence and development of tumors. In some cancers, the high expression of AURKB is related to the proliferation of tumor cells and poor prognosis [24]. NDC1 promotes hepatocellular carcinoma tumorigenesis by targeting BCAP31 to activate PI3K/AKT signaling [25]. UHRF2 promotes hepatocellular carcinoma progression by upregulating the ErbB3/Ras/Raf signaling pathway [26], and it also promotes intestinal tumorigenesis through stabilization of TCF4 mediated Wnt/β-catenin signaling [27]. CDCA8 facilitates tumor proliferation and predicts a poor prognosis in hepatocellular carcinoma. Additionally, KIF18B promotes the proliferation of pancreatic ductal adenocarcinoma via activating the expression of CDCA8. Moreover, a cell cycle-regulated chromosomal passenger protein with aberrant expression and nuclear accumulation is linked to poor prognosis for gastric cancer [28-30]. However, there are few studies on KIAA1586, and we have carried out key analysis and exploration.

The importance of KIAA1586 in cancer biology is further supported by its association with key cancer-related pathways, particularly the cell cycle and DNA damage repair pathways. Cancer is a group of diseases in which cells continue to divide excessively. Cancer-related mutations that disrupt cell cycle control achieve continuous cell division mainly by impairing the ability of cells to exit the cell cycle [31]. Gene Set Enrichment Analysis shows that high expression of KIAA1586 is significantly correlated with increased cell cycle activity. Through spatial transcriptomics and single-cell transcriptomics analyses, we found that KIAA1586 is mainly expressed in malignant tumor cells and has a significant positive correlation with proliferation-related genes such as MKI67, CENPF, and PCNA. This indicates that KIAA1586 may influence cancer progression by promoting the proliferation of tumor cells. In addition, the high expression of KIAA1586 in the malignant regions of tumors further supports its central role in cancer development.

The JAK-STAT signaling pathway serves as a pivotal regulator of immune homeostasis [32]. NF-κB transcription factors play a central role in regulating immunity and inflammation [33]. Anticancer immune surveillance and immunotherapies initiate the activation of cytotoxic cytokine signaling, encompassing the tumor necrosis factor alpha (TNF-α) and TNF-related apoptosis-inducing ligand (TRAIL) pathways [34]. Conversely, KIAA1586 is negatively correlated with immune-related pathways such as JAK-STAT, NF-κB, TNF-α, and Trail. This suggests that KIAA1586 may affect cancer progression by suppressing the immune response. In terms of immune regulation, our study shows that the expression of KIAA1586 is widely and significantly negatively correlated with various immune-related features, indicating that KIAA1586 may play an important role in the process of immune regulation. According to previous research, in tumors enriched with functional cytotoxic T lymphocytes (CTLs), the median MeTIL score is significantly higher, indicating that the MeTIL score serves as a measure of CTL function [16]. Conversely, the MeTIL score is lower in the group with high KIAA1586 expression. This finding implies a close association between KIAA1586 and weakened CTL function, further underscoring the potential of KIAA1586 as a target for immunotherapy. Through further analysis of the expression profiles of immunomodulatory molecules, we provided more evidence for the role of KIAA1586 in immunosuppression.

Notably, our study also revealed the possibility of HDAC2 being a potential transcriptional regulator of KIAA1586. This regulatory relationship was not only verified at the mRNA level but also further supported by the correlation between copy number variations and the expression of KIAA1586. This finding provides a new perspective for understanding the abnormal expression of KIAA1586 in cancer and offers potential intervention targets for future research.

In summary, through multi - dimensional bioinformatics analysis, this study has deeply revealed the crucial role of KIAA1586 in SARC and its potential immunosuppressive mechanisms. These findings not only provide a new perspective for understanding cancer progression but also offer a scientific basis for the development of novel therapeutic strategies. In the future, research on KIAA1586 and its regulatory network may bring new breakthroughs in cancer treatment.

Conclusions

In this study, bioinformatics analysis showed that KIAA1586 was an important risk gene for SARC, and its high expression was associated with poor prognosis of patients. KIAA1586 affects sarcoma progression by promoting cell proliferation and inhibiting immune response. Spatial transcriptomics and single-cell analysis showed that KIAA1586 was mainly expressed in malignant tumor cells and positively correlated with proliferation genes. In addition, we found that HDAC2 may be a transcriptional regulator of KIAA1586. These findings provide new ideas for the treatment of sarcomas and a scientific basis for the development of new therapeutic

strategies for KIAA1586.

Acknowledgements

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Author Contributions

Juehua Jing, Yuyao Liu and Haoxue Zhang designed the whole project and wrote the manuscript.
Jinhao Cheng performed the most of the analysis.
Zhitao He contributed to the investigation.
Jinhao Cheng and Yuyao Liu contributed to the methodology.
All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data Availability Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Abbreviations

Sarcoma: SARC; The Cancer Genome Atlas: TCGA; Molecular Signatures Database: MSigDB; Overall Survival: OS; Progression-Free Survival: PFS; Disease-Free Survival: DFS; Relapse-Free Survival: RFS; ; Receiver Operating Characteristic: ROC ; Area Under the Curve: AUC ; Hazard Ratio: HR ; CRISPR Essentiality Screen: CERES ; Cancer Dependency Map: DEPMAP ; Kyoto Encyclopedia of Genes and Genomes: KEGG; Gene Ontology: GO; Principal Component Analysis: PCA; Methylation-based Immune Response Signature: MeTIL; Tumor Immunophenotype Profiling: TIP; Uniform Manifold Approximation and Projection: UMAP; Somatic Copy Number Alterations: SCNA; Cytotoxic T Lymphocytes: CTLs; Post - translational protein modification: PTM; Kaplan-Meier: KM: Tumor Microenvironment: TME

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Short Communication

Life Conflux

ATPexGen: ATP-induced cell death database

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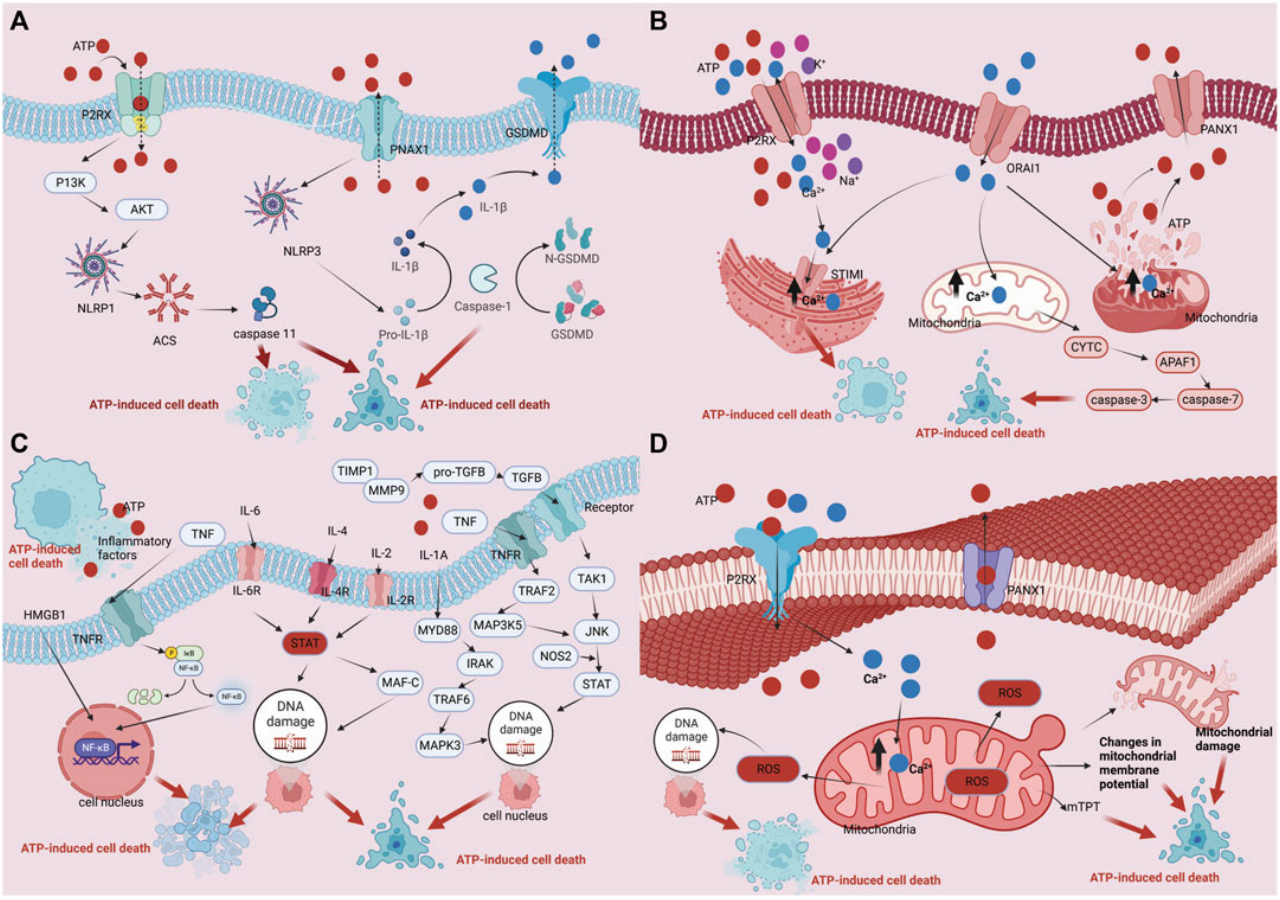
Graphical Abstract



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Short Communication Life Conflux

ATPexGen: ATP-induced cell death database

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Abstract: ATPexGen aims to establish a comprehensive database dedicated to ATP-induced cell death, addressing a critical gap in biomedical research. While ATP is the primary energy source for cells, its role in regulating cell death remains underexplored. Existing databases lack the systematic and detailed data required to support advanced research in this area. ATPexGen will integrate cutting-edge experimental data and literature, creating a multi-dimensional information platform to enhance understanding of ATP's role in cell death mechanisms. This platform will not only advance fundamental research but also facilitate drug development and therapeutic innovations. Given the global prevalence of cell death-related diseases, the development of ATPexGen is both urgent and impactful, offering robust support and valuable references for advancing research and clinical applications in this critical field.

Key words: ATPexGen: ATP-induced cell death; Database; Gene silencing; CTD; BioGRID.

Introduction

The ATPexGen project aims to build a comprehensive database dedicated to ATP-induced cell death, highlighting ATP's pivotal role in this process. The project encompasses key components such as data integration and standardization, mechanistic analysis, user platform development, and application promotion. By providing an efficient and comprehensive information platform, ATPexGen seeks to support researchers in exploring the mechanisms underlying ATP-induced cell death [1-4]. This integrated resource will facilitate significant advancements in both fundamental research and clinical applications, fostering progress in related scientific and medical fields [5-7].

To achieve its objectives, ATPexGen will actively collaborate with experts in biomedicine and drug development, fostering the application of data and the translation of research findings. The project aims to deepen understanding of ATP's role in cell death, providing novel insights for treating related diseases and driving advancements in the biomedical field. By bridging scientific research, drug development, and clinical application, ATPexGen seeks to accelerate progress in translational medicine, paving the way for innovative therapies and improved healthcare outcomes.

ATP-induced Cell Death Signaling

ATP-induced cell death manifests as either apoptosis or necrosis, each characterized by distinct morphological and biochemical features influenced by factors such as cell type, ATP concentration, and environmental conditions. Apoptosis is marked by cellular shrinkage, loss of cellular connectivity, mitochondrial membrane potential damage, cytochrome C (CYTC) release, nucleolar fragmentation, and DNA degradation into fragments of 180–200 base pairs. Apoptotic bodies are formed without inducing inflammation, as they are phagocytosed by surrounding cells. In contrast, necrosis is characterized by increased membrane permeability, cellular swelling, organelle deformation, rupture, and subsequent inflammatory responses. Following necrosis, tissue healing often results in fibrosis and scar formation. The mode of ATP-induced cell death is determined by a combination of cellular and environmental factors (Figure 1-4), primarily including: (A) activation of membrane-bound purinergic P2 receptors; (B) Ca2+ signaling pathways that induce cell death; (C) ATP-induced release of immune-inflammatory factors, which activate immune pathways; and (D) ATP-induced loss of mitochondrial membrane potential, disruption of mitochondrial integrity, production of reactive oxygen species (ROS), and alterations in mitochondrial membrane permeability, all of which contribute to cell death[1-7].

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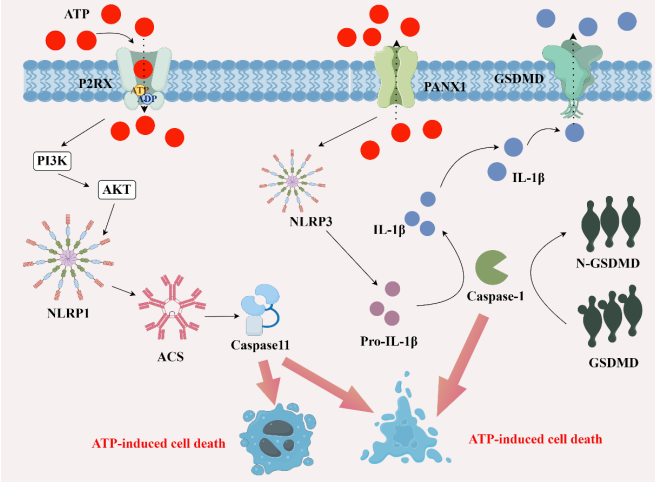

Figure 1. P2 receptor activation pathway

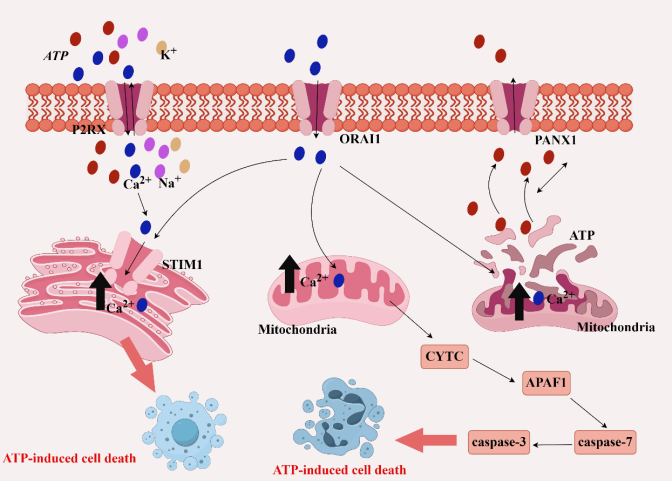

Figure 2. Ca2+ pathway induces cell death pathways

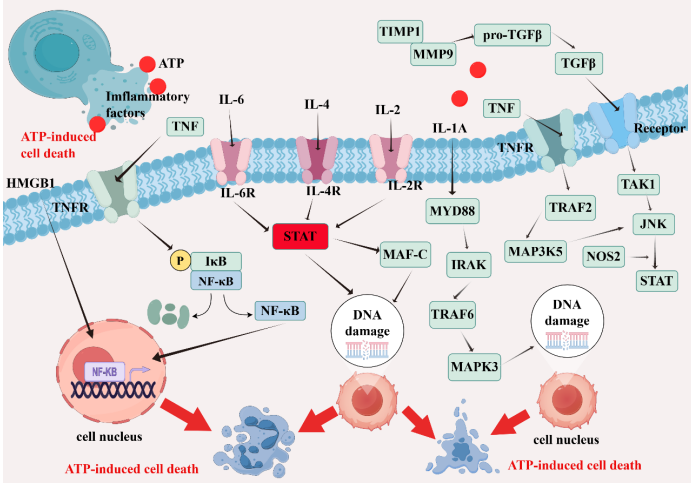

Figure 3. ATP triggers the release of immune-inflammatory factors from cells, activating immune pathways

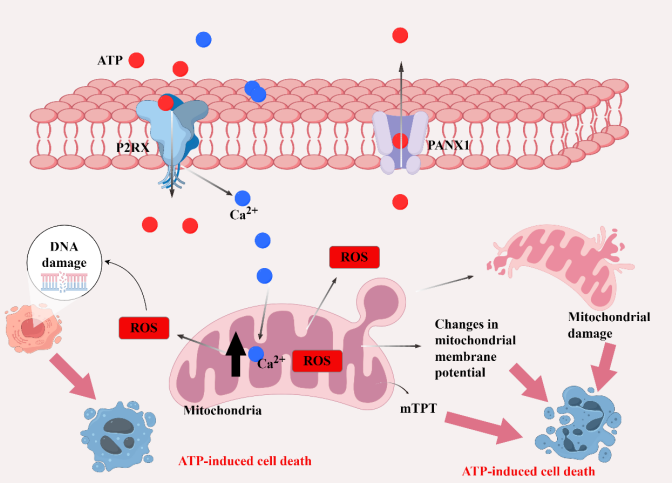

Figure 4. ATP causes the loss of mitochondrial membrane potential, the disruption of mitochondrial integrity, the production of ROS and alterations in mitochondrial membrane permeability, collectively leading to cell deaths

Main Functions of Database

ATPexGen is the world's first dedicated database focusing on ATP-induced cell death regulators and their associations with diseases. It categorizes ATP-induced cell death regulators into two main groups: (1) genes and (2) substances. Gene regulators include drivers, suppressors, markers, and unclassified regulators. Substance regulators encompass a diverse array of chemical entities, ranging from pure substances (e.g., ATP analogs, small molecule inhibitors) to mixtures (e.g., synthetic compounds, natural product extracts). These are further classified as inducers or inhibitors of ATP-induced cell death. Built on this framework, ATPexGen comprises seven independently curated datasets, offering a comprehensive resource for advancing research in this emerging fiel

Gene Silencing

CRISPR-Cas9 technology is a powerful and efficient gene-editing tool vital for functional genomics research. The GeCKO v2 library, developed by Feng Zhang's team and available via Addgene (IDs: #1000000048, #1000000049), comprises 123,411 gRNAs targeting approximately 19,050 human genes. This library enables high-throughput screens to identify key genes involved in biological processes and diseases by precisely disrupting target genes. Its accuracy and scalability make CRISPR particularly well-suited for studying complex genetic networks, especially in cancer research(Supplementary material:Table 1). Part of the show (see: https://grswsci.top/ATPexGen/).

RNA interference (RNAi) employs small RNA molecules to silence genes and is a widely used tool in biological research. Moffat et al. developed lentiviral shRNA libraries targeting transcription factor regulators, available through the GPP Web Portal. These shRNAs reduce mRNA expression, lowering protein levels and inhibiting target gene function. Unlike CRISPR, RNAi offers a reversible approach to gene regulation,

making it particularly suited for time-dependent or long-term studies. Part of the show (see: https://grswsci.top/ATPexGen/).

CTD

The Comparative Toxicogenomics Database (CTD) is a comprehensive, publicly accessible resource designed to enhance understanding of how environmental exposures impact human health. It offers manually curated data on chemical–gene/protein interactions, integrated with functional and pathway information. This integration supports hypothesis generation regarding the mechanisms driving environmentally influenced diseases (Supplementary material:Table 2). Part of the show (see: https://grswsci.top/ATPexGen/).

BioGRID

The Comparative Toxicogenomics Database (CTD) incorporates gene–gene and protein–protein interaction data from BioGRID(Supplementary material:Table 3).Part of the show (see: https://grswsci.top/ATPexGen/).

Primer Design

ATPexGen contains primers for all genes, which can be referenced in later experiments (Supplementary material: Table 4).

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Author Contributions

Wei Wang was responsible for drafting the primary manuscript and conducting the data analyses;Yanfei Wang, Xuqiang Yang, Rui Zhao, DOBLIN SANDAI were involved in the data collection, and preparation of tables and charts, ZhiJing Song,HaoLing Zhang, Rui Zhao had pivotal roles in the research design, guiding the research group, and orchestrating the collaborative efforts of all authors; ZhiJing Song and HaoLing Zhang gave detailed guidance on the paper; All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate

The research did not involve any human participants or animals, and therefore did not require approval from an ethics committee. All data used in this study were obtained from publicly available sources and were analyzed in accordance with ethical guidelines and regulations.

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Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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Research on the Impact of PiCCO-guided Precise Fluid Resuscitation on Hemodynamics and Organ Perfusion in Patients with Extremely Severe Burns

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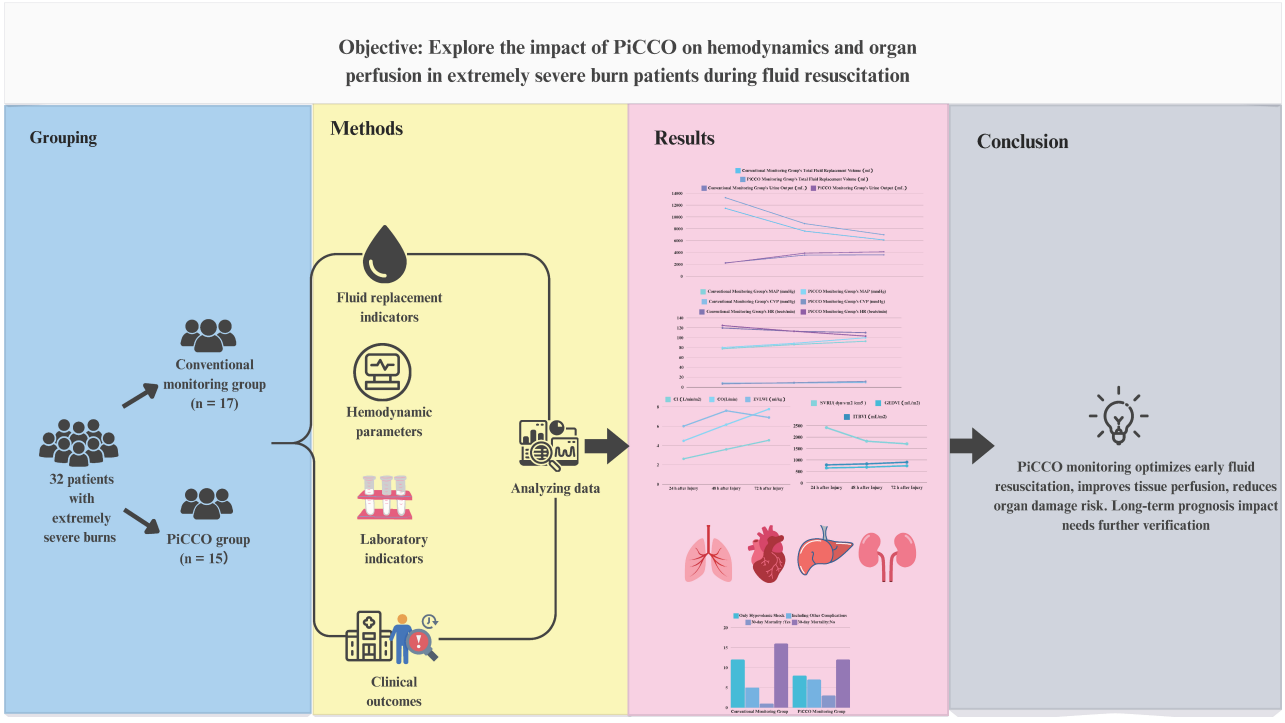
Graphical Abstract



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Research on the Impact of PiCCO-guided Precise Fluid Resuscitation on Hemodynamics and Organ Perfusion in Patients with Extremely Severe Burns

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Objective: To explore the impact of Pulse Indicator Continuous Cardiac Output (PiCCO) on hemodynamics and organ perfusion during fluid resuscitation in patients with extremely severe burns.

Methods: This study adopted a retrospective cohort study design. A total of 32 patients with extremely severe burns admitted to the Burn Department of the First Affiliated Hospital of Anhui Medical University from June 2022 to July 2024 were included. According to the monitoring methods, they were divided into a conventional monitoring group (n = 17) and a PiCCO group (n = 15). The basic data, fluid replacement indicators (total fluid replacement volume, urine output, fluid replacement coefficient, urine output per hour per kilogram of body weight), hemodynamic parameters [mean arterial pressure (MAP), central venous pressure (CVP), heart rate (HR), cardiac index (CI), cardiac output (CO), systemic vascular resistance index (SVRI), extravascular lung water index (EVLWI), global end-diastolic volume index (GEDVI), intrathoracic blood volume index (ITBVI)], laboratory indicators [lactate (Lac), base excess (BE), creatine kinase (CK), creatine kinase - MB (CK - MB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), serum creatinine (Scr)] and clinical outcomes (complication incidence, 30 - day mortality, antibiotic use time, hospital stay and cost) of the two groups were compared. T-test, repeated-measures analysis of variance, Mann-Whitney U test, Bonferroni correction, chi-square test, and Fisher's exact probability method were used for statistical analysis.

Results: A comparison of the relevant indicators of patients with extremely severe burns between the PiCCO monitoring group and the conventional monitoring group showed no difference in the general conditions between the two groups (P>0.05). The total fluid replacement volume in the PiCCO monitoring group was higher in the first, second, and third 24h periods after injury (P<0.05), with no difference in urine output. The fluid replacement coefficient in the first and second 24h periods after injury was also higher (P<0.05), and the urine output per hour per kilogram of body weight was higher than the planned value, but there was no difference between the groups. At 72 hours after injury, the MAP and CVP in the PiCCO monitoring group were higher, and the HR was lower (P<0.05). At 72 hours after resuscitation, Lac and BUN in the PiCCO monitoring group were lower, and BE was higher (P<0.05), with no differences in other indicators. In the PiCCO monitoring group, multiple hemodynamic indicators such as CI, CO, SVRI, EVLWI, GEDVI, and ITBVI showed obvious changing trends over time after injury, and these changes were statistically significant (P<0.05), and these indicators returned to normal at different time points after injury. There were no significant differences in complications, 30-day mortality, antibiotic use days, hospital stay, and hospitalization costs between the two groups (P>0.05).

Conclusion: PiCCO monitoring can optimize the early fluid resuscitation of patients with extremely severe burns by precisely regulating the volume status, improving tissue perfusion, and reducing the risk of organ damage. However, its impact on long-term prognosis needs to be further verified by multi-center and large-sample studies.

Keywords: Extremely Severe Burns; Hemodynamics; PICCO; Fluid Resuscitation.

