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Original Article

Screening of autophagy genes as prognostic indicators for glioma patients

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Abstract: Although autophagy is reported to be involved in tumorigenesis and cancer progression, its correlation with the prognosis of glioma patients remains unclear. Thus, the aim of this study was to identify prognostic autophagy-related genes, analyze their correlation with clinicopathological features of glioma, and further construct a prognostic model for glioma patients. After 139 autophagy-related genes were obtained from the GeneCards database, their expression data in glioma patients were extracted from the Chinese Glioma Genome Atlas database. Univariate and multivariate COX regression analyses were performed to identify prognostic autophagy-related genes. Ten hub autophagy-related genes associated with prognosis were identified. The autophagy risk score (ARS) was only positively correlated with histopathology (P = 0.000) and World Health Organization grade (P = 0.000). Kaplan-Meier analysis showed that the overall survival of patients with a high ARS was significantly worse than that of patients with a low ARS (hazard ratio = 1.59, 95% confidence interval = 1.25-2.03, P = 0.0001). In addition, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses revealed several common biological processes and signaling pathways related to the 10 hub genes in glioblastoma. A prediction model was developed for glioma patients, which demonstrated high prediction efficiency on calibration. Moreover, the area under the receiver operating characteristic curve values for 1-, 3- and 5-year survival probabilities were 0.790, 0.861, and 0.853, respectively. In conclusion, we identified 10 autophagy-related genes that can serve as novel prognostic biomarkers for glioma patients. Our prediction model accurately predicted patient outcomes, and thus, may be a valuable tool in clinical practice.

Keywords: Neoplasms, survival, nomograms

Introduction

Glioma of the brain is a common human malignancy that is harmful to human health globally. With the development of imaging technologies, the overall incidence of gliomas in the past decades has continued to increase [1]. According to an analysis of the Central Brain Tumor Registry of the United States (CBTRUS) data, an estimated 87,240 cases of primary central nervous system (CNS) tumors will be diagnosed in the United States in 2020, including 25,800 malignant tumors and 61430 non-malignant tumors [2]. At present, the main treatment strategy for gliomas worldwide remains surgery followed by postoperative adjuvant radiotherapy and chemotherapy. Although the treatment of gliomas has been improved to some extent, the prognosis of glioma patients continues to not be satisfactory. Notably, overall survival (OS) varies greatly among patients with the same pathological diagnosis [3], which shows that the traditional pathological diagnosis is insufficient for judging patients' prognosis. In addition, the common clinical prognostic markers (e.g., isocitrate dehydrogenase [IDH] mutation 1p/19q co-deletion) only distinguish two subgroups of patients with the same histopathology. These markers are still not sufficient for more subtle stratification and cannot reflect the individual prognosis of each patient. Therefore, there is an urgent need for a new prediction model that offers greater accuracy for glioma patient prognosis. Moreover, such a model

would be valuable for guiding personalized medicine for glioma patients [4].

Research in recent years has demonstrated that autophagy is a process of degradation and reuse of cellular components that plays a key role in tumorigenesis, cancer development, and metastasis [5]. Studies have also shown that tumor cells, especially under stress conditions, can obtain the energy and substances necessary for survival through autophagy, and thus, autophagy is a type of survival mechanism for tumor cells [6]. Thus, the inhibition of autophagy can reduce the tolerance of tumor cells to stress, increase the sensitivity of tumor cells to anti-tumor drugs, and improve the effect of anticancer therapy [7]. Notably, multiple preclinical studies have found that knockout of the key autophagy genes (e.g., beclin-1, Atg12, Atg5) can improve the killing ability of antineoplastic drugs, which indicates that autophagy can protect cancer cells and inhibit the efficacy of antineoplastic drugs [8, 9].

These findings further suggest that autophagy-related genes play a key role in tumorigenesis and development and have a significant impact on the prognosis of cancer patients. As such, these genes may also provide prognostic markers. However, autophagy is a complex biological process involving hundreds of genes that is common in the life processes of eukaryotes. The prognostic value of autophagy genes in glioma patients remains unclear, and investigation of which autophagy-related genes have the potential to become prognostic markers is worthwhile.

