DOI: 10.53469/jcmp.2025.07(09).42

Identification of Autophagy-Related Biomarkers in Ischemic Stroke

Yaoyao Dai[1,#], Rui HaoGeng Wang[1,#], Mengqi Shu[2], Chen Zhang[1,*]

[1]Department of Neurology, Shahe City Hospital of Traditional Chinese Medicine, Shuhe 054100, Hebei, China
[2]The Key Research Laboratory of Benefiting Qi for Acting Blood Circulation Method to Treat Multiple Sclerosis of State Administration of Traditional Chinese Medicine, Research Center of Neurobiology, Shanxi University of Chinese Medicine, Jinzhong 030619, Shanxi, China
[#]*Co-first authors: Dai Yaoyao and Wang Rui Haogeng contributed equally to this work.*
**Correspondence Author*

Abstract: ***Objective:*** ***This study utilizes bioinformatics technology to screen and predict potential diagnostic autophagy related biomarkers in CIS.*** ***Methods:*** ***By analyzing genes in the GEO dataset GSE16561 and the autophagy database HADb, differentially expressed genes (DEGs) related to autophagy in CIS were identified. GO and KEGG enriched and analyzed these autophagy related DEGs. Subsequently, module analysis was conducted using LASSO and SVM-REF algorithms, and finally, the diagnostic value of autophagy related biomarkers in CIS was evaluated through subject operating characteristic curve (ROC) univariate analysis.*** ***Results:*** ***After intersection of differential genes and autophagy gene data, a total of 31 DEGs related to autophagy were identified. GO and KEGG analysis showed that these autophagy related DEGs are mainly enriched in the reactions of autophagy, apoptosis, inflammation, and aging. They have molecular functions such as binding to cadherin and cell adhesion molecules, and are involved in utilizing autophagy mechanisms, biological processes of apoptosis, and cellular components of lysosomes, whole membranes, cytoplasmic colloids, and cytoplasmic vesicles. And it is related to the FoxO signaling pathway, NOD like receptor signaling pathway, chemokine signaling pathway, PI3K Akt signaling pathway, IL-17 signaling pathway, etc. In addition, LASSO and SVM-REF identified 10 autophagy related biomarkers, namely CASP4, FOXO3, HSPA8, EIF2AK2, PRKCQ, GAA, NLRC4, TNFSF10, CXCR4, RAB1A. ROC univariate analysis showed that CASP4, FOXO3, EIF2AK2, NLRC4, and CXCR4 have good expression and diagnostic value in CIS.*** ***Conclusion:*** ***CASP4, FOXO3, EIF2AK2, NLRC4, and CXCR4 genes may be potential autophagy related biomarkers for the diagnosis and treatment of CIS, and provide some evidence for the important role of autophagy in CIS.***

Keywords: Bioinformatics, Cerebral ischemic stroke, Autophagy, Biomarkers**.**

1. Introduction

Cerebral ischemic stroke (CIS) is a condition characterized by the sudden loss of blood flow to a specific area of the brain. It is not only a major cause of permanent disability but also one of the leading causes of death worldwide [1]. Once CIS occurs, it triggers a cascade of harmful signals, including excitotoxicity, calcium overload, inflammatory responses, apoptosis, and mitochondrial dysfunction. These factors interact with each other, creating a vicious cycle that ultimately leads to irreversible brain damage [2]. To date, there is no specific cure for CIS. Thrombolytic therapy is the most widely used treatment for CIS and cerebral infarction, but its clinical efficacy is only confirmed for patients within 4.5 hours of stroke onset, which is time-limited [3]. Therefore, it is necessary to identify effective biomarkers for CIS diagnosis and therapeutic targets, as there is currently no definitive method for effectively treating CIS.

Autophagy, a cellular "self-eating" process, is involved in the development of various diseases, including cancer [4], Parkinson's disease [5], neurodegenerative diseases [6], Crohn's disease [7], and cerebral ischemic stroke (CIS). As a lysosome-mediated catabolic process, autophagy involves the cytoplasm, organelles, and proteins. Its function is to recycle misfolded proteins and damaged organelles by forming autophagosomes that fuse with lysosomes to degrade the recycled proteins and organelles, thereby maintaining cellular metabolism and homeostasis [8]. In diseases such as cancer and tumors, autophagy-related genes can serve as biomarkers and potential therapeutic targets for diagnosis, treatment, and prognosis [9]. Studies have shown that autophagy regulation is impaired in CIS. Moreover, ample evidence indicates that autophagy acts as a "double-edged sword" in CIS. However, its role in the pathogenesis of CIS has not been fully elucidated, suggesting that autophagy regulation may be a novel therapeutic target for CIS [10]. However, the role of autophagy-related biomarkers in the diagnosis and treatment of CIS remains unexplored.