Abbreviations: PiCCO, Pulse Indicator Continuous Cardiac Output; TBSA, Total Body Surface Area; CI, Cardiac Index; CO, Cardiac output; EVLWI, Extravascular Lung Water Index; SVRI, Systemic Vascular Resistance Index; GEDVI, Global End Diastolic Volume Index; ITBVI, Intrathoracic Blood Volume Index; Lac, Lactic acid; BE, Base Excess; Scr, Serum Creatinine; BUN, Blood urea nitrogen; MAP, Mean Arterial Pressure; CVP, Central venous Pressure; HR, Heart Rate; CK, Creatine Kinase; CK - MB, Creatine Kinase - Myocardial Band; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; RAAS, Renin - Angiotensin - Aldosterone System; MODS, Multiple Organ Dysfunction Syndrome.

Introduction

Burn injury is a global public health problem. Approximately 265,000 people die from burns each year. Especially in low- and middle-income countries, the incidence and mortality rates of burns are higher[1-3]. The pathological process after a burn starts with the capillary leakage syndrome mediated by the inflammatory cascade reaction, which leads to hypovolemic shock and may further trigger multiple organ dysfunction syndrome or even death. As the burn area gradually expands and the burn depth continuously increases, the damage to the body becomes more severe. The incidence of shock significantly rises, and the time of its occurrence is also notably advanced. Extremely severe burns refer to burns with an area of more than 50% of the total body surface area (TBSA); or third-degree burns with an area of more than 20% of the TBSA. These burns often cause extremely serious damage to the body, triggering a series of complex and serious pathophysiological changes, such as systemic inflammatory

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reaction, massive loss of body fluids, tissue and organ damage, which greatly increases the difficulty of treatment and the risk of patient death. Therefore, for the early treatment of patients with extremely severe burns, timely and effective fluid resuscitation is of crucial importance. Early fluid resuscitation for patients with extremely severe burns faces a dual dilemma. The traditional protocol, relying on the formula from the Third Military Medical University and empirical monitoring indicators, has blind spots in hemodynamic assessment. Excessive fluid replacement is likely to cause serious complications such as pulmonary edema and cerebral edema[4], while insufficient fluid replacement will exacerbate organ hypoperfusion and may even lead to acute kidney injury, myocardial injury, etc [5, 6]. Thus, how to scientifically monitor early fluid replacement after injury and reduce related complications has become an urgent problem that needs to be solved.

In clinical practice, the evaluation of early hypovolemic shock after burns has long relied on traditional indicators such as urine output, electrocardiogram monitoring, blood oxygen saturation, and mental status[7, 8]. However, these indicators have obvious limitations. Clinical research and practice have shown that they may appear stable even when tissue perfusion and oxygenation have not been effectively improved, making it difficult to detect subtle changes in the condition, and they are insufficient in sensitivity[9]. This means that even if the vital signs and urine output are normal, it cannot be determined that the resuscitation is sufficient. There may be potential insufficient resuscitation, which can lead to the insidious progression of the condition, increase the risk of complications, and affect the prognosis of patients. Therefore, relying solely on traditional indicators cannot meet the clinical needs for accurate assessment and guidance of fluid resuscitation.

In recent years, hemodynamic monitoring techniques have been widely applied in the fluid management of critically ill patients [10]. The Pulse Indicator Continuous Cardiac Output (PiCCO) monitoring technique, with its advantages of simple operation and minimal invasiveness, has been widely used in clinical practice [11-13]. By integrating the transpulmonary thermodilution method and arterial waveform analysis, this technique overcomes the limitations of the traditional central venous pressure (CVP) that is interfered with by thoracic and abdominal pressure, heart diseases, and mechanical ventilation [14-16]. It can comprehensively and accurately monitor multiple key hemodynamic parameters [17, 18], including Cardiac Output (CO), Extravascular Lung Water Index (EVLWI), Cardiac Index (CI), and Systemic Vascular Resistance Index (SVRI), etc. [19, 20]. Among them, the Global End-Diastolic Volume Index (GEDVI) monitored by PiCCO has been confirmed by numerous studies to be an indicator with high sensitivity and good reproducibility [21, 22]. Moreover, since it is not affected by respiratory movements and myocardial compliance, it can more accurately reflect the preload of the heart. Currently, the PiCCO monitoring technique, with its unique advantages, has been widely applied in the fields of critical care medicine such as sepsis and heart diseases [23-25]. However, in the special and highly challenging clinical scenario of extremely severe burns, its application research is relatively limited. Although this technique is expected to provide more accurate guidance for the treatment of patients with extremely severe burns, the current number of relevant studies is insufficient, and both the depth and breadth need to be expanded. Therefore, an in-depth exploration of the impact of PiCCO-guided precise fluid resuscitation on the hemodynamics and organ perfusion of patients with extremely severe burns is of great significance for optimizing treatment strategies and improving the success rate of treating patients with extremely severe burns.

This study compares the effects of the PiCCO monitoring technique and the conventional monitoring technique in guiding fluid resuscitation for patients with extremely severe burns, explores the clinical value of the PiCCO monitoring technique in fluid resuscitation for patients with extremely severe burns and its role in preventing resuscitation-related complications, and provides a scientific basis for more accurate fluid replacement treatment in clinical practice.

Methods

This study was a retrospective analysis. After being approved by the hospital ethics committee according to the pre-set inclusion and exclusion criteria, the relevant clinical data of 32 patients with extremely severe burns admitted to the Burn Department of the First Affiliated Hospital of Anhui Medical University from June 2022 to July 2024 were collected. On the premise of ensuring the security of patient identity information without leakage, the clinical data could be used for analysis and research.

Inclusion and Exclusion Criteria

Inclusion criteria:

Total burn area > 50% TBSA or third - degree burn area > 20% TBSA; Age between 18 and 65 years old; Admitted to the hospital within 24 hours after injury and received fluid resuscitation; Complete fluid replacement and laboratory data within 3 days after injury.

Exclusion criteria:

Severe cardiovascular diseases (such as aortic aneurysm) or a history of vascular surgery; Puncture contraindications (local burns, infections, or coagulation abnormalities); Complicated with important organ dysfunction or pregnancy; Delayed resuscitation (> 24h) or incomplete data.

Clinical Data and Grouping

A total of 32 patients were enrolled in this clinical study, and were naturally grouped according to the actual clinical monitoring means: the routine monitoring group (n=17) received routine vital signs monitoring, while the PiCCO monitoring group (n=15) used PICCO detection technology for hemodynamic monitoring. The grouping was based on the clinical interventions actually received by the patients, and a non-randomized observational study design was used to minimize artificial selection bias. There were no statistically significant differences between the two groups in terms of general baseline data such as gender, age, weight, cause of injury, burn area, admission time, degree of inhalation injury and past history (P>0.05), indicating that the two groups were comparable. See Table 1.

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Table 1. Comparison of general data at admission between the two groups of patients with extremely

Group	Number of Cases	Gender		Cause of Injury		Age (years, $\bar{X}$±s)	Weight (kg, $\bar{X}$±s)	Admission Time (h, $\bar{X}$±s)	Total Burn Area (%TBSA, $\bar{X}$±s)	Inhalation Injury			Past Medical History	
		Male	Female	Flame	Hot Liquid					Mild	Moderate	Severe	Yes	No
Conventional Monitoring Group	17	16	1	16	1	47.59 ±9.88	73.82 ±11.71	5.18±3.13	83.06±9.37	8	2	7	3	14
PiCCO Monitoring Group	15	11	4	13	2	47.67 ±11.57	69.00 ±9.83	7.10±5.53	88.57±9.29	3	1	11	5	10
t value	-	-		-		-0.021	1.252	-1.190	-1.666	-			-	
P value	-	0.161*		0.589*		0.984	0.220	0.247	0.160	0.225*			0.423*	

Note: PiCCO denotes Pulse Contour Cardiac Output, and TBSA represents Total Body Surface Area. "—" signifies the absence of a statistical value, while "*" indicates the application of the Chi-square test.

Treatment Methods

General Treatment

Upon admission, intravenous access was immediately established for fluid resuscitation and anti-shock treatment in both groups of patients with extremely severe burns. Vital signs and fluid intake and output were closely monitored. Tracheotomy or fasciotomy was performed when necessary. The dressing - change or exposure therapy was selected according to the wound surface, and dressing changes were carried out regularly. Early esophagectomy (tangential excision) and skin grafting or debridement and skin grafting were performed. Broad-spectrum antibiotics (such as imipenem) were empirically used and then adjusted according to the culture results. Enteral and parenteral nutritional support was provided. Stress ulcers (such as omeprazole) and myocardial injury were prevented. Multidisciplinary cooperation and early rehabilitation intervention were carried out to ensure comprehensive treatment.

Fluid Replacement Method

Fluid resuscitation was carried out in both groups of patients with extremely severe burns according to the fluid replacement formula for the shock stage of the Third Military Medical University. The total fluid replacement volume = burn area (%)×body weight (kg)×1.5 (the sum of crystalloid and colloid) + 2000 ml (basic water). For patients with second-degree to third-degree burns, 1.5 ml of fluid was replenished per 1% burn area per kilogram of body weight, and the ratio of crystalloid to colloid was 2:1. At the same time, 2000 ml of 5% glucose was supplemented. Within the first 24 hours, half of the total volume was replenished in the first 8 hours, and then one-fourth of the total volume was replenished in the second 8-hour period and the third 8-hour period respectively; the total fluid replacement volume in the second 24 - hour period was half of the actual fluid replacement volume in the first 24 - hour period, the ratio of crystalloid to colloid remained unchanged, and 2000 ml of physiological requirement was infused at a constant speed.

Conventional Monitoring Group

A single-lumen central venous catheter from Arrow Company in the United States was used. After catheterization via the subclavian vein or femoral vein, the CVP was dynamically monitored through the GE DASH4000 monitoring system, and the pressure zero point was regularly calibrated.

PiCCO Monitoring Group

The prepared equipment and materials included a multi-function monitor (equipped with a PiCCO module), a double-lumen central venous catheter, a PiCCO thermodilution catheter device, a pressure sensor, 100 mL of sterile normal saline at 4℃ - 8℃, 100 mL and 500 mL of sterile normal saline, a pressure bag, etc. Bedside deep-vein puncture and catheterization were performed. The right internal jugular vein was the first choice, followed by the subclavian vein or femoral vein. After inserting the double-lumen central venous catheter, the CVP lead wire was connected to continuously monitor the change of CVP, and the temperature probe was connected to the CO module through a three-way connector. After femoral artery catheterization, the PiCCO thermodilution catheter was inserted, and the PiCCO module was connected to the wire. Before monitoring, fluid replacement was stopped, the arterial and venous zero points were calibrated, and strict aseptic operation was performed. 15 mL of sterile normal saline at 4℃ - 8℃ was uniformly injected into the central venous catheter,

with an injection time of 4 - 7 seconds, and repeated 3 times. The monitor automatically calculated the average value to obtain parameters such as EVLWI, GEDVI, ITBVI, and CI. After the monitoring system was set up, the circulatory status at the time of admission was immediately measured and recorded. Thereafter, monitoring was carried out every 8 hours, and the average value was taken from 3-time monitoring per day on the 1st - 3rd day after injury. The fluid replacement plan was dynamically adjusted based on PiCCO monitoring parameters (CI, CO, GEDVI, ITBVI, EVLWI, SVRI).

Statistical Indicators

(1) Fluid resuscitation indicators: The total fluid replacement volume and urine output of the two groups of patients in the first, second, and third 24h periods after injury were statistically analyzed. The fluid replacement coefficients in the first and second 24h periods after injury were calculated (formula: fluid replacement coefficient = [actual total fluid replacement volume - basic water]÷body weight÷total burn area), as well as the urine output per hour per kilogram of body weight. The above-measured data were compared with the standardized fluid replacement plan formulated by the Third Military Medical University (hereinafter referred to as the "planned value"). Among them, the planned values of the fluid replacement coefficients in the first and second 24h periods were 1.50 $mL \cdot kg^{-1} \cdot \%TBSA^{-1}$ and 0.75 $mL \cdot kg^{-1} \cdot \%TBSA^{-1}$ respectively, and the planned value of the urine output per hour per kilogram of body weight was 1.00 $mL \cdot kg^{-1} \cdot h^{-1}$.

(2) Vital sign indicators: MAP, CVP, and HR at 24h, 48h, and 72h after injury.

(3) Key resuscitation indicators and organ function indicators: Blood samples were collected at admission and after resuscitation (72h after injury) to detect the following indicators: Metabolism-related indicators: Lac and BE; Cardiac function indicators: CK and CK - MB; Liver function indicators: ALT and AST; Renal function indicators: Scr and BUN.

(4) Hemodynamic indicators: CI, CO, GEDVI, ITBVI, EVLWI, SVRI, etc. at 24h, 48h, and 72h after injury.

(5) Prognosis-related indicators: The complications, 30-day mortality after injury, antibiotic use days, hospital stay, and hospitalization costs of the two groups of patients were statistically analyzed.

Statistical Processing

In this study, key data were cross-validated against multiple sources of data, and the various data recorded were compared with the nursing records and medical records in the electronic medical record system to ensure data consistency. For missing data, we first assessed the missing mechanism, used multiple interpolation to process random missing data, and assessed the robustness of the processing method by sensitivity analysis. All reference time points (e.g., admission time, PiCCO monitoring start time) were aligned on a uniform timeline, and clinical parameters (e.g., CO, SVRI) were aligned with the PiCCO device output files to ensure consistency and accuracy of records.

Data were analyzed using SPSS 26.0 software to obtain data. Measurement data conforming to the normal distribution were expressed as $\overline{X}\pm s$. Repeated-measures analysis of variance was performed for the overall comparison between groups at multiple time points. The independent-sample t-test was used for the comparison between the two groups at each time point and between the two groups. The - sample t-test was used for the comparison between the data at each time point and the planned value and Bonferroni correction was performed for all; measurement data not conforming to the normal distribution were expressed as M (P25, P75), and the Mann - Whitney U test was used and Bonferroni - corrected; count data were expressed as frequencies and percentages, and the X2 test or Fisher's exact probability method was used (the software automatically ignored the statistical value). A difference was considered statistically significant when $P<0.05$, and a significant difference was considered to exist when $P<0.01$.

Table 2. Comparison of total fluid intake and urine output levels at various time points post-injury between the two groups of severely burned patients. ($\overline{X}\pm s$, ml)

Group	Number of Cases	Total Fluid Replacement Volume (ml)			Urine Output (ml)		
		1st 24 h	2nd 24 h	3rd 24 h	1st 24 h	2nd 24 h	3rd 24 h
Conventional Monitoring Group	17	11456.47 ±1741.62	7610.06 ±1516.02	6114.12 ±743.73	2249.12 ±691.37	3551.47 ±1254.11	3599.12 ±1509.90
PiCCO Monitoring Group	15	13260.00 ±1496.48	8878.33 ±1398.46	6990.67 ±1084.35	2198.00 ±351.22	3878.67 ±1210.92	4109.53 ±1283.67
t value	-	-3.120	-2.448	-2.694	0.258	-0.748	-1.023
P value	-	0.004	0.020	0.011	0.798	0.460	0.315

Note: PiCCO stands for Pulse Contour Cardiac Output.

Results

Fluid Resuscitation Indicators

The total fluid replacement volume in the first, second, and third 24h periods after injury in the PiCCO monitoring group was significantly higher than that in the conventional monitoring group (t=-3.120, -2.448, -2.694, P=0.004, 0.020, 0.011), but there was no statistically significant difference in urine output (t=0.258, -0.748, -1.023, P=0.798, 0.460, 0.315), as shown in Table 2. The fluid replacement coefficients in the first and second 24h periods in the PiCCO monitoring group were higher than those in the conventional monitoring group (t1=-2.263, -2.149, P1=0.031, 0.040). The fluid replacement coefficient in the first 24- h period in the conventional monitoring group was close to the planned value (t2=0.947, P2=0.358), and it was significantly higher than the planned value in the second 24h period (t2=3.016, P2=0.008). The fluid replacement coefficients in the first and second 24h periods in the PiCCO monitoring group were significantly higher than the planned values (t3=4.019, 4.542, P3 both<0.001). There was no statistically significant difference in urine output per hour per kilogram of body weight between the two groups in the first and second 24h periods (t1=-0.307, -1.115, P1=0.761, 0.274), but it was significantly higher than the planned value in both groups (in the conventional group, t2=2.758, 4.646, P2=0.014<0.001; in the PiCCO group, t3=5.618, 6.064, P3 both<0.001), as shown in Table 3.

Vital sign indicators

At 24h and 48h after injury, there were no statistically significant differences in the levels of MAP, CVP, and HR between the two groups (P>0. 05). At 72h after injury, the MAP and CVP in the PiCCO monitoring group were higher than those in the conventional monitoring group, and the HR was lower than that in the conventional monitoring group, with statistically significant differences (P<0. 05). See Table 4.

Key resuscitation indicators and organ function indicators

There were no statistically significant differences in the levels of Lac and BE between the two groups upon admission (P>0.05). After resuscitation (72 hours after admission), the level of Lac in the PiCCO monitoring group was significantly lower than that in the conventional monitoring group (P<0.001), and the level of BE was significantly higher than that in the conventional monitoring group (P<0.05). See Table 5. There was no statistically significant difference in the level of BUN between the two groups upon admission (P>0.05). After resuscitation (72 hours after admission), the level of BUN in the PiCCO monitoring group was significantly lower than that in the conventional monitoring group (P<0.05). There were no statistically significant differences in the levels of CK, CK - MB, ALT, AST, and Scr between the two groups upon admission and after resuscitation (72 hours after admission) (P>0.05). See Table 6.

Table 3. Comparison of fluid resuscitation coefficients ($\bar{X}$±s, mL·kg^{-1}·%TBSA^{-1}) and urine output per kilogram of body weight per hour ($\bar{X}$±s, ml/(kg·h) at various time points post-injury between the two groups of severely burned patients.

Group	Number of Cases	Fluid replacement coefficient (mL·kg^{-1}·%TBSA-1)		Urine output per hour per kilogram of body weight (ml/(kg·h)	
		1st 24 h	2nd 24 h	1st 24 h	2nd 24 h
Conventional Monitoring Group	17	1.59±0.38	0.93±0.25	1.31±0.46	2.07±0.89
PiCCO Monitoring Group	15	1.89±0.37	1.17±0.36	1.35±0.24	2.42±0.91
t1 value	-	-2.263	-2.149	-0.307	-1.115
P1 value	-	0.031	0.040	0.761	0.274
t2 value	-	0.947	3.016	2.758	4.646
P2 value	-	0.358	0.008	0.014	<0.001
t3 value	-	4.019	4.542	5.618	6.064
P3 value	-	0.001	<0.001	<0.001	<0.001

Note: PiCCO stands for Pulse Contour Cardiac Output, and TBSA stands for Total Body Surface Area. The fluid replacement plan values of the Third Military Medical University for the fluid replacement coefficients in the first and second 24-hour periods are 1.50 and 0.75 mL·kg^{-1}·%TBSA^{-1} respectively. The fluid replacement plan values of the Third Military Medical University for the urine output per hour per kilogram of body weight in the first and second 24-hour periods are both 1.00 mL. The t1 values and P1 values are obtained by comparing each index of the PiCCO monitoring group with that of the conventional monitoring group at each time point. The t2 values, P2 values, t3 values, and P3 values are obtained by comparing each index of the conventional monitoring group and the PiCCO monitoring group with the planned values at each time point respectively.

Hemodynamic indicators

In the PiCCO monitoring group, the CI, CO, SVRI, EVLWI, GEDVI, and ITBVI of patients showed obvious changing trends over time after injury, and these changes were statistically significant($P<0.05$). Multiple hemodynamic indices of patients in the PiCCO monitoring group showed specific changing trends after injury. The GEDVI was lower than normal at 24 h after injury, then gradually increased, and returned to normal at 48 h and 72 h. The ITBVI was lower than normal at 24 h and 48 h after injury, then gradually increased, and reached the normal level at 72 h. The CI was lower than normal at 24 h after injury, then gradually increased, and returned to normal at 48 h and 72 h. The SVRI was higher than normal at 24 h after injury, then gradually decreased, and returned to normal at 48 h and 72 h. The CO continuously increased after injury, and the average values at the three time points were all within the normal range. The EVLWI increased at 24 h and 48 h after injury and decreased at 72 h, and the mean values at each time point did not exceed 10 mL/kg. See Table 7.

Table 4. Comparison of MAP ($\bar{X}\pm s$, mmHg), CVP ($\bar{X}\pm s$, mmHg), and HR ($\bar{X}\pm s$, beats/min) levels at various time points post-injury between the two groups of severely burned patients.

Group	Number of Cases	MAP (mmHg) at 24 hours after injury	MAP (mmHg) at 48 hours after injury	MAP (mmHg) at 72 hours after injury	CVP (mmHg) at 24 hours after injury	CVP (mmHg) at 48 hours after injury	CVP (mmHg) at 72 hours after injury	HR (beats/min) at 24 hours after injury)	HR (beats/min) at 48 hours after injury	HR (beats/min) at 72 hours after injury
Conventional Monitoring Group	17	77.55 ±6.30	86.10 ±8.57	92.77 ±8.02	8.11 ±3.33	8.15 ±3.20	9.24 ±3.07	119.65 ±8.90	113.06 ±7.47	110.24 ±9.32
PiCCO Monitoring Group	15	79.91 ±9.25	88.53 ±7.86	99.94 ±4.88	6.53 ±2.47	8.87 ±3.18	11.4 ±2.73	124.73 ±10.05	113.00 ±5.13	103.20 ±6.89
T value	-	-0.856	-0.835	-3.005	1.512	-0.637	-2.097	-1.518	0.026	2.399
P value	-	0.399	0.410	0.005	0.141	0.529	0.044	0.139	0.980	0.023

Note: PiCCO stands for Pulse Contour Cardiac Output. 1 mmHg = 0.133 kPa. The range of mean arterial pressure (MAP) is 70-105 mmHg; the range of central venous pressure (CVP) is 8-12 mmHg; the range of heart rate (HR) is 60-100 beats/min.

Table 5. Comparison of the levels of Lac ($\bar{X}\pm s$, mmol/L) and BE ($\bar{X}\pm s$, mmol/L) between the two groups of patients with extremely severe burns upon admission and after resuscitation (72 hours after admission).

Group	Number of Cases	Lac at Admission (mmol/L)	Lac after Resuscitation (mmol/L)	BE at Admission (mmol/L)	BE after Resuscitation (mmol/L)
Conventional Monitoring Group	17	4.95±2.05	2.79±0.86	-5.52(-7.10, -3.21)	-1.60(-2.94, 0.25)
PiCCO Monitoring Group	15	5.05±2.32	1.62±0.54	-5.00(-7.00, -2.50)	1.50(-1.30, 4.50)
T value / Z value	-	-0.121	4.533	-0.567	-2.476
P value	-	0.904	<0.001	0.571	0.013

Note: PiCCO stands for Pulse Contour Cardiac Output. Lac stands for blood lactate, and BE stands for base excess.

Table 6. Comparison of the Levels of CK and CK - MB, ALT and AST, BUN and Scr between the two groups of severely burned patients upon admission and after resuscitation (72 hours after admission).

Group	Number of Cases	CK at Admission (U/L)	CK after Resuscitation (U/L)	CK - MB at Admission (U/L)	CK - MB after Resuscitation (U/L)	ALT at Admission (U/L)	ALT after Resuscitation (U/L)	AST at Admission (U/L)	AST after Resuscitation (U/L)	BUN at Admission (mmol/L)	BUN after Resuscitation (mmol/L)	Scr at Admission (μmol/L)	Scr after Resuscitation (μmol/L)
Conventional Monitoring Group	17	265.60 (118.00, 669.50)	130.00 (87.50, 468.00)	77.00(51.50, 145.00)	38.00 (21.00, 54.00)	38.00 (25.50, 65.50)	16.00 (13.00, 21.50)	72.00 (55.50, 92.00)	19.00 (17.00, 24.50)	8.13 ±1.45	5.79 ±1.87	91.75±17.79	68.36±12.45
PiCCO Monitoring Group	15	1166.00 (208.70, 18761.00)	532.00 (145.70, 4835.00)	110.00 (64.10,140.00)	49.00 (28.00, 88.00)	28.00 (21.00, 49.00)	19.00 (16.00, 51.00)	72.20 (55.00, 280.00)	19.30 (19.00, 126.00)	8.07 ±3.35	4.55 ±0.77	114.14 ±47.34	63.18±13.82
T value /Z value	-	-1.454	-1.605	-0.548	-0.737	-1.171	-1.684	-0.718	-1.805	0.067	2.508	-1.727	1.116
P value	-	0.146	0.108	0.584	0.461	0.242	0.092	0.473	0.071	0.947	0.020	0.102	0.273

Note: PiCCO stands for Pulse Contour Cardiac Output; CK stands for Creatine Kinase, and CK - MB stands for Creatine Kinase - MB Isoenzyme (Myocardial Type); ALT stands for Alanine Aminotransferase; AST stands for Aspartate Aminotransferase; Scr stands for Serum Creatinine, and BUN stands for Blood Urea Nitrogen.

Table 7. Comparison of hemodynamic indicators at various time points post-injury in the PiCCO monitoring group ($\bar{X}$±s)

Time	Number of Cases	CI(L/min/m2)	CO(L/min)	EVLWI(ml/kg)	SVRI/ (dyn·s·m^2 /cm^5)	GEDVI (mL/m^2)	ITBVI (mL/m^2)
24 h after Injury	15	2.62±0.72	4.48±1.07	6.0±1.6	2415.19 ±515.07	653±109	786±137
48 h after Injury	15	3.60±0.71	6.16±1.06	7.6±1.1	1821.34 ±344.62	688±102	831±128
72 h after Injury	15	4.54±1.06	7.77±1.63	6.9±1.5	1705.53 ±315.07	747±140	904±175
F value	-	56.615	55.075	6.901	28.759	13.210	15.387
P value	-	<0.001	<0.001	0.004	<0.001	0.001	<0.001

Note: PiCCO represents Pulse Contour Cardiac Output. The normal value of Global End-Diastolic Volume Index (GEDVI) is 680-800 mL/m², the normal value of Intrathoracic Blood Volume Index (ITBVI) is 850-1000 mL/m², the normal value of Cardiac Index (CI) is 3-5 L·min^{-1}·m^{-2}, the normal range of Cardiac Output (CO) is 4-8 L/min, the normal value of Extravascular Lung Water Index (EVLWI) is 3.0-7.0 mL/kg, and the normal value of Systemic Vascular Resistance Index (SVRI) is 1700-2400 dyn·s·cm^{-5}·m^2.

