New Autophagy-Ferroptosis Gene Signature Predicts Survival in Glioma

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Background: Ferroptosis plays an important role in glioma and significantly affects the prognosis, but the specific mechanism has not yet been elucidated. Recent studies suggest that autophagy regulates the process of ferroptosis. This study aimed to find potential autophagy-ferroptosis genes and explore the prognostic significance in glioma.

Methods: Ferroptosis and autophagy genes were obtained from two online databases (zhounan.org/ferrdb and autophagy.lu/). The RNAseq data and clinical information were obtained from the Chinese Glioma Genome Atlas (CGGA) database (http://www.cgga.org.cn/). Univariate, multivariate, lasso and Cox regression analysis screened out prognosis-related genes, and a risk model was constructed. Receiver operating characteristic (ROC) curve analysis evaluated the predictive efficiency of the model. Finally, a nomogram was constructed to more accurately predict the prognosis of glioma.

Results: We developed a Venn diagram showing 23 autophagy-ferroptosis genes. A total of 660 cases (including RNA sequences and complete clinical information) from two different cohorts (training group n = 413, verification group n = 247) of the CGGA database was acquired. Cohorts were screened to include five prognosis-related genes (MTOR, BID, HSPA5, CDKN2A, GABARAPLA2). Kaplan-Meier curves showed that the risk model was a good prognostic indicator (p < 0.001). ROC analysis showed good efficacy of the risk model. Multivariate Cox analysis also revealed that the risk model was suitable for clinical factors related to prognosis, including type of disease (primary, recurrence), grade (III-IV), age, temozolomide treatment, and 1p19q state. Using the five prognosis-related genes and the risk score, we constructed a nomogram assessed by C-index (0.7205) and a calibration plot that could more accurately predict glioma prognosis.

Conclusion: Using a current database of autophagy and ferroptosis genes, we confirmed the prognostic significance of autophagy-ferroptosis genes in glioma, and we constructed a prognostic model to help guide treatment for high grade glioma in the future.

Keywords: ferroptosis, autophagy, glioma, prognosis, risk model
INTRODUCTION

Glioma is the most common primary malignant tumor in the brain. Current treatments for glioma include surgical resection, chemotherapy, radiotherapy, immunotherapy, and electric field therapy. Although many treatments exist, prognosis has not significantly improved (Duffau and Taillandier, 2015; Tan et al., 2020). Traditionally, gliomas have been divided into grades I-IV in pathological classification, of which grades I-II belong to low grades, and grades III-IV belong to high grades. According to the latest WHO classification in 2016, molecular pathology is now included in the classification of gliomas (Louis et al., 2016; White et al., 2020). This change shows the importance of molecular pathology for the diagnosis and treatment of glioma. Currently, molecular markers that affect prognosis have been identified for gliomas, but no exact and efficient target for clinical application yet exists.

Studies have found that ferroptosis plays an important role in nervous system tumors and notably affects the prognosis of gliomas (Buccarelli et al., 2018; Wan et al., 2021). Ferroptosis was initially described as a unique type of regulated cell death that is observed in oncogenic RAS-mutated cancer cells and that is, distinct from apoptosis, necrosis, and autophagy at the morphological, biochemical, and genetic levels (Dixon et al., 2012; Xie et al., 2016). However, increasing evidence challenges these early observations and suggests that the autophagic machinery, at least under certain conditions, contributes to ferroptosis.

BECN1 is a key regulator of autophagy. The BECN1-SLC7A11 complex directly inhibits the activity of systemXc (-) and promotes ferroptosis (Kang et al., 2018). Nrf2 is a key anti-ferroptosis transcription factor in liver cancer, and it can inhibit ferroptosis induced by sorafenib and erastin through the p62-keap1-Nrf2 pathway (Sun et al., 2016). RAB7A can mediate the degradation of lipid droplets by the lipophagy pathway, increase the level of intracellular lipids, promote lipid peroxidation, and promote the ferroptosis of liver cancer cells induced by RSL3. Knockdown of the RAB7A gene inhibits lipophages and the degradation of lipid droplets, which can reverse RSL3-induced cell ferroptosis (Bai et al., 2019). NCOA4 targets ferritin to the lysosome for autophagic degradation, increases unstable iron levels in cells, promotes reactive oxygen species production, and leads to cell ferroptosis. Knockout of the NCOA4 gene inhibits ferritin autophagy, alleviates iron overload, and reverses erastin-induced cell ferroptosis (Mancias et al., 2014; Mancias et al., 2015).