After performing a comprehensive analysis of the least absolute shrinkage and selection operator (LASSO) regression and support vector machine (SVM), we constructed receiver operating characteristic (ROC) curves for biomarkers based on key genes from an independent database and compared their diagnostic capabilities for CIS between gene pairs and single genes. Importantly, we further explored the potential related biomarkers from the perspectives of CIS and autophagy. This study provides a starting point for further investigation into the molecular mechanisms of CIS and may lead to new intervention strategies targeting autophagy in CIS patients.

2. Materials and Methods

2.1 Data Collection

GEO (http://www.ncbi.nlm.nih.gov/geo) is a high-throughput functional genomics database that includes microarray, microarray, and gene expression data [11].

2.2 Identification of DEGs

The GSE16561 dataset was processed using the "affy" and "limma" packages in R (version 4.0.5) to calculate the adjusted P - value and |log2FC|. DEGs were identified using a

threshold of adjusted P - value < 0.05 and |log2FC|≥ 1. The autophagy - related DEGs in CIS were identified by intersecting the DEGs with the autophagy dataset, and a Venn diagram was constructed.

2.3 GO and KEGG Enrichment Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were used to identify significant pathways. The DAVID bioinformatics resource (version 6.8) was used to perform GO and KEGG enrichment analyses on autophagy-related differentially expressed genes (DEGs) and to distinguish and enrich the biological attributes of significant DEGs, including biological processes, cellular components, molecular functions, and pathways (https://david.ncifcrf.gov/).A P-value of <0.05 was set as the threshold for significant enrichment, and the results were visualized using Cytoscape.

2.4 Diagnostic Value of Autophagy-Related Genes in CIS: LASSO, SVM-REF, and ROC

The LASSO binomial logistic regression model was employed, and the "glmnet" package was used to select the optimal feature genes from DEGs, with the optimal penalty parameter λ determined by the minimum binomial deviance. The SVM-RFE algorithm was used, and the "e1071," "kernlab," and "caret" packages were employed to select the optimal feature genes by cross - validating the point with the minimum error. The receiver operating characteristic (ROC) curve was constructed using the "survival" package in R to evaluate the diagnostic value of autophagy - related biomarkers in CIS.

3. Results

3.1 Identification of Autophagy-Related Differentially Expressed Genes

After intersecting the screened CIS differentially expressed genes (DEGs) with autophagy-related genes, a total of 31 autophagy-related DEGs were identified (Figure 1), including NLRC4, EIF2AK2, RAB1A, GAA, TNFSF10, PRKCQ, CASP4, PTEN, FOXO3, NAMPT, VAMP3, LAMP2, RAB24, HSPA8, FOS, BNIP3, GNAI3, BAG3, RB1CC1, CANX, SH3GLB1, BID, USP10, EEF2, ATIC, CD46, RAF1, GABARAP, PPP1R15A, HSP90AB1, and CXCR4.

3.2 Enrichment Analysis of Autophagy-Related DEGs: GO and KEGG Analysis

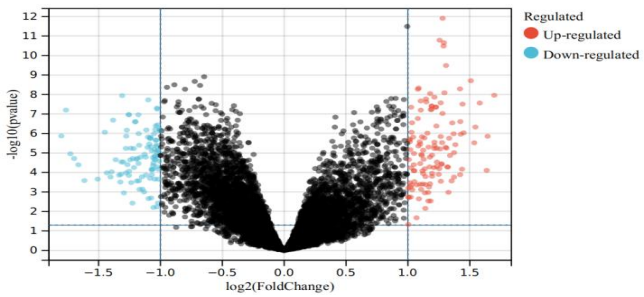


Figure 1(a): Volcano plot of DEGs in the GSE16561 dataset
Note: Red dots represent upregulated differentially expressed genes (DEGs), and blue dots represent downregulated DEGs.