Prognosis - related indicators

There were no statistically significant differences in the complications, 30-day mortality, number of days of antibiotic use, length of hospital stay, and hospitalization expenses between the two groups of patients (P > 0.05). See Table 8.

Discussion

Early treatment of extremely severe burns hinges on swiftly restoring the effective circulating blood volume during the shock phase and enhancing microcirculation and oxygen delivery. As burn area enlarges and depth deepens, fluid replacement requirements surge [26]. Proactive prevention and treatment of early burn shock are pivotal for successful outcomes. Currently, the "timely, rapid, and sufficient" principle should be adhered to in early burn fluid resuscitation. Nevertheless, accurately controlling the fluid replacement volume via traditional formulas remains challenging. Clinically, reliable hemodynamic evaluation metrics are urgently needed for precise fluid resuscitation. Given the complex pathophysiology and unstable hemodynamics of patients with extremely severe burns, establishing a dependable monitoring system is of great significance. Non-invasive monitoring is user-friendly but prone to interference, while invasive monitoring offers precise parameters at the cost of complex operations, high costs, and potential complications[27, 28].

In recent years, the PiCCO monitoring technology has emerged as a minimally invasive approach with remarkable advantages. By integrating the transpulmonary thermodilution method and arterial pulse contour analysis, it enables continuous and dynamic hemodynamic monitoring, providing parameters such as cardiac output and EVLWI. It is easy to operate and has few complications [29, 30]. PiCCO has enhanced hemodynamic stability and reduced the incidence of Multiple Organ Dysfunction Syndrome(MODS) in sepsis patients. However, its benefits in extremely severe burn patients are yet to be fully validated. Early provision of accurate hemodynamic data can assist clinicians in refining treatment plans.

Blood lactate and base excess are vital for assessing shock and resuscitation, as they can sensitively reflect tissue hypoperfusion and changes in effective circulating blood volume [31]. The initial 24 hours post-burn is the "golden window" for fluid resuscitation [32]. This study compared the PiCCO and conventional monitoring groups and found that in the first 3 post - injury 24 - hour periods, the total fluid replacement volume in the PiCCO group was significantly higher. The difference in the fluid replacement coefficient was more pronounced in the first 2 24-hour periods. Both groups presented with ischemia and hypoxia upon admission. After 72-hour fluid resuscitation, blood lactate and base excess values improved in both groups, with more significant improvements in the PiCCO group. This could be because traditional - indicator - guided resuscitation may lead to insufficient fluid replacement, while PiCCO can accurately assess volume status and offers more advantages in managing early shock correction in extremely severe burns. The study also found that the fluid replacement coefficient in the PiCCO group exceeded the standard protocol value in the first 2 24-hour periods, and the conventional group also surpassed it in the second 24-hour period. This might be due to the current protocol's failure to fully account for burn depth. Since deep burns demand more fluid, and the standard value is a theoretical estimate with large individual variations, clinically, multiple factors should be considered, and individualized resuscitation strategies should be combined with dynamic monitoring[26, 33]. Multiple studies have shown that shock patients often require more fluid than calculated by traditional formulas, and this study indicates that the PiCCO group better meets the needs of extremely severe burn patients.

Table 8. Comparison of complications, 30-day mortality, days of antibiotic use, length of hospital stay, and hospitalization costs between the two groups of patients with extremely severe burns.

Group	Number of Cases	Complications		30-day Mortality		Antibiotic Use Duration (days)	Hospitalization Duration (days)	Hospitalization Expenses (10,000 yuan)
		Only Hypovolemic Shock	Including Other Complications	Yes	No			
Conventional Monitoring Group	17	12	5	1	16	42(30, 58)	54(33, 73)	56.70(30.10, 73.05)
PiCCO Monitoring Group	15	8	7	3	12	47(6, 70)	47(6, 72)	71.10(39.90, 126.80)
Z value	-	-		-		-0.132	-0.434	-1.152
P value	-	0.467*		0.319*		0.895	0.664	0.249

Note: PiCCO represents Pulse Contour Cardiac Output; * indicates the Chi-square test; "—" indicates that there is no value for this statistic.

Urine output, a traditional shock resuscitation indicator, can reflect renal perfusion and systemic circulation. However, normal absolute urine output does not rule out tissue hypoperfusion. Using urine output per kilogram of body weight per hour as a standardized parameter can more sensitively evaluate tissue perfusion and is a more reliable resuscitation endpoint indicator. In the first 2 24-hour periods, this indicator was higher than the protocol value in both groups, possibly due to diuretic use. In the second 24-hour period, the PiCCO group had a slightly higher value, suggesting its advantage in shock improvement.

Hemodynamic indicators are crucial for evaluating patient condition and resuscitation efficacy. At 24 and 48 hours post-injury, the two monitoring methods had similar hemodynamic effects. However, at 72 hours, the PiCCO group had significantly higher mean arterial pressure (MAP) and central venous pressure (CVP) and a significantly lower heart rate (HR), indicating its potential in fluid resuscitation to ensure blood flow stability and enhance cardiac function.

Extremely severe burns can damage the myocardium, kidneys, and liver. The sudden drop in effective circulating blood volume activates the Renin - Angiotensin - Aldosterone System (RAAS), leading to the "shock heart", i.e., the heart may experience accelerated heart rate, altered myocardial contractility, and decreased cardiac output, accompanied by cardiomyocyte ischemia and hypoxia, impaired energy metabolism, and even cardiomyocyte damage, apoptosis, and impaired cardiac function. A state of the heart in which the function and structure of the heart are abnormally altered in a state of shock. Thus, myocardial protection should be emphasized during early shock fluid resuscitation [34]. A moderate increase in heart rate can improve coronary perfusion, while excessive increases can worsen myocardial damage. The study showed that at 72 hours post-injury, the PiCCO group had a lower and more rapidly decreasing heart rate ($p < 0.05$), which was beneficial for cardiac function, possibly due to individualized fluid management and myocardial protection drugs. There was no significant difference in traditional myocardial injury indicators like CK - MB between the two groups ($p > 0.05$), possibly due to their lack of specificity [35]. Renal function impairment is a common and serious complication, and preventing acute kidney injury is crucial. After severe burns, factors such as fluid loss and RAAS activation exacerbate renal insufficiency [36, 37]. Timely fluid resuscitation is the key. Abnormal increases in blood urea nitrogen and serum creatinine can diagnose AKI [38, 39]. There was no significant difference in these indicators between the two groups upon admission ($P > 0.05$), and they normalized after 72-hour resuscitation. The PiCCO group had lower blood urea nitrogen ($P < 0.05$), suggesting that PiCCO can precisely guide resuscitation and improve renal perfusion and function. Extremely severe burns can damage liver cells, causing elevated liver enzymes. In this study, there was no significant difference in ALT and AST levels between the PiCCO and conventional groups upon admission and 72 hours after resuscitation ($P > 0.05$), indicating similar liver function protection effects. Liver injury is mainly influenced by burn severity and systemic inflammation, and ALT/AST is insensitive to early-stage resuscitation strategies.

The PiCCO group initially exhibited "low cardiac output and high peripheral resistance" shock characteristics. The cardiac index (CI) was below normal at 24 hours post-injury and gradually recovered after 48 hours. The systemic vascular resistance index (SVRI) was above normal at 24 hours and decreased after 48 hours. The global end-diastolic volume index reflects the recovery of effective circulating blood volume. The extravascular lung water index increased at 48 and 72 hours post-injury, suggesting possible excessive fluid replacement in the second 24-hour period. Its decrease from 48 to 72 hours indicates recovering pulmonary vascular endothelial barrier function, necessitating optimization of the fluid replacement strategy. Based on this, comprehensive treatment during the shock period should include using positive inotropic drugs for myocardial contraction dysfunction and appropriate vasodilators. During the absorption - return period, vasoactive drugs should be used rationally, diuresis enhanced, and fluid input controlled. The PiCCO monitoring system provides valuable references for individualized treatment.

Prognosis-related indexes showed that the differences in complication rates and mortality rates between the two groups were not statistically significant. In conclusion, this study has the limitation of a small sample size, and at the same time, as a retrospective study, it is difficult to completely avoid bias. In addition, potential confounding factors such as patients' comorbidities may have affected the results of this study to different degrees. In future studies, we will consider enlarging the sample size and extending the follow-up period, conducting prospective studies, and strengthening the control and analysis of potential confounders to more accurately assess the clinical value of PiCCO monitoring in the treatment of patients with very severe burns.

Conclusion

This study compared PiCCO and conventional monitoring in extremely severe burn fluid resuscitation and confirmed the PiCCO group's advantages in improving tissue perfusion and organ function protection. PiCCO provides precise data for formulating individualized fluid resuscitation strategies by continuously monitoring key parameters such as CI and GEDVI. Although there were no significant differences in endpoint indicators like complication incidence and mortality, PiCCO significantly optimized the resuscitation process. Based on current evidence, PiCCO monitoring is recommended for patients with extremely severe burns and unstable hemodynamics. Future multi-center large-sample studies are needed to further validate its clinical value in improving long-term patient prognosis.

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Author Contributions

Lulu Jiang: Writing – original draft, Writing–review & editing, Visualization, Conceptualization, Formal analysis, Software, Methodology, Investigation. Yindong Wu: Data curation, Validation. Zijian Zhang: Project administration, Resources. Youxin Yu: Project administration, Resources. Yi Hu: Supervision. Delin Hu: Supervision.

Ethics approval and consent to participate

All data used in this study were analyzed in accordance with ethical guidelines and regulations.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data generated or analyzed in this study are available from the corresponding authors upon reasonable request.

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Research Article

Life Conflux

Inflammatory Responses of High Mobility Group Protein B1 in Disease: Current Trends, novel Insights, and challenges

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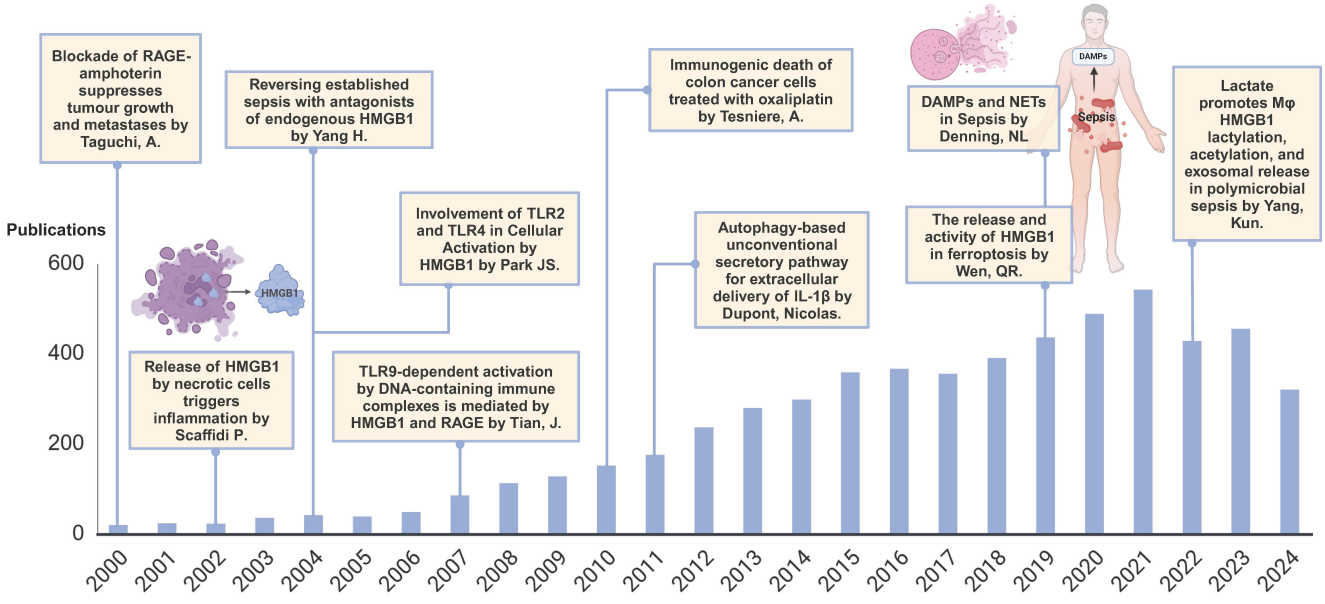
Graphical Abstract



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Research Article

Life Conflux

Inflammatory Responses of High Mobility Group Protein B1 in Disease: Current Trends, novel Insights, and challenges

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Abstract: HMGB1, a key member of the HMG protein family, stabilizes nucleosomes and mediates infection, injury, and late inflammatory responses. Its related research is crucial in critical care medicine, cancer, and neurological diseases, yet current reports inadequately present its status. This review makes a comprehensive bibliometric analysis of the past hmgb1 research, and makes a graphical analysis of the trends and hotspots of HMGB1-related research. From 1976 to 2024, a total of 5992 articles were included, and the number of published papers and citations showed an increasing trend. China and the United States are leading contributors in this field, with the University of Pittsburgh being the most prominent research institution. Among the authors, Billiar, Timothy R. is the most prolific author, while Yang, H. ranks first in co-citation frequency. Inspection of the literature and keywords reveals that disease and its clinical diagnosis and treatment, cell death, mechanism, and molecular pattern are popular research topics. About disease frontiers, critical care medicine (sepsis, endotoxemia, severe viral hemorrhagic fever, multiple organ failure) is the earliest identified and very popular direction of HMGB1, and neurological diseases-related research are at the forefront of the field. In summary, this study employs bibliometrics to identify, visualize, and discuss trends and hotspots in HMGB1 research. Through comprehensive analysis of extensive literature, the aim is to clarify the current research status of HMGB1-related diseases, understand major trends, find potential collaborators, and discover promising and innovative research directions.
Keywords: HMGB1, disease, inflammatory responses.

Introduction

1973 marked the first extraction and identification of the high mobility group (HMG) from calf thymus, the name of which derived from its high mobility in polyacrylamide gel electrophoresis[1,2]. As the most abundant HMG protein, the evolution of High Mobility Group Protein B1 (HMGB1) is highly conserved, which is also known as amphoterin[3]. HMGB1 is believed to be a nuclear protein that occurs in virtually all eukaryotic cells, binds loosely to chromatin, and serves to stabilize nucleosome formation[4-6]. It functionally like a transcription factor which regulates the encoding of multiple genes[7-9]. As research has progressed, it has become increasingly clear that when cells are subjected to stress, injury, or inflammatory stimuli, HMGB1 can be actively secreted or passively released into the extracellular environment, transforming it into a potent pro-inflammatory mediator[10]. In the first case, HMGB1 is released following acetylation in the nucleus during active modification and can be actively secreted by activated macrophages, mature dendritic cells, and activated N.K. cells[11,12]. This process is dependent on lipopolysaccharide-mediated (LPS-mediated) signaling via the TLR4-CD14 complex as well as TNF and TGF-β, which elicit HMGB1 migration from the nucleus and initiate the release of hyperacetylated HMGB1[13]. The second place is the passive departure of active HMGB1 from the cell, which frequently occurs during cell death, such as necrosis[10]. After its release, HMGB1 can transduce cellular signals by binding to at least three receptors: RAGE, TLR2, and TLR4. Signaling via RAGE activates the NF-κB signaling pathway, triggering cell survival and generating cytokines, including TNF, IL-6, and IFN-γ via signaling by ERK and p38[14]. TLR2 and TLR4 binding to HMGB1 activates the NF-κB pathway through MyD88-dependent mechanism[15,16]. These receptor-mediated signaling pathways can promote leukocyte chemotaxis, increase vascular permeability, and upregulate the expression of other pro-inflammatory factors, ultimately leading to enhanced inflammatory responses both locally and systemically.

In addition, HMGB1 has been shown to have a regulatory effect on the immune system[17,18]. Extracellular HMGB1, acting as one of the damage-associated molecular pattern (DAMP) molecules, can activate the innate immune system and has been recognized in recent years for its cytokine-like functions[19]. Under specific conditions, it also influences adaptive immune responses, such as by modulating the maturation of dendritic cells and the function of T cells. Therefore, it has been causally linked to a wide spectrum of diseases, including sepsis[20], myocardial infarction[21],

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atherosclerosis[22], ischemia-reperfusion injury[23], diabetes mellitus[24], rheumatoid arthritis[25], systemic lupus erythematosus[26], liver[27] and pulmonary fibrosis[28], SARS-CoV-2[29], cancer[30] and neurodegeneration[31].

Since the 1960s, with the popularization of the concept of open science, bibliometrics has ushered in remarkable development. The Internet has facilitated the open exchange and free sharing of scientific research results, and this revolution has had a profound impact on bibliometrics, from data analysis to support systems, to evaluation criteria and access to statistical information. By means of analyzing research in a specific field over a specific period, bibliometric analysis employs qualitative and quantitative methods, along with mathematical and statistical approaches[32]. This method focuses on disclosing the structure of countries, institutions, journals, authors, and keywords associated with research in that field, thereby presenting readers with an unbiased portrayal of trends and frontiers in the subject[33,34]. Bibliometric analysis has found utility across various research domains, such as innate immunity[35], pyroptosis[36], ferroptosis[37] , etc. Despite the recent exponential growth in HMGB1-related research, only limited efforts have been made to systematically assess the global scientific outcomes and status of this field.

As a result, a suitable visualization manner is urgently required to show the current state, potential trends, and hotspots of HMGB1-related research. Therefore, this study aimed to evaluate the overall picture of HMGB1-related research from 1976-2024 using VOSviewer, CiteSpace, and Bibliometrix (R-Studio's R-Tool) to identify the journals, institutions, and authors with the highest impact to enhance collaboration and learning. More importantly, these data will provide clinicians and researchers with future directions on the inflammatory response to HMGB1-related diseases to more fully present the current state of the field, potential trends, and hot spots.

Materials and Methods

Data source

The data for the Bibliometrics in this study were sourced from the Web of Science Core Collection database (WOSCC), a standardized database that is frequently utilized in academia for scientific research and analysis. In WOSCC, T.I. denotes title, and A.K. denotes author keywords. The search strategy used in this research was TI=("HMGB1" OR "HMG1" OR "FM1 Gene Product" OR "HMG-1 Protein" OR "Box Protein 1, High Mobility Group" OR "Amphoterin" OR "HMGB1 Protein" OR "HMG 1 Protein" OR "Heparin-Binding Protein p30" OR "Heparin Binding Protein p30" OR "p30, Heparin-Binding Protein" OR "high-mobility group box 1") OR AK=("HMGB1" OR "HMG1" OR "FM1 Gene Product" OR "HMG-1 Protein" OR "Box Protein 1, High Mobility Group" OR "Amphoterin" OR "HMGB1 Protein" OR "HMG 1 Protein "OR "Heparin-Binding Protein p30" OR "Heparin Binding Protein p30" OR "p30, Heparin-Binding Protein" OR "high-mobility group box 1"). The search period is set to start from September 21, 1976 until November 7, 2024, with the inclusion criteria set to only Articles and Reviews, and the language confined to English. Ultimately, 5992 documents were subordinated in total. The results were exported in txt and xlsx format according to the above WOSCC search formula. In order to prevent data bias from database changes, the search was finished on November 7, 2024. Article citation analysis uses global citation counts, and co-citation analysis for countries, institutions, journals, keywords, etc., uses local citation counts from 5992 articles. The specific literature screening process is shown in Figure 1A.

Data Analysis

For bibliometric analysis, CiteSpace, developed by Chaomei Chen, is now the most used program[38]. CiteSpace 6.1.R2 advanced visualization was utilized to examine international collaboration, journal dual map coverage, institutional distribution, topic area distribution, reference collaboration, and literature explosion. Bibliometric cooperation network graph analysis was the major use of VOSviewer developed by Nees Jan van Eck et al.[39]. Visual analysis of the distribution and collaboration among institutions, authors, journals, keyword collaboration and temporal evolution were performed using VOSviewer 1.6.18. Automatic clustering using VOS mapping techniques and similarity matrices is carried out, and subsequent addition of the corresponding labels is based on the content. With the help of Bibliometrix (an R-Tool from R-Studio), an open-source R package developed by Massimo Aria and Corrado Cuccurullo, we were able to visually analyze the annual average citation data, nation distribution, publishing collaborations, and reference collaborations[40]. Additionally, to visualize HMGB1-related publications and citation trends, we used Microsoft Excel 365.

Results

Annual publication and citation trends

From 1976, when the first article on HMGB1 was published, to 2024, the number of publications and citations in the literature relevant to HMGB1 are depicted in Figure 1A. The number of publications showed an overall upward trend, with a particularly noticeable increase in the number of articles after 2006. Among them, the most significant increase was from 2017 to 2021, with 542 articles published in 2021. Regarding the change in citations, the number generally showed an escalating trend year by year, with a significant increase in 2019-2021. Until 2021, the citations reached 23749, with the highest annual growth rate of 15.91%.

Inter-country distribution

As of November 7, 2024, 66 countries or regions have published a total of 5992 HMGB1-related studies (Supplementary Table 1). In terms of the number of publications, China is the first (2759), followed by the United States (1202) and Japan (626), with the account of China and the United States which is more than half of all. Regarding citations, the United States ranked first (95984), followed by China (68067) and Italy (33038), displaying an obvious distinction from the rest countries. In terms of total link strength, the United States is the first (743), followed by China (427) and Germany (196). Vertically, among the top ten countries, the publications of top 2 are above 1200 while the rest are under 700, and the citations of top 2 are above 60000 while the rest are under 40000, showing a significant gap.

International collaborations for HMGB1-related research are shown (Figure 2A), mainly concentrated in the northern hemisphere, with collaborations between the United States

and Europe, the United States and China, and the United States and Japan dominating. The chord diagram shows the collaborations between countries or regions (Figure 2B). The total number of publications from the United States and China accounted for 66.1% of HMGB1-related studies. The cooperation between countries was dominated by the United States and China, followed by collaborations between the U.S. and Japan, Germany, and Italy. In terms of the ratio between domestic and international collaborative publications (Figure 2C), most European countries such as the Belgium (62.5%), Sweden (47.8%), United Kingdom (34.8%), France (35.5%) have a higher share of international collaborative publications, while the United States (28.2%) and Italy (18.1%) has a balanced development, and Japan (13%), China (9.8%), and Korea (8.0%) are relatively low.

Institutional distribution

Supplementary Table 2 lists the top 10 institutions of publications, citations, and total link strength. In terms of publications, 7 of the top 10 institutions are in China, followed by 2 in the United States and 1 in Japan. The institution with the highest number of publications is Cent South Univ (190), followed by Univ Pittsburgh (185), and Huazhong Univ Sci & Technol (133). The most cited institution is under Univ Pittsburgh (23383) and Feinstein Inst Med Res (14480) in the U.S., followed by Ist Sci San Raffaele (10742) in Sweden. In terms of total link strength, it is still Univ Pittsburgh (157), Feinstein Inst Med Res (140) in the U.S., and Cent South

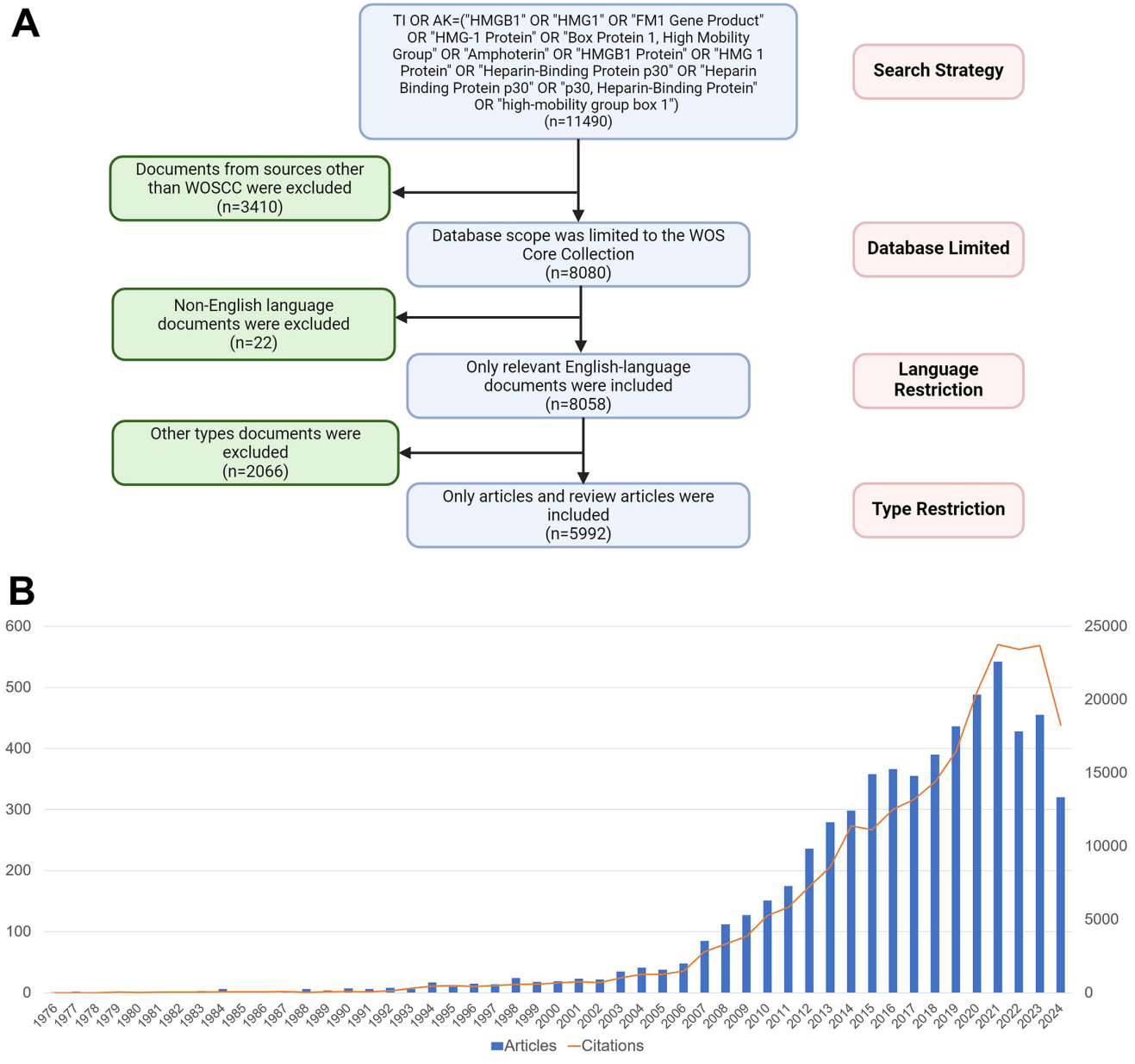

Figure 1 (A) Flow chart of HMGB1-related research screening process. **(B)** Description of HMGB1-related publications and citations. The blue bar represents the number and trend of articles, and the orange line represents the number and trend of citations.