However, the relationship between autophagy and ferroptosis in tumors is complex; Autophagy not only promotes but also inhibits ferroptosis. Adjusting autophagy activity to promote ferroptosis of tumor cells is of great significance to cancer treatment. Little research on the autophagy-ferroptosis connection in glioma has been conducted. In this study, we screened autophagy-ferroptosis genes using RNA sequences and clinical data in the Chinese Glioma Genome Atlas (CGGA) database. The purpose of this study was

![Gene expression and clinical data](image)

**FIGURE 1** | Study process.

| Clinical characteristics | Training group (n = 413) | Verification group (n = 247) | P |
|--------------------------|------------------------|-----------------------------|---|
| Age                      |                        |                             |   |
| < 50                     | 294                    | 164                         | 0.2|
| ≥ 50                     | 119                    | 83                          |   |
| Gender                   |                        |                             |   |
| Female                   | 182                    | 98                          | 0.27|
| Male                     | 231                    | 149                         |   |
| Type                     |                        |                             |   |
| Primary                  | 248                    | 194                         | < 0.05|
| Recurrence               | 165                    | 53                          |   |
| Grade                    |                        |                             |   |
| II                       | 47                     | 81                          | < 0.05|
| III                      | 87                     | 64                          |   |
| IV                       | 279                    | 102                         |   |
| IDH mutation             |                        |                             |   |
| Yes                      | 228                    | 125                         | 0.25|
| No                       | 185                    | 122                         |   |
| 1p19q codeletion         |                        |                             |   |
| Yes                      | 87                     | 50                          | 0.8|
| No                       | 326                    | 197                         |   |
| MGMT                     |                        |                             |   |
| Yes                      | 244                    | 125                         | 0.03|
| No                       | 169                    | 122                         |   |
| Radiation therapy        |                        |                             |   |
| Yes                      | 328                    | 198                         | 0.82|
| No                       | 85                     | 49                          |   |
| TMZ therapy              |                        |                             |   |
| Yes                      | 324                    | 158                         | < 0.05|
| No                       | 89                     | 89                          |   |
to investigate and verify the expression characteristics of autophagy-ferroptosis genes to predict the prognosis of glioma. This study also established a new predictive nomogram for related genes to more accurately assess the prognosis of glioma. These selected genes also provide a basis for subsequent research.

**MATERIALS AND METHODS**

**Patient Data**

The RNAseq data and clinical information of a training group and a verification group were obtained from the CGGA database (http://www.cgga.org.cn/); all data were complete and uniform (Zhao et al., 2017). We normalized gene expression by the RPKM (transcriptome reads per kilobase reads per million reads) method (Mortazavi et al., 2008). The study process is shown in Figure 1.

**Construction and Verification of Risk Models**

Univariate and lasso regression analysis screened for the best prognosis-related genes with \( p < 0.01 \). A prognostic risk model for predicting overall survival (OS) was established. Patients with glioma were divided into high and low expression groups according to their median expression levels of prognosis-related genes. Kaplan-Meier survival analysis evaluated the relationship between the expression levels of prognostic-related genes and OS. Then, the correlations between the risk model and clinical characteristics were analyzed.

**Enrichment Analysis and Protein-Protein Interaction Network Construction**

Gene Set Enrichment Analysis (GSEA) was used to enrich signal pathways between the low- and high-risk groups of patients with glioma (Subramanian et al., 2005). The cutoff criteria were a normalized enrichment score (NES) \( > 1.5 \) and a nominal \( p < 0.05 \). A protein-protein interaction (PPI) network of autophagy-ferroptosis genes was constructed to understand the relationship among genes.