Toward this end, we obtained the expression profile data for autophagy-related genes and the clinicopathological data of patients from the Chinese Glioma Genome Atlas (CGGA) dataset in order to search for independent prognostic factors by analyzing the correlations between autophagy-related genes and patients' prognosis. Additionally, from the identified genes, we constructed a new prediction model for glioma patients' prognosis.

Materials and methods

Screening of autophagy-related genes

The genes related to autophagy were searched in the GeneCards database (https://www.genecards.org/). Genes with a relevance score >8 were screened as autophagy-related genes in this study.

Patient selection

First, we extracted the data for all patients in the dataset 4 cohort of the CGGA (http://cgga.org.cn/index.jsp) [10], which is a database for storing clinical and pathological information on glioma patients in China. Second, patients with missing data were excluded. Finally, we included data for 269 glioma patients in our analysis.

Identification of prognostic genes

First, univariate Cox regression analysis was performed to screen autophagy-related genes significantly associated with the prognosis of glioma patients. The autophagy-related genes for which $P<0.05$ were further included in multivariate regression analysis, and independent prognostic autophagy-related genes were identified.

Oncomine database and human protein atlas (HPA) analysis

The Oncomine gene expression array database (www.oncomine.org) was used to assess the mRNA expression levels of the identified prognostic genes. In addition, the expression levels of the corresponding proteins in gliomas and normal tissues were reviewed in the HPA (http://www.proteinatlas.org/) [11].

Pathway analysis

From the prognostic autophagy-related genes, we determined the nine most relevant coexpression genes in the TCGA-CNS/Brain database (http://www.cbioportal.org/). Gene Ontology (GO) analysis of all prognostic genes and coexpression genes was performed using the DAVID [12] database (https://david.ncifcrf.gov/), including biological process, cellular component, and molecular function. The Cytoscape tool [13] was used to implement the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. We used the String database [14, 15] to observe protein-protein interaction between the proteins encoded by the prognostic genes (https://string-db.org/).

Correlations between autophagy risk score (ARS) and clinicopathological features of patients

The ARS was constructed according to the expression of a prognostic gene and a correlation risk coefficient for the gene. The formula for the ARS is as follows: ARS = gene1*β1 + gene2*β2 + gene3*β3.....gene (n)*β (n). Chi-square test was performed to explore correlations between clinical parameters and the ARS. The relationship of the ARS to the prognosis of glioma patients was analyzed by Kaplan-Meier survival curve analysis.

Development and validation of nomogram

Based on clinical indicators and the ARS, a predictive model was constructed using the "Survival" package and "rms" package. A calibration curve and time-dependent receive operating characteristic (ROC) curve were generated to test the accuracy of the prognostic model. In addition, the "survivalROC" package was used to draw the ROC curve to compare the predictive efficiencies among the nomogram, traditional predictive models, and common clinical prognostic indicators.

Statistical analysis

All data were analyzed using SPSS software (version 23.0, Corp., Armonk, NY, USA) and R software version 3.6.1. Chi-square test was used to identify differences in classified variables. Differences in continuous variables were analyzed by the independent sample t-test. Univariate and multivariate Cox regression analyses were used to identify independent prognostic factors. The Kaplan-Meier method was applied to draw the survival curves, and the log-rank test was used to compare the survival curves. Time-dependent ROC curve analysis was performed using R package "survivalROC". All tests were two-sided, and *P*-values <0.05 were considered to be statistically significant. For multiple testing, we use the Bonferroni correction method to correct the *P*-value.

Results

Glioma patients' characteristics

A total of 269 patients with glioma were included in this study. The clinicopathological data of patients are summarized in Table S1. The average age of all patients was 42.58±11.83 years. The incidence of glioma was higher in males than in females, and the median survival time of all patients was 31.2 months (interquartile range, 0.7-138.1 months). The 1-, 3-, and 5-year survival rates for all glioma patients were 77.32%, 46.84%, and 34.94%, respectively.

