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Research article

Identification of disulfidptosis-related genes and analysis of immune infiltration characteristics in ischemic strokes

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Abstract: Immune infiltration plays a pivotal role in the pathogenesis of ischemic stroke. A novel form of cell death known as disulfidptosis has emerged in recent studies. However, there is currently a lack of research investigating the regulatory mechanism of disulfidptosis-related genes in immune infiltration during ischemic stroke. Using machine learning methods, we identified candidate key disulfidptosis-related genes (DRGs). Subsequently, we performed an analysis of immune cell infiltration to investigate the dysregulation of immune cells in the context of ischemic stroke. We assessed their diagnostic value by employing receiver operating characteristic (ROC) curves. To gain further insights, we conducted functional enrichment analyses to elucidate the signaling pathways associated with these seven DRGs. We identified two distinct subclusters based on the expression patterns of these seven DRGs. The unique roles of these subclusters were further evaluated through KEGG analysis and immune infiltration studies. Furthermore, we validated the expression profiles of these seven DRGs using both single-cell datasets and external datasets. Lastly, molecular docking was performed to explore potential drugs for the treatment of ischemic stroke. We identified seven DRGs. The seven DRGs are related to immune cells. Additionally, these seven DRGs also demonstrate potential diagnostic value in ischemic stroke. Functional enrichment analysis highlighted pathways such as platelet aggregation and platelet activation. Two subclusters related to disulfidptosis were

defined, and functional enrichment analysis of their differentially expressed genes (DEGs) primarily involved pathways like cytokine-cytokine receptor interaction. Single-cell analysis indicated that these seven DRGs were primarily distributed among immune cell types. Molecular docking results suggested that genistein might be a potential therapeutic drug. This study has opened up new avenues for exploring the causes of ischemic stroke and developing potential therapeutic targets.

Keywords: ischemic stroke; disulfidptosis; immune infiltration; single-cell; transcriptome

1. Introduction

Strokes cause long-term disability worldwide, but there are no effective methods to improve functional recovery [1]. Stroke is a medical condition caused by lesions in the cerebral arteries, resulting in the formation of a thrombus or blood clot that leads to changes in blood components and ultimately causes cerebrovascular damage [2]. Multiple studies have reported an increase in stroke incidence, with up to 25% of the global population expected to experience this condition during their lifetime [3]. Strokes are primarily classified into two types: hemorrhagic stroke and ischemic stroke. It is estimated that approximately 87% of strokes are ischemic in nature [4]. The risk factors for stroke include atrial fibrillation, hypertension, hyperlipidemia, diabetes, smoking and alcohol consumption [5]. At present, treatment options for ischemic strokes are extremely limited. The current treatment plan for stroke aims primarily to minimize damage and facilitate recovery. The major treatment strategies available today include intravenous thrombolysis and arterial thrombectomy, both of which are often accompanied by cerebral ischemia-reperfusion injury during treatment, further exacerbating brain damage [6]. Identifying new and effective therapeutic targets is critical for treating ischemic stroke, as developing viable treatment options is essential for improving outcomes in clinical practice.

Recent studies have revealed a new form of cell death, which has been named disulfidptosis. Disulfide stress is a rapid form of cell death that occurs when intracellular cysteine accumulates excessively, resulting in the formation of disulfide molecules. In cancer cells with high levels of *SLC7A11* that are deprived of glucose, the excessive accumulation of disulfide molecules causes abnormal disulfide bond formation between actin cytoskeleton proteins. This leads to the disruption of their organization and ultimately results in the collapse of the actin network, leading to cell death [7]. Reducing the formation of disulfide compounds forms the basis for improving ischemic stroke [8]. In addition, correcting the imbalance of thiol-disulfide can be beneficial for ischemic stroke [9, 10]. Currently, the role of disulfidptosis in ischemic stroke is not clear. Therefore, blocking or reversing disulfidptosis may become an important strategy for the future treatment of ischemic stroke.

Ischemic stroke occurs when blood vessels that supply the brain become blocked, leading to a deprivation of oxygen and nutrients in the affected area. This can cause brain damage and cell death [11]. An increasing body of evidence indicates that following acute ischemic stroke, immune cells are recruited to engage in neuroinflammatory processes and maintain homeostasis in the central nervous system [12, 13]. Immune cells from the peripheral system infiltrate into the affected brain tissue during ischemic stroke, where they play a crucial role in maintaining homeostasis through neuroinflammatory processes [14]. Upon experiencing such injuries, the body's immune system will be triggered into action, summoning immune cells to the affected site. Although the immune response in ischemic stroke is essential for clearing dead cells and promoting tissue restoration, it may also induce inflammation

that can worsen the damage done to the brain tissue [15]. Therefore, it is crucial to investigate the relationship between the immune system and ischemic stroke further in order to develop effective prevention or treatment strategies for this disabling condition.

We explored the role of disulfidptosis-related genes (DRGs) and immune cells in ischemic stroke. We employed the least absolute shrinkage and selection operator (LASSO) method to identify key DRGs and calculated the area under the curve (AUC) using receiver operating characteristic (ROC) curves. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis revealed that DRGs were primarily enriched in the regulation of actin cytoskeleton, adherens junction, tight junction and motor proteins signaling pathways. Using consensus clustering, we classified ischemic stroke patients into two clusters. Further analysis was conducted on the immune cell variations between these clusters. We considered the relationship between DRGs and immune infiltration. Through single-cell analysis, it was observed that in the ischemic stroke group, DRGs were primarily expressed in immune cells compared to the control group. Validation of DRGs expression using external datasets confirmed the predictive value of the DRGs prediction model. Finally, molecular docking was performed between predicted drugs and DRGs. This study is expected to enhance our understanding of ischemic stroke and contribute to the development of innovative approaches for its prevention or treatment.

2. Materials and methods

2.1. Data acquisition

We collected ten DRGs from the Pubmed database. Liu et al. had confirmed the existence of ten DRGs [7]. Transcriptome and single-cell datasets on ischemic stroke were obtained from the Gene Expression Omnibus (GEO) database. GSE58294 comprises 69 samples of ischemic stroke and 23 control samples [16]. The GSE16561 dataset comprises 39 stroke samples and 24 control samples [17]. The GSE122709 dataset consists of 10 stroke samples and 5 control samples [18]. GSE174574 comprises three sham operation samples and three ischemic stroke samples [19].

2.2. LASSO and ROC

To identify key DRGs in ischemic stroke, we performed screening using the LASSO method with the R package “glmnet” [20]. The diagnostic efficacy of DRGs in ischemic stroke was assessed by utilizing the “pROC” package to determine AUC [21]. The DRGs were then visualized using boxplots created with the “ggplot2” package.

2.3. Immune infiltration analysis

We investigated the relationship between DRGs and 22 immune cells using the CIBERSORT algorithm. We also constructed a correlation heatmap of DRGs and immune cells.