**Construction and Verification of Nomogram**

A nomogram was constructed according to the five prognosis-related genes to more accurately predict the prognosis for patients with glioma (Iasonos et al., 2008). This constructed allowed investigation of the 1-, 3-, 5-, 7-, and 9-years survival rates of...
FIGURE 3 | (A) The risk model of the experimental group and the validation group ($p < 0.001$). (B) The receiver operating characteristic curve suggests that the risk model has good short-term and long-term predictive values between the training and verification groups. (C) Component analysis suggests that the model can accurately distinguish high- and low-risk groups. (D) The risk curve and risk status show the survival status of the patient as the score increases. The risk heat map shows the expression of prognosis-related genes in the high- and low-risk groups.
patients with glioma. The concordance index (C-index) was calculated, and a calibration curve was plotted to assess the discrimination and accuracy of the nomogram.

Statistical Analysis
All statistical analysis were performed with R (version 3.63, http://www.r-project.org/).

RESULTS

Patient Database
Data from 660 patients with glioma were collected after missing values (RNAseq data and clinical characteristics) were excluded. Of this total, data from 413 patients were collected as a training group, and data from 247 patients were collected as a verification group. The clinical characteristics of the two cohorts are shown in Table 1.

Construction and Verification of Risk Models
Overall, 147 autophagy genes were obtained from an online database (http://www.autophagy.lu/) (Wang et al., 2019), and 150 ferroptosis genes were obtained from an online database (http://www.zhounan.org/) (Zheng et al., 2021). A constructed Venn diagram displayed 23 autophagy-ferroptosis genes (Figure 2A). Univariate and lasso regression analysis screened for five prognosis-related genes (MTOR, BID, HSPA5, CDKN2A, and GABARAPL2) (Figures 2B–D). The risk score was defined as $0.40 \times HSPA5 + 0.34 \times MTOR - 0.33 \times BID - 0.08 \times CDKN2A - 0.33 \times GABARAPL2$. Kaplan-Meier survival analysis using the survival package estimated the associations between the expression levels of the prognosis-related genes and OS (Figure 3A). The prognostic performance was evaluated using time-dependent receiver operating characteristic (ROC) curve.
FIGURE 5 | The relationship between risk model and clinical characters. The model is not applicable to WHO grade II glioma ($p > 0.05$).
FIGURE 6 | (A) Nomogram based on five prognosis-related genes (left) and calibration curves for 1, 5, and 9 years (right). (B) The main pathway enrichments were in basal cell carcinoma, linoleic acid metabolism, and mature-onset diabetes of the young. (C, D) Protein-protein interaction network suggesting that MTOR has the most extensive links. (E) Positive and negative correlations among 23 autophagy-ferroptosis genes.
analysis within 1, 3, 5, 7, and 9 years to evaluate the predictive accuracy of the prognostic model (Figure 3B). The risk curve and risk status, risk heat maps, risk scatter plots, and PCA were drawn to evaluate the model’s ability to distinguish between high- and low-risk groups (Figures 3C,D). Then, the relationship between risk models and clinical characteristics was explored. Type, grade, age, temozolomide use, and 1p19q state were the risk factors associated with prognosis in glioma (Figures 3C,D). This risk model is applicable to these clinical risk factors except WHO grade II (Figure 5). The expression of the five prognosis-related genes was explored in relation to different clinical characteristics as well (Figure 4B).

GSEA Enrichment Analysis and PPI Construction
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed no significant enrichment in the high-risk group ($p > 0.05$); in the low-risk group, the main pathways of enrichment were in basal cell carcinoma, linoleic acid metabolism, and mature-onset diabetes of the young (Figure 6B). MTOR had the strongest correlation of 23 autophagy-ferroptosis genes in PPI (Figures 6C–E).