Screening of prognostic autophagy-related genes in 269 glioma patients

To search for prognostic autophagy-related genes of glioma patients, all candidate genes related to autophagy were searched through the GeneCards database. A total of 139 autophagy-related genes with a relevance score >8 were selected for further investigation. The IRGM and BECN2 genes were subsequently excluded, because expression of these genes was not found by mRNA expression microarray analysis for glioma patients. Finally, the remaining 137 genes were entered in the univariate Cox regression analysis. On this analysis, 86 autophagy-related genes were significantly associated with the prognosis of glioma patients (Table S2). These 86 genes were further incorporated into the multivariate analysis, and 10 hub autophagy-relate genes associated with prognosis were finally identified (**Table 1**). The detailed information for the 10 hub autophagy-related genes is presented in **Table 2**. The expression data for the 10 autophagy-related genes were obtained from the mRNA array data of dataset 4. The differential expression levels of the autophagy-related genes between high- and low-grade glioma cases were compared (**Figure 1**). Furthermore, we also analyzed the mRNA levels of autophagy-related genes based on data from the Oncomine database and the protein expression levels based on data from the HPA database (**Table 3**; Figure S1).

Biological processes related to hub autophagy-related genes

To identify the biological functions of these genes in glioma, we searched for the nine most relevant coexpression genes in the TCGA-CNS/Brain (http://www.cbioportal.org/). Then, GO enrichment analysis and KEGG analysis were conducted. GO enrichment analysis revealed that these genes were mostly enriched in autophagy, macroautophagy, regulation of MAP

Table 1. Results of univariate and multivariate Cox analyses of prognostic genes for OS in glioma

Features	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ULK1	0.67	0.52-0.87	0.002	2.27	1.14-4.54	0.020
ATG10	0.51	0.34-0.77	0.001	2.59	1.31-5.13	0.006
ATG16L2	0.57	0.45-0.73	0.000	0.48	0.27-0.86	0.013
RB1CC1	0.65	0.53-0.79	0.000	2.03	1.06-3.88	0.032
RUBCNL	1.12	1.01-1.24	0.038	0.71	0.54-0.94	0.016
PRKN	0.46	0.34-0.62	0.000	0.45	0.23-0.85	0.015
GSK3B	0.49	0.35-0.69	0.000	3.20	1.42-7.24	0.005
TBC1D5	0.30	0.23-0.41	0.000	0.27	0.13-0.56	0.000
PIK3CB	0.70	0.6-0.82	0.000	0.57	0.34-0.96	0.034
RAB33B	1.40	1.19-1.64	0.000	1.54	1.14-2.07	0.004

HR, hazard ratio; OS, overall survival; CI, confidence interval.

Table 2. Functional details of the 10 independently prognostic autophagy-related genes

Gene symbol	Full name	Category	Relevance score
ULK1	Unc-51 Like Autophagy Activating Kinase 1	Protein Coding	37.03
ATG10	Autophagy Related 10	Protein Coding	28.09
ATG16L2	Autophagy Related 16 Like 2	Protein Coding	21.84
RB1CC1	RB1 Inducible Coiled-Coil 1	Protein Coding	20.16
RUBCNL	Rubicon Like Autophagy Enhancer	Protein Coding	16.07
PRKN	Parkin RBR E3 Ubiquitin Protein Ligase	Protein Coding	11.71
GSK3B	Glycogen Synthase Kinase 3 Beta	Protein Coding	11.21
TBC1D5	TBC1 Domain Family Member 5	Protein Coding	9.99
PIK3CB	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Beta	Protein Coding	9.16
RAB33B	RAB33B, Member RAS Oncogene Family	Protein Coding	8.48

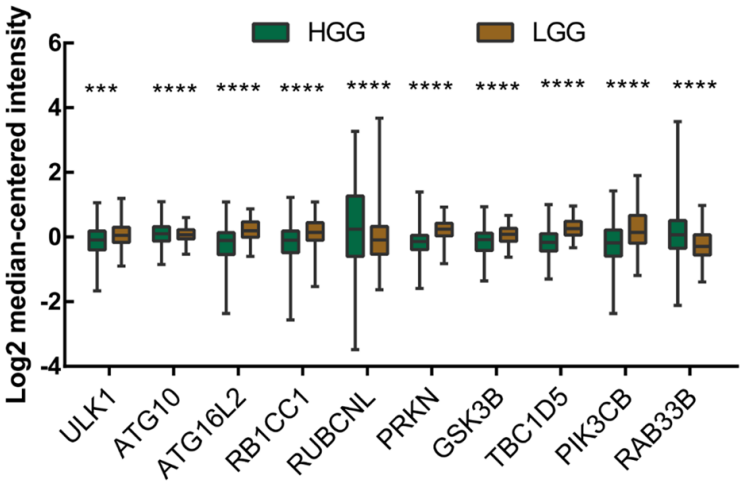

Figure 1. Differential mRNA expression of autophagy-related genes between low-grade glioma and high-grade glioma groups. ***P<0.001, ****P<0.0001.