2.4. Functional analysis and protein-protein interactions (PPI)

In order to gain a deeper understanding of the mechanisms driving ischemic stroke, we conducted an extensive investigation, focusing on seven DRGs. Employing a systematic analytical approach,

Gene Ontology (GO) and KEGG analyses were conducted. Through GO analysis, gene functions were delved into from three distinct angles: molecular function, biological process and cellular component. Utilization of the KEGG database enabled a methodical exploration of genetic information, enriching the understanding of gene functions, biological processes and pathways. These meticulous analyses were carried out using R packages, including "ClusterProfile" and "org.Hs.eg.Db".

2.5. Consensus clustering analysis

We performed consensus clustering using the "ConsensusClusterPlus" package [22] to stratify ischemic stroke patients into two clusters.

2.6. Processing of single-cell RNA-sequencing (scRNA-seq) measurement data

We obtained scRNA-seq data of ischemic stroke from the GEO database and analyzed it with the "Seurat" [23] and "SingleR" packages. The "Seurat" package was employed to apply the cross-dataset normalization (CCA) method for integrating stroke and control samples. Subsequently, cell profiles were filtered based on criteria such as nCountRNA < 5000 and percent.mt < 15. Marker genes were then determined for each cluster based on the obtained clustering results, and cell annotation was performed using the "SingleR" package and PanglaoDB database.

2.7. Predicting potential drug candidates

The DRGs were uploaded to the Drug Signatures Database (DSigDB) [24] for further candidate drug prediction. Access to DSigDB is acquired through the Enrichr platform. Candidate drugs are sorted in ascending order based on their adjusted P-values, with those having an adjusted P-value less than 0.01 considered statistically significant.

2.8. Molecular Docking

The crystal structures of proteins associated with the DRGs were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [25]. Additionally, the three-dimensional structure of drugs was sourced from PubChem [26]. We performed molecular docking of predicted drugs and DRGs using the CB-Dock2 tool [27].

2.9. Statistical analysis

Data processing was carried out with R software (version 4.2.2), and significant differences between two independent groups were identified using the Wilcoxon rank-sum test. A two-sided P-value threshold of < 0.05 was considered statistically significant.

3. Results

3.1. DRGs in ischemic stroke

The flowchart illustrates the sequential steps performed in our study with clear and concise visual representations (Figure 1). Based on the GSE58294 dataset, which includes 23 control samples and 69 ischemic stroke samples, we conducted an analysis of the expression of 10 DRGs. In the ischemic stroke group, when compared to the control group, the expression of *IQGAP1*, *TLN1*, *CAPZB*, *INF2* and *SLC7A11* was upregulated and statistically significant (Figure 2A). LASSO can effectively select the most relevant predictor variables, thereby simplifying the model and improving its interpretability. LASSO regression with tenfold cross-validation was further used to screen for DRGs significantly associated with ischemic stroke patients. The optimal lambda value was obtained from the minimum partial likelihood deviation. Subsequently, the best model with seven DRGs, including *ACTB*, *IQGAP1*, *FLNA*, *PDLIM1*, *MYH10*, *INF2* and *SLC7A11*, was obtained (Figure 2B-C). Then, a risk score was constructed based on the coefficients and categorical values of expression levels as follows: riskscore = (-0.08957191 * *ACTB*) + (0.50853064 * *IQGAP1*) + (-0.49537563 * *FLNA*) + (0.07030566 * *PDLIM1*) + (-0.25041386 * *MYH10*) + (0.10204431 * *INF2*) + (0.05109314 * *SLC7A11*). In the discovery GSE58294 cohort, the riskscore for each ischemic stroke patient was calculated, and the patients were divided into a high-risk group and a low-risk group based on the median risk score (Figure 2D). We generated ROC curves for the seven identified DRGs, demonstrating their strong diagnostic performance. The AUC values for *IQGAP1*, *FLNA* and *SLC7A11* were 0.901, 0.849 and 0.838, respectively (Figure 2E). We've generated an correlation heatmap to visually depict the associations between DRGs and immune cells. In this context, *PDLIM1* is closely linked to CD8+ T cells, *ACTB* is associated with CD8+ T cells, neutrophils, M2 macrophages and eosinophils, *MYH10* primarily correlates with naive B cells and *IQGAP1* encompasses a wide range of associations, including CD8+ T cells, naive B cells, naive CD4+ T cells, M2 macrophages, eosinophils, neutrophils and resting mast cells. *SLC7A11* specifically associates with CD8+ T cells, *INF2* is related to naive B cells and *FLNA* is correlated with neutrophils, CD8+ T cells and naive CD4+ T cells. This heatmap provides a clear representation of the relationships between these genes and various immune cell types (Figure 2F).

3.2. Functional analyses and PPI network of seven DRGs

We performed GO, KEGG and PPI network construction for seven DRGs. The findings from the GO enrichment analysis revealed that these DRGs were primarily associated with processes such as fibroblast migration, platelet aggregation, homotypic cell-cell adhesion, platelet activation and regulation of protein localization to membrane (Figure 3A). Based on the gene ratios observed in the KEGG enrichment results, we have identified the top five pathways. These pathways consist of the regulation of actin cytoskeleton, adherens junction, tight junction, motor proteins and pathogenic *Escherichia coli* infection (Figure 3B). The PPI network analysis underscores potential interactions among the seven DRGs. Yellow lines illustrate "textmining" gene interactions, whereas black lines indicate "co-expression". Interactions represented by purple lines are based on experimental data, while those depicted by blue lines arise from curated databases (Figure 3C).

3.3. Consensus clustering based on seven DRGs

Consensus clustering divides the disease samples of a dataset into several subtypes, thereby discovering new disease subtypes or conducting comparative analysis on different subtypes. We

utilized a consensus clustering approach to partition the samples into two distinct clusters based on the differential expression patterns of seven DRGs. There are 34 patient samples in cluster 1, and 35 patient samples in cluster 2 (Figure 4A-C). Between the two clusters, there are differential expressions of *FLNA, MYH10* and *SLC7A11*. Specifically, the expression levels of *MYH10* and *SLC7A11* are higher in cluster 2 compared to cluster 1 (Figure 4D). In order to examine the disparities in immune cell infiltration between the two clusters, we performed an analysis on immune infiltration. Our results unveiled variations in the presence of naive B cells, resting mast cells and monocytes across the two clusters (Figure 4E). To gain insights into the functional enrichment of DEGs between the two clusters in various pathways, we conducted the KEGG analysis. The DEGs between the two clusters can be found in the Supplementary Materials. The findings demonstrated that these DEGs were predominantly enriched in key pathways such as taste transduction, microRNAs in cancer, intestinal immune network for IgA production and cytokine-cytokine receptor interaction (Figure 4F).