Prognosis of Different Glioma Subgroups
There were significant prognostic differences in 4 glioma subgroups (GBM IDH wildtype, GBM IDH mutant, Oligodendrogloma II–III, Astrocytoma II–III) (Figure 7). 15 autophagy-ferroptosis genes were analyzed (Figure 8). The prognostic genes of GBM IDH mutant were HSPA5 and NFE2L2; GBM IDH wildtype were ATG7 and MAPK9; A/AA group includes BID, LAMP2 and MAPK3; ATG5, BECN1, GABARAPL1 and HSPA5 were prognostic genes in the O/AO subgroup; In the clinical risk assessment (Figure 8), Type (primary/recurrence), radiotherapy, and TMZ were prognostic factors for GBM IDH wildtype; Type (primary/recurrence) and Grade were prognostic factors for O/AO.

Construction and Verification of Nomogram
A nomogram was built for accurately predicting 1-, 3-, 5-, 7-, and 9-years OS. The five prognosis-related genes were added to the prediction model (Figure 6A). The discrimination and accuracy of the nomogram were evaluated by C-index and calibration. The C-index was 0.72 in the training group and was 0.74 in verification group. Calibration curves of 1-, 5-, and 9-years survival rates were relatively close between prediction and observation.

DISCUSSION
Previous study explains the specific mechanism of ferroptosis in glioma. ATF4 increases tumor angiogenesis and vascular structure shaping in an xCT activity-dependent manner, and downregulating ATF4 expression can enhance the sensitivity of nerve tumor cells, which control the proliferation and vasculature of tumors, to ferroptosis (Chen et al., 2017). The overexpression of Nrf2 and the knockout of Keap1 can promote the proliferation and migration of tumor cells by upregulating the activity of xCT, thus changing the tumor microenvironment and inhibiting ferroptosis (Fan et al., 2017). The combined use of temozolomide and ferroptosis inducers can improve the therapeutic effect on glioma cells (Sehm et al., 2016).
However, the mechanism of ferroptosis remains unclear. Although ferroptosis differs from other types of regulated cell death, studies have found that autophagy can regulate the process of ferroptosis. Few studies on autophagy-ferroptosis in gliomas have been conducted. In one study, amentoflavone treatment led to reduced cell viability and cell death by triggering ferroptosis in gliomas. The figure shows the autophagy-ferroptosis prognostic gene and clinical risk assessment in different subgroups of glioma.

FIGURE 8 | Autophagy-ferroptosis prognostic gene (A) and clinical risk assessment (B) in different subgroups of glioma.
an autophagy-dependent manner in glioma (Chen et al., 2020). Additional study of autophagy-ferroptosis may provide new concepts to treat glioma in the future.

In this study, we first identified 23 genes with dual functions of autophagy and ferroptosis. We designated these genes as autophagy-ferroptosis genes to distinguish them from the autophagy-dependent ferroptosis pathway, and because the relationship between the autophagy and ferroptosis genes has not been elucidated in previous studies, to our knowledge. We conducted various methods of statistical analysis, such as lasso regression, Cox regression, ROC curve analysis, and GSEA. We found that the risk model constructed by autophagy-ferroptosis genes are independently related to glioma prognosis. Summarized in Figure 9. Our model can improve the therapeutic effect of TMZ and the prognosis of high-grade gliomas, except for low-grade gliomas. Among the prognostic-related molecular subtypes included in the 2016 WHO guidelines, IDH1 mutation/wild type, MGMT unmethylation/unmethylation, 1p19q deletion/no deletion, female/male, WHO III-IV glioma, except WHO II glioma; On the left is the expression of the five genes that constructing the model in different clinical characters. The express difference can provide a direction for future basic study.

CONCLUSION

Using databases of autophagy and ferroptosis genes, we explored the prognostic significance of autophagy-ferroptosis genes in glioma.
glioma and constructed a prognostic model to help improve care for patients with high grade glioma in the future.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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**AUTHOR CONTRIBUTIONS**

LV contributed to conception, design and the first draft of the manuscript of the study. ZY, ZJ, HW, and WP performed the statistical analysis. ZW, YY, and BC performed the figures and table. GW and ZX reviewed drafts and edits. All authors contributed to manuscript revision, read, and approved the submitted version.

**Data availability**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.
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