kinase activity, and intracellular protein transport (**Figure 2A-C**). From the pathway analysis, these genes were mainly involved in autophagy signal, the B cell receptor signaling pathway, and the prolactin signaling pathway (**Figure 2D**). In addition, a protein-protein interaction network of the proteins encoded by these 10 autophagy-related genes was constructed (Figure S2).

Correlations between ARS and clinicopathological features

To identify any correlations between autophagy-related genes

Table 3. Significant differences in transcription levels of autophagy-related genes between glioma and normal brain tissues (Oncomine)

Gene	Type of Glioma vs. Normal	T-test	*P*-value	Reference
ULK1	Glioblastoma	-7.45	5.94E-06	Bredel Brain 2
ATG10	Glioblastoma	3.725	0.007	Bredel Brain 2
RB1CC1	Glioblastoma	-13.189	1.34E-09	Bredel Brain 2
RUBCNL	Glioblastoma	4.789	9.51E-05	Bredel Brain 2
GSK3B	Glioblastoma	-3.857	0.001	Bredel Brain 2
PIK3CB	Glioblastoma	-7.286	4.28E-04	Bredel Brain 2
PRKN	Anaplastic Astrocytoma	-4.988	6.61E-06	Sun Brain
TBC1D5	Glioblastoma	-7.22	1.12E-09	Sun Brain
RAB33B	Glioblastoma	5.097	2.52E-04	TCGA Brain
ATG16L2	Glioblastoma	-2.568	0.005	TCGA Brain 2

and clinicopathological features, the ARS was calculated for each patient according to the formula based on the expression of autophagy-related genes and their correlation coefficients. The formula for calculating the ARS was as follows: Autophagy Risk Score (ARS) = ULK1* (0.820) + ATG10* (0.952) + ATG16-L2* (-0.734) + RB1CC1* (0.708) + RUBCNL* (-0.342) + PRKN* (-0.799) + GSK3B* (1.163) + TBC1D5* (-1.309) + PIK3CB* (-0.562) + RAB-33B* (0.432).

According to the median ARS value, the 269 patients were divided into a high-risk group (n = 135) and a low-risk group (n = 134), and the correlations between the ARS and the individual clinicopathological features were analyzed by chi-square test. The results showed that the ARS was significantly correlated with World Health Organization (WHO) grade (*P* = 0.000) and histopathology (*P* = 0.000), but not with age (*P* = 0.668), gender (*P* = 0.055), IDH mutation (*P* = 0.672), radiotherapy (*P* = 0.239), chemotherapy (*P* = 0.059), or recurrence (*P* = 0.362) (**Table 4**). We also further assessed the distribution of ARS values among the 269 patients stratified by WHO grade and histopathology. Consistently, the results revealed that the ARS was significantly associated with WHO grade and histopathology (**Figure 3**).

Correlation between ARS and OS of patients

To assess the prognostic value of autophagy-related genes in glioma patients, Kaplan-Meier analysis was performed to compare patients' OS between the high- (n = 135) and low-risk score groups (n = 134). The results indicated that the OS of patients with a high ARS was significantly worse than that of patients with a low ARS (hazard ratio [HR] = 1.59, 95% confidence interval [CI] = 1.25-2.03, *P* = 0.0001), and the median survival times of the two groups were 53.43 months and 18.20 months, respectively (**Figure 4**). To further assess the prognostic value of ARS in subgroups of glioma patients, all patients were further stratified by age, gender, WHO grade, IDH mutation, and recurrence. The results similarly showed that the OS of patients with a high ARS was significantly worse than that of patients with a low ARS, except in the recurrence subgroup (Figure S3A-J).