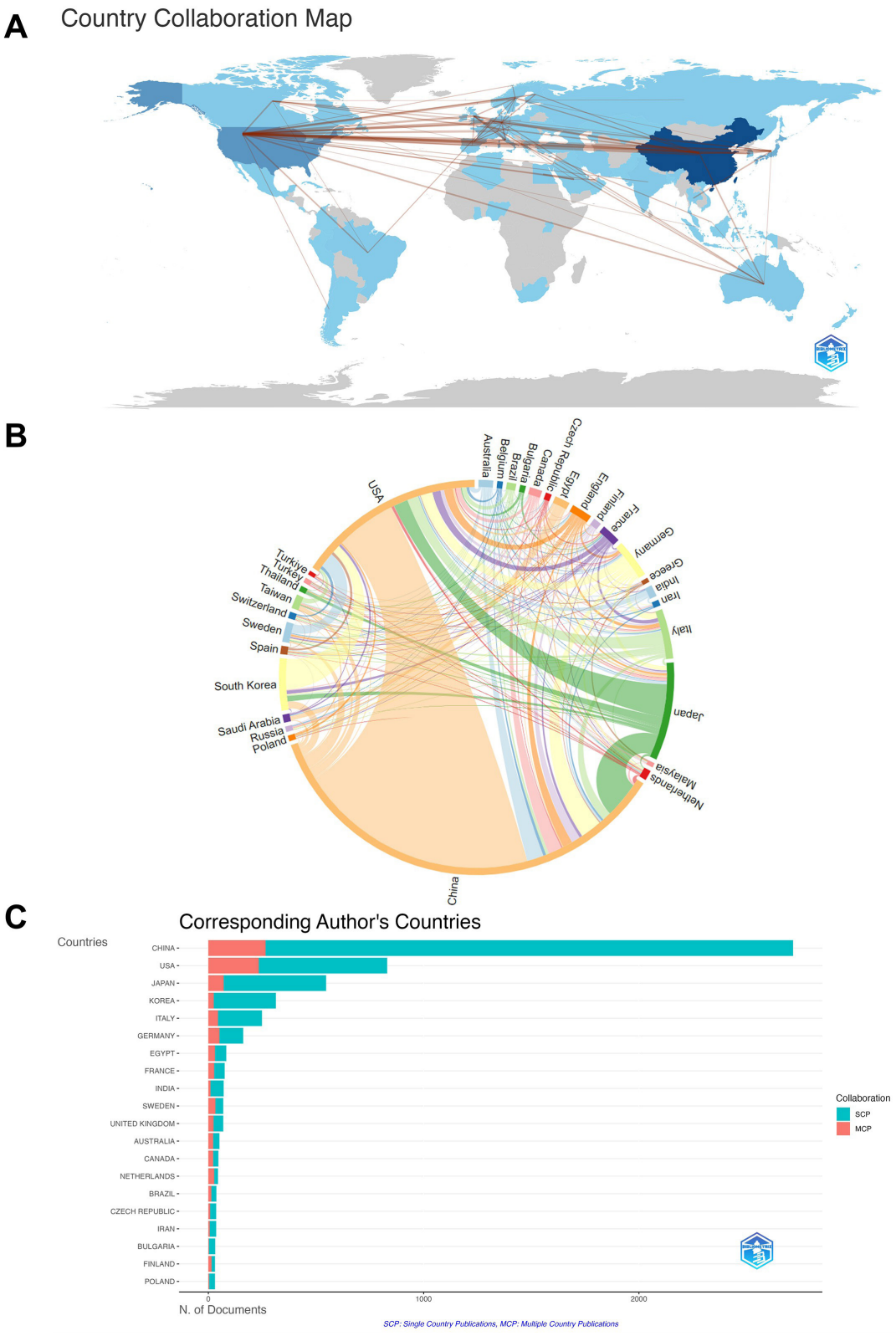

Figure 2 (A) Countries/regions collaboration map. On the map of world administrative divisions, the lines between countries/regions represent collaborations, and more lines mean more frequent collaborations. **(B)** The chord diagram of collaboration between countries/regions. The arc is proportional to the total number of publications, the width of the arc is proportional to the total number of publications, and the width of the shading is proportional to collaborative publications between the two countries. **(C)** Analysis of the corresponding author's country. Blue represents the number of single country publications (SCP) and red represents the number of multiple collaborative publications (MCP). (Country analysis includes nations with a publication volume greater than 25.)

Univ (139) in China are in the lead. Longitudinally, among the top ten institutions in terms of publications, the highest two are more than 180, followed by the third are less than 140; in terms of citations, there is a clear gap between the top (both more than 23000) and the bottom (all less than 5000). From a horizontal perspective, Univ Pittsburgh is at the top of both publications and citations, which implies that the institutions have an important position in research in HMGB1-related fields. Notably, the number of Feinstein Inst Med Res publications did not even make the top 5, but the number of citations was significantly high, which proves the outstanding quality of the research published at this institution in the field of HMGB1.

We used VOSviewer to cluster the inter-institutional collaborations into eight closely related clusters (Figure 3A). The cluster analysis of these research institutions aimed to understand global distribution and collaboration. Overall, institutions within clusters cooperated closely showed a balanced distribution, such as China (red, e.g., Huazhong Univ Sci & technol, Wuhan Univ), American (light blue; e.g., Feinstein Inst Med Res, Univ Pittsburgh), France (brown; e.g., Univ Paries, Inserm), Korea (yellow; e.g., Kyungpook Natl Univ, Yonsei Univ), and Japan (dark blue; e.g., Kagoshima Univ, Okayama Univ). In contrast, in the United States, HMGB1-related research has the characteristic of radiating outward mainly from Univ Pittsburgh, Feinstein Inst Med Res. It is worth noting that the cluster (light blue; e.g., Univ Pittsburgh, Cent S Univ) does not belong to a single country, and the University of Pittsburgh and Cent S Univ included in it play an essential role in linking collaborative research publications from multinational research institutions. Figure 3B presents a visual analysis of the institutional affiliations and their associated collaborative networks pertaining to articles in the field from 2012 to 2020. Red nodes indicate institutions that have been more active recently, such as Fujian Med Univ, Nanchang Univ, and Kunming Med Univ, highlighting their forefront positions in recent HMGB1-related research. Blue nodes represent institutions that were more active in the past, including Kagoshima Univ, San Raffaele Univ, and NYU. As one of the core nodes, Univ Pittsburgh has published a significant number of papers and has collaborated closely with many other institutions.

Author Distribution

By analyzing the authors of the sample reference, we listed the top 10 authors in publications and co-citations (Supplementary Table3) to display the research strengths of the authors and the research hotspots related to HMGB1. The most published author was Bianchi, Marco E., followed by Tracey, Kevin J., and Nishibori, Masahiro. The most co-cited was Yang, H, followed by Andersson, U, and Wang, Hc.

The collaboration between authors is shown in Figure 4A, which can guide the search for research partners and the judgment for authoritative institutions in this field. The results of the VOSviewer cluster analysis indicate that collaborations among Tracey, Kevin J., Yang, Huan, Wang, Haichao, is are significantly close. Interestingly, the collaboration among authors seems to exhibit a geographical clustering pattern, with affiliations broadly reflecting their respective countries or regions. For instance, green (the United States), light blue (Europe), brown and red (China), light purple (Japan), and orange (South Korea). The co-cited authorship network map using VOSviewer (Figure 4B) shows that the citation profile of HMGB1-related studies is pronounced clustering, forming four major clusters: the red cluster mainly includes Yang, H, Andersson, U, Tang, Dl, Kang, R, the blue cluster mainly includes Bianchi, Me, Scaffidi, P, the green cluster mainly includes Wang, Hc, and the yellow clusters, in which internal distribution is relatively balanced. Figure 4C presents an analysis of the popularity of articles published by various authors over the past five years. The heat value for each author is calculated by dividing the number of papers they have published in the recent five years by their total number of publications. The results indicate that the works of Bianchi, Marco E., Yang, Huan, Andersson, Ulf, and Tang, Daolin, among others, are highly popular among readers. In contrast, the popularity of articles authored by Bae, Jong-Sup, Yamada, Shingo, and Maruyama, Ikuro has relatively declined during this period.

Journal Distribution

The top 10 journals in terms of publications and citations were compiled by taking into account the journals that have been published in the literature on HMGB1 (Supplementary Table 4). The table's impact factor (IF) and Journal Citation Reports (JCR) quartiles show how influential the journals are. International Immunopharmacology (104; 4.8, Q1), International Journal of Molecular Sciences (160; 98, Q1), Plos One (92; 2.9, Q1), Frontiers in Immunology (88; 5.7, Q1), and Biochemical and Biophysical Research Communications (87; 2.5, Q3) are the journals with the most publications. Among the top ten journals, seven journals are in the Q1 division, and five of them have an IF above 4. These data indicate that HMGB1 is highly regarded and influential, in which results are primarily published in well-known journals. Journals with the most co-citations are J Biol Chem and J Immunol. The Q1 division has seven of the top ten journals by co-citations, and three of these journals had an IF of more than 40. Notably, International Immunopharmacology and Journal of Biological Chemistry rank among the top journals for publications and co-citations, demonstrating their authority in the field of HMGB1.

In Figure 5A, all of the journals are generally divided into five clusters based on publications: The red cluster is concerned with cancer and its treatment (Biomedicine & Pharmacotherapy, Oncotarget, Oncoimmunology, etc.); the green cluster is concerned with immune function and cell biology; the yellow cluster is concerned with critical care medicine (Shock, Critical Care Medicine); and the blue cluster is concerned with neurology (Journal of Neuroinflammation, Neurosciance, etc.); the purple Cluster focuses on Biochemistry (Biochemistry and Biophysical Research communications). In Figure 5B, all of the journals were grouped into five clusters based on the co-citations, which have a propensity for sharing research trajectories. The red cluster focuses on blood-related fields (Circulation, Arterioscl Throm Vas, J Cerebr Blood F Met, etc.); the green cluster favors various clinical diseases such as immunology (J Immunol, J Clin Invest, etc.); the yellow cluster mainly deals with cancer, cell death (Cancer Res, Oncogene, Cell Death Differ, Autophagy, etc.); the blue cluster focuses on cell biology (J Biol Chem, Cell, Mol Cell Biol, etc.); the purple cluster involves critical care medicine (Crit Care Med, etc.). Through

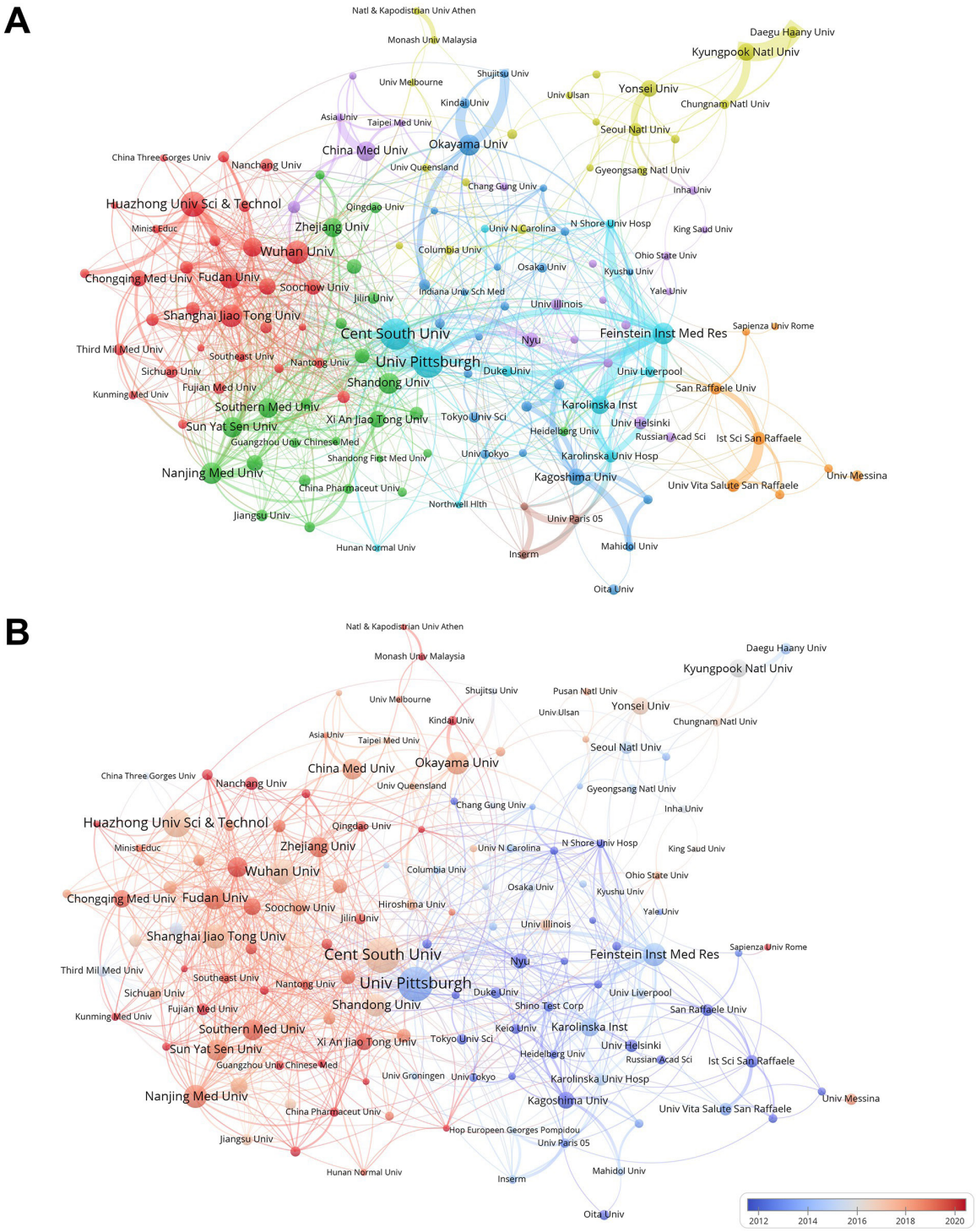

Figure 3 (A) Analysis of HMGB1-related collaborative network visualization of institutions in VOSviewer. Vosviewer automatically clusters all institutions into different colored clusters based on inter-institutional collaboration. Each dot represents an institution, the size of the dot reflects the number of publications, and the thickness of the line between the dots reflects the closeness of the collaboration between institutions. **(B)** Institutional analysis of published articles from 2012 to 2020. (Institutional analysis includes organizations with a publication volume greater than 15.)

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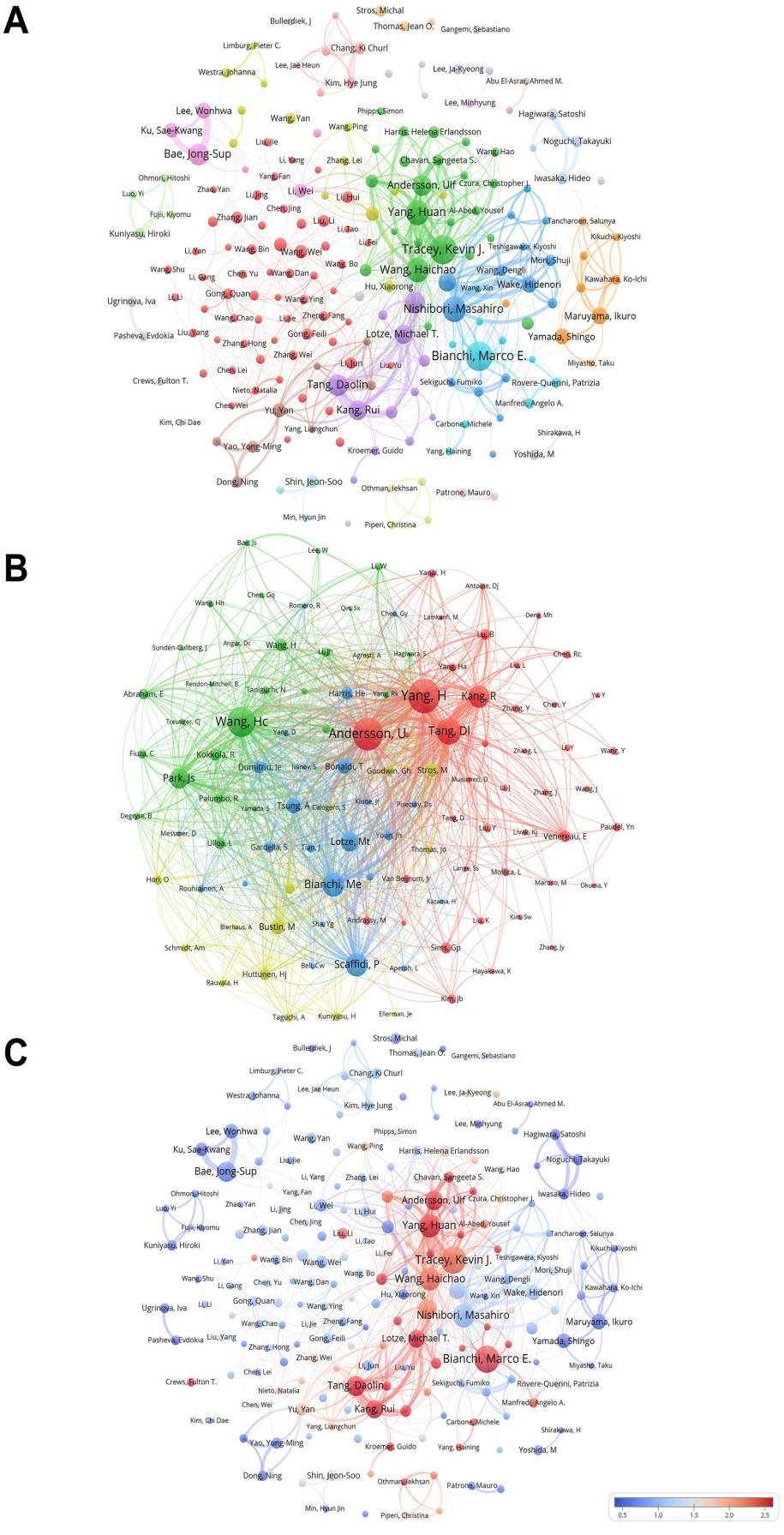

Figure 4 (A) Analysis of HMGB1-related collaborative network visualization of authors in VOSviewer. VOSviewer divides authors into different colored clusters based on their collaboration status. Different colors represent closer collaboration relationships. Each dot in the graph represents an author, the size of the dot reflects the number of publications of the author, and the thickness of the line between the dots reflects the degree of cooperation. **(B)** Analysis of collaborative network visualization of co-cited authorship in VOSviewer. The analysis relates to one or more future works simultaneously citing two or more authors. Higher co-citation frequency denotes more concentrated academic attention and research. Vosviewer automatically clusters authors into different colored clusters based on their co-cited status, where each dot represents an author, the size of the dot reflects the co-cited strength, and the thickness of the line between the dots reflects the co-cited relationship of the two authors. **(C)** Analysis of authors that publish articles. The heat value for each keyword over the past five years is calculated by dividing the number of publications in the last five years by the total number of publications. (Author analysis includes authors with a publication volume greater than 10. Co-cited author analysis includes authors with citation counts greater than 175.)

knowledge flow analysis, we investigated how published and cited journals relate to one another, which is displayed on the dual-overlay journal map (Figure 5C). The most frequently published journals are those from forefront fields including MOLECULAR, BIOLOGY, IMMUNOLOGY, NEUROLOGY, SPORTS, OPHTHALMOLOGY, MEDICINE, and MEDICAL CLINICAL. The majority of the referred journals are from authoritative information sources like MOLECULAR, BIOLOGY, GENETICS, HEALTH, NURSING, MEDICINE, DERMATOLOGY, DENTISTRY, and SURGERY. Notably, the most frequently published and most commonly cited journals overlap highly, which means that these journals are at the forefront of HMGB1-related research.

Keyword analysis

The principal themes of the article are encapsulated by its keywords, and the analysis of keyword co-occurrences can be employed to evaluate the developmental status of research pertaining to HMGB1. We listed the top 10 keywords by frequency of occurrence (Supplementary Table 5). Except for "HMGB1" (3990), the keyword with the highest frequency is "inflammation" (758), followed by "RAGE" (the Receptor of Advanced Glycation Endproducts, 379), "sepsis"(305), and "toll-like receptor 4"(257), which indicates that they are hot issues in the field of HMGB1 counterpart research.

A co-occurrence network map of keywords is displayed in VOSviewer (Figure 6A). According to the concerned areas, the keywords were grouped into seven categories, based on the obtained results. The red cluster includes the topics covered by cancer and cell death (breast cancer, NSCLC, hepatocellular carcinoma, autophagy, apoptosis, migration, metastasis). The blue cluster is related to neurological damage (neuroinflammation, epilepsy, stroke, microglia). The green cluster is related to acute injury and oxidative stress (sepsis, acute lung injury, oxidative stress, cytokine). The yellow cluster is associated with cardiovascular disease (atherosclerosis, myocardial infarction, and angiogenesis); the purple and cyan sections are related to mechanisms of immune diseases (rheumatoid arthritis, endothelial cells, dendritic cells, innate immunity, and diabetic nephropathy). The orange cluster mainly involves inflammatory molecules (Toll-like receptor, IL-6; TNF-α). Attentionally, the cyan cluster (immunity) is extensively connected to all other clusters, as it involves nodes that are key focuses in HMGB1 research.

Figure 6B shows the Thematic evaluation map of related research, which visualizes four different periods of themes according to two dimensions (density and centrality). In terms of motor themes (Q1), from 1990 to 2010, the focus was primarily on foundational studies of molecular structures, such as DNA bending, mRNA localization. Entering the period from 2010 to 2015, the interest shifted towards inflammatory factors and cell types, including damage-associated molecular patterns (DAMPs), alarmins, and dendritic cells. Subsequently, between 2015 and 2022, the research emphasis expanded to encompass diseases closely linked with the immune system, particularly focusing on hepatocellular carcinoma, rheumatoid arthritis, and neurological disorders. In the most recent phase, from 2022 to 2024, there has been an increased concentration on exploring neurological conditions (neuroinflammation and microglia functions) along with DAMPs, hypoxia, and proliferation. Regarding niche topics (Q2), these involve diseases that are less studied (diabetic nephropathy, melanoma) and molecular mechanisms (immunogenic cell death, vascular endothelial growth factor, in situ hybridization). For emerging or declining topics (Q3), these include fibrosis, asthma, thrombomodulin, acetylation, and other themes that may develop in the future or are currently in decline. In basic themes (Q4), sepsis and pyroptosis have been relevant to this area over the past two years but have not yet been fully developed. They are expected to become future hotspots (Figure 6D).

Figure 7A extends the keyword analysis by adding a temporal layer, illustrating that research before 2019 was mainly concentrated on critical illnesses such as HMGB1-associated malignancies, sepsis, myocardial infarction, and cerebral ischemia as. Since 2019, the research has branched into three distinct streams: firstly, HMGB1-related receptor proteins and active components, such as NLRP3, NRF2, glycyrrhizic acid, and toll-like receptor 4; secondly, neurological conditions such as depression, epilepsy, ischemic strokes, spinal cord injuries, and neuroinflammation; and thirdly, the modes of programmed cell death include autophagy, as well as recent research hotspots such as ferroptosis and pyroptosis. Figure 7B analyzes and depicts a heatmap of the co-occurrence frequencies among the top 100 keywords. Pronounced red areas indicate strong correlations between keywords such as necroptosis, ferroptosis, and neuroinflammation, which are highly related to spinal cord injury, depression, epilepsy, and exosomes. Systemic lupus erythematosus and severe acute pancreatitis are linked to immune responses, sirtuin 1, S100, toll-like receptor and monocyte. Crucially, HMGB1 emerges as the keyword with the highest frequency of occurrence, predominantly connected to critial care medicine such as sepsis, ischemia-reperfusi, acute liver injury, and breast cancer, with implicated mechanisms involving innate immunity, TNF-α, NF-κB, cytokines, amphoterin, and ethyl pyruvate. To better define the temporal classification of these keywords for trend analysis, we manually categorize them into three groups: basic (prior to 2004), median (2005–2014), and hotspots (2015–2024). The results of the principal component analysis (PCA) (Figure 7C) indicate that while the "intermediate" group partially overlaps with the other two groups, there is a clear separation between the groups. This suggests that the internal similarity within each group has changed significantly over time, validating the effectiveness of the classification and indicating substantial changes in the evolution of keywords in this field. Subsequently, the analysis of keyword heat using the random forest method (Figure 7D) shows that the keywords with higher recent heat values include SARS-CoV-2, microglia, NLRP3, pyroptosis, neuroinflammation, depression, exosome, Parkinson's disease, and epilepsy.