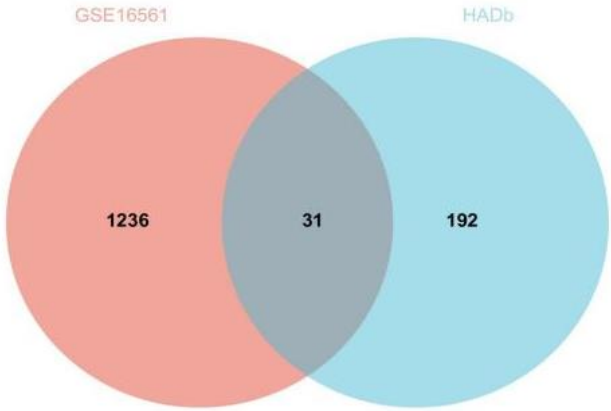
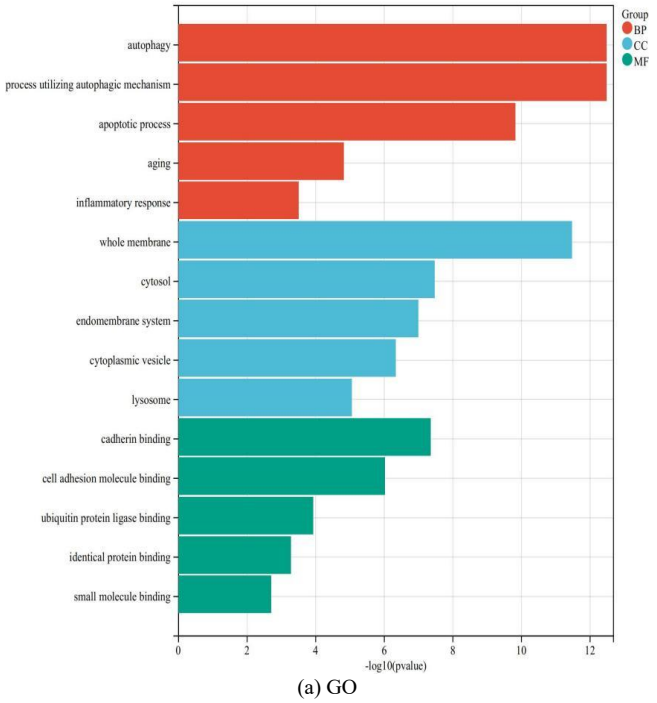
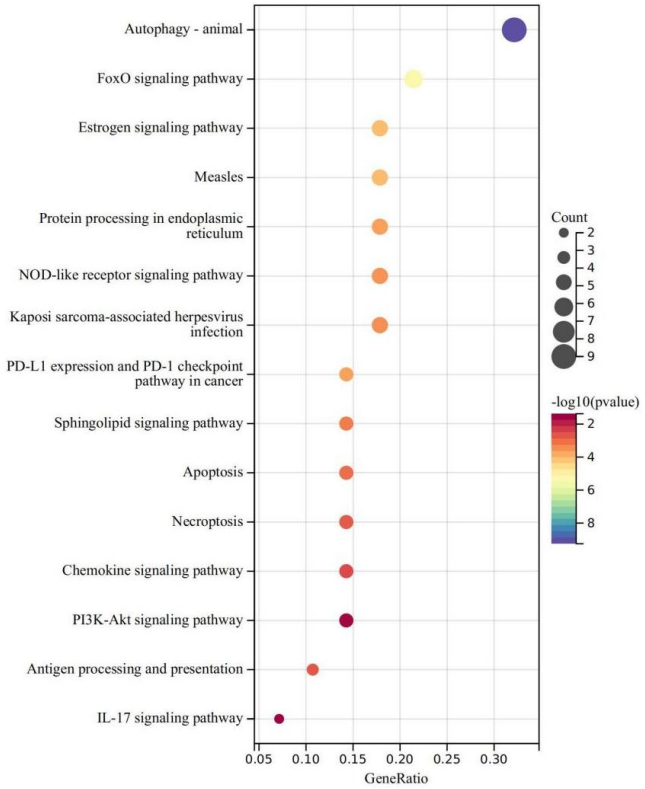


Figure 1(b): Venn diagram showing the intersection of GSE16561 dataset and autophagy database HADb

GO and KEGG enrichment analyses were performed on these autophagy-related DEGs. The GO analysis results showed (Figure 2a) that these autophagy-related DEGs were mainly enriched in responses to autophagy, apoptosis, inflammation, and aging, with molecular functions such as cadherin binding, cell adhesion molecule binding, ubiquitin ligase binding, identical protein binding, and small molecule binding. They are involved in biological processes like autophagy mechanisms and apoptosis, and cellular components such as lysosomes, plasma membrane, cytosol, and cytoplasmic vesicles. The KEGG analysis results showed (Figure 2b) that these DEGs were mainly enriched in pathways such as autophagy (animal), FoxO signaling pathway, estrogen signaling pathway, measles, protein processing in endoplasmic reticulum, NOD-like receptor signaling pathway, Kaposi sarcoma-associated herpesvirus infection, PD-L1 expression and PD-1 checkpoint pathway in cancer, sphingolipid signaling pathway, apoptosis, necroptosis, chemokine signaling pathway, PI3K-Akt signaling pathway, antigen processing and presentation, and IL-17 signaling pathway.