In addition, we also analyzed the effect of each autophagy gene on the prognosis of glioma patients in the GEPIA database. The results are presented in Figure S4A-J.

To clarify the role of the hub autophagy-related genes in glioblastoma, we analyzed the expression changes of these 10 genes through the TCGA dataset of Cbioportal database. The glioblastoma dataset (TCGA, cell 2013) [16] included 543 cases, of which 152 cases had mRNA expression profile data, that could be used to analyze genetic changes and their correlation with pathological features. Changes in the expression of autophagy-related genes we-re found in 40 (26.3%) of the 152 cases (Figure S5). High-frequency genetic changes often suggested that these genes could play an important role in tumorigenesis and the development of gliomas.

A personalized prognostic prediction model

To filter the clinicopathological factors for use in developing the prediction model, univariate

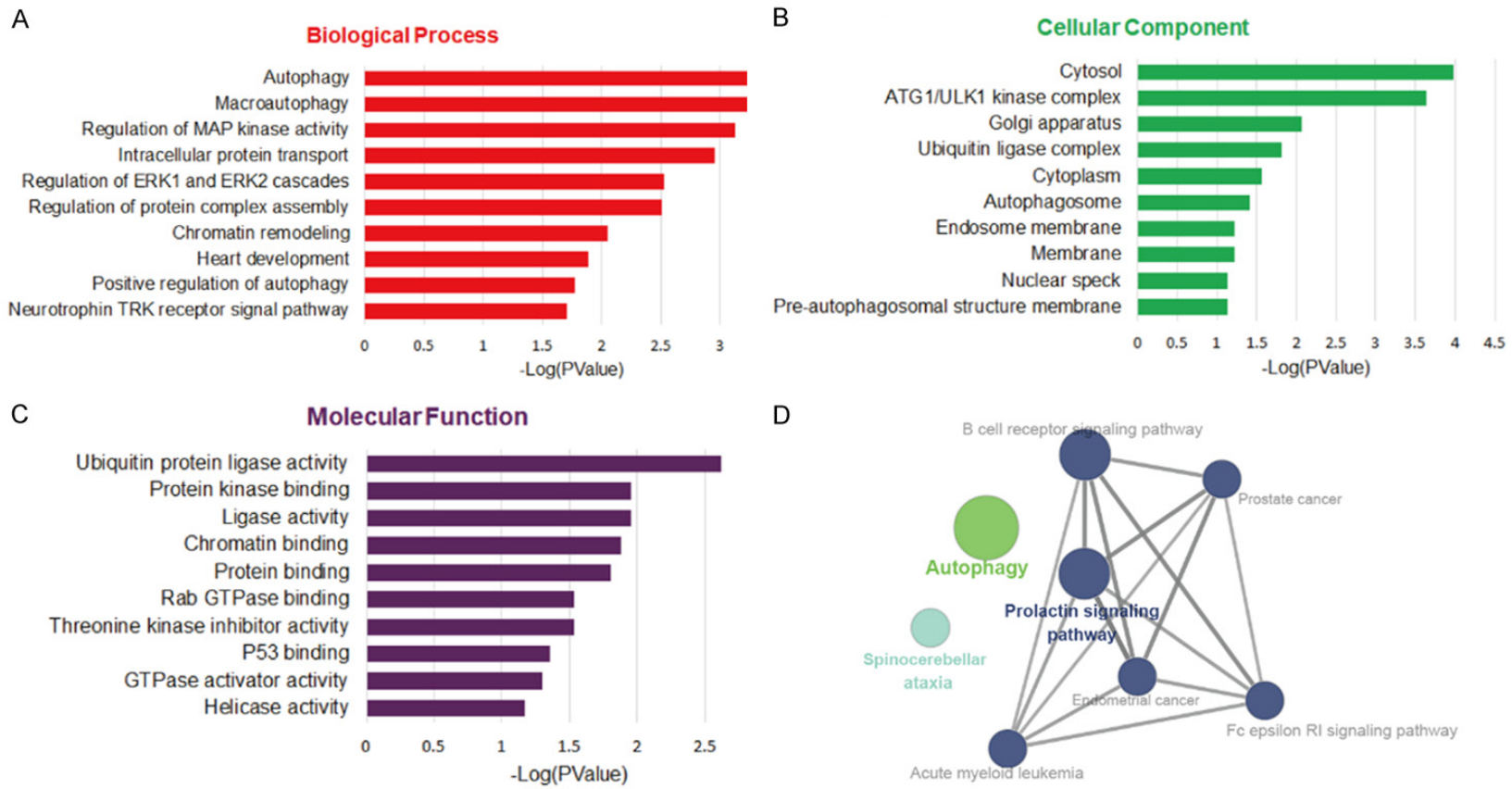

Figure 2. Functional enrichment analysis and significantly enriched terms of 10 prognostic autophagy-related genes in gliomas. A. GO biological process analysis. B. Cellular component analysis. C. Molecular function analysis. D. KEGG pathway analysis of prognostic autophagy-related genes. The size represents the gene number in this KEGG term.

Table 4. Correlations between autophagy risk score (ARS) and clinicopathological features in patients with glioma

Characteristics	Autophagy Risk Score		*P*-value
	Low-risk Score	High-risk Score	
Age, years			
≥42	71	68	0.668
<42	63	67	
Gender			
Male	85	69	0.055
Female	49	66	
WHO grade			
II	68	32	0.000
III	15	38	
IV	51	65	
Histopathology			
O	9	7	0.000
OA	18	16	
A	41	9	
AO	2	11	
AOA	9	17	
AA	4	10	
GBM	51	65	
IDH			
Mutation	62	59	0.672
Wild-type	72	76	
Radiotherapy			
Yes	123	118	0.239
No	11	17	
Chemotherapy			
Yes	65	81	0.059
No	69	54	
Recurrence			
Yes	8	12	0.362
No	126	123	

WHO, World Health Organization; O, oligodendroglioma; OA, oligoastrocytoma; A, astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma; IDH, isocitrate dehydrogenase.

and multivariate Cox regression analyses were performed. Multivariate analysis revealed five independent prognostic risk factors, of which age ($P = 0.010$), ARS ($P = 0.004$), histopathology ($P = 0.012$), and recurrence ($P = 0.004$) predicted a poor prognostic and radiotherapy ($P = 0.003$) predicted a good prognosis (**Table 5**).