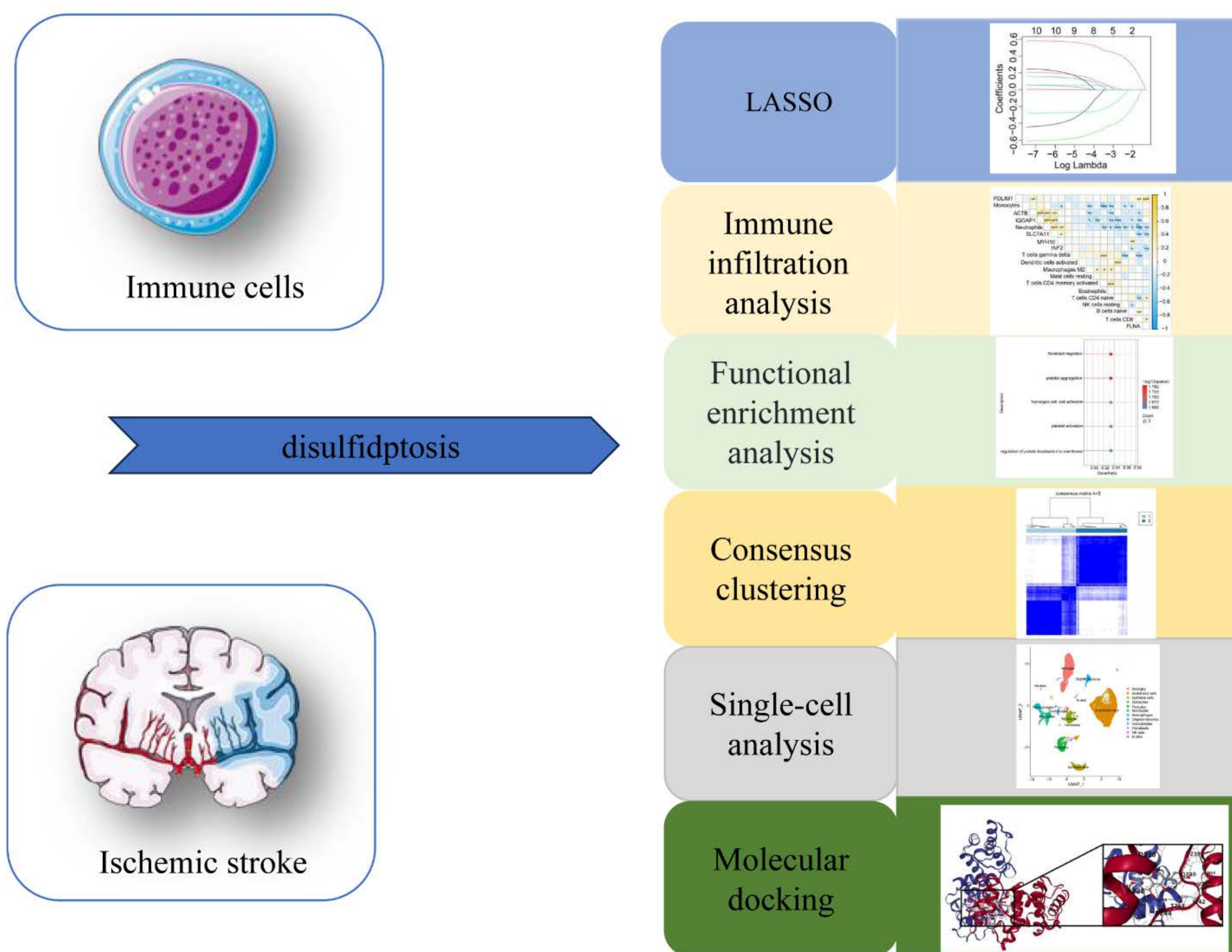

Figure 1. The flow chart for this study.

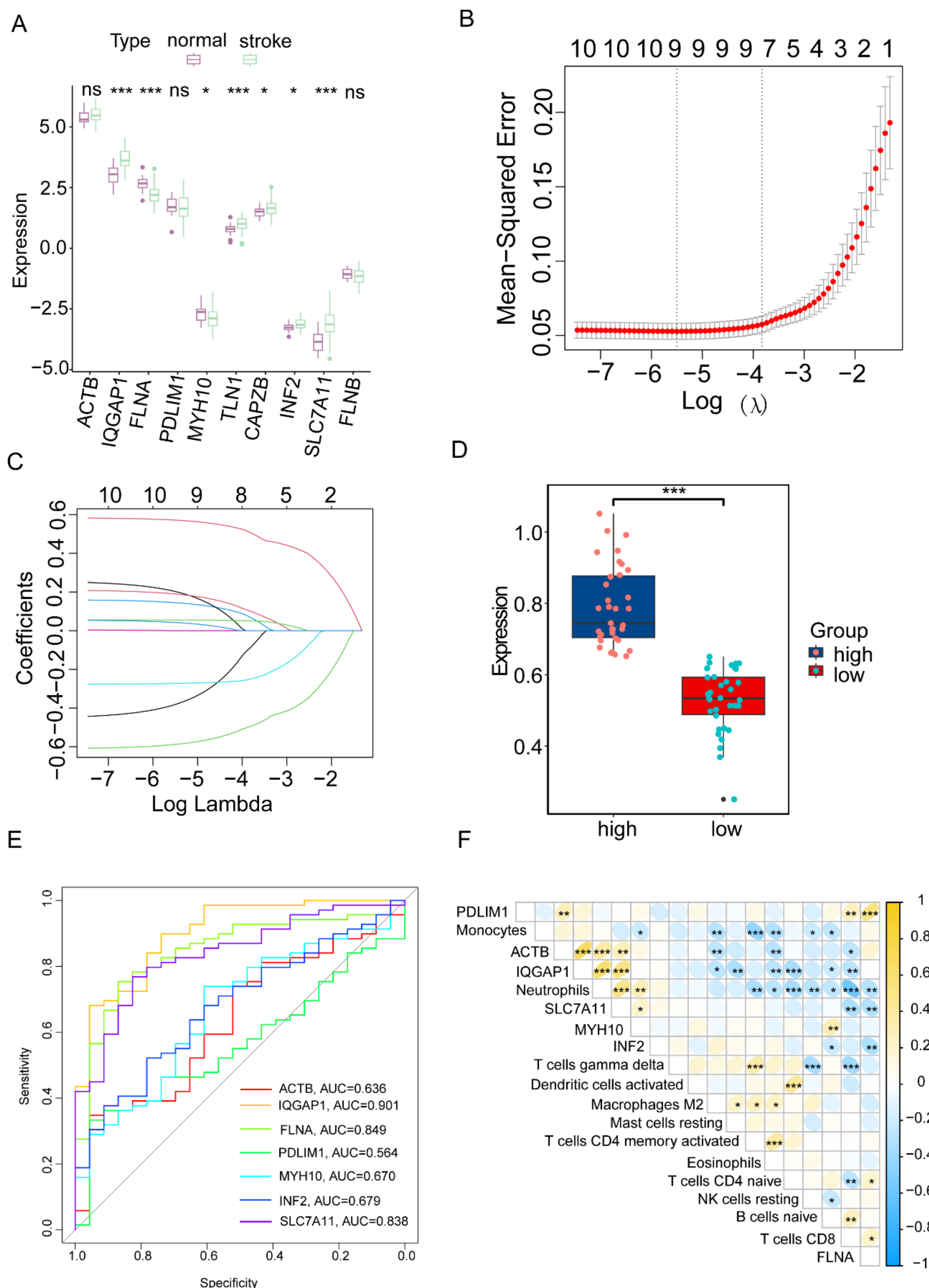

Figure 2. Identification of DRGs in ischemic stroke. (A) Boxplots of ten DRGs between ischemic stroke and control group. (B–C) Constructed disulfidptosis-signatures using LASSO regression. (D) The expression of disulfidptosis-relative riskscores in high and low group of ischemic stroke patients. (E) ROC curves of seven DRGs. (F) The correlation heatmap of seven DRGs and immune cells (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