(a) GO

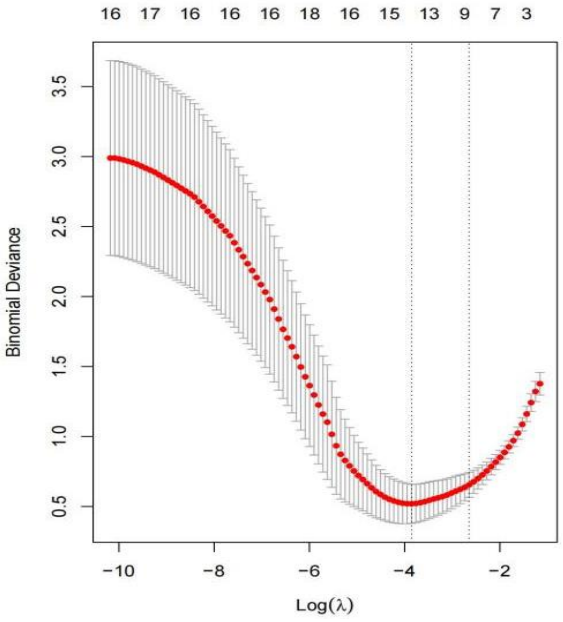


(b) KEGG

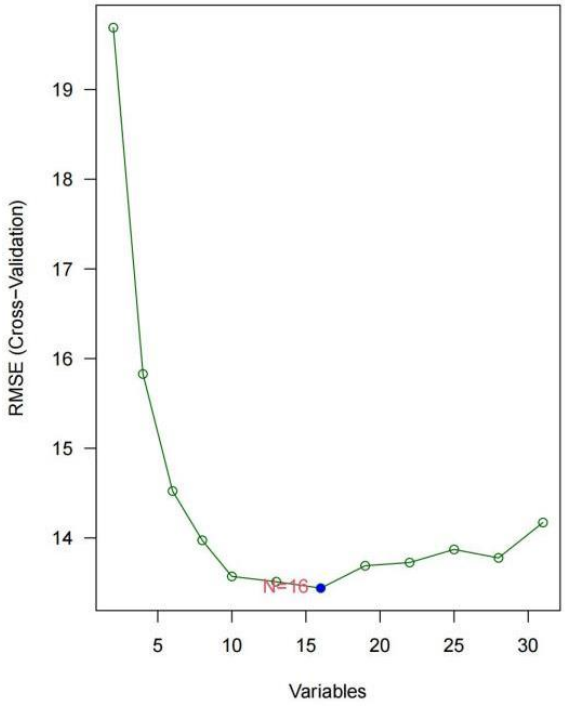
Figure 2: Enrichment Analysis of Autophagy-Related DEGs

3.3 Identification of Optimal Autophagy-Related Diagnostic Biomarkers in CIS: LASSO, SVM-REF, and ROC

LASSO and SVM-REF algorithms identified 10 autophagy-related biomarkers (Figure 3): CASP4, FOXO3, HSPA8, EIF2AK2, PRKCQ, GAA, NLRC4, TNFSF10, CXCR4, and RAB1A.The area under the receiver operating characteristic curve (AUC) for autophagy-related biomarkers, calculated using R, showed that CASP4, FOXO3, EIF2AK2, NLRC4, and CXCR4 had AUC scores greater than 0.7, indicating accurate diagnostic value and good expression and diagnostic potential in CIS (Figure 4).