Using the five identified prognostic indicators, a prediction model was constructed using R software. As shown in **Figure 5**, the first row includes the score information, totaling 100 points. The second to eighth rows are age, histopathology, recurrence, radiotherapy, and ARS. The scores for individual indicators were obtained by comparing the first row, and the total points were calculated. Then, the 1-, 3-, and 5-year survival rates of the patients were obtained by comparing the survival rates in rows 8-10. A higher point total indicated a worse prognosis.

Evaluation of prediction model by calibration and time-dependent ROC curve analyses

A calibration curve and time-dependent ROC curve were prepared to test the accuracy of the prognostic model. We could see from the calibration curves that the 1-, 3- and 5-year survival curves predicted by the model were very close to the real survival curves, which indicated that the prediction power of the model was high (**Figure 6A-C**).

Ultimately, we further generated the time-dependent ROC curve to evaluate the accuracy of the prediction model. The area under the ROC curve (AUC) values for 1-, 3-, and 5-year survival were 0.790, 0.861, and 0.853, respectively (**Figure 6D**).

Comparison of predictive efficiency among different prognostic models

To further evaluate the superiority of the clinical application value of the nomogram developed in this study, we compared the prediction accuracies among the nomogram, the traditional prediction model (i.e., age, WHO classification and histopathology), a gene-model based on 10 autophagy-related genes, the IDH mutation clinical biomarker, and the WHO grade through ROC curve analysis. The results showed that the accuracy of the nomogram for predicting the prognosis of patients was significantly better than that of the traditional predictive model, IDH mutation, and WHO grade (**Figure 7**).

Discussion

Glioma is a common malignant disease with high morbidity and mortality, and the poor prognosis of glioma patients has always been the most intractable problem faced by clinicians.