Analysis of critical literature and references

We listed the top 15 cited references (Supplementary Table 6). The most highly cited reference is "Release of chromatin protein HMGB1 by necrotic cells triggers inflammation"[41]. It proposes that necrotic cells signal the surrounding environment by releasing HMGB1 and activate the inflammatory response by binding to receptors such as RAGE, revealing the dual role of HMGB1 in the cell nucleus and its involvement in the inflammatory process as a signaling molecule in the extracellular environment. The second highly

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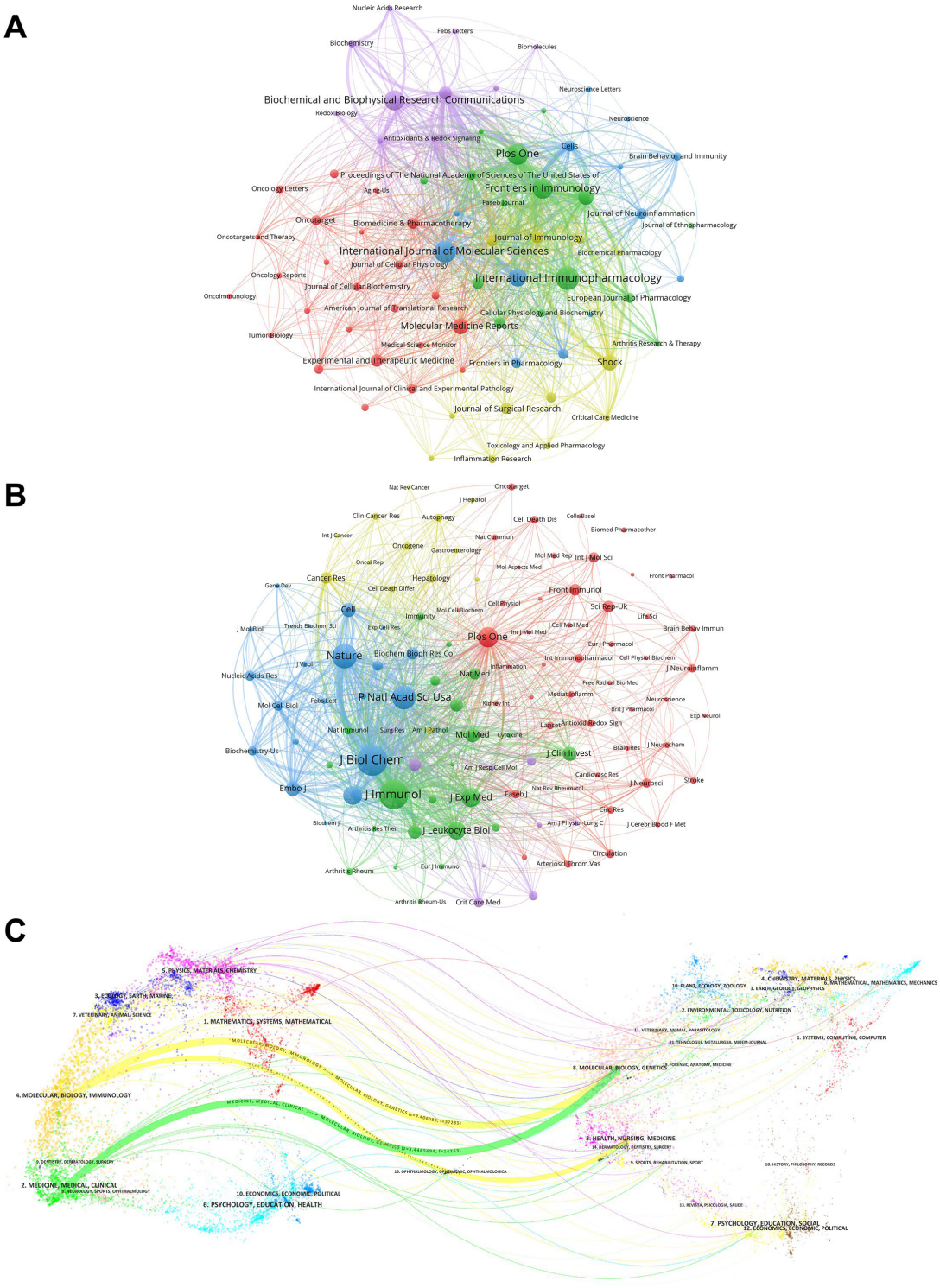

Figure 5 (A) Analysis of HMGB1-related collaborative network visualization of journals in VOSviewer. The visualization in VOSviewer displays the journals and their relationship based on the proximity of the journals. Different dots represent different journals, the color reflects the corresponding clusters, and the connecting line indicates the closeness of the connection. **(B)** Analysis of collaborative network visualization of journal citations in VOSviewer. Different dots represent different journals, the colors reflect the corresponding clusters, and the lines indicate the closeness of the connections. **(C)** The dual-map overlay of journals. The distribution of themes, variations in citation trajectories, and changes in research centers are all displayed. The published journals are labeled on the left side of the double map, while the cited journals are labeled on the right. The context of the citation connection is indicated by the colored arrows pointing from the published journal to the referenced journal. (Journal analysis includes journals with a publication volume greater than 15. Co-cited journal analysis includes journals with citation counts greater than 500.)

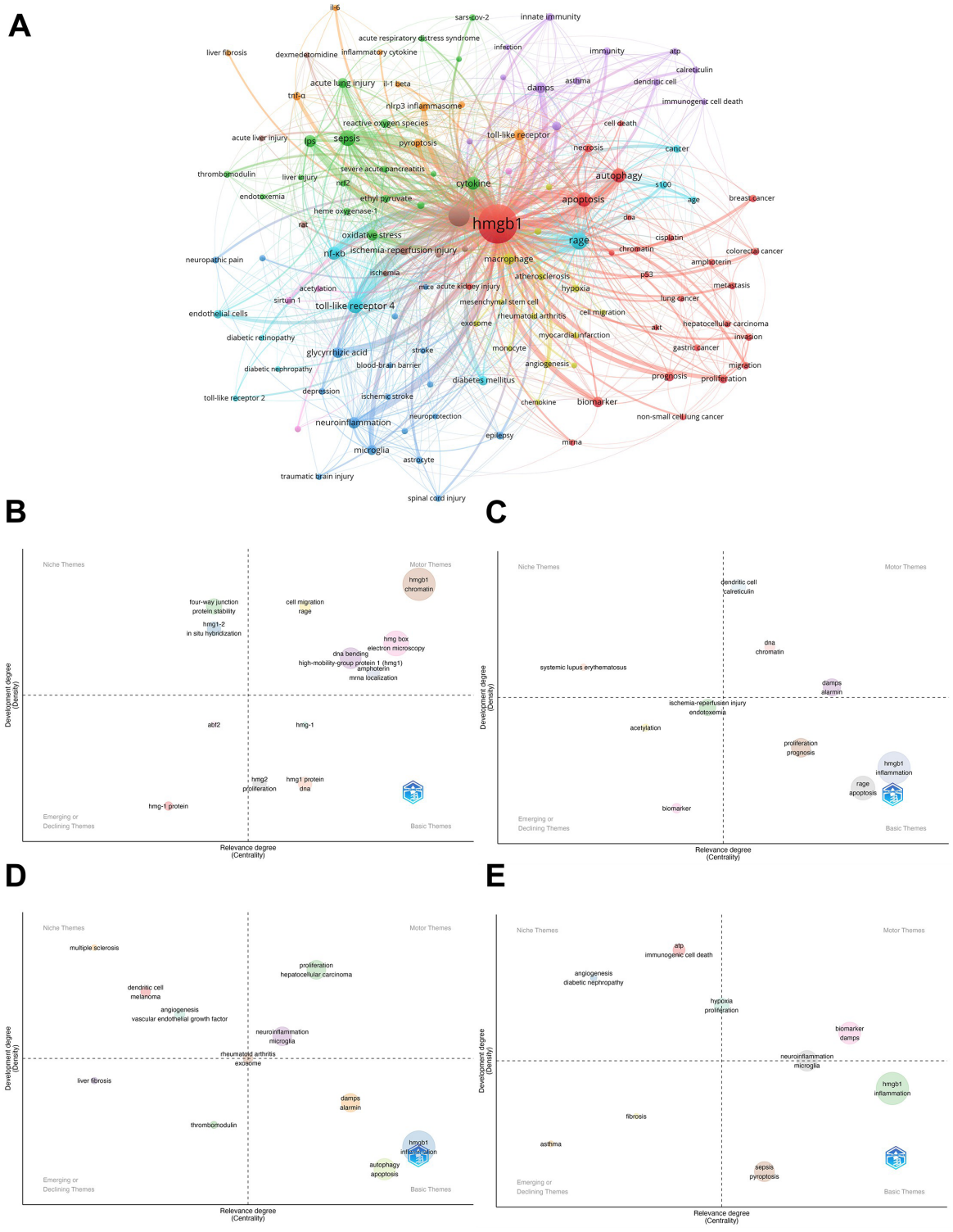

Figure 6 (A) Analysis of HMGB1-related collaborative network visualization of keywords in VOSviewer. Clusters are formed based on keyword co-occurrence frequency, with colors distinguishing clusters. Node size indicates keyword frequency, and line thickness represents relationship strength. (Keyword analysis includes terms that appear more than 20.) **(B)** Thematic evaluation of the research field during 1990-2010. **(C)** Thematic evaluation of the research field during 2010-2015. **(D)** Thematic evaluation of the research field during 2015-2022. **(E)** Thematic evaluation of the research field during 2022-2024. **(B-E)** The thematic evaluation map visualizes four different periods of themes according to two dimensions (density and centrality). Motor themes are the keywords in the upper right corner of the first quadrant. Niche themes are located in the upper left (second quadrant), emerging or declining themes are in the lower left (third quadrant), and basic themes are in the lower right (fourth quadrant). The Motor Themes in the upper right quadrant represent directions that are relevant and already rapidly developing in the field of HMGB1, which is the focus of current research. The Basic Themes in the lower right quadrant represent topics that are relevant to the field of HMGB1 but are not yet well developed and may be the focus of future research in the field of HMGB1.

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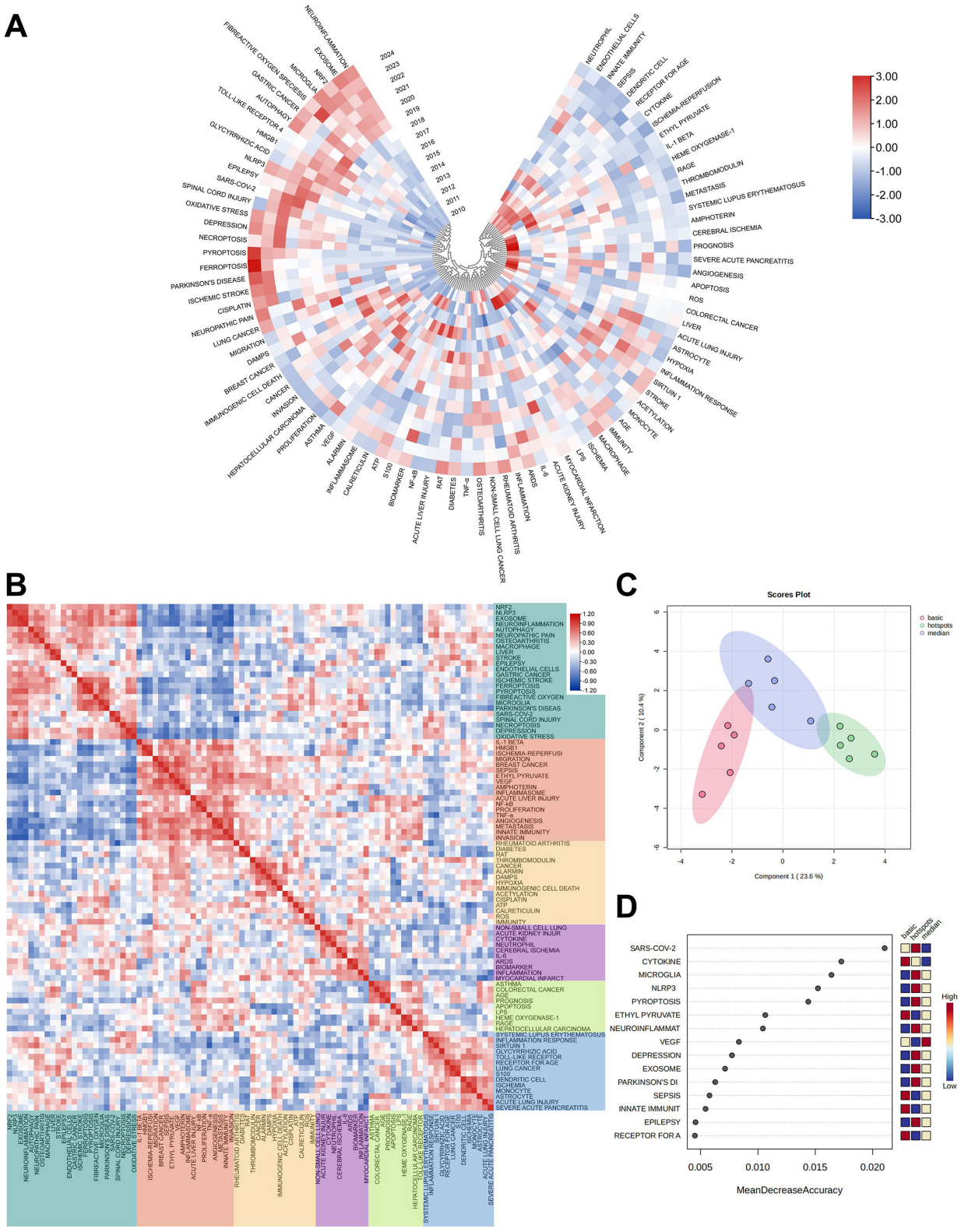


Figure 7 (A) Heatmap of keywords in temporal dimension. Each radial line corresponds to a specific keyword, while each concentric circle denotes a successive year. A color block with a more intense red indicates a higher frequency of the keyword's occurrence in that year's literature. **(B)** Heatmap of the top100 keywords. It depicts a keyword heatmap where each cell's color intensity correlates with the co-occurrence frequency of the two respective keywords it represents. A deeper red hue within a cell signifies a higher co-occurrence rate, indicating that extensive red areas are often indicative of a high degree of relevance between the associated keywords. **(C)** Annual heat map spanning from 1976 to 2024. The annual heat value for each keyword is calculated by dividing the citation count for that year by the total citations made in the same year. **(D)** Heat map illustrating keyword correlation. Keywords with high visibility are divided into different categories and distinguished by different colors.

cited reference is "Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein"[42]. This study investigates how HMGB1 enhances the nuclear translocation of NF-κB and the expression of pro-inflammatory cytokines by activating Toll-like receptors 2 and 4 (TLR2 and TLR4), thus playing a role in acute inflammatory responses. In comparison, RAGE has a more limited role in HMGB1's activation of macrophages, and the response it elicits differs from that caused by lipopolysaccharide (LPS).

Figure 8A shows the citation relationship between the top 25 documents. The results show that "Release of chromatin protein HMGB1 by necrotic cells triggers inflammation", published by Scaffidi, P et al. in 2002 received the most citations of other articles, which firstly reported HMGB1 released from necrotic cells promotes inflammation by signaling cell death to neighboring cells. In contrast, apoptotic cells are programmed to retain the signals that would otherwise be spread by damaged or dying cells, thus they do not release HMGB1 and consequently do not promote inflammation. Subsequently, "Extracellular role of HMGB1 in inflammation and sepsis." published by Yang, H et al. 2004, "Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein" published by Park, JS et al. 2004 and "Nuclear factor HMGB1 mediates liver injury after murine liver ischemia-reperfusion" published by Tsung, A et al. 2005, were the most cited articles, which served as a bridge between the previous stage and the next stage. Finally, "HMGB1 in health and disease" by Kang et al. 2014 cited almost all of the articles in Figure 8A, providing a comprehensive review of HMGB1-related research.

Through CiteSpace, we analyzed the relationship between co-citations among the literature in the field of HMGB1 (Figure 8B). The clusters were divided into 18 categories and shown in various colors. Cluster #0 is involved in Ferroptosis. According to publication years of literature, we observed the development of HMGB1-related study from the time dimension. The earliest areas of HMGB1-related literature from 1999-2003 were #9 (amphotericin), #15 (HMG1), #14(cholesterol-3-sulfate) and #13 (transfection), a period in which the infrastructure and binding sites of HMGB1 itself was mainly explored. 2004-2010, studies focused on #12 (colorectal cancer), #10 (NMR spectroscopy), #3 (cytokines), and #4 (high mobility group box 1 protein). In 2010, the number of studies on the relevance of autophagy, invasion and HMGB1 proliferated, and then emerged #8 (autophagy), #6 (systemic lupus erythematosus), #5 (asthma), #11 (sepsis), #2 (invasion), etc. In 2015 and beyond, the intensity between studies decreased, with the emergence of several clusters: #1 (neuroinflammation), #0 (ferroptosis), #16 (metformin), and #18 (polycystic ovarian syndrome). It is worth noting that the #1 (neuroinflammation) cluster has the most significant number among those clusters.

Figure 8C shows the top 25 references with the strongest citation bursts. The initial citation bursts occurred in 2000. The title of the article is "HMG-1 as a Late Mediator of Endotoxin Lethality in Mice." Notably, "HMGB1 Is a Therapeutic Target for Sterile Inflammation and Infection" by Andersson, Ulf et al. in ANNU REV IMMUNOL 2011 was the most explosive paper (intensity = 84.05), followed by " HMGB1 in health and disease" by Kang, R et al. in MOL ASPECTS MED 2014 (intensity = 73.04). These two high-outbreak review articles also illustrate the authoritative status of Andersson, Ulf, and Kang, R in the field of HMGB1-related research. 2015 and beyond, the outbreak continues until 2022, mainly involving new molecular patterns such as Caspase dependence of HMGB1 in driving the inflammatory process, and in infections, endotoxemia, and other inflammatory diseases as well as cancer, targeting the secretion and release of HMGB1 provides effective therapeutic strategies, suggesting that these directions still have a good scope for development.

Discussion

The analysis in this study is based on 5992 HMGB1-related papers from 66 countries/regions that were archived in the WOSCC database from 1976 to 2024. The 5992 HMGB1-related literature was analyzed using CiteSpace 6.1.R2 Advanced, VOSviewer 1.6.18, and Rbibiometrix. Based on these outcomes, the region's geographic and temporal distribution, author contributions, core articles, research hotspots, and field frontiers were evaluated.

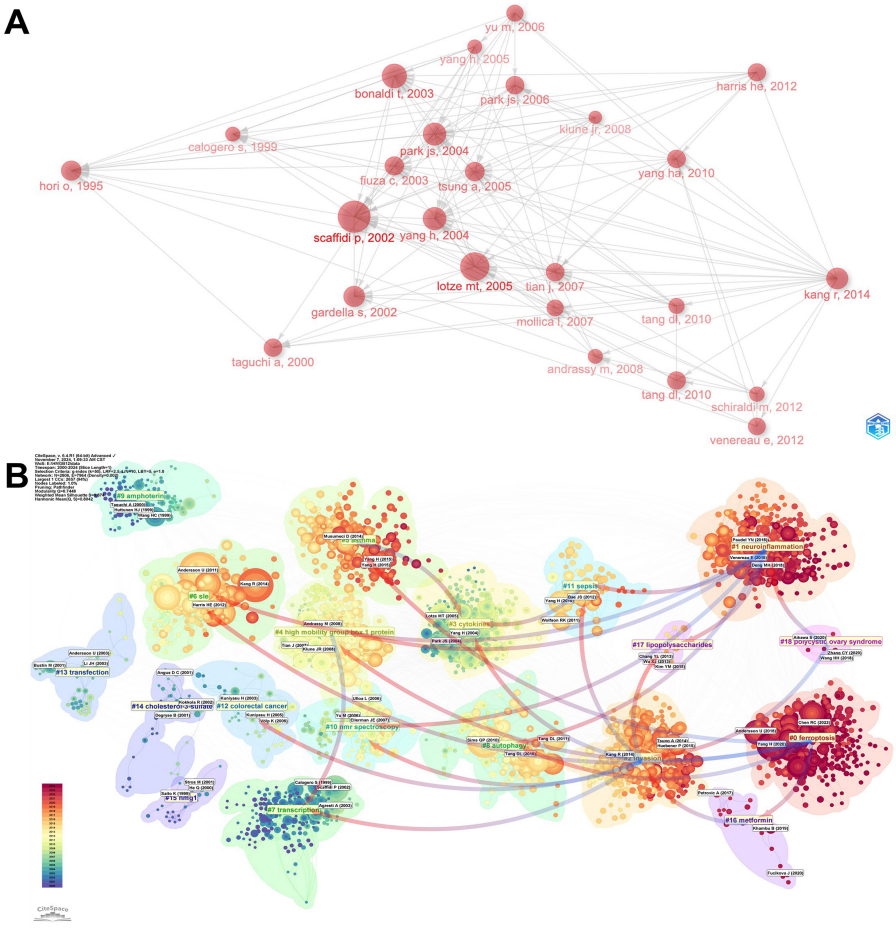
General Distribution

In the general trend analysis, the rapid increase in publications and citations indicates that HMGB1 is receiving increasing attention. Over the past 15 years, the quantity of HMGB1-related studies has continuously increased, with the most significant growth occurring between 2017 and 2021. By 2021, the number of HMGB1-related publications will be approximately ten times higher than in 2004. This substantial rise is largely attributed to the emergence of the COVID-19 pandemic, which sparked considerable academic interest in the role of HMGB1 in pulmonary inflammatory diseases. The overall trend in the number of publications indicates a steady growth of research. Attentionally, a sudden increase of the citation index emerged in 2021-2022 which means a possible breakthrough in the area. The total link strength and the number of publications are two key metrics in the country/region analysis. These nations/regions with high total link strength serve as "bridges" in the global cooperation network. Horizontally, China and the United States are in the top either publications or citations, which proves their strong overall strength. It is noteworthy that Italy and Germany have a high number of citations despite the low number of publications, which proves the high quality of research published in the field in these countries.

In the examination of research institutions, seven of the top ten institutions in terms of publications are from China, followed by two in the United States and one in Japan. The United States leaders in terms of publication, citation, and total link strength are Univ Pittsburgh and Feinstein Inst Med Res. In addition, research institutions from China, Sweden, Germany, Italy, France, Japan, and Korea are also widely involved in HMGB1-related collaborations. In the author analysis, as shown in Supplementary Table 3, Yang, H, and Andersson, U ranked first in co-citations, far exceeding other scholars. It is also evidenced by 4 of the 25 highest outbreak literature published by Yang, H and 3 by Andersson, U., demonstrating their tremendous influence in HMGB1-related fields. In addition, Andersson, U (second place in co-citations) has co-authored several high burst articles with Yang, H. (first place in co-citations). Interestingly, Andersson, U ranked second in terms of co-citations but was only top 9 publications, probably

because his research focus is on inflammation rather than specifically on HMGB1. The quality of its early publications at the intersection of these two fields is authoritative, with a gradual citation blast. Inferred from the central position within the collaboration map, Tracey, Kevin J., Wang, Haichao, and Yang, Huan are productive authors. In the co-citation author network, due to the multi-disciplinary and multi-field nature of HMGB1 research, authors from different clusters also maintain close collaborative relationships. Kang, R., from the red cluster, along with several prominent authors from other clusters, comprehensively summarized the structure, function, regulatory mechanisms, and roles of HMGB1 in various diseases, particularly in inflammatory conditions such as sepsis. They also explored the potential of HMGB1 as a biomarker and discussed therapeutic strategies targeting HMGB1, providing valuable references and directions for future research and clinical applications[43].

Among the top 10 journals, journals with high volumes of publication and co-citations include PLoS ONE and the Journal of Biological Chemistry. Among them, Journal of Biological Chemistry had the most co-citations but only the top 10 publications, principally because it comprised a great deal of pertinent and highly cited papers. Notably, the majority of publications in this research field cover a broad spectrum of



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Top 25 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2000 - 2024
Wang HC, 1999, SCIENCE, V285, P248, DOI 10.1126/science.285.5425.248, DOI	1999	37.11	2000	2004	
Scaffidi P, 2002, NATURE, V418, P191, DOI 10.1038/nature00858, DOI	2002	62.69	2002	2007	
Yang H, 2004, P NATL ACAD SCI USA, V101, P296, DOI 10.1073/pnas.2434651100, DOI	2004	56.98	2004	2009	
Park JS, 2004, J BIOL CHEM, V279, P7370, DOI 10.1074/jbc.M306793200, DOI	2004	45.52	2004	2009	
Bonaldi T, 2003, EMBO J, V22, P5551, DOI 10.1093/emboj/cdg516, DOI	2003	41.23	2004	2008	
Lotze MT, 2005, NAT REV IMMUNOL, V5, P331, DOI 10.1038/nri1594, DOI	2005	62.26	2006	2010	
Park JS, 2006, AM J PHYSIOL-CELL PH, V290, PC917, DOI 10.1152/ajpcell.00401.2005, DOI	2006	32.77	2006	2011	
Tsung A, 2005, J EXP MED, V201, P1135, DOI 10.1084/jem.20042614, DOI	2005	32.22	2006	2010	
Tian J, 2007, NAT IMMUNOL, V8, P487, DOI 10.1038/ni1457, DOI	2007	33.92	2008	2012	
Andrassy M, 2008, CIRCULATION, V117, P3216, DOI 10.1161/CIRCULATIONAHA.108.769331, DOI	2008	32.14	2010	2013	
Sims GP, 2010, ANNU REV IMMUNOL, V28, P367, DOI 10.1146/annurev.immunol.021908.132603, DOI	2010	61.15	2011	2015	
Tang DL, 2010, BBA-GENE REGUL MECH, V1799, P131, DOI 10.1016/j.bbagrm.2009.11.014, DOI	2010	43.76	2011	2015	
Yang HA, 2010, P NATL ACAD SCI USA, V107, P11942, DOI 10.1073/pnas.1003893107, DOI	2010	39.99	2011	2015	
Andersson U, 2011, ANNU REV IMMUNOL, V29, P139, DOI 10.1146/annurev-immunol-030409-101323, DOI	2011	84.05	2012	2016	
Harris HE, 2012, NAT REV RHEUMATOL, V8, P195, DOI 10.1038/nrrheum.2011.222, DOI	2012	41.93	2013	2017	
Venereau E, 2012, J EXP MED, V209, P1519, DOI 10.1084/jem.20120189, DOI	2012	35.85	2013	2017	
Yang H, 2013, J LEUKOCYTE BIOL, V93, P865, DOI 10.1189/jlb.1212662, DOI	2013	39.1	2014	2018	
Musumeci D, 2014, PHARMACOL THERAPEUT, V141, P347, DOI 10.1016/j.pharmthera.2013.11.001, DOI	2014	35.16	2015	2019	
Kang R, 2014, MOL ASPECTS MED, V40, P1, DOI 10.1016/j.mam.2014.05.001, DOI	2014	73.04	2016	2019	
Andersson U, 2018, EXPERT OPIN THER TAR, V22, P263, DOI 10.1080/14728222.2018.1439924, DOI	2018	41.69	2019	2024	
Andersson U, 2018, SEMIN IMMUNOL, V38, P40, DOI 10.1016/j.smim.2018.02.011, DOI	2018	35.84	2019	2024	
Yang H, 2020, FRONT IMMUNOL, V11, P0, DOI 10.3389/fimmu.2020.00484, DOI	2020	68.82	2021	2024	
Xue JM, 2021, J CELL PHYSIOL, V236, P3406, DOI 10.1002/jcp.30125, DOI	2021	33.61	2021	2024	
Chen RC, 2022, EXP MOL MED, V54, P91	2022	58.36	2022	2024	
Wang SM, 2020, J HEMATOL ONCOL, V13, P0, DOI 10.1186/s13045-020-00950-x, DOI	2020	32.26	2022	2024	

Figure 8 (A) Association between the top highly cited references. Each node represents a highly cited paper, and arrows indicate citation relationships, pointing from the citing paper to the cited paper. The size of the nodes is proportional to the number of co-citations the paper has received. **(B)** Clustering of references based on the similarity between references, which shows the authors and the year of publications with an explosive increase in co-citations. The category with the most articles is 0, followed by 1, and so forth. **(C)** The top 25 references with the strongest citation bursts. The blue line indicates the timeline, and the red segments represent burst periods. A citation burst represent a sharp increase in the number of articles cited and some of the key issues in the field raised or addressed in the article.

disciplines, including immunology, neurology, sports science, ophthalmology, dermatology, nursing, and clinical medicine. However, recent studies on HMGB1, particularly in the context of neuroinflammation within neurology, have become increasingly in-depth, suggesting that HMGB1 in neurology will be a research hotspot in the coming period.