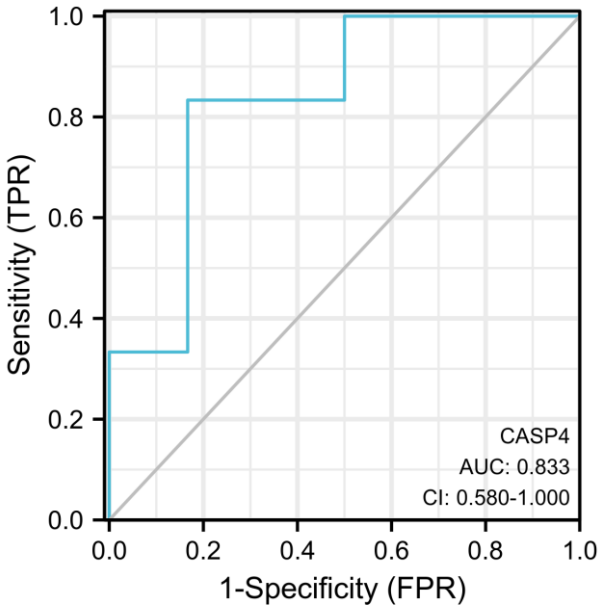
(a) LASSO

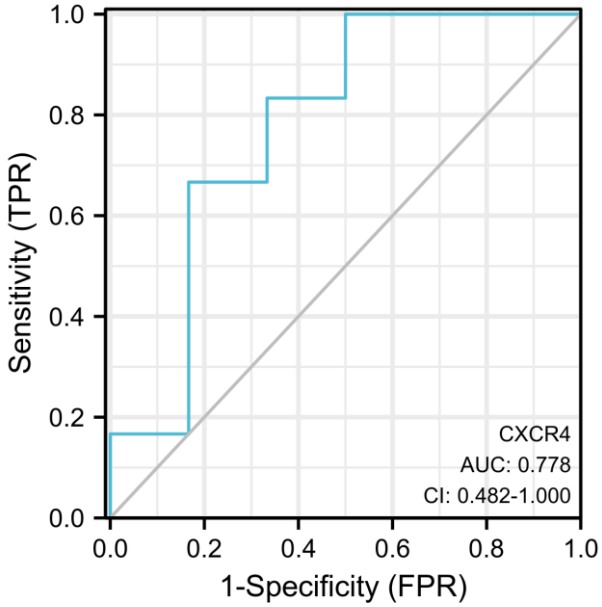


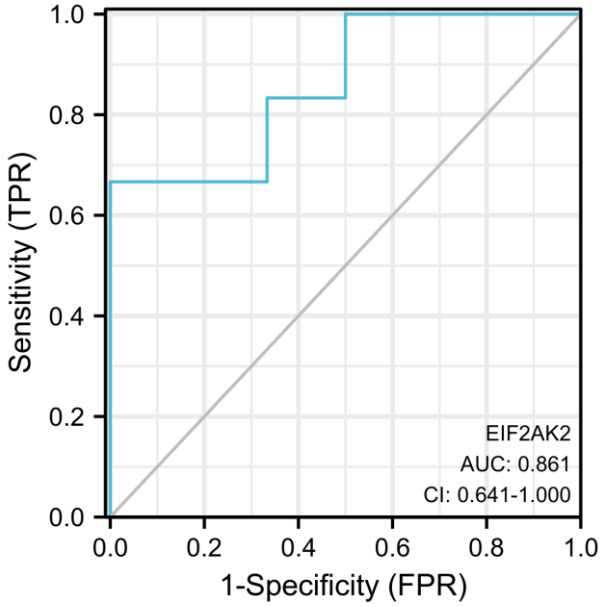
(b) SVM-REF

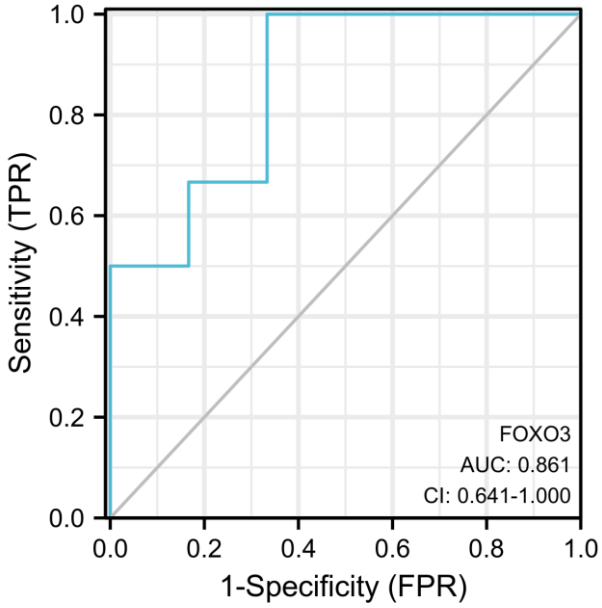
(c) Intersection Venn Diagram

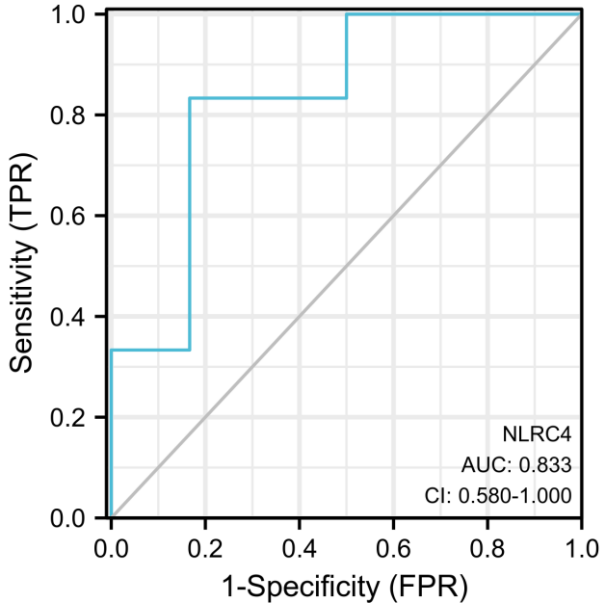
Figure 3: Autophagy-Related Biomarkers Identified by LASSO and SVM-REF Algorithms











(d) ROC Curve

Figure 4: Validation of ROC Curves for Optimal Autophagy-Related Diagnostic Biomarkers

4. Discussion

In recent years, against the backdrop of the high incidence and mortality rates of CIS, significant advancements in bioinformatics have enhanced our understanding of the mechanisms underlying CIS development and enabled timely adjustments to subsequent treatment strategies. Autophagy, a conserved intracellular catabolic degradation pathway, can be activated under conditions of starvation and stress [12]. This process maintains homeostasis by transporting misfolded proteins and other cytoplasmic components to lysosomes for digestion and recycling degraded metabolic components, which is crucial for cell survival [13]. However, it is well-known that autophagy has a dual effect. Studies have shown that excessive activation of autophagy can have adverse effects on cell function, leading to cell damage or even death [14]. Similarly, the role of autophagy in CIS remains unclear. Acting as a double-edged sword, it can protect or damage neurons during ischemic injury [15]. Accumulating evidence indicates that autophagy is associated with pathological cell death related to neurodegenerative diseases, carcinogenesis, stroke, and traumatic brain injury, and it plays a crucial role in the progression of CIS [16]. Elucidating the correlation between autophagy and CIS can provide new biomarkers and therapeutic strategies for the diagnosis and treatment of this disease.