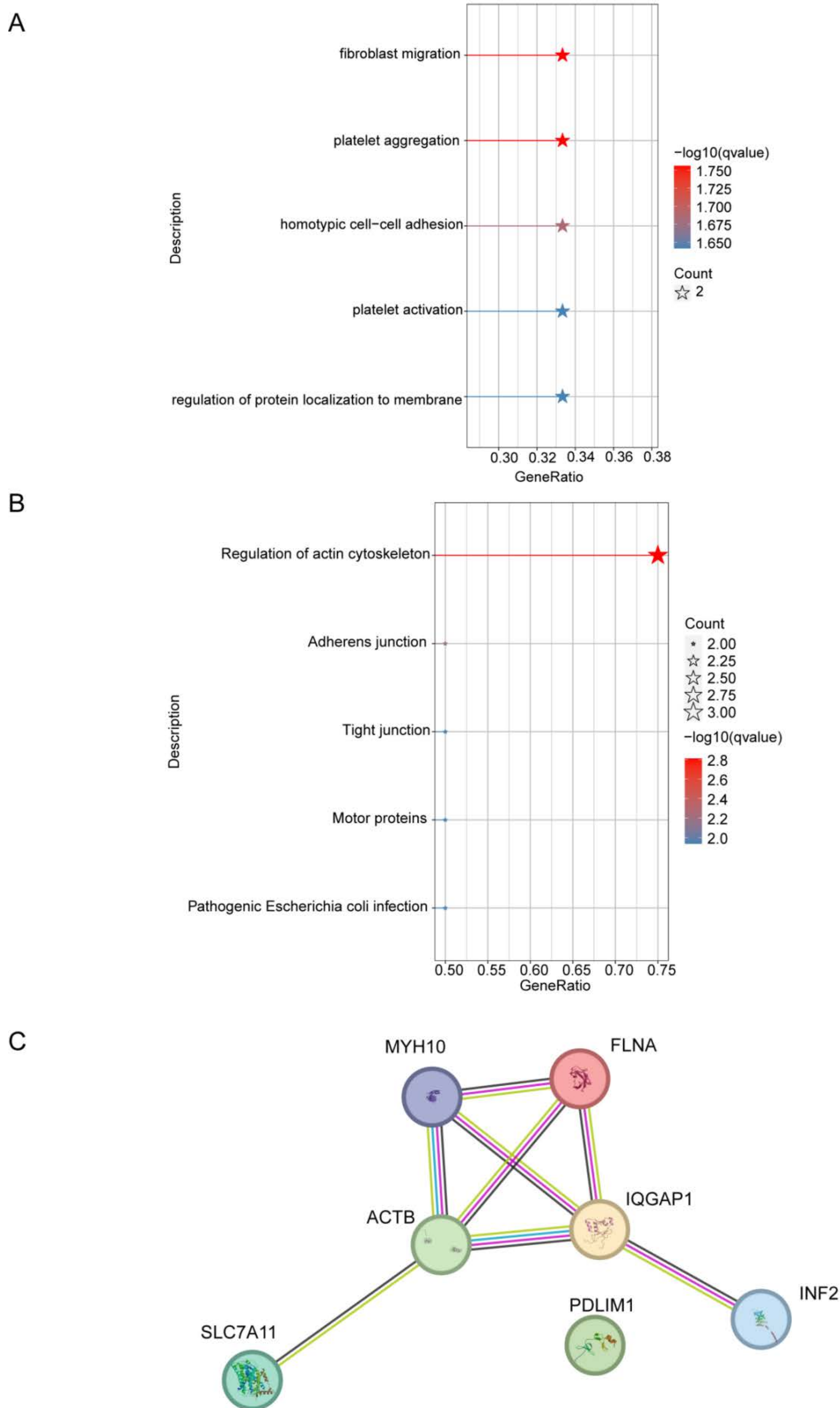

Figure 3. Functional analysis and PPI network of seven DRGs. (A) GO enrichment analysis of seven DRGs. (B) KEGG analysis of seven DRGs. (C) PPI network of seven DRGs.