The analysis of the top ten co-cited references reveals their profound impact on subsequent research in the HMGB1 field. The groundbreaking study by Scaffidi, P., et al. reshaped our understanding of sterile inflammation and cell death signaling. This discovery spurred transformative research into HMGB1's dual nuclear/extracellular roles and its pathological implications in autoimmune diseases (SLE), cancer (glioblastoma chemoresistance), and ischemia-reperfusion injuries. This paper experienced a significant burst in citations between 2002 and 2007, significantly influencing subsequent studies. Another influential work by Park, JS, et al. demonstrated that HMGB1 activates TLR2 and TLR4 to enhance NF-κB-driven pro-inflammatory cytokine release, providing a theoretical basis for studying disease mechanisms across various conditions and developing therapeutic strategies. However, the multifunctionality of HMGB1 and the complexity of its signaling networks remain key challenges and focal points in current research. Future studies need to integrate multi-omics technologies and clinical translational research to resolve mechanistic controversies and achieve precise interventions.

Hot spots and frontiers

Keyword analysis and citation analysis are helpful in understanding the cutting-edge and hot topics within the HMGB1 field. Based on the ranking analysis of high-frequency keywords, it can be observed that the popular research topics related to HMGB1 primarily concern molecular patterns and mechanisms of injury associated with HMGB1. Co-occurrence network maps also highlight several trending research directions from past studies, including cancer and cell death, neurological damage, cardiovascular disease, mechanisms of immune diseases, acute injury and oxidative stress, inflammatory molecules. The results incorporating time variation show that keywords are concentrated in areas such as diseases and their clinical diagnosis and treatment, cell death, mechanisms, and molecular patterns, among which neurological disorders have received significant attention in recent years. Critical literature and reference analysis reveals that the development trend of HMGB1-related research has become more diversified since around 2015. Meanwhile, new clusters have emerged: #1 (neuroinflammation), #0 (ferroptosis), #16 (metformin), and #18 (polycystic ovarian syndrome). These data reflect that these areas represent promising research directions in HMGB1-related studies.

HMGB1-associated cell death

HMGB1, which is typical of DAMPs and frequently leads to serious cell damage[44]. Necrosis, characterized by cell swelling and plasma membrane rupture, typically involves the passive release of HMGB1, which also plays a positive feedback role, promoting inflammation[45]. Consequently, HMGB1 is often used as a marker for necrosis[46]. In 2002, Scaffidi et al.'s pioneering study first elucidated HMGB1's role in necrotic cells, leading to increased attention on how HMGB1 is passively released from these cells to trigger sterile inflammation (Supplementary Table 6).

As shown in Figure 6A, HMGB1-related autophagy and apoptosis research is currently a hot field. The role of HMGB1-related autophagy in tumors is twofold. On the one hand, HMGB1 acts as a critical factor in cellular autophagy and mitosis[47,48], which inhibits tumors. A deficiency of HMGB1 can trigger autophagy defects causing inflammation and genomic instability, leading to tumorigenesis[49,50]. On the other hand, autophagy mediated by HMGB1 enhances chemoresistance in cancer cells, such as colon, osteosarcoma, pancreatic, leukemia, gastric, and ovarian malignancies[51]. Numerous studies have demonstrated that inhibiting the expression of HMGB1 by RNAi increases the anticancer activity of drugs, and overexpression of HMGB1 by gene transfection increases drug resistance[50,52,53], which may be related to the promotion of lactic acid production and glutamine metabolism[54]. It has been identified that HMGB1 acts as an anti-apoptotic protein within cells by regulating Bcl-2 family protein expression (transcription-dependent way), autophagy, and p53 localization (non-transcription-dependent way)[55,56], in response to a variety of apoptotic stimuli including ultraviolet (UV) radiation, CD95, TRAIL, Casp-8, and Bax[57,58]. It is vital to note that HGBM1's ability to regulate apoptosis depends on its redox status[59]. Reducible HMGB1 inhibits apoptosis through binding to RAGE, but oxidized HMGB1 increases the drug's cytotoxicity and triggers apoptosis through the mitochondrial path[55].

Keyword hotspot analysis further reveals that pyroptosis, ferroptosis, and immunogenic cell death represent cutting-edge research directions within the HMGB1 field (Figure 6, 7). Pyroptosis is a form of programmed cell death mediated by the Gasdermin family, characterized by membrane pore formation and cellular lysis, followed by the release of pro-inflammatory components such as HMGB1[60,61]. Pyroptosis can be mediated by caspase1, a core molecule of inflammatory vesicle activation[62] in which the inhibition of inflammatory vesicles reduces serum HMGB1 levels and protects against inflammatory damage in diabetic nephropathy[63](70). Pyroptosis can be mediated by caspase1, a core molecule of inflammatory vesicle activation[62]in which the inhibition of inflammatory vesicles reduces serum HMGB1 levels and protects against inflammatory damage in diabetic nephropathy[63](70). In ferroptosis, HMGB1 promotes iron accumulation through multiple mechanisms [64] and indirectly or directly increases iron death damage, such as by MAPK-dependent transferrin receptor (TFRC) expression[65] or acting as an autophagy regulator[66]. HMGB1 promotes ferroptosis through different mechanisms: by promoting MAPK-dependent TFRC expression, or acting as a regulator of autophagy that indirectly increases iron accumulation during ferroptosis injury. In addition, during ferroptosis, the inhibition of histone deacetylase (HDAC) facilitated the acetylation of HMGB1, thus promoting its release[67]. Immunogenic cell death is a type of cell death that occurs through the activation of adaptive immunity. This type of cell death occurs when tumor cells produce HMGB1, which then binds to TLR4 and RAGE[68], thereby stimulating the body's immunological response to tumor cells, a mechanism often used in cancer treatment strategies[69,70].

HMGB1-related diseases

Based on the results of the bibliometric analysis, as shown in Figure 6, research on HMGB1-related diseases initially focused primarily on critical care medicine, followed by cardiovascular diseases. During endotoxemia and sepsis, elevated levels of HMGB1 in tissues and circulation induce intestinal barrier dysfunction[71] and acute pulmonary injury[72], and even fatal multi-organ failure[73]. In a manner analogous, HMGB1 acts as an intermediary of multiple organ failure during ischemia-reperfusion injury and is regarded as a biomarker of injury after liver and kidney transplantation[74,75]. HMGB1 levels are also associated with the urgency of several respiratory diseases, including asthma, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and pneumonia[76]. Available data suggest that the release of HMGB1 is associated with the progression of SARS-CoV-2 from acute respiratory failure to sepsis[77]. In terms of cardiovascular disease, patients who suffer from atherosclerosis, myocardial infarction, cardiovascular inflammation, or heart failure and have elevated levels of HMGB1 in their serum are more likely to have a negative outcome from their disease[78,79]. Most studies imply that HMGB1 mediates injury through HMGB1-RAGE, with a particular focus on its role in atherosclerosis, where it is overexpressed in vascular smooth muscle cells, endothelial cells, foam cells, and macrophages[80,81]. Moreover, HMGB1 has been found to induce angiogenesis in tumor cells, involving the upregulation of neuropeptide 1, VEGF receptors 1 and 2, vascular endothelial growth factor A (VEGFA), platelet-derived growth factors, leukocyte adhesion molecules, and angiogenesis factors[82,83]. Similar literature clustering and topic evaluation of research fields indicate that as research progresses, the association of HMGB1 with cancer, autoimmune diseases, liver fibrosis, and diabetes is receiving increasing attention. Although HMGB1 is linked to a wide range of cancers[84,85], such as hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colorectal cancer, melanoma, esophageal cancer, mesothelioma, and others, it actually plays a dual role in the development of cancer[86]. On one hand, HMGB1 serves an anti-tumor role due to its contribution in the ICD process as well as the fact that it helps maintain the structure and integrity of the genome[87,88]. In contrast, a high level of HMGB1 production is linked to the development and spread of malignant tumors[84]. As a kind of autoimmune disease associated with HMGB1, rheumatoid arthritis is first reported, with elevated levels of HMGB1 in both the serum and the joint where inflammation occurred[89,90]. In one study, injection of HMGB1 into healthy joints resulted in NF-κB activation, IL-1β production, and arthritic symptoms in 80% of animals[91]. It may be hypothesized that HMGB1's complex formation with IL-1a, IL-1b, and LPS[92] is the pathogenic mechanism that causes the immunological and inflammatory response at the joints to be amplified. Serum HMGB1 levels are correlated with disease index and anti-dsDNA levels in SLE patients[93]. Notably, liver fibrosis, a common pathway of regression in chronic liver disease, also shows a high correlation with HMGB1, which may be related to its promotion of hepatic stellate cell proliferation and induction of fibronectin and collagen deposition[94,95]. In addition, HMGB1 has been found to promote the progression of both type 1 and type 2 diabetes mellitus[96,97], which may be related to its mediated inflammatory and immune responses.

In recent years, research on HMGB1 in relation to neurological disorders has been at the forefront and has received considerable attention from a large number of scholars (Figure 8B). HMGB1 mediates neuroinflammation by binding to mediators such as RAGE and TLR4, and is associated with early brain injury (EBI) after traumatic brain injury (TBI) [98,99]and subarachnoid hemorrhage (SAH) [100]. In addition, HMGB1 is associated with various neurodegenerative illnesses such as Alzheimer's disease[101], Huntington's disease[102], Parkinson's disease[103], and amyotrophic lateral sclerosis[104]. The results of keyword analysis showed that HMGB1 was closely related to the occurrence of epilepsy. It induces tissue damage and inflammatory responses through the TLR4-dependent pathway[105] and other complex receptor interactions such as IL-1R[106], TLR2[107], RAGE[108], and NMDAR[109], leading to seizures. At the same time, HMGB1, released from damaged neurons and astrocytes, may induce pain hypersensitivity by activating RAGE or TLR4 receptors, which may be an important factor in neuropathic pain [110,111]. After ischemic brain injury, HMGB1 is involved in the upregulation of hepcidin in astrocytes through ferroptosis and causes a sharp increase of cerebral iron levels[112]. In depression models, HMGB1 induces depressive-like behaviors by activating the TLR4/NF-κB signaling pathway in the hippocampus[113], and sustained production of HMGB1/RAGE in microglia may increase susceptibility to depression[114]. However, the role and specific mechanisms of HMGB1 in these diseases still lack consistency and require further research to uncover its complex functions and potential therapeutic targets.

HMGB1-related clinical treatment

HMGB1 has become a focal point of research due to its detrimental roles in various diseases, particularly within the realm of critical care medicine. Highly cited literature analysis shows that therapeutic strategies for HMGB1 are rapidly developing, including anti-HMGB1 antibodies[115], inhibitors[116], insulin[117], vasoactive intestinal peptides [118] and natural compounds such as quercetin[119] and Chinese herbal extracts [120] [121]. These methods have been shown to protect laboratory animals from endotoxemia or sepsis. Interestingly, HMGB1 antibodies attenuated septic injury dose-dependently[122]. In the Intensive Care Unit (ICU), HMGB1 serves as a biomarker for assessing the severity of sepsis through scoring systems like the Disseminated Intravascular Coagulation (DIC) score and Sequential Organ Failure Assessment (SOFA) score[123]. Furthermore, HMGB1 has been identified as a therapeutic target for acute-on-chronic liver failure (ACLF)[124]. In vivo studies have shown that treating mouse tumor models with anti-HMGB1 antibodies or inhibitors (e.g., BoxA) resulted in reduced tumor burden and improved survival rates[125]. Meanwhile, autophagy inhibitors like chloroquine (C.Q.) can slow down asbestos-induced mesothelioma carcinogenesis by inhibiting HMGB1-induced autophagy[126]. Treatment with TLR4 antagonist Eritoran reduced serum HMGB1 levels and attenuated hepatic ischemia-reperfusion injury[127]. For rheumatoid arthritis, oxaliplatin, glucocorticoids, and soluble RAGE, among others, have shown inhibitory effects on disease progression[90]. In severe cases of COVID-19, blocking the HMGB1-Ager pathway

may help prevent the formation of ACE2[128], and different HMGB1 gene polymorphisms and protein isoforms have been associated with varying disease progression and outcomes in patients[129]. In addition, inhibiting the TLR4 receptor with HMGB1 antibodies has been demonstrated to alleviate seizures in an erythropoietin-induced epilepsy model[130].

In conclusion, basic medical research is currently working on the production of pharmaceuticals to treat related disorders by targeting the expression, release, or activation of HMGB1. In the future, firstly, additional clinical trials are required to elucidate the therapeutic impact of these treatments. Secondly, research in this field may concentrate on drugs that target the synthesis of HMGB1 but not limited to the expression, release, or activation. Thirdly, the biological effects of HMGB1 often vary due to different receptors and participating cells. Therefore, the development of drugs with higher specificity and effectiveness is warranted.

Limitations

The current work is to initially apply bibliometric visualization analysis to systematically review the research journey from the publication of the first article in the hgbm1-related field up to 2024. Nevertheless, this study has certain shortcomings unavoidably. First off, this study solely used data from the WOSCC database; it did not incorporate information from other databases like PubMed, the Cochrane Library, or Google Scholar. Despite WOSCC's thoroughness and dependability, the data in its database might contain some missing articles. Secondly, this study only included works in English, which could have skewed the findings. Thirdly, the data in this study could also be contradictory in other ways, such as if the same institution used different names at different times.

Conclusion

This study reviewed trends, hotspots, and frontiers of HMGB1-related research from 1976 to 2024 using bibliometric analysis. Influential journals in this area include International Immunopharmacology, Journal of Biological Chemistry, etc. In the field, reputable authors include Bianchi, M E, Tracey, Kevin J, Yang, H, and Andersson, U. The association between HMGB1 and neurological damage may be a route for future research. Immunology, inflammatory injury, cancer, and neurological damage are hot areas in the field. Our study elucidates the fundamental scientific knowledge and different interrelationships of HMGB1 and offers significant insights regarding current and emerging areas of HMGB1-related research. The results of this study should aid academics in better understanding current general trends, locating collaborators, and spotting more promising and innovative research topics.

Abbreviations

ACLF: acute-on-chronic liver failure; C.Q.: chloroquine; DAMP: damage-associated molecular pattern; DAMPs: damage-associated molecular patterns; DIC: Disseminated Intravascular Coagulation; EBI: early brain injury; HCC: hepatocellular carcinoma; HMG: high mobility group; HMGB1: High Mobility Group Protein B1; HDAC: histone deacetylase; IF: impact factor; ICU: Intensive Care Unit; JCR: Journal Citation Reports; LPS: lipopolysaccharide; LPS-mediated: Lipopolysaccharide-mediated; NSCLC: non-small cell lung cancer; PCA:principal component analysis; SOFA: Sequential Organ Failure Assessment; SAH: subarachnoid hemorrhage; TLR2 and TLR4: Toll-like receptors 2 and 4; TFRC: transferrin receptor; TBI: traumatic brain injury; UV: ultraviolet; VEGFA: vascular endothelial growth factor A; WOSCC: Web of Science Core Collection database

Author Contributions

JW, LQ, GL, PD and YW: study conception, design and conduct, and full access to all the data in the study. LQ: draft the manuscript. YW: accept accountability for the correctness of the data analysis and interpretation. The manuscript was critically revised by JW, LQ, GL, PD and HL for key intellectual substance. The article's submission was reviewed and approved by all authors.

Ethics Approval and Consent to Participate

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Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The article material contains the original contributions that were presented in this study. Further inquiries can be directed to the corresponding authors.

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Short Communication Life Conflux

A Mendelian randomization study investigating the causal relationship between 35 blood and urine metabolite biomarkers and postmenopausal osteoporosis

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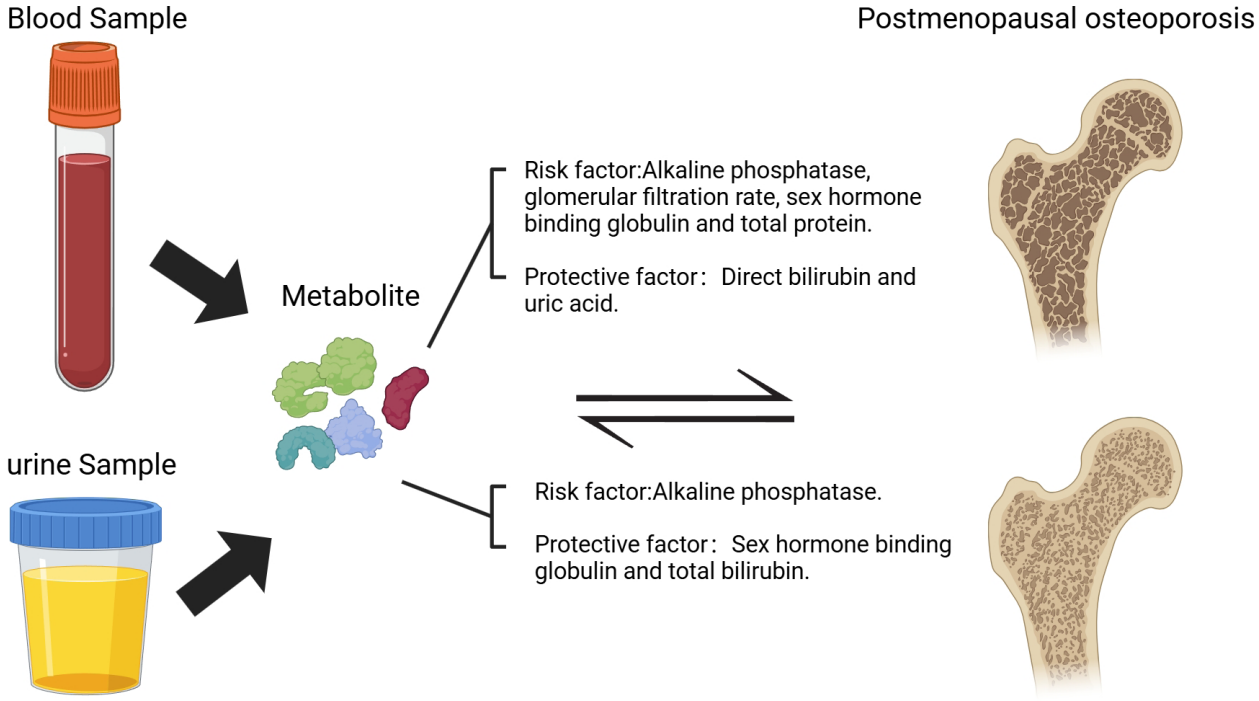
Graphical Abstract



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Short Communication Life Conflux

A Mendelian randomization study investigating the causal relationship between 35 blood and urine metabolite biomarkers and postmenopausal osteoporosis

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Objective: This study intends to investigate the causal association between 35 blood and urine biomarkers and postmenopausal osteoporosis (PMOP) through two-way Mendelian randomization analysis.

Methods: This study adopted a two-way Mendelian randomization analysis, with data sourced from the UK Biobank and the Finnish Biobank Study. Among them, the R12 dataset of the Finnish Biobank Study was used as the test set, and the R11 dataset as the validation set. The study regarded 35 biomarkers as exposure factors and PMOP (a condition characterized by decreased bone density after menopause) as the outcome variable. It was analyzed through methods such as the inverse variance weighting method, the weighted median method, and MR-Egger regression, and combined with the MR-PRESSO test to exclude the influence of pleiotropy.

Results: In the positive direction analysis, alkaline phosphatase, glomerular filtration rate, sex hormone-binding globulin, and total protein showed statistical significance in both the test set and the validation set, and they were all risk factors for PMOP. Direct bilirubin and uric acid demonstrated statistical significance in both the test and validation sets, and they served as protective factors against PMOP. In the negative direction analysis, alkaline phosphatase showed statistical significance in both the test set and the validation set, being a positive result for PMOP; sex hormone-binding globulin and total bilirubin showed statistical significance in both the test set and the validation set, being negative results for PMOP.

Conclusion: Employing bidirectional Mendelian randomization methodology, this investigation elucidated the causal relationships between multiple hematological and urinary biomarkers and PMOP. The results provide promising biomarker candidates for future diagnostic and therapeutic strategies targeting PMOP, while simultaneously establishing a robust framework for subsequent exploration of its underlying pathophysiological mechanisms.

Keywords: Postmenopausal osteoporosis; Blood and urine biomarkers; Mendelian randomization; Causal association; Metabolite.

Introduction

Postmenopausal osteoporosis (PMOP) represents a prevalent metabolic bone disorder predominantly affecting women following menopause, marked by a reduction in bone mineral density and deterioration of bone microarchitecture. This condition substantially elevates fracture susceptibility, profoundly compromising patients' quality of life and longevity [1]. As global demographic trends shift toward an aging population, the prevalence of PMOP continues to escalate annually, emerging as a critical public health concern worldwide [2].

The pathogenesis of PMOP is complex and involves multiple factors, including estrogen deficiency, imbalance in bone metabolism, genetic factors, and lifestyle, etc. [1-3]. In recent years, biomarkers in blood and urine have demonstrated potential application value in the diagnosis, risk assessment, and treatment monitoring of osteoporosis [4]. For example, biomarkers such as alkaline phosphatase and sex hormone-binding globulin (SHBG) have been confirmed to be closely related to bone metabolism [5, 6]. However, the current research on the causal relationship between these biomarkers and PMOP is still insufficient. Most studies are only based on cross-sectional or observational designs, making it difficult to clarify the causal direction.

Mendelian Randomization (MR) represents a robust analytical approach that leverages genetic variants as instrumental variables to infer causal associations, thereby minimizing the impact of confounding variables and bidirectional causation [7]. This methodology has gained significant traction in contemporary research, particularly in elucidating the etiological links between various biomarkers and disease phenotypes. The application of MR has become increasingly prevalent in epidemiological investigations, offering a powerful tool for establishing causal inference in complex biological systems. For example, some studies have revealed the causal associations between various metabolites and chronic

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diseases such as cardiovascular diseases and diabetes through MR analysis [8-10]. However, there is currently a lack of systematic research on the causal relationship between blood and urine biomarkers and PMOP.

This research employs bidirectional MR to investigate potential causal relationships between 35 circulating and urinary biomarkers and PMOP. Leveraging comprehensive datasets from the UK Biobank and Finnish Biobank studies, this investigation seeks to validate established biomarker-PMOP associations while potentially identifying novel diagnostic indicators. The findings are expected to contribute significantly to advancing our understanding of PMOP pathogenesis, offering valuable insights for diagnostic strategies and therapeutic interventions in this prevalent condition.

Materials and Methods

Study Design

This investigation employed a two-stage analytical approach to examine potential causal relationships between 35 hematological and urinary biomarkers (n=363,228) obtained from the UK Biobank (UKB) [11] and PMOP data derived from the Finnish Biobank Study (FinnGen) [12]. The R12 dataset from FinnGen served as the primary test cohort, while the R11 dataset functioned as the validation cohort. Initial screening for significant associations was conducted through MR analysis using inverse variance weighting (IVW), with biomarkers as exposure variables and PMOP as the outcome measure (significance threshold: $P < 0.05$). To address potential pleiotropic effects, MR-Egger regression was implemented, retaining associations with $P > 0.05$. Result consistency was subsequently verified through complementary analyses using the weighted median approach and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) testing ($P > 0.05$) [13]. Associations satisfying these rigorous criteria were considered to provide robust evidence of causality [14].

Instrumental Variables

This investigation employed a rigorous selection process for genome-wide significant single nucleotide polymorphisms (SNPs) associated with the exposure, applying a stringent significance threshold ($P < 5 \times 10^{-8}$). To minimize potential confounding effects, SNPs exhibiting linkage disequilibrium (LD) were systematically excluded based on established criteria ($R^2 < 0.001$, with a clustering distance of 10,000 kb), while maintaining consistency in the effect allele direction. The instrumental variables were required to meet three fundamental criteria: demonstrating robust association with the exposure, independence from confounding variables, and exerting influence on the outcome exclusively through the exposure pathway [7, 15]. Instrumental variable strength was quantitatively assessed using the F statistic ($F > 10$) to eliminate weak associations [16]. Additionally, minor allele frequency (MAF) was derived from effect allele frequency (EAF) calculations, with SNPs demonstrating $MAF < 0.01$ being excluded to mitigate the impact of rare genetic variations. The final analytical framework incorporated comprehensive SNP formatting and LD pruning procedures (LD threshold 0.001, distance 10,000 kb) to ensure optimal analytical precision.

Statistical analysis

The causal associations between biomarkers and PMOP were investigated using three primary approaches: IVW method, weighted median method, and MR-Egger regression [7, 17]. Heterogeneity among the genetic instruments was assessed through the Cochrane Q test, with a significance threshold set at $P < 0.05$, and the appropriate model (fixed-effect or random-effect) was selected based on the results. Horizontal pleiotropy was evaluated using the MR-Egger intercept test ($P < 0.05$), while potential pleiotropic SNPs were identified through leave-one-out sensitivity analysis. To examine reverse causality, reverse MR analysis was conducted. Additionally, the MR-PRESSO framework was applied to detect and remove outliers associated with horizontal pleiotropy ($P < 0.05$), complemented by the MR-Egger method to assess global pleiotropic effects. All statistical procedures were performed using the R programming environment (version 4.4.1).