In this study, we first investigated the autophagy-related DEGs between CIS and normal controls and compared the results of GSE16561 with the normal control group, identifying a total of 31 autophagy-related DEGs. Additionally, GO and KEGG analyses revealed that these 31 autophagy-related DEGs are mainly involved in responses to autophagy, apoptosis, inflammation, and aging. They possess molecular functions such as cadherin binding, cell adhesion molecule binding, ubiquitin protein ligase binding, identical protein binding, and small molecule binding. They participate in biological processes like autophagy mechanisms and apoptosis and are components of cellular structures such as lysosomes, plasma membranes, cytosol, and cytoplasmic

vesicles. Moreover, these DEGs are primarily enriched in pathways such as autophagy (animal), FoxO signaling pathway, estrogen signaling pathway, measles, protein processing in the endoplasmic reticulum, NOD-like receptor signaling pathway, Kaposi's sarcoma-associated herpesvirus infection, PD-L1 expression and PD-1 checkpoint pathway in cancer, sphingolipid signaling pathway, apoptosis, necrosis, chemokine signaling pathway, PI3K-Akt signaling pathway, antigen processing and presentation, and IL-17 signaling pathway. Interestingly, many of these pathways are associated with CIS. The upregulation of the FoxO signaling pathway and the downregulation of the chemokine signaling pathway have positive effects on cerebral ischemia [17]. IL-17 plays a crucial role in promoting inflammatory responses and inducing secondary injury after stroke through hypoxic preconditioning, and the cascading IL-17 signaling pathway can induce stroke tolerance [18]. NLRP3, a member of the NOD-like receptor signaling pathway, plays an important role in the progression of inflammatory responses in CIS [19]. The PI3K-Akt signaling pathway regulates neuroinflammation, neuronal apoptosis, and autophagy following CIS [20-21]. Therefore, these 31 autophagy-related DEGs may also function in CIS through these pathways.

By constructing a random forest model and performing ROC univariate analysis, we identified five optimal autophagy-related biomarkers: CASP4, FOXO3, EIF2AK2, NLRC4, and CXCR4.These five autophagy-related biomarkers demonstrated good performance in the diagnosis of CIS. In fact, previous studies have reported that these autophagy-related biomarkers may play crucial roles in CIS or serve as biomarkers for CIS and other diseases.

Caspase-4 (CASP4) is a member of the caspase protein family. As a key molecule in the non-canonical cell death pathway, CASP4 is a core component of the non-canonical inflammasome and plays a role in the coordination of immune, inflammatory, and cellular processes, including cellular homeostasis, apoptosis, and pyroptosis [22]. Studies have shown that CASP4 may be a potential cancer biomarker, and its expression levels may be associated with the occurrence and development of various cancers, including rectal cancer and esophageal squamous cell carcinoma [23]. Recent studies have found that CASP4 can participate in autophagosome formation [24]. Forkhead box O3 (FOXO3) is a transcription factor that plays a central role in various cellular functions and is also known as FOXO3a.Studies have shown that FOXO3 is a key regulator of ischemia-reperfusion injury and can promote ischemia-reperfusion injury by inducing inflammatory responses, apoptosis, autophagy, mitophagy, pyroptosis, and oxidative damage [25]. Del [26] et al. found that FOXO3 expression was increased in the penumbra of rats with cerebral ischemia, which may have a protective effect against ischemic injury. Additionally, Deng A [27] et al. demonstrated that in a rat model of cerebral ischemia-reperfusion, autophagy could be activated and brain damage alleviated via the Akt/FOXO3 signaling pathway. A bioinformatics study indicated that FOXO3 may be a key target gene regulating the development of acute CIS [28]. Eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2) is an IFN-stimulated gene that shows higher expression in systemic lupus erythematosus (SLE) probands, which may account for the impaired translation and proliferative responses of T cells to mitogens in SLE patients [29]. In the quantitative proteomics analysis of the brain by Zhang X [30] et al., the differentially expressed protein EIF2AK2 was found to be mainly related to autophagy and inflammatory responses, and it changes between cerebral ischemia-reperfusion injury and drug effects. The NLR-family CARD-containing protein 4 (NLRC4) inflammasome, a member of the nucleotide-binding and oligomerization domain-like receptor family, amplifies inflammation by promoting the processing of caspase-1, interleukin (IL)-1β, and IL-18. Studies have found that the NLRC4 inflammasome is involved in inflammation induced by intracerebral hemorrhage. However, the molecular mechanisms of NLRC4 inflammasome activation in intracerebral hemorrhage are poorly understood [31]. Another study showed that the NLRC4 inflammasome is a biomarker for the progression of coronary artery disease in type 1 diabetes and is positively correlated with neutrophils, making it a potential therapeutic target [32]. Poh L [33] et al. demonstrated that the NLRC4 inflammasome can mediate microglial cell death in CIS.C-X-C motif chemokine receptor 4 (CXCR4) is a G-protein-coupled receptor involved in homing and chemotaxis in the hematopoietic and immune systems [34]. Zou R [35] et al. bioinformatics studies have shown that CIS may be associated with atrial fibrillation, which is a risk factor for ischemic stroke, and CXCR4 may be related to atrial fibrillation.