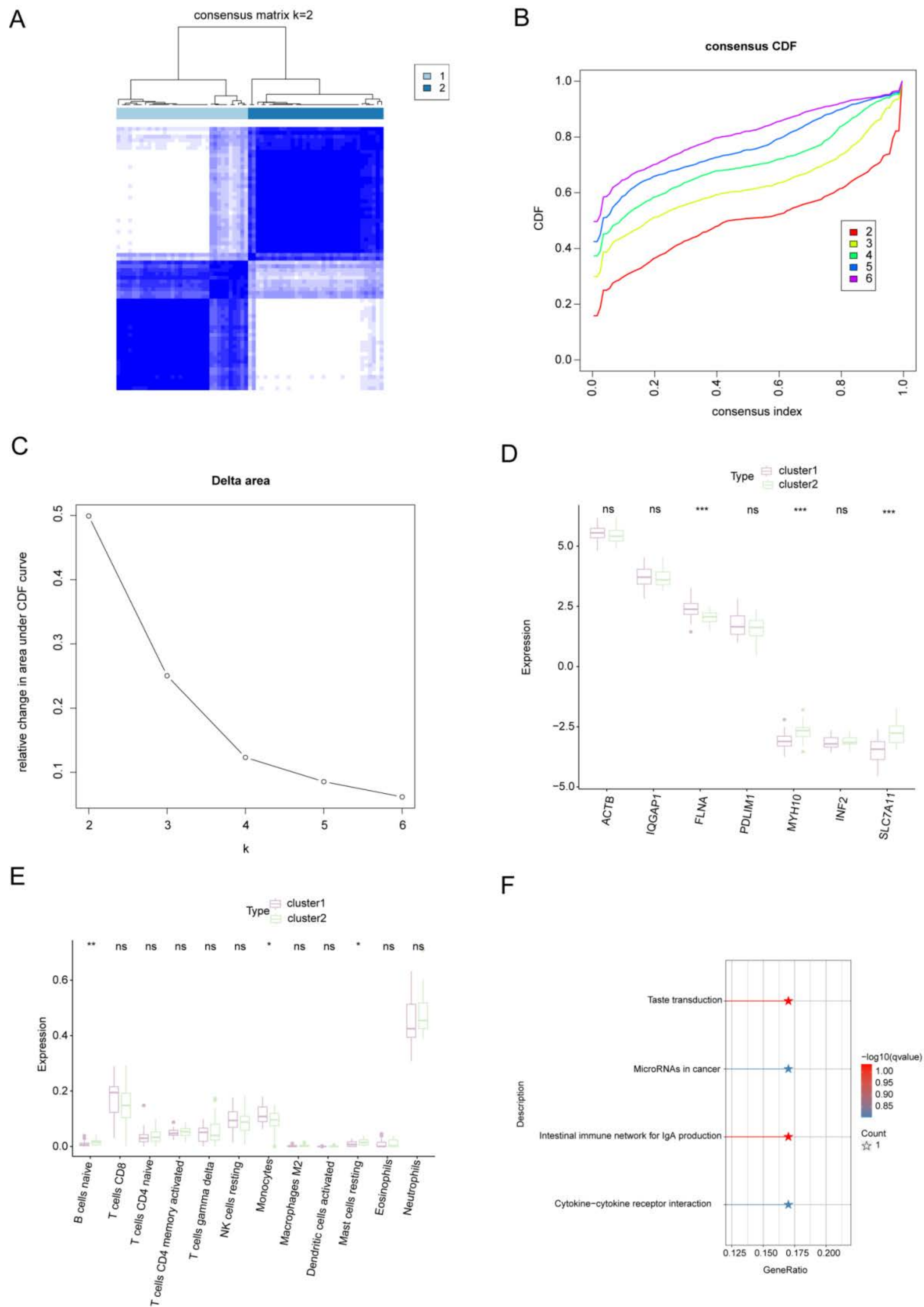

Figure 4. Consensus clustering and immune cell infiltration analysis. (A) Consensus clustering plot with k = 2. (B) Cumulative distribution function curves. (C) Delta area. (D) Display the differential expression of DRGs between two clusters. (E) Illustrate the differential expression of immune cells between two clusters. (F) KEGG enrichment analysis of DEGs between the two clusters. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

3.4. Validation of seven DRGs using single-cell dataset

We validated the expression of seven DRGs using the single-cell dataset GSE174574 and further investigated the primary cell types where these seven DRGs are predominantly distributed. The stroke samples consisted of 11,772 cells, while the control samples consisted of 9,980 cells. Quality control was performed on the single-cell dataset, filtering out dead cells and doublets. Subsequently, we identified 2,000 highly variable genes and merged them using the CCA method (Figure 5A). These cells were later divided into 30 clusters, primarily encompassing 12 cell types: Microglia, Endothelial cells, Epithelial cells, Astrocytes, Pericytes, Monocytes, Macrophages, Oligodendrocytes, Granulocytes, Fibroblasts, NK cells and B cells (Figure 5B). The heatmap displays the top one marker gene for each cluster (Figure 5C).

Compared to the control samples, seven DRGs showed predominant expression in the following cell types of stroke samples. *Actb* exhibited expression in various cell types, while *Iqgap1* was predominantly expressed in NK cells, monocytes and microglia. *Flna* showed primary expression in NK cells and microglia. *Pdlim1* displayed primary expression in fibroblasts cells. *Myh10* demonstrated low expression in these cell types. *Inf2* was mainly expressed in granulocytes and monocytes, while *Slc7a11* showed predominant expression in granulocytes, monocytes and microglia (Figure 5D). The specific distribution of these seven DRGs across twelve cell types can be seen in control and stroke samples (Figure 5E-F, Figure 6).

3.5. Validation with external dataset

To further understand the expression profiles of seven DRGs in stroke samples and control samples, we validated our findings using external datasets, namely GSE16561 and GSE122709. In order to explore the diagnostic efficacy of these seven DRGs, we conducted ROC curves analysis, where DRGs with an AUC value greater than 0.7 were considered to have higher diagnostic value. In the GSE16561 dataset, the AUC values for *ACTB* and *IQGAP1* exceeded 0.7. Similarly, in the GSE122709 dataset, the AUC values for *ACTB*, *IQGAP1*, *FLNA*, *PDLIM1*, *MYH10*, *INF2* and *SLC7A11* surpassed the 0.7 threshold (Figure 7A–B).

Within the GSE16561 dataset, the expression levels of *ACTB*, *IQGAP1* and *FLNA* were found to be elevated in stroke samples as compared to control samples. In the GSE122709 dataset, the expression levels of *ACTB*, *FLNA*, *PDLIM1* and *INF2* were observed to be higher in the stroke samples (Figure 7C–D).