Results

Positive MR results: 35 blood/urine metabolites as causal validation of the association between exposure and PMOP as an outcome

The results of the study showed a batch Mendelian randomisation analysis with a p-value of less than 0.05 for the inverse variance-weighted results and selection of the appropriate mode of interpretation based on heterogeneity. In the test cohort, six significant positive associations were identified: alkaline phosphatase (odds ratio [OR]=1.26, 95% confidence interval [CI]=1.09-1.45, P=0.002), glomerular filtration rate (OR=1.21, 95% CI=1.01-1.45, P=0.046), sex hormone binding globulin (OR=1.38, 95% CI=1.16-1.65, P<0.001), total protein (OR=1.26, 95% CI=1.10-1.56, P=0.038), direct bilirubin (OR=0.84, 95% CI=0.71-0.99, P=0.035), and uric acid (OR=0.76, 95% CI=0.64-0.91, P=0.003). In the validation cohort, seven significant positive associations were observed: alkaline phosphatase (OR=1.25, 95% CI=1.07-1.47, P=0.005), glomerular filtration rate (OR=1.31, 95% CI=1.08-1.60, P=0.007), sex hormone binding globulin (OR=1.42, 95% CI=1.18-1.72, P<0.001), total protein (OR=1.27, 95% CI=1.01-1.61, P=0.042), creatinine (OR=0.77, 95% CI=0.64-0.93, P=0.007), direct bilirubin (OR=0.83, 95% CI=0.70-0.99, P=0.027), and uric acid (OR=0.81, 95% CI=0.67-0.92, P=0.003). In particular, alkaline phosphatase, glomerular filtration rate, SHBG, and total protein were statistically significant in both the test and validation sets and were risk factors for promoting the development of PMOP, while direct bilirubin and uric acid were statistically significant in both the test and validation sets and were protective factors for inhibiting the development of PMOP (Figure 1). In the MR Egger's method test, all results did not show pleiotropy (P>0.05), whereas MR-Presso showed statistically significant P-values (P<0.05) before and after correction for SNP at the level of pleiotropy. Detailed data are shown in the Supplementary Material.

Inverse MR results: Causal validation between PMOP as exposure and 35 blood/urine metabolites as outcome

The results were subjected to batch MR analysis, employing a significance threshold of $P < 0.05$ for inverse variance weighted estimates, with the interpretation method adjusted according to heterogeneity levels. In the initial testing cohort, six significant associations were identified: alkaline phosphatase (OR=1.02, 95% CI=1.01-1.03, P=0.003), cystatin C (OR=1.20, 95% CI=1.01-1.02, P=0.017), direct bilirubin

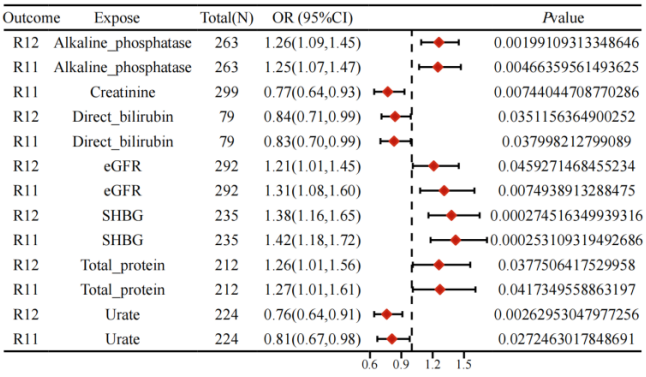

Figure 1. Forest plot of forward MR results

(OR=0.99, 95% CI=0.98-0.99, *P*=0.041), non-albumin proteins (OR=1.01, 95% CI=1.01-1.03, *P*=0.038), SHBG (OR=0.99, 95% CI=0.98-0.99, *P*=0.033), and total bilirubin (OR=0.98, 95% CI=0.97-0.99, *P*=0.002). In the validation cohort, four significant associations were confirmed: alkaline phosphatase (OR=1.02, 95% CI=1.01-1.03, *P*=0.0057), C-reactive protein (OR=1.01, 95% CI=1.01-1.02, *P*=0.034), and SHBG (OR=0.98, 95% CI=0.95-0.99, *P*=0.029). Among them, alkaline phosphatase was statistically significant in both the test and validation sets and was a positive outcome for the occurrence of PMOP, while SHBG and total bilirubin were statistically significant in both the test and validation sets and were a negative outcome for the occurrence of PMOP (Figure 2).None of the results showed multiplicity in the test of the MR Egger's method (*P* > 0.05), and the MR-Presso showed statistically significant P-values before and after correction for SNP at the level of pleiotropy (*P* < 0.05). Detailed data are shown in the Supplementary Material.

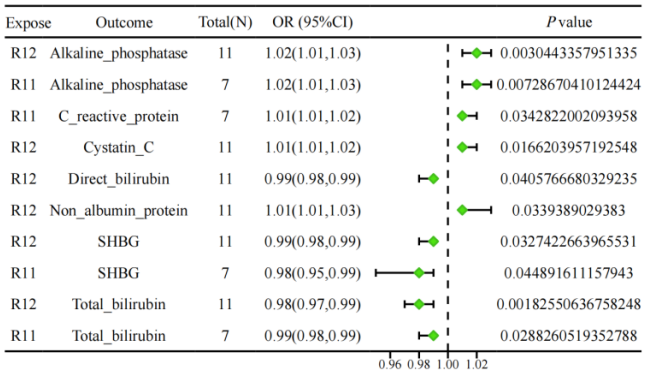

Figure 2. Forest plot of inverse MR results

Discussion

This research represents the inaugural investigation into the bidirectional causal relationships between 35 distinct blood and urine biomarkers and PMOP (PMOP) through MR analysis. The study identified significant causal associations between specific biomarkers and PMOP development, offering novel insights into the disease's pathogenesis and establishing a foundation for identifying potential biomarker targets.

Through comprehensive analyses, alkaline phosphatase, glomerular filtration rate, SHBG, and total protein were identified as significant risk factors for PMOP. Conversely, direct bilirubin and uric acid demonstrated protective effects against PMOP development. These findings align with existing literature on biomarker involvement in bone metabolism regulation. Specifically, alkaline phosphatase serves as a crucial marker of bone turnover, with elevated concentrations typically indicating enhanced bone resorption and formation activities, potentially predisposing individuals to PMOP . Furthermore, alterations in SHBG concentrations may influence the biological activity of sex hormones, which are pivotal in the pathogenesis of PMOP [5, 6].

Emerging evidence from clinical investigations has identified several protective biomarkers, including direct bilirubin and uric acid, which potentially influence skeletal homeostasis through their roles in oxidative stress modulation and inflammatory pathway regulation. Among these biomarkers, bilirubin, a potent endogenous antioxidant metabolite, exerts its protective effects by neutralizing reactive oxygen species and reducing oxidative damage, a critical pathogenic factor implicated in the progression of osteoporotic conditions [18, 19]. The molecular mechanisms underlying bilirubin's protective effects involve its capacity to scavenge free radicals and mitigate oxidative stress-mediated bone resorption, thereby contributing to the maintenance of bone mineral density and structural integrity. In addition, uric acid, as a purine metabolite, also possesses antioxidant capacity, and its protective effect on bone may be related to the inhibition of inflammatory response and modulation of osteoblast activity [20, 21].

In reverse analyses, alkaline phosphatase, SHBG and total bilirubin showed causal associations with PMOP. This suggests that these biomarkers may not only be risk factors for PMOP, but may also be influenced by osteoporotic status. This bidirectional causality suggests that we need to consider the dynamics of biomarkers and their complex interactions with bone health in a comprehensive manner in clinical practice [22].

The bidirectional relationship between SHBG and PMOP may reflect a feedback loop: elevated SHBG reduces bioavailable estrogen, exacerbating bone loss, while osteoporosis-induced inflammatory signals (e.g., IL-6) may further suppress SHBG synthesis in the liver. This hypothesis aligns with recent experimental evidence demonstrating IL-6-mediated downregulation of SHBG in hepatocyte models [23].

Notably, alkaline phosphatase was a risk factor for PMOP in the forward analysis and increased with PMOP in the reverse analysis, suggesting a possible positive feedback mechanism between alkaline phosphatase and PMOP. SHBG was a risk factor for promoting the development of PMOP in the forward analysis, whereas the opposite was true in the reverse analysis, and its level may be suppressed with the development of PMOP.

Although this study used multiple Mendelian randomisation methods to reduce the effects of pleiotropy and confounding, several limitations remain. Firstly, Mendelian randomisation analyses rely on the strength and validity of genetic instrumental variables, and although we used methods such as the F-statistic and MR-PRESSO to assess this, there may still be pleiotropy that has not been fully excluded [16]. Secondly, only 35 biomarkers were analysed in this study, while other potential metabolites or biomarkers may also be causally associated with PMOP, and future studies need to further extend the analysis. While our findings provide valuable insights into the causal associations between biomarkers and PMOP, the generalizability of results may be limited by the

predominantly European ancestry of participants in both UK Biobank and FinnGen datasets. Future studies incorporating diverse populations (e.g., Asian or African cohorts) are warranted to validate these associations across ethnic groups. Furthermore, it is imperative to conduct validation studies across multiple independent cohorts to confirm the robustness and reproducibility of the results, thereby enhancing their generalizability and reliability.

Conclusion

In this study, the causal associations between multiple blood and urine biomarkers and PMOP were revealed by bidirectional Mendelian randomization analysis. These findings not only provide potential biomarker targets for the diagnosis and treatment of PMOP, but also provide new directions for further research on its pathogenesis. Future studies should further explore the potential mechanisms of these biomarkers and validate their causal associations in more populations, with the aim of providing a stronger basis for the prevention and treatment of postmenopausal osteoporosis.

Abbreviations

PMOP, Postmenopausal osteoporosis; MR, Mendelian Randomization; UKB, UK Biobank; FinnGen, Finnish Biobank Study; IVW, Inverse Variance Weighting; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; SNP, Single Nucleotide Polymorphism; MAF, Minor Allele Frequency; EAF, Effect Allele Frequency; LD, Linkage Disequilibrium; OR, Odds Ratio; CI, Confidence Interval; SHBG, Sex Hormone-binding Globulin.

Acknowledgments

Not applicable.

Authors Contributions

Yimin Liu designed the whole project and was responsible for the software. Runtong Liu curated the data and provided resources. Yuhan Zhao conducted the formal analysis and supervised the project. Yongheng Wang carried out the investigation. Xiaoli Hou was in charge of the methodology. Lei Xing administered the project. Fuyuan Cao was responsible for the validation. Xinhao Fan and Binbin An handled the visualization. All authors contributed to writing the original draft and the review and editing of the manuscript.

Ethics Approval and Consent to Participate

The research did not involve any human participants or animals, and therefore did not require approval from an ethics committee. All data used in this study were obtained from publicly available sources and were analyzed in accordance with ethical guidelines and regulations.

Funding Information

Not applicable.

Competing Interests

All authors declare no conflict of interest.

Data Availability

The data supporting this study are included in the manuscript or its supplementary materials. Publicly accessible datasets were utilized for this analysis and are available at the following repositories: (https://www.finngen.fi/en) and (https://www.ukbiobank.ac.uk/).

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Global research status and hotspots of squamous cell carcinoma endothelial cells in the last 20 years: A bibliometric analysis

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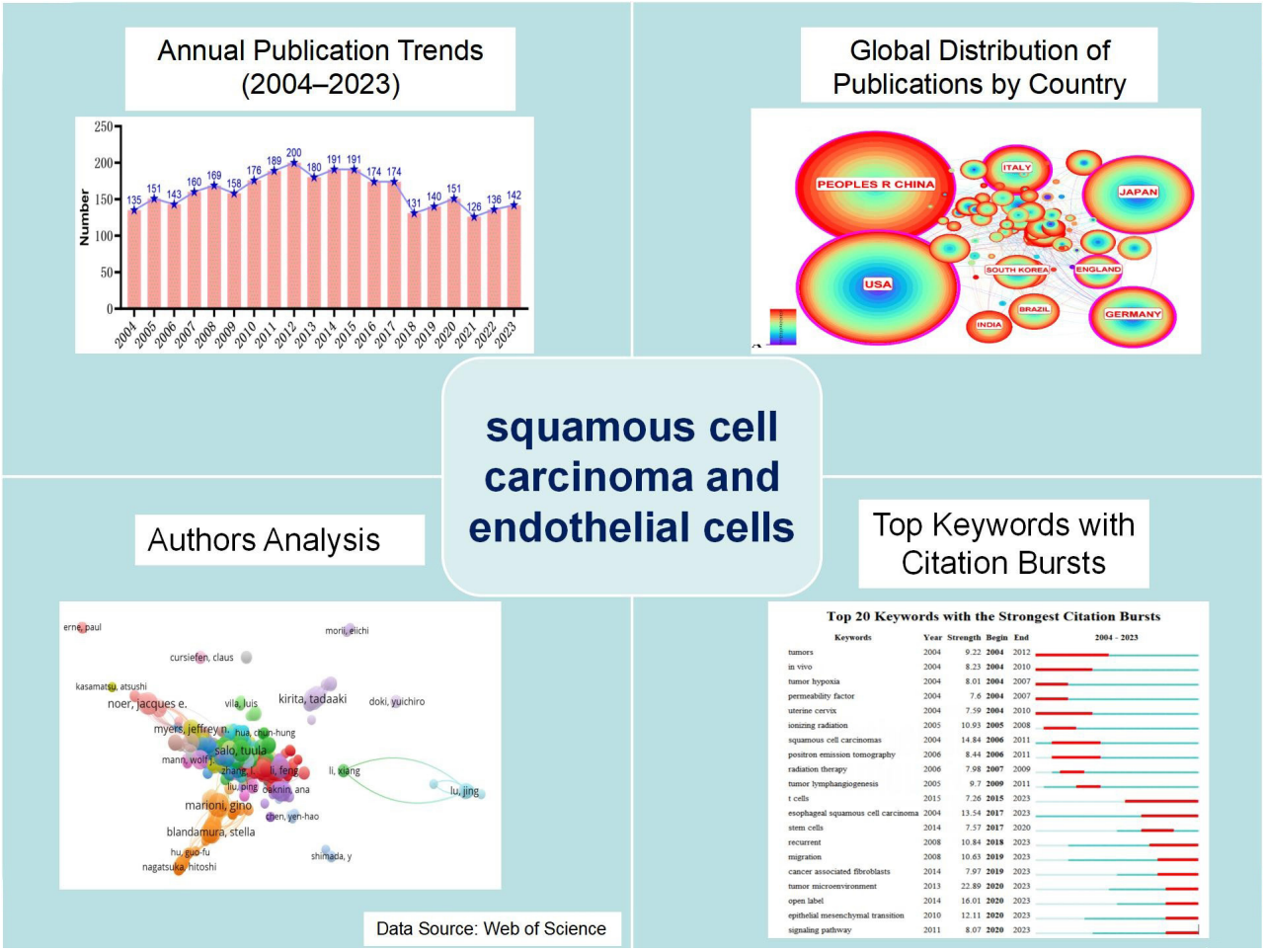
Graphical Abstract



doi: https://doi.org/10.71321/m8qh8d83

Global research status and hotspots of squamous cell carcinoma endothelial cells in the last 20 years: A bibliometric analysis

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Objective: The aim is to analyze the current research status and hotspots of squamous cell carcinoma endothelial cells and to provide a reference for the following fundamental research and clinical treatment.

Methods: The Web of Science Core Collection (WOSCC) database was searched for literature on squamous cell carcinoma endothelial cells from January 1, 2004, to December 31, 2023. The results were analyzed for research trends, authors, countries, research institutions, and keywords using CiteSpace, VOSviewer, and the bibliometrixc data package in R language.

Results: 3217 articles were included in the analysis, and the number of articles published in the past five years is relatively stable. The number of publications from 89 countries and 3,163 institutions has been relatively stable in the past five years, of which 800 are from China, 195 are from the University of Texas System, which is higher than that of other countries and institutions, and there is a big difference in the number of publications between countries and institutions. Prof. Marioni Gino has published 16 relevant papers, and 713 citations have been given to Forkman J, so there is more frequent cooperation between scholars. There are more frequent collaborations among scholars. Eight hundred sixty-eight journals published relevant papers, with Oral Oncology having the highest number of articles and Cancer Research having the highest number of citations. The most cited reference is "Hallmarks of cancer: the next generation," DOI:10.1016/j.cell.2011.02.013 (intensity 21.75). In the last five years, keywords with high intensity are migration, tumor microenvironment, open-label, epithelial-mesenchymal transition, t cells, esophageal squamous cell carcinoma, and recurrent.

Conclusion: The development of squamous cell carcinoma endothelial cell research is uneven among different countries, institutions, and authors, and the journal Oral Oncology publishes the most relevant papers, with current research hotspots including metastasis, recurrence, tumor microenvironment, epithelial-mesenchymal transition, and open labelling.

Keywords: Squamous cell carcinoma; Endothelial cells; Research progress; Bibliometric analysis; hotspot.

Introduction

Squamous cell carcinoma (SCC) is an aggressive malignant tumor commonly found in epithelial tissues of the skin, oral cavity, esophagus, and lungs[1]. As the constitutive cells of blood vessels, endothelial cells play a key role in maintaining tissue homeostasis and play a complex role in tumor angiogenesis, immune escape, and tumor microenvironment regulation[2].

In recent years, the role of endothelial cells in the development of squamous cell carcinoma has been gradually revealed with the in-depth study of the tumor microenvironment[3]. Endothelial cells are not only involved in tumor angiogenesis but also influence the invasiveness of tumor cells and the infiltration of immune cells by secreting various cytokines and chemokines[4-5]. In addition, aberrant activation of endothelial cells is closely related to tumor metastasis and prognosis, and the exact mechanism of endothelial cells in squamous cell carcinoma is still not fully understood despite the progress of existing studies[6-7].

Bibliometric analysis is based on the analysis of existing published literature to assess the intrinsic relationships and dissemination patterns in the literature, enabling researchers to quickly understand the hotspots in the field and promote the dissemination and sharing of knowledge[8]. In this study, we used bibliometric analysis to analyze the literature on endothelial cells in squamous cell carcinoma in the last 20 years to provide a reference for future basic research and clinical treatment.

Materials and Methods

Data collection

This data was collected by searching the core database in Web of Science (https://www.webofscience.com/wos/woscc/basic-search) for literature on squamous cell carcinoma

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endothelial cells. The search strategy was "TS=(squamous cell carcinoma) AND (Endothelial Cells)," and the literature type was selected as a treatise or review. The period was set from January 1, 2004, to December 31, 2023, and the retrieval was conducted on September 24, 2024. Plain text files, tab-delimited files, and BIBTEX formats were selected for downloading in WOSCC, and the primary information included article title, year of publication, country/region, institution, publication, author, keywords, and cited literature. Two researchers checked and screened the literature after downloading it.

Data analysis

This study used CiteSpace (Version 6.4.R1 Advanced), VOSviewer (1.6.10), and R 4.4.1, GraphPad Prism 10.0 software, to organize and analyze the information in the literature. Where CiteSpace set the period (2004-2023), year slice (1 year), pruning (none), and all other defaults were used, R 4.3.3 chose the bibliometrix packet, and VOSviewer chose the default options.

Results

Annual publication of papers related to squamous cell carcinoma endothelial cells

From January 1, 2004, to December 31, 2023, 3217 squamous cell carcinoma endothelial cell treatises and reviews were published. The annual publication volume was generally greater than 120, of which the highest publication volume was 200 in 2012, and the last five years were more stable at 126 to 151 articles (Figure 1).

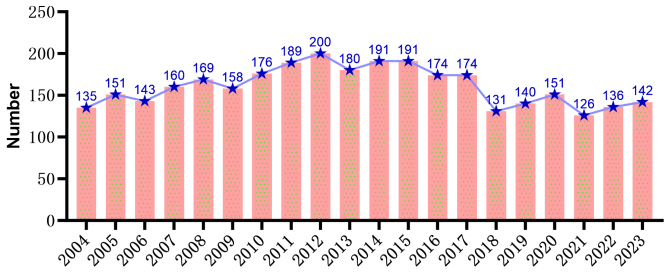

Figure 1. Annual number of publications related to endothelial cells in squamous cell carcinoma

Distribution of countries and institutions

There are 3217 papers from 89 countries and 3163 institutions. The top 3 countries are China (800 papers, centrality 0.13), the United States (795 papers, centrality 0.48), and Japan (408 papers, centrality 0.10), among which China has the highest number of papers, the United States has the highest centrality, and the cooperation among countries is relatively close. The top 3 institutions are the University of Texas System (195 papers, the University of Michigan System (172 papers), and UTMD Anderson Cancer Center (165 papers), and there is a big difference in the number of papers published among institutions. University of Texas System (195 papers), University of Michigan System (172 papers), UTMD Anderson Cancer Center (165 papers), and there is a significant difference in the number of papers published among institutions (Figure 2).

Authors and co-cited authors

A total of 18,298 authors have published related papers, the top 3 authors are Marioni Gino (16 articles), Neor Jacques e (14 articles), and Kirita Tadaaki (13 articles), and the top 3 authors with the highest number of citations are Forkman J (713), Ferrara N (510), and Hanahan D (356). Three hundred fifty-six times), with more frequent collaborations between individual scholars, Marioni Gino has the highest number of publications, and Forkman J has the highest number of citations (Figure 3).

Journals and co-cited journals

A total of 868 journals published papers related to squamous cell carcinoma endothelial cells, with the top 3 journals ranked in the order of 72 articles in Oral Oncology, 64 articles in Anticancer Research, and 59 articles in Oncology Letters, and the top 3 journals ranked in the order of the total number of citations were Cancer Research, Clinical Cancer Research, and Journal of Clinical Oncology, with the top 3 journals ranked in the order of the total number of citations. Clinical Cancer Research, Journal of Clinical Oncology (Figure 4).

Co-cited references

There are 126,764 cited references, and the top 3 cited references highlighted are, in order, "Hallmarks of cancer: the next generation," DOI:10.1016/j.cell.2011.02.013 (intensity 21.75), "Thyroid cancer management: from a suspicious nodule to targeted therapy," DOI:10.1097/CAD.0000000000000617 (intensity 19.33), "Radiotherapy plus cetuximab for squamous -cell carcinoma of the head and neck," DOI:10.1056/NEJMoa053422 (intensity 18.22) (Figure 5).

Keyword analysis

There are 10608 keywords in this analysis, of which the top 3 keywords in order of occurrence are squamous-cell carcinoma (1154 times), endothelial growth factor (1137 times), and expression (810 times); the top 3 keywords in terms of the intensity of keyword emergence display are tumor microenvironment (intensity 22.89), open label (intensity 16.01), squamous cell carcinomas (intensity 14.84), and in the past five years, the keywords with higher intensity are migration, tumor microenvironment, open label, epithelial mesenchymal label, and epithelial mesenchymal label. , epithelial mesenchymal transition, t cells, esophageal squamous cell carcinoma, recurrent (Figure 6).

Discussion

General Information

A total of 3217 squamous cell carcinoma endothelial cell related literature were included for analysis, with an annual publication volume of more than 120 articles, among which the highest publication volume was 200 articles in 2012, which was reduced in recent years compared with the previous one. The volume of publication volume was relatively stable in the past five years.

Among different countries, China has published 800 articles, and the United States has published 795 articles, far more than other countries. There is active cooperation among countries. However, the difference in the number of articles between countries is more prominent. The University of Texas

https://doi.org/10.71321/m8qh8d83

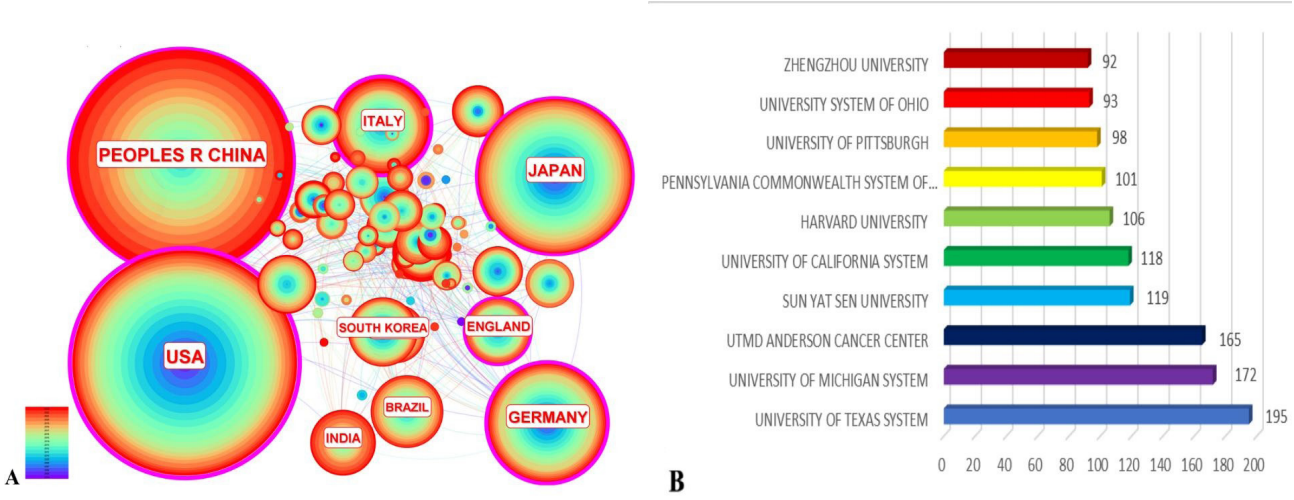

Figure 2. Volume of communications from countries and institutions. **(A)** Volume of communications from different countries and partnerships. **(B)** The Volume of communications from different institutions

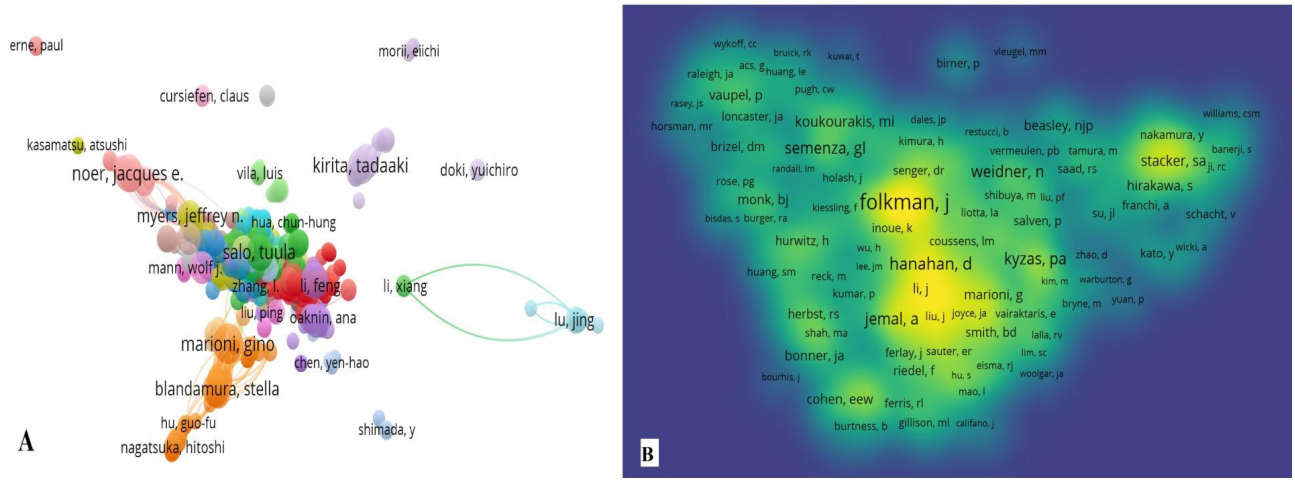

Figure 3. Authors and cited authors. **(A)** Clustering plot of authors' publications. **(B)** Density plot of cited authors.