In summary, our results indicate that CASP4, FOXO3, EIF2AK2, NLRC4, and CXCR4 are potential diagnostic biomarkers for CIS and provide further evidence for the significant role of autophagy in CIS. Our study has certain limitations. First, the autophagy-related genes included in our study may not be comprehensive. Additionally, these findings need to be validated through in vivo and in vitro experiments.

Funding

Graduate Education Innovation Program of Shanxi University of Chinese Medicine (2023CX024); Graduate Research and Practice Projects of Shanxi Province (2023KY677, 2023KY678).

References

[1] FARINA M, VIEIRA LE, BUTTARI B, et al. The Nrf2 Pathway in Ischemic Stroke: A Review [J]. Molecules. 2021; 26(16):5001.
[2] LIU S, LIN F, WANG J, et al. Polyphenols for the Treatment of Ischemic Stroke: New Applications and Insights [J]. Molecules. 2022;27(13):4181.
[3] ZHAO Y, ZHANG X, CHEN X, et al. Neuronal injuries in cerebral infarction and ischemic stroke: From mechanisms to treatment (Review) [J]. Int J Mol Med. 2022; 49(2):15.
[4] HUANG T, SONG X, YANG Y, et al. Autophagy and Hallmarks of Cancer [J]. Crit Rev Oncog. 2018; 23(5-6): 247-267.
[5] HOU X, WATZLAWIK JO, FIESEL FC, et al. Autophagy in Parkinson's Disease [J]. J Mol Biol. 2020; 432(8):2651-2672.
[6] MALIK BR, MADDISON DC, SMITH GA, et al. Autophagic and endo-lysosomal dysfunction in