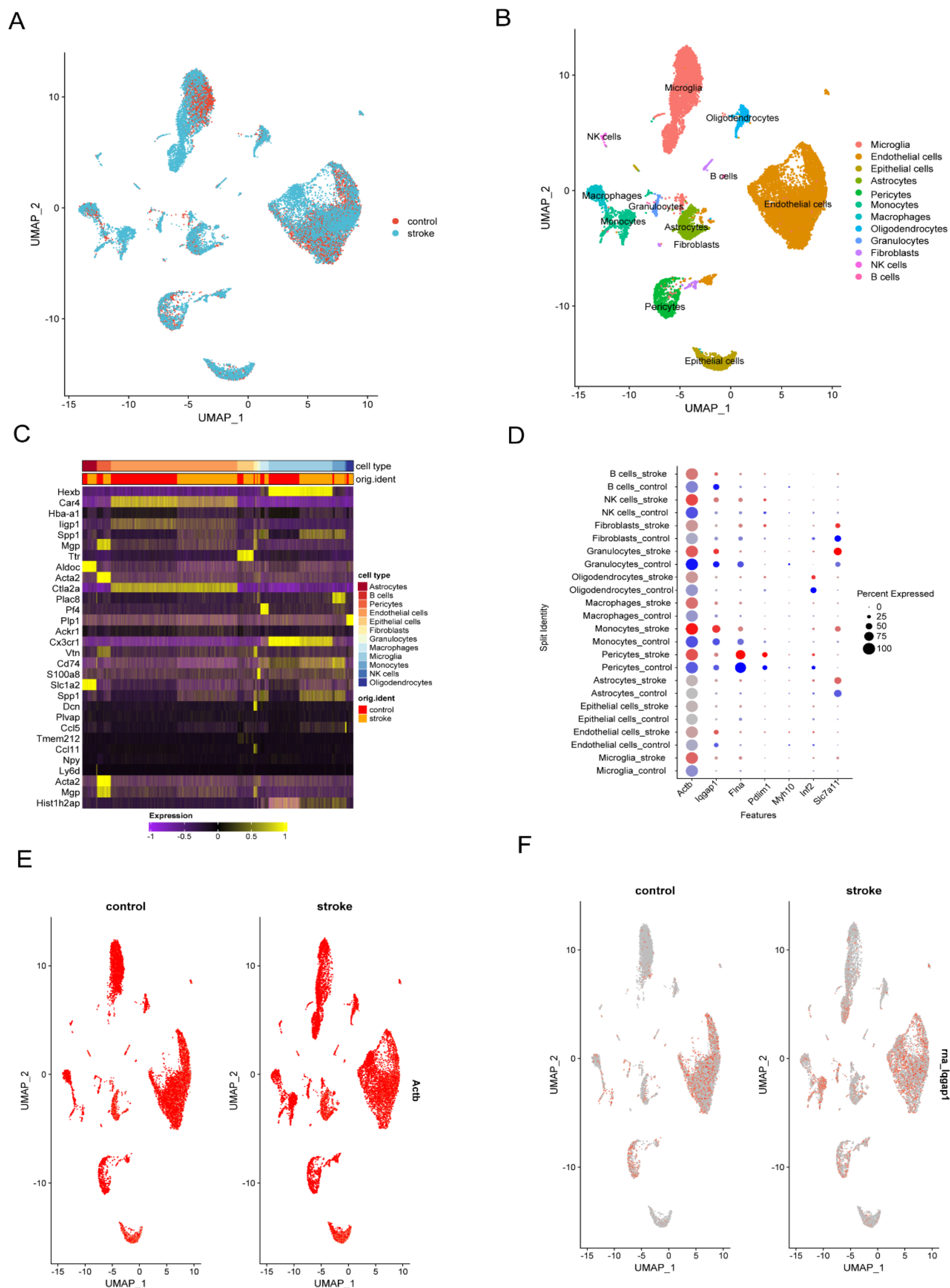

Figure 5. Integration of one stroke sample and one control sample using the CCA method. (A) Integrating UMAP of stroke and control samples using CCA method. (B) Identified 12 cell types. UMAP, Uniform Manifold Approximation and Projection. (C) Heatmap displaying the top one marker for each cluster. (D) The dot plot represents the expression of seven DRGs in 12 cell types for stroke and control samples. (E-F) UMAP displays the expression levels of *Actb* and *Iqgap1*.

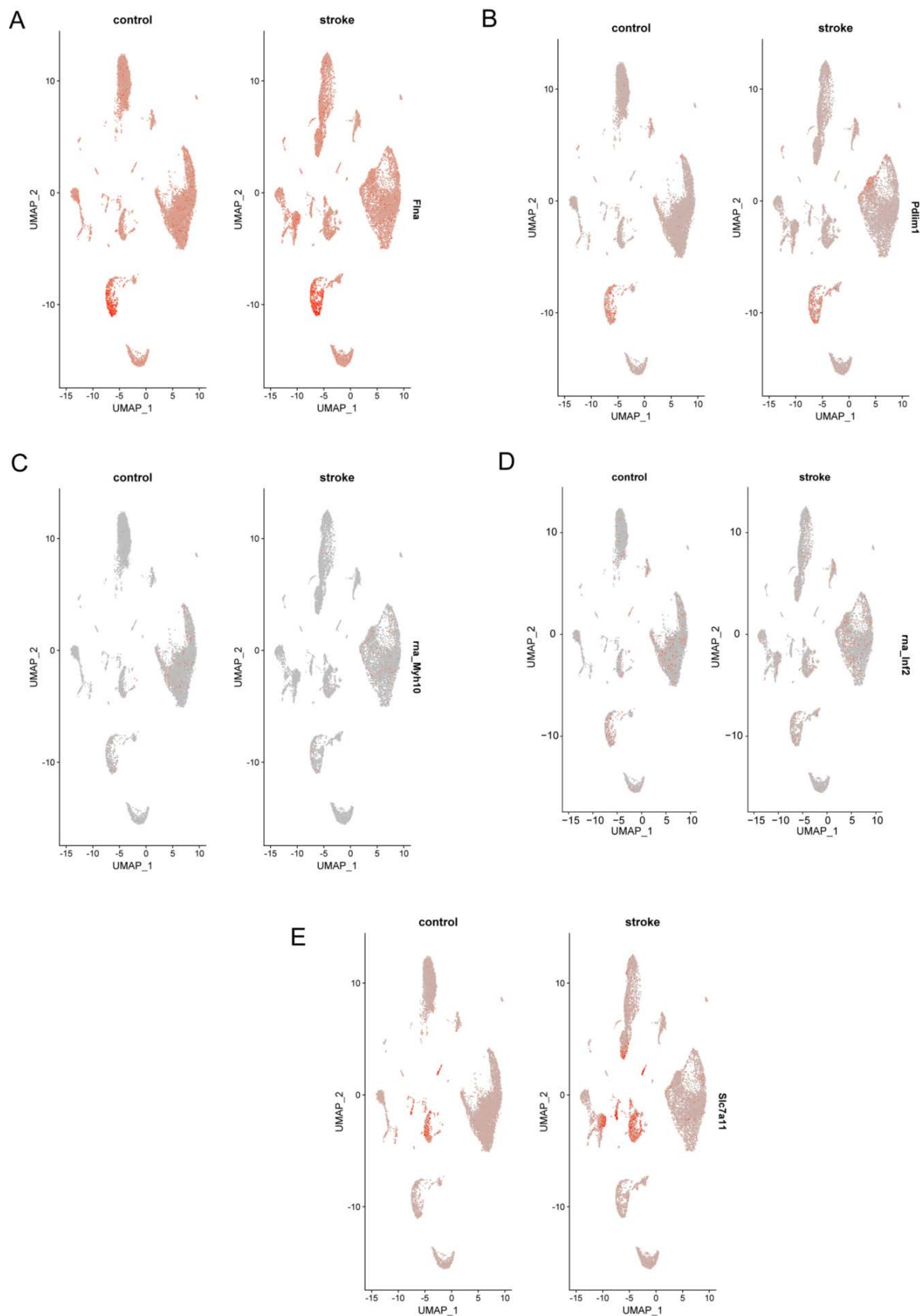

Figure 6. UMAP displays the expression levels of DRGs. (A) *Flna*. (B) *Pdlim1*. (C) *Myh10*. (D) *Inf2*. (E) *Slc7a11*.