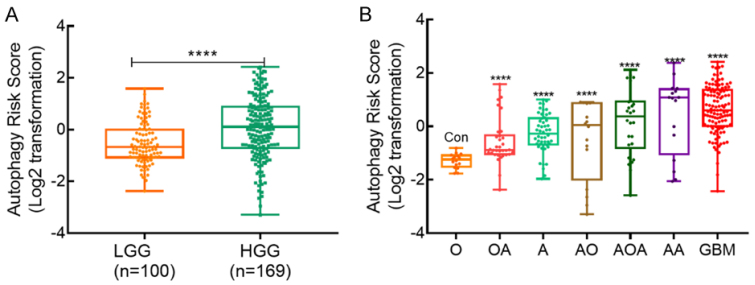

Figure 3. Correlations between autophagy risk score (ARS) and clinicopathological features in gliomas. A and B. Differential distribution of ARS in glioma patients stratified by grade and histopathology, separately. ****P<0.0001.

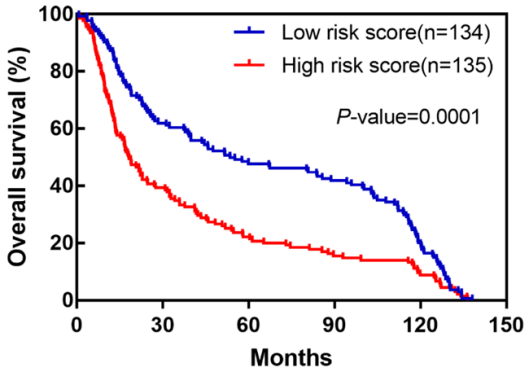

Figure 4. Kaplan-Meier survival analysis of the predictive ability of ARS for OS in glioma. The OS of patients with a low-risk score was significantly longer than that of patients with a high-risk score (P = 0.0001).

Thus, a better understanding of the molecular pathological mechanisms of glioma is urgently needed, along with the identification of potential therapeutic targets and new prognostic biomarkers.

In recent years, autophagy of tumor cells has gradually attracted considerable attention as a potential mechanism that could be employed in novel treatments. So far, it has been accepted that during autophagy, a common biological process in the life activities of eukaryotes, large amounts of cytoplasmic macromolecules and organelles are degraded in membrane vesicles [17]. Overall, it is a complex biological process involving hundreds of molecules, and each step is regulated by different autophagy-related genes [18]. As described, mounting evidence demonstrates that many autophagy-related genes (e.g., ULK1, ATG10, GSK3B, and ATG16L2) are up-regulated in tumor tissues where they act as oncogenes to regulate tumor proliferation, apoptosis, and metastasis [19-21]. For example, Zhu et al. confirmed that autophagy is necessary for the regulation of epithelial-mesenchymal transition (EMT) by hypoxia-inducible factor 1α (HIF-1α) as well as the metastatic ability of prostate cancer stem cells [22]. Likewise, endometrial carcinomas harbor frequent alterations in components of the autophagy pathway, including changes in gene copy number and mutations, in particular in the oncogene PIK3CA, the gene that encodes the PI3K catalytic subunit p110α, and the tumor suppressor PTEN. PIK3CB, which encodes the other ubiquitously expressed class I isoform p110β, is less frequently altered, but the few mutations identified to date have all been shown to be oncogenic [23].

In the present study, we first screened 139 genes significantly associated with autophagy. Of these, univariate following by multivariate Cox analysis identified 10 autophagy-related genes (ULK1, ATG10, RB1CC1, GSK3B, PIK3CB, RUBCNL, PRKN, TBC1D5, and RAB33B) as potentially independent prognostic risk biomarkers for glioma patients. We calculated an ARS for each patient and found that the ARS was significantly correlated with WHO grade and histopathology, making it useful for distinguishing the prognosis of patients in different subgroups.

Accumulating research has continued to suggest that autophagy-related genes can act as oncogenes and result in poor prognosis of tumor patients. In one example, Chen et al. discovered that ULK1 is upregulated in gastric cancer and associated with tumor T stage and recurrence [19]. In 2015, Ruvolo et al. first reported that glycogen synthase kinase (GSK)-3β is regulated by phosphorylation of AKT kinase, and phosphorylated GSK-3β is an important poor prognostic factor in patients with acute myeloid leukemia [24]. Similarly, ATG10 is reported to be involved in the proliferation and migration of lung cancer cells, and its overexpression is a poor prognostic biomarker in patients with lung cancer [25].