neurodegenerative disease [J]. Mol Brain. 2019; 12(1): 100.
[7] AZZMAN N. Crohn's Disease: Potential Drugs for Modulation of Autophagy [J]. Medicina (Kaunas). 2019; 55(6):224.
[8] HUANG GZ, LU ZY, RAO Y, et al. Screening and identification of autophagy-related biomarkers for oral squamous cell carcinoma (OSCC) via integrated bioinformatics analysis [J]. J Cell Mol Med. 2021; 25(9): 4444-4454.
[9] USMAN RM, RAZZAQ F, AKBAR A, et al. Role and mechanism of autophagy-regulating factors in tumorigenesis and drug resistance [J]. Asia Pac J Clin Oncol. 2021; 17(3):193-208.
[10] SHI Q, CHENG Q, CHEN C. The Role of Autophagy in the Pathogenesis of Ischemic Stroke [J]. Curr Neuropharmacol. 2021;19(5):629-640.
[11] BARRETT T, WILHITE SE, LEDOUX P, et al. NCBI GEO: archive for functional genomics data sets--update [J]. Nucleic Acids Res. 2013; 41(Database issue): D991-D995.
[12] DIKIC I, ELAZAR Z. Mechanism and medical implications of mammalian autophagy [J]. Nat Rev Mol Cell Biol. 2018; 19: 349–364.
[13] MIZUSHIMA N, LEVINE B. Autophagy in human diseases [J]. N Engl J Med. 2020; 383: 1564–1576.
[14] GALLUZZI L, BRAVO-SAN PEDRO JM, BLOMGREN, et al. Autophagy in acute brain injury [J]. Nat Rev Neurosci. 2016; 17: 467–484.
[15] TOOZE SA, DIKIC I. Autophagy captures the Nobel prize [J]. Cell. 2016; 167: 1433–1435.
[16] MAO R, ZONG N, HU Y, et al. Neuronal Death Mechanisms and Therapeutic Strategy in Ischemic Stroke [J]. Neurosci Bull. 2022;38(10):1229-1247.
[17] ZHANG Y, LIU X, LONG J, et al. Exploring Active Compounds and Mechanisms of Angong Niuhuang Wan on Ischemic Stroke Based on Network Pharmacology and Molecular Docking [J]. Evid Based Complement Alternat Med. 2022; 2022: 2443615.
[18] ZHANG Q, LIAO Y, LIU Z, et al. Interleukin-17 and ischaemic stroke [J]. Immunology. 2021; 162(2): 179-193.
[19] HU R, LIANG J, DING L, et al. Edaravone dexborneol provides neuroprotective benefits by suppressing NLRP3 inflammasome-induced microglial pyroptosis in experimental ischemic stroke [J]. Int Immunopharmacol. 2022;113(Pt A):109315.
[20] CHEN S, PENG J, SHERCHAN P, et al. TREM2 activation attenuates neuroinflammation and neuronal apoptosis via PI3K/Akt pathway after intracerebral hemorrhage in mice [J]. J Neuroinflammation. 2020; 17(1):168.
[21] YANG B, LI Y, MA Y, et al. Selenium attenuates ischemia/reperfusion injury-induced damage to the blood-brain barrier in hyperglycemia through PI3K/AKT/mTOR pathway-mediated autophagy inhibition [J]. Int J Mol Med. 2021;48(3):178.
[22] TIAN G, LI Q, NIU L, et al. CASP4 can be a diagnostic biomarker and correlated with immune infiltrates in gliomas [J]. Front Oncol. 2023; 12: 1025065.
[23] MENG L, TIAN Z, LONG X, et al. Prognostic autophagy model based on CASP4 and BIRC5 expression in patients with renal cancer: independent datasets-based study [J]. Am J Transl Res. 2020; 12(11): 7475-7489.
[24] KRAUSE K, CAUTION K, BAHR A, et al. CASP4/caspase-11 promotes autophagosome formation in response to bacterial infection [J]. Autophagy. 2018; 14(11): 1928-1942.
[25] OMOROU M, HUANG Y, GAO M, et al. The forkhead box O3 (FOXO3): a key player in the regulation of ischemia and reperfusion injury [J]. Cell Mol Life Sci. 2023; 80(4):102.
[26] DELRIO C, MONTANER J. Hypoxia Tolerant Species: The wisdom of nature translated into targets for stroke therapy [J]. Int J Mol Sci. 2021; 22(20):11131.
[27] DENG A, MA L, JI Q, et al. Activation of the Akt/FoxO3 signaling pathway enhances oxidative stress-induced autophagy and alleviates brain damage in a rat model of ischemic stroke [J]. Can J Physiol Pharmacol. 2023; 101(1):18-26.
[28] ZHENG J, LUO S, LONG Y. Bioinformatic analysis and clinical diagnostic value of hsa_circ_0004099 in acute ischemic stroke [J]. PLoS One. 2022; 17(11): e0277832.
[29] GE L, ZHANG Y, ZHAO X, et al. EIF2AK2 selectively regulates the gene transcription in immune response and histones associated with systemic lupus erythematosus [J]. Mol Immunol. 2021; 132:132-141.
[30] ZHANG X, WANG X, KHURM M, et al. Alterations of Brain Quantitative Proteomics Profiling Revealed the Molecular Mechanisms of Diosgenin against Cerebral Ischemia Reperfusion Effects [J]. J Proteome Res. 2020; 19(3):1154-1168.
[31] GAN H, ZHANG L, CHEN H, et al. The pivotal role of the NLRC4 inflammasome in neuroinflammation after intracerebral hemorrhage in rats [J]. Exp Mol Med. 2021; 53(11):1807-1818.
[32] ZHOU Y, LIU C, ZHANG Z, et al. Identification and validation of diagnostic biomarkers of coronary artery disease progression in type 1 diabetes via integrated computational and bioinformatics strategies [J]. Comput Biol Med. 2023; 159: 106940.
[33] POH L, KANG SW, BAIK SH, et al. Evidence that NLRC4 inflammasome mediates apoptotic and pyroptotic microglial death following ischemic stroke [J]. Brain Behav Immun. 2019; 75:34-47.
[34] KAWAGUCHI N, ZHANG TT, NAKANISHI T. Involvement of CXCR4 in Normal and Abnormal Development [J]. Cells. 2019; 8(2):185.
[35] ZOU R, ZHANG D, LV L, et al. Bioinformatic gene analysis for potential biomarkers and therapeutic targets of atrial fibrillation-related stroke [J]. J Transl Med. 2019;17(1):45.

Author Profile

Yaoyao Dai (1998-), female, Han nationality, from Xingtai, Hebei Province, Master's student, Research direction: Integrated Traditional Chinese and Western Medicine for the Prevention and Treatment of Cerebrovascular Diseases, E-mail: 291577411@qq.com.

Rui Haogeng Wang (2000-), male, Han nationality, from Xingtai, Hebei Province, Master's student, Research direction: Integrated Traditional Chinese and Western Medicine for the Prevention and

Treatment of Cerebrovascular Diseases, E-mail: q18330981516@163.com.

Chen Zhang (1995-), male, Han nationality, from Xingtai, Hebei Province, Research direction: Integrated Traditional Chinese and Western Medicine for the Prevention and Treatment of Cerebrovascular Diseases, Email: 571834498@qq.com