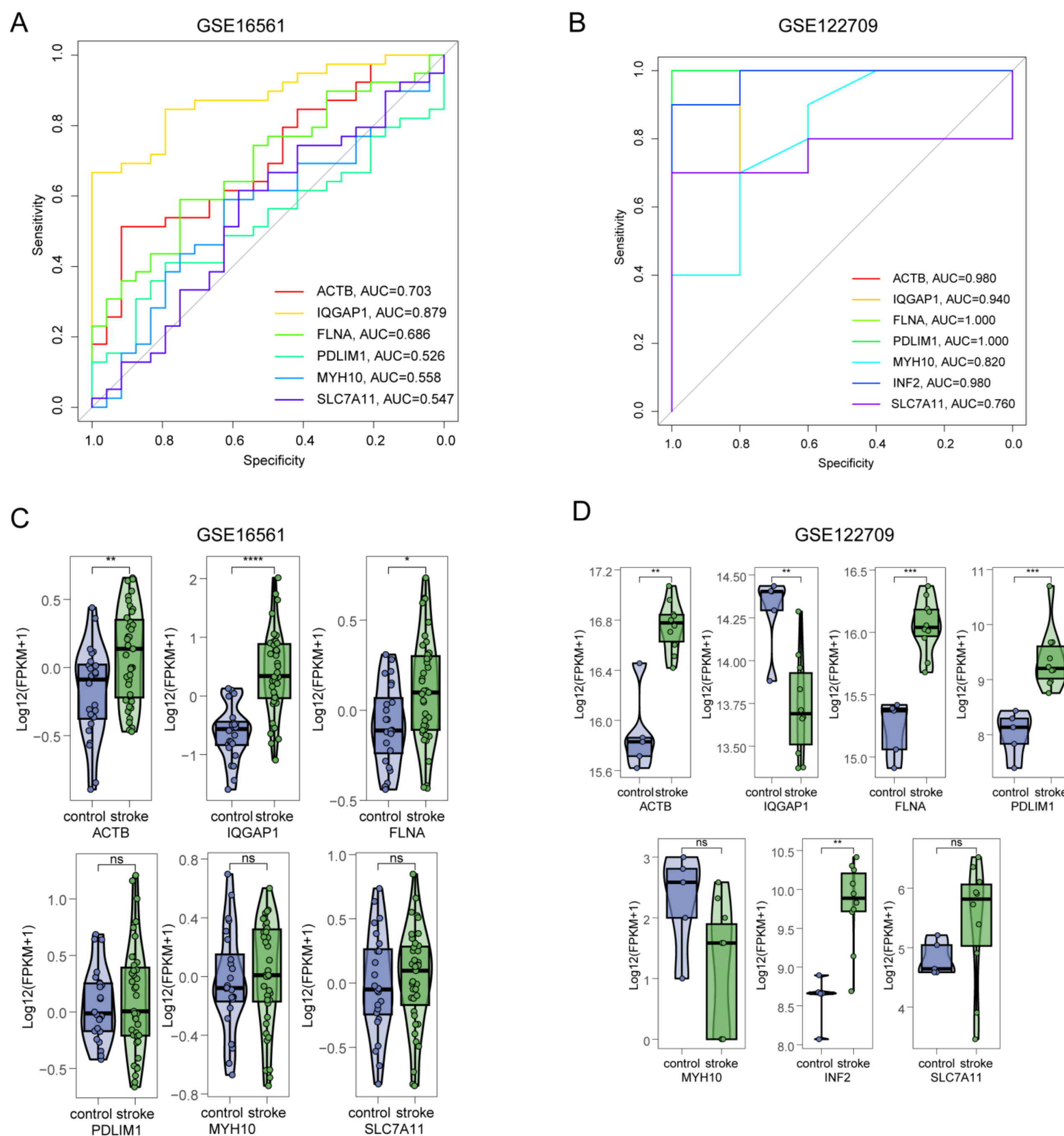

Figure 7. Validation of DRGs expression and ROC curves analysis using external datasets. (A) ROC curves were employed to analyze the expression of DRGs in the GSE16561 dataset. (B) ROC curves were employed to analyze the expression of DRGs in the GSE122709 dataset. (C) Expression pattern of DRGs within the GSE16561 dataset. (D) Expression pattern of DRGs within the GSE122709 dataset. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

3.6. Molecular docking of genistein with DRGs

Using the DSigDB database within the Enrichr platform, we employed a drug prediction approach

to identify potential compounds for the seven DRGs. Through adjusted *P*-values, we extracted the top 15 candidates. Importantly, genistein has been identified as a drug molecule that interacts with the genes *ACTB*, *FLNA* and *MYH10*. Genistein has been reported to emphasize its potential significance in preventing and alleviating strokes [28]. Thus, we performed molecular docking experiments to assess the binding of genistein with these three DRGs. The results demonstrated binding energies of -8.9 kJ/mol, -9.1 kJ/mol and -9.0 kJ/mol for genistein with *ACTB*, *FLNA* and *MYH10*, respectively (Figure 8). These findings strongly indicate that genistein exhibits a robust binding affinity with *ACTB*, *FLNA* and *MYH10*.

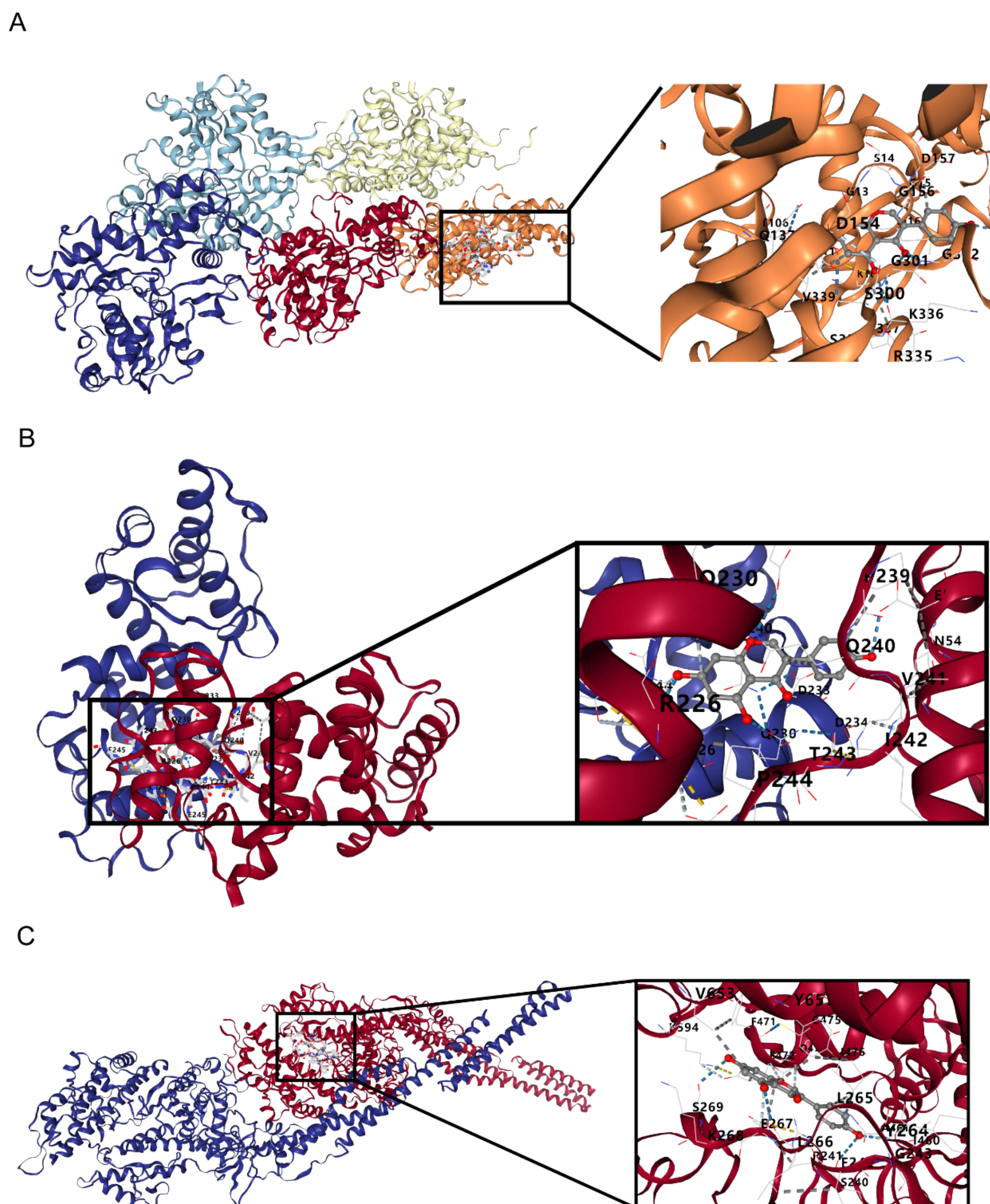

Figure 8. Molecular docking analysis was performed for three proteins. (A) *ACTB*, (B) *FLNA*, (C) *MYH10*.