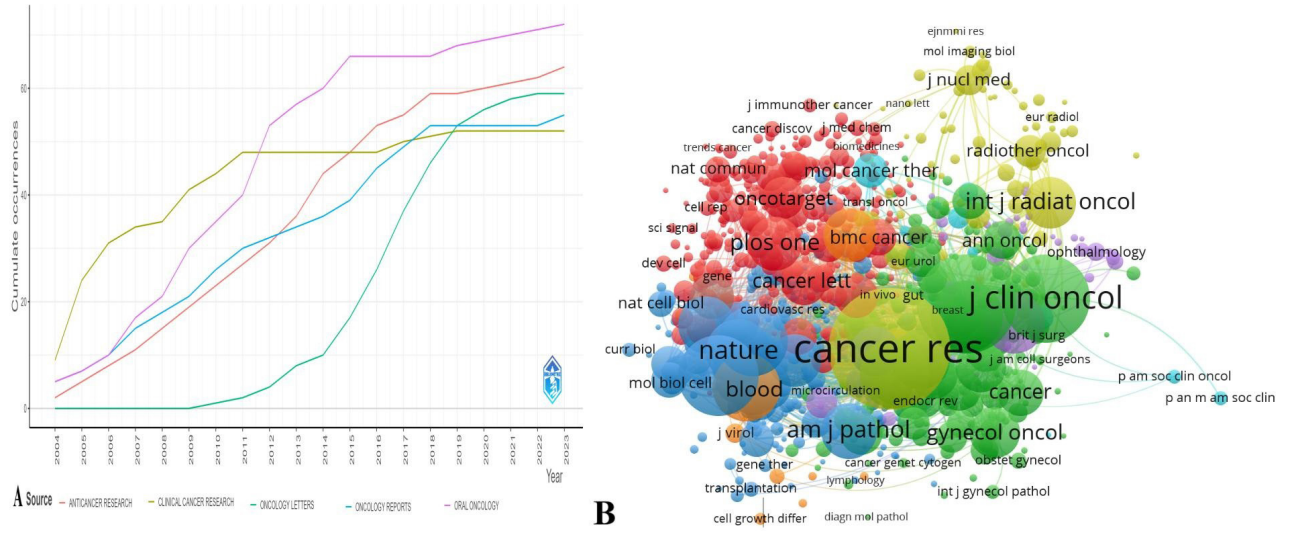

Figure 4. Journals and co-cited journals. (A) Annual publication volume of the top five journals. (B) Density plot of journals.

System has published the most 195 articles among different organizations. In a comprehensive analysis, research teams led by China and the United States have led in squamous cell carcinoma endothelial cell research. However, there are still significant differences between countries and institutions.

In terms of authors, Prof. Marioni Gino has published 16 relevant papers. At the same time, Prof. Forkman J has been cited 713 times, with active collaborations between multiple scholars to promote the common development of the field.

Among the journals, Oral Oncology has the most articles, and Cancer Research has the most citations. The literature Hallmarks of Cancer: The Next Generation has the highest number of citations[9]. More journals are publishing papers on squamous cell carcinoma endothelial cells, indicating that this field has received attention from researchers.

Research hotspot

There are 10,608 keywords, and the keywords with higher

Top 20 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2004 - 2023
Bergers G, 2003, NAT REV CANCER, V3, P401, DOI 10.1038/nrc1093, DOI	2003	7.8	2004	2008	
Hurwitz H, 2004, NEW ENGL J MED, V350, P2335, DOI 10.1056/NEJMoa032691, DOI	2004	13.55	2005	2009	
Franchi A, 2004, CANCER, V101, P973, DOI 10.1002/cncr.20454, DOI	2004	8.78	2005	2009	
Shintani S, 2004, ORAL ONCOL, V40, P13, DOI 10.1016/S1368-8375(03)00127-1, DOI	2004	6.75	2005	2009	
Gombos Z, 2005, CLIN CANCER RES, V11, P8364, DOI 10.1158/1078-0432.CCR-05-1238, DOI	2005	10.23	2006	2010	
Hirakawa S, 2005, J EXP MED, V201, P1089, DOI 10.1084/jem.20041896, DOI	2005	8.39	2006	2010	
Martone T, 2005, ORAL ONCOL, V41, P147, DOI 10.1016/j.oraloncology.2004.08.001, DOI	2005	5.1	2006	2010	
Bonner JA, 2006, NEW ENGL J MED, V354, P567, DOI 10.1056/NEJMoa053422, DOI	2006	18.22	2007	2011	
Jemal A, 2007, CA-CANCER J CLIN, V57, P43, DOI 10.3322/canjclin.57.1.43, DOI	2007	6.63	2008	2012	
Cohen EEW, 2009, LANCET ONCOL, V10, P247, DOI 10.1016/S1470-2045(09)70002-6, DOI	2009	11.03	2010	2014	
Monk BJ, 2009, J CLIN ONCOL, V27, P1069, DOI 10.1200/JCO.2008.18.9043, DOI	2009	8.81	2010	2014	
Lohela M, 2009, CURR OPIN CELL BIOL, V21, P154, DOI 10.1016/j.ceb.2008.12.012, DOI	2009	4.84	2010	2014	
Pàez-Ribes M, 2009, CANCER CELL, V15, P220, DOI 10.1016/j.ccr.2009.01.027, DOI	2009	4.84	2010	2014	
Hanahan D, 2011, CELL, V144, P646, DOI 10.1016/j.cell.2011.02.013, DOI	2011	21.75	2012	2016	
Unknown -, 2018, ANTI-CANCER DRUG, V0, P0	2018	19.33	2018	2019	
Leemans CR, 2011, NAT REV CANCER, V11, P9, DOI 10.1038/nrc2982, DOI	2011	8.73	2012	2016	
Carmeliet P, 2011, NATURE, V473, P298, DOI 10.1038/nature10144, DOI	2011	7.81	2012	2016	
Siegel R, 2013, CA-CANCER J CLIN, V63, P11, DOI 10.3322/caac.21166, DOI	2013	10.7	2014	2018	
Tewari KS, 2014, NEW ENGL J MED, V370, P734, DOI 10.1056/NEJMoa1309748, DOI	2014	7.12	2014	2018	
Ferlay J, 2015, INT J CANCER, V136, PE359, DOI 10.1002/ijc.29210, DOI	2015	15.09	2016	2020	

Figure 5. Cited reference highlighting.

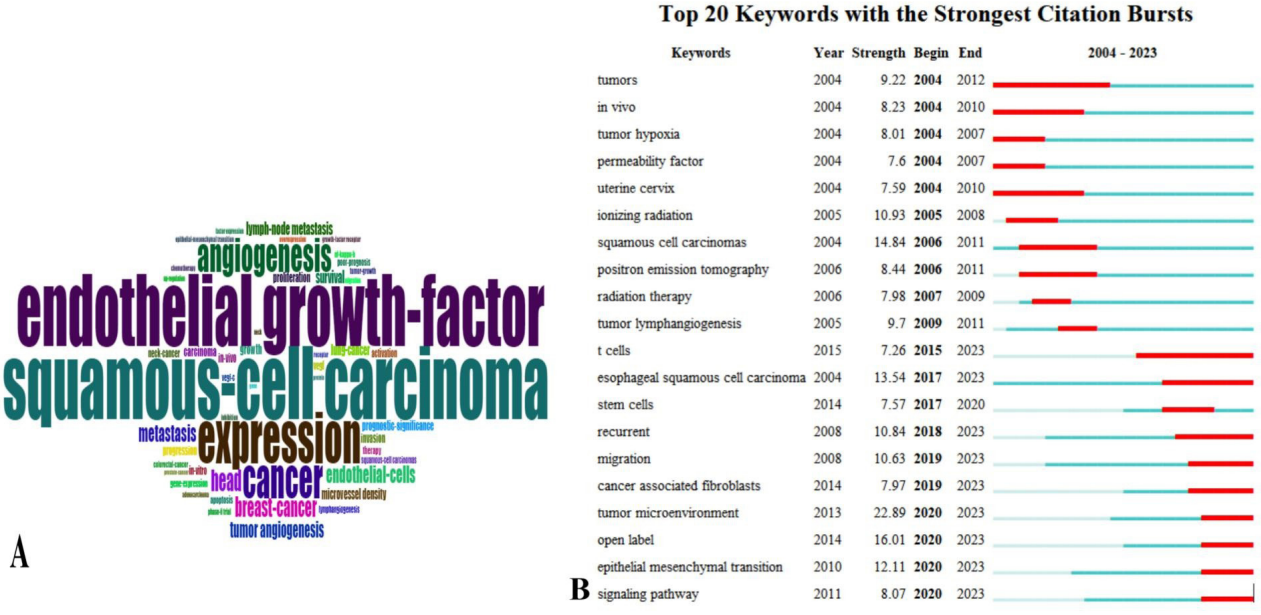

Figure 6. Keywords as well as keyword emergence. **(A)** Keyword word cloud map **(B)** Keyword emergence.

intensity in the last five years are migration, recurrent, tumor microenvironment, epithelial mesenchymal transition, t cells, open label, and esophageal squamous cell carcinoma.

Endothelial cells play an important role in squamous cell development, and it has been shown that specific circRNA (circFNDC3B) is significantly upregulated in oral squamous cell carcinoma (OSCC) and positively correlates with lymph node metastasis. circFNDC3B can accelerate the migration and invasion of OSCC cells and enhance human umbilical vein endothelial cells and lymphatic vessel endothelial cell tube formation ability[10]. Other studies have shown that endothelin on endothelial cells may play a role in paracrine signalling between cells, leading to the proliferation or migration of squamous cell carcinoma[11-12]. Moreover, in squamous cell carcinoma, circulating endothelial progenitor cells may play a role in regulating anti-tumour immune responses and angiogenesis, thus affecting tumor recurrence and prognosis[13]. Various other cytokines may regulate endothelial cell expression and thus cause tumor recurrence[14].

The tumor microenvironment plays an important role in squamous cell carcinoma endothelial cells, which can promote tumor angiogenesis by secreting a variety of cytokines and growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF)[15-16]. This process is crucial for tumor growth and metastasis as it provides essential nutrients and oxygen to the tumor and helps the tumor cells to evade the immune system surveillance[17]. Epithelial-mesenchymal transition is an important biological process associated with a reduction in intercellular adhesion and an increase in the ability of cells to invade and migrate. In contrast, interactions with endothelial cells may impact the tumor's angiogenic, invasive, and metastatic capacities[18-20]. T cells play an important role in anti-tumor immunity. However, their function may be inhibited by the tumor microenvironment[21]. Modulating T cells' function and endothelial cells' angiogenic capacity may provide new strategies for treating squamous cell carcinoma[22].

There are many studies on esophageal squamous cell carcinoma and endothelial cells, in which angiogenesis is one of the key factors in tumor growth and metastasis[23]. In esophageal squamous cell carcinoma, VEGF and its signalling pathway are important in promoting angiogenesis. The interactions between esophageal squamous cell carcinoma and endothelial cells are complex and varied, involving various signalling pathways and molecular mechanisms, which provide potential targets for developing new therapies as the pathogenesis continues to be understood[24]. The treatment of squamous cell carcinoma is also a significant concern for researchers, and some of the development and application of new drugs require open-label trials, which can improve the transparency of clinical trials but need to be alert to potential bias[25].

Limitations

Only papers and reviews published on the Web of Science were included in this study, and not all the data of all the research papers in the world could be included. Meanwhile, this analysis is based on machine learning and natural language processing methods, which may cause operational bias.

Conclusion

In conclusion, the development of squamous cell carcinoma endothelial cell research is uneven among different countries, institutions, and authors, and the journal Oral Oncology publishes the most relevant papers. The current research hotspots include metastasis, recurrence, tumor microenvironment, and open labelling.

Abbreviations:

WOSCC: Web of Science Core Collection; SCC: Squamous cell carcinoma; OSCC: Oral squamous cell carcinoma; VEGF: Vascular endothelial growth factor; FGF: Fibroblast growth factor; PDGF: Platelet-derived growth factor.

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This study was conducted using the web of science database resource. We want to thank all participants.

Author Contributions:

Research design: Jiang Li, Zhe dong, Wei Ren; Data analysis: Jiang Li, Zhe dong, Sibo Bi, Tianwen Gao; Draft Writing and Revision: Jiang Li, Zhe dong Wei Ren, Junyi Zhou.

Ethics Atatement and Consent to Participate:

The research did not involve any human participants or animals, and therefore did not require approval from an ethics committee. All data used in this study were obtained from publicly available sources and were analyzed in accordance with ethical guidelines and regulations.

Funding information:

Not applicable.

Competing Interests:

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data availability:

The research did not involve any human participants or animals, and therefore did not require approval from an ethics committee. All data used in this study were obtained from publicly available sources and were analyzed in accordance with ethical guidelines and regulations.

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Deciphering the multifaceted significance of MPZL2 in pancreatic cancer via in-depth bioinformatics analyses

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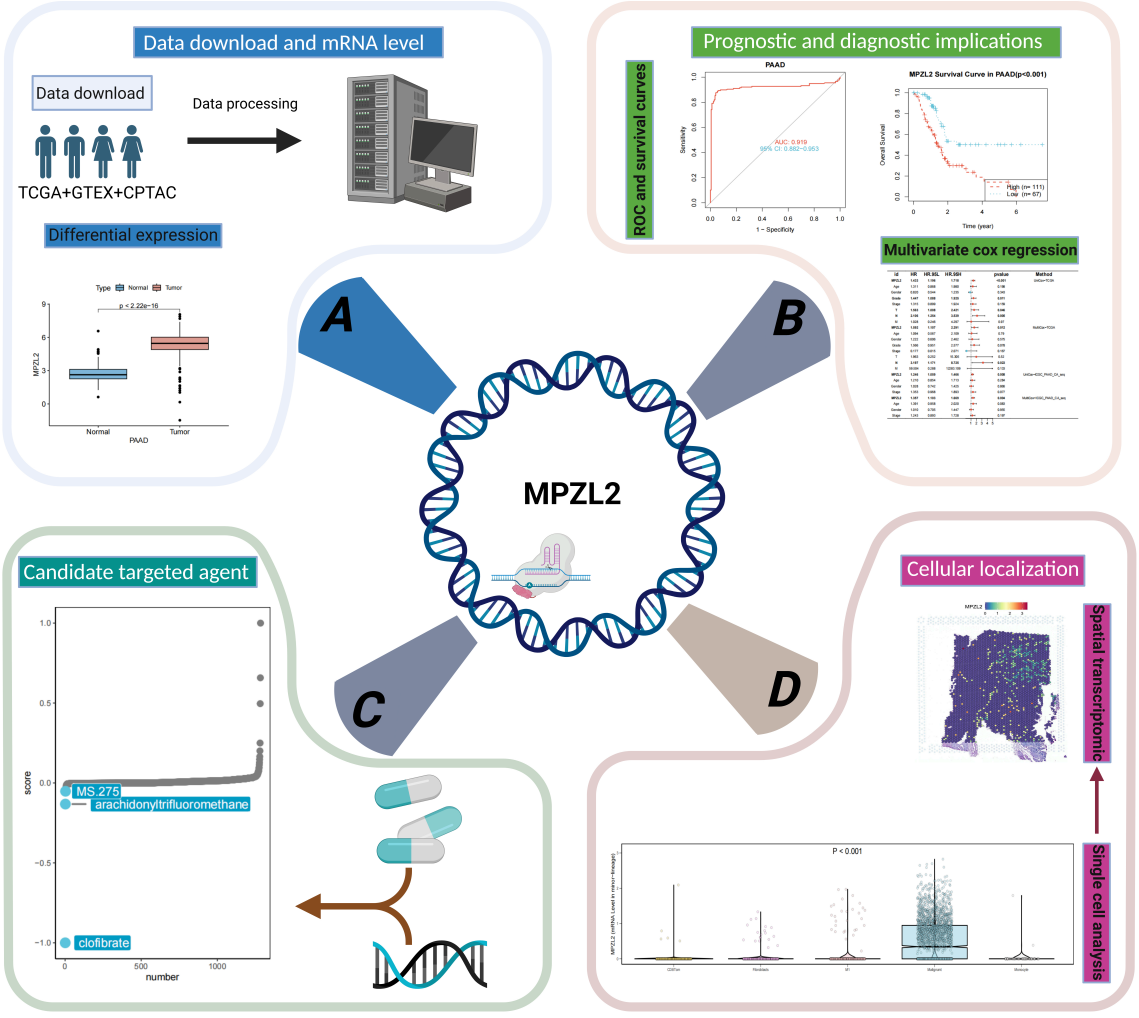
Graphical Abstract



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Deciphering the multifaceted significance of MPZL2 in pancreatic cancer via in-depth bioinformatics analyses

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Abstract: Pancreatic cancer, a deadly malignancy with a median survival of less than 1 year, urgently requires biomarkers for prognosis prediction. MPZL2, associated with poor outcomes in other cancers, was analyzed in pancreatic cancer using TCGA and GTEX data. Our study found increased MPZL2 expression in PAAD, which can distinguish patients from healthy individuals with an AUC of 0.919. High MPZL2 levels correlate with worse survival outcomes and identify malignant tumor cells as the primary source. CMap analysis suggests clofibrate may reverse MPZL2 - driven molecular changes. In conclusion, MPZL2 is a promising prognostic biomarker and therapeutic target in PAAD.

Dear Editor,

Pancreatic cancer, a malignancy challenging to diagnose and treat, ranks as the third - leading cause of cancer - related mortality. Since 2000, the median survival duration of this disease has remained less than 1 year [1]. It is emerging as an increasingly prevalent cause of cancer - related mortality. When the cancer is in its confined stage, patients often present with advanced - stage disease owing to the absence or ambiguity of symptoms [2]. Therefore, there is an urgent need to identify a biomarker capable of more precisely predicting the prognosis and immunotherapy effectiveness in pancreatic cancer patients.

MPZL2 has been demonstrated to have a close association with multiple types of cancers. MPZL2, expressed in lymphoid organs, the thymus, and other epithelial structures, is capable of maintaining the stem-like properties of glioblastoma [3] It has been reported that elevated MPZL2 expression in acute myeloid leukemia and hepatocellular carcinoma is linked to adverse prognosis and recurrence [4,5]. However, the expression of MPZL2 and its implications in pancreatic cancer have never been clarified.

In this study, we primarily utilized the pancreatic cancer dataset from TCGA and the normal sample data from the GTEX database to perform in - depth bioinformatics analyses of MPZL2. The proteomic data of MPZL2 were sourced from the CPTAC database. We evaluated the prognostic and diagnostic implications using Kaplan - Meier survival curves, COX regression models, and ROC curves. Single - cell and spatial transcriptomic analyses determined the cell populations that exhibited significant MPZL2 expression. Additionally, the eXtreme Sum (XSum) method was employed for CMap analysis to identify drugs sensitive to patients with high MPZL2 expression.

Our findings demonstrate that MPZL2 exhibits increased mRNA and protein levels in PAAD (Figure 1A-B). It can accurately distinguish between the pancreatic cancer patient group and the normal group, with an AUC of 0.919 (Figure 1C). Simultaneously, we discovered that elevated MPZL2 expression was significantly associated with poorer overall survival (OS), disease - specific survival (DSS), disease - free interval (DFI), and progression - free interval (PFI) (Figure 1D). Both univariate and multivariate Cox analyses indicated that MPZL2 was detrimental to the overall survival of PAAD patients (Figure 1E) and served as an independent risk factor for PAAD. CMap analysis revealed that clofibrate might reverse the molecular signatures resulting from MPZL2 expression dysregulation, thus counteracting the tumor - promoting effects mediated by MPZL2 (Figure 1F). Single - cell and spatial transcriptomic analyses demonstrated that malignant tumor cells were the specific cell types where MPZL2 was located (Figure 1G-J), and the content of these cells was positively correlated with MPZL2 expression (Figure 1K). Furthermore, spatial transcriptomic analysis revealed that the average expression of MPZL2 in the tumor core and tumor boundary regions was significantly higher than that in the normal regions, and this difference was statistically significant (Figure 1L).

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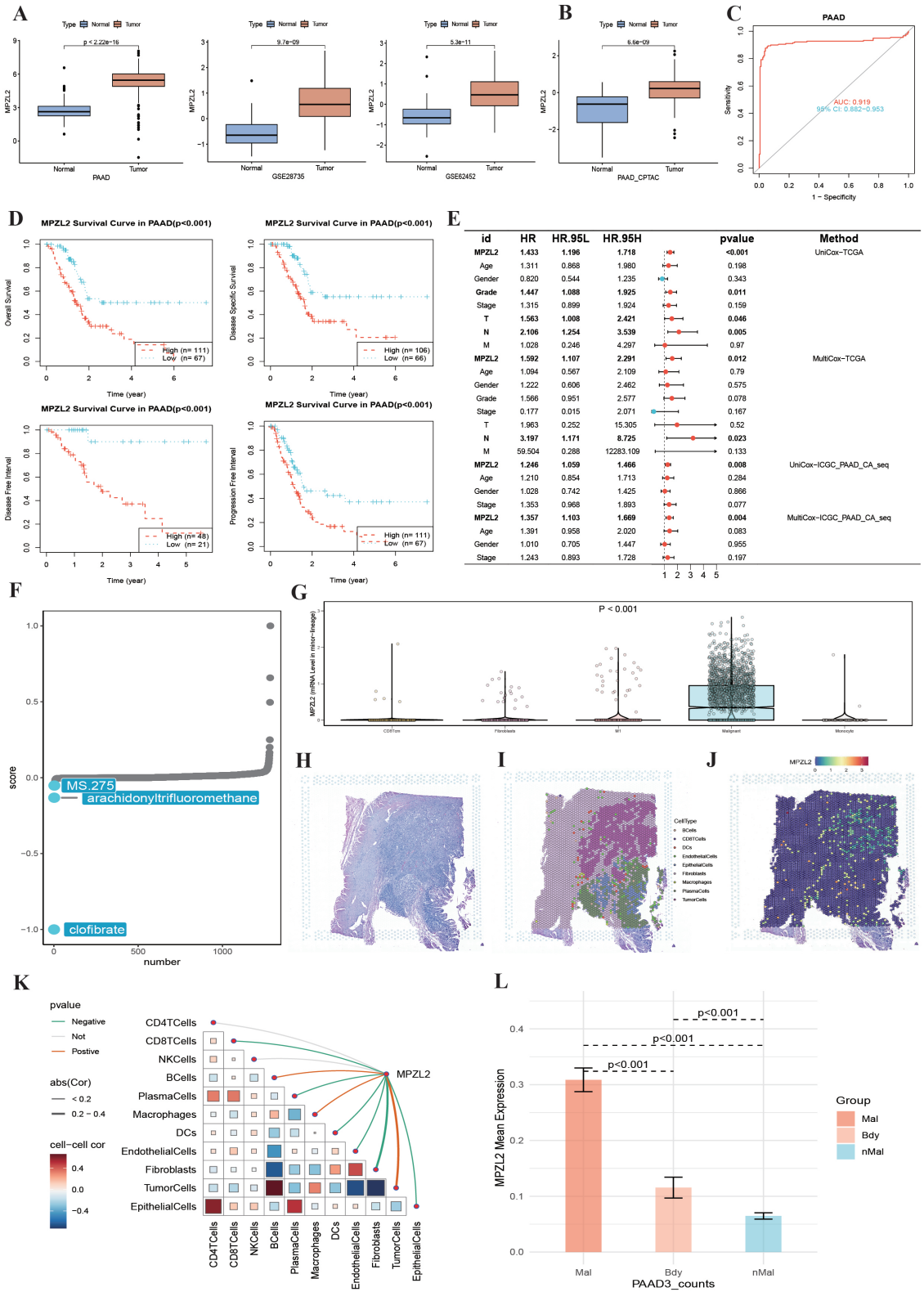

Figure 1 Expression and Prognostic Significance of MPZL2 in Pancreatic Cancer. **(A - B)** MPZL2 expression in normal and tumor tissues. **(C)** Diagnostic efficiency of the ROC curve of MPZL2 based on TCGA data; **(D)** Survival curves of MPZL2 in the TCGA dataset; **(E)** Univariate and multivariate analyses of MPZL2; **(G)** Potential small - molecule compounds and drugs predicted by the XSum algorithm to reverse the biological effects induced by MPZL2 gene - expression dysregulation; **(G - J)** Cellular localization of MPZL2. **(K)** Spearman's correlation between MPZL2 expression and microenvironmental components at the spatial - transcriptomic resolution. **(L)** Differences in MPZL2 expression levels among malignant regions (Mal), tumor - boundary regions (Bdy), and normal regions (nMal) at the spatial - transcriptomic resolution.

In conclusion, our study highlights MPZL2 as a valuable prognostic biomarker and a potential therapeutic target in PAAD, bearing great significance for guiding clinical drug development.

Acknowledgments

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Author Contributions

ZZ and YK was responsible for writing and authoring the article, ZZ,LJ and YF processed the data, and HC were in charge of reviewing the article.

Ethics Approval and Consent to Participate

The research did not involve any human participants or animals, and therefore did not require approval from an ethics committee. All data used in this study were obtained from publicly available sources and were analyzed in accordance with ethical guidelines and regulations.

Funding information

Not applicable.

Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data Availability

All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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