4. Discussion

Stroke is a frequent and severe manifestation of cerebrovascular disease that can result in significant disability or death [29]. Early diagnosis and effective treatment of ischemic strokes are crucial in improving clinical outcomes. Hence, our study establishes a connection between disulfidptosis and the pathogenesis of ischemic stroke. Through bioinformatics analysis, we have identified potential DRGs and explored prospective therapeutic targets, thus fostering the development of more efficacious treatment strategies and novel pharmaceuticals aimed at potential therapeutic targets for ischemic stroke.

In our study, according to GO analysis, it was found that seven DRGs are associated with platelet aggregation and platelet activation. The aggregation of platelets has been established as a prognostic indicator for unfavorable clinical outcomes in individuals suffering from acute ischemic stroke [30]. Platelet activation is linked to a neutrophil-dominated inflammatory response during a stroke [31]. After dividing ischemic stroke patients into two clusters, a KEGG analysis was performed on the DEGs from these clusters. The results demonstrated significant enrichment, particularly in the cytokine-cytokine receptor interaction pathway. Studies have shown that cytokine-cytokine receptor interaction is an important pathway following a stroke [32].

We employed machine learning to select seven DRGs, with the majority of them demonstrating high diagnostic capability for ischemic stroke patients. *ACTB* is a cytoskeleton protein involved in maintaining cellular structure and activity [33-35]. *ACTB* can induce vascular hypertrophy and hypertension [36]. The actin cytoskeleton plays a crucial role in neuronal development and activity [37]. Prior research has demonstrated the significance of downregulating *ACTB* expression in the prevention and treatment of ischemic stroke [38]. *IQGAP1* is a widely expressed protein in cells that plays an important role in regulating cellular activity [39]. Studies have shown that *IQGAP1* can regulate multiple signaling pathways, such as the p38 MAPK and JNK pathways, both of which are involved in the development of ischemic stroke [40-43]. *FLNA* can reduce macrophage activity and atherosclerosis [44]. However, the specific mechanism of *FLNA* in ischemic stroke needs further research.

PDLIM1 can promote neurite growth, but its role in ischemic stroke is not yet clear [45]. Although there is no specific research to support this, it is suggested that *MYH10* may contribute to the recovery of neuronal damage following stroke by promoting neuronal homeostasis [46].The activation of *INF2* is a key mediator of the neuronal pro-survival cytoskeletal response [47]. Based on the study results, the signaling pathway associated with *SLC7A11* appears to be a promising target for therapeutic intervention in the treatment of ischemic stroke [48]. *SLC7A11* can inhibit ferroptosis and reduce neuronal damage [49]. Exploring the mechanism of DRGs in ischemic stroke can improve our understanding of the molecular basis of this condition and identify novel therapeutic targets for its management.

In the analysis of immune cell infiltration, seven DRGs are closely linked to various immune cell types. Within the two clusters observed in ischemic stroke patients, variations in immune cell levels were also detected. At the single-cell level, these seven DRGs are predominantly present in different immune cell types. The inflammatory response in the ischemic hemisphere of the brain is mediated by both microglia and peripheral immune cells, including mononuclear/macrophage cells, neutrophils and lymphocytes. Microglia are a key regulator of homeostasis in the central nervous system and play a dual role in mediating both neuronal damage and survival [50, 51]. Neutrophils, specifically circulating granulocytes, are the first immune cells to infiltrate stroke-affected tissues [52]. Research indicates that the neutrophil-to-lymphocyte ratio may be a useful prognostic marker for ischemic stroke [53].

Following the stroke, macrophages can have anti-inflammatory effects and produce growth factors that facilitate functional recovery [54, 55]. Studies have shown that monocytes can promote functional recovery after ischemic stroke [56]. In the pathophysiology of stroke, T cells play a crucial role. Beyond their detrimental effects in the acute phase following a stroke, T cells also have a significant role in regulating inflammation and promoting post-stroke recovery [57-59]. Research suggests that eosinophil count may have a significant impact on the prognosis of stroke patients [60]. Previous research has found that NK cells accelerate the progression of brain infarction in the acute phase of ischemic stroke [61]. Oligodendrocytes have the capacity to stimulate peripheral T cells, potentially paving the way for functional recovery following an ischemic stroke [62]. This further underscores the significance of immune cells in the progression of ischemic strokes.

By employing molecular docking techniques, we have identified that genistein demonstrates robust binding affinity towards *ACTB*, *FLNA* and *MYH10* proteins. Genistein is a natural isoflavone that can reduce oxidative stress and inflammation damage to the central nervous system [63]. Homocysteine has been established as a risk factor for strokes, while Genistein has shown to mitigate the effects induced by homocysteine [64, 65]. Increased homocysteine levels during the acute phase of ischemic strokes can serve as a prognostic marker for mortality, particularly in patients with the large-vessel atherosclerosis subtype of stroke [66]. Genistein holds promise as a potential therapeutic intervention for the prevention and treatment of stroke patients.

Currently, our research primarily centers around bioinformatics analysis, with plans to enhance the precision of our analyses through experimental means. Our goal is to explore the association between immune infiltration, DRGs and ischemic stroke, aiming to unveil the underlying pathophysiological mechanisms and advance disease management. Utilizing transgenic techniques, we will evaluate ischemic damage and DRGs expression in mouse brain tissue. Through methods like immunocell labeling or flow cytometry, we will validate the relationship between DRGs and immune cells. Clinical samples will corroborate the association between DRGs and clinical traits. Single-cell transcriptomics will be employed to verify changes in DRGs expression. *In vitro* experiments will utilize techniques to assess the interaction between genistein and DRGs, evaluating their affinity and kinetic properties.

5. Conclusions

Through bulk transcription and single-cell transcription technologies, we revealed the association between DRGs and infiltrated immune cells. Selecting the characteristics of disulfidptosis based on seven DRGs as the best machine learning model, this model can accurately assess the diagnosis of ischemic stroke patients. Ischemic stroke patients with different subgroups of disulfidptosis exhibit varying immune cell expressions. At the single-cell level, compared to the control group, the seven DRGs are primarily expressed in immune cell types from ischemic stroke samples. Molecular docking results demonstrate that genistein exhibits a strong binding affinity with *ACTB*, *FLNA* and *MYH10*. Our research findings have revealed the role of disulfidptosis in the progression of ischemic stroke and provided new insights into potential pathogenic processes and therapeutic strategies for ischemic strokes.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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