Furthermore, in the present study, GO and KEGG analyses further found that the 10 prog-

Table 5. Results of univariate and multivariate Cox analyses identifying clinicopathological factors associated with the prognosis of glioma patients

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age	1.03	1.02-1.04	0.000*	1.02	1.01-1.03	0.010*
Gender	0.75	0.59-0.96	0.022*	0.77	0.60-1.00	0.050
WHO grade	2.03	1.75-2.36	0.000*	1.04	0.62-1.75	0.891
Histopathology	1.35	1.27-1.44	0.000*	1.31	1.06-1.62	0.012*
IDH mutation	0.53	0.42-0.68	0.000*	0.84	0.64-1.11	0.211
Radiotherapy	0.54	0.36-0.80	0.002*	0.53	0.35-0.80	0.003*
Chemotherapy	1.39	1.09-1.78	0.008*	0.80	0.60-1.07	0.131
Recurrence	1.98	1.25-3.14	0.004*	2.11	1.28-3.47	0.004*
ARS	1.22	1.08-1.36	0.001*	1.19	1.06-1.34	0.004*

*significant variables. HR, hazard ratio; CI, confidence interval; WHO, World Health Organization; IDH, isocitrate dehydrogenase; ARS, autophagy risk score.

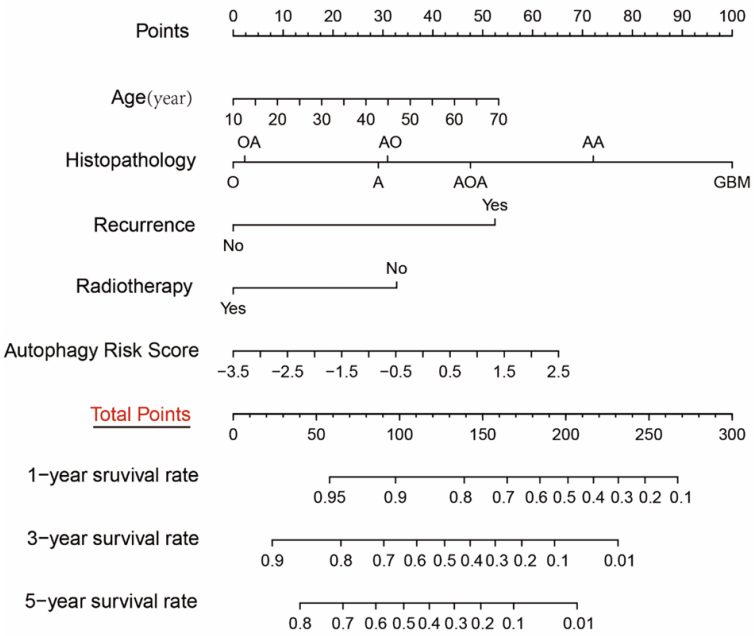

Figure 5. Nomogram for predicting 1-, 3-, and 5-year survival rates in glioma patients.

nostic genes were mainly involved in tumor autophagy, regulation of MAP kinase activity and intracellular protein transport, and Erk tumor signaling. Previous research has confirmed that activated ULK1 forms a complex with p38MAPK, which is found at significantly increased levels under a tumor burden and participates in the activation of autophagy [26]. Jiang et al. provided evidence that autophagy is involved in the MEK1/ERK1/2 signaling pathway [27]. Additionally, several recently identified autophagy substrates reveal novel functions of autophagy early in the metastatic cascade in the direct regulation of the EMT as well as tumor cell migration and invasion [28]. Our results suggest that these autophagy-related oncogenes are involved in the biological processes of tumorigenesis and development, which is consistent with the previously reported results [29].

With the development of genomics technology, greater attention has been given to the molecular markers, such as IDH mutation and 1p/19q codeletion, related to the pathological features of tumors in order to judge the prognosis of patients more accurately. Admittedly, we often also find that the prognosis of some patients with the same pathological diagnosis can vary greatly, and even some glioblastoma patients with long-term survival have a better prognosis than patients with diffuse astrocytoma [30]. Thus, it is evident that the traditional histopathology and existing prognostic markers are still insufficient for judging the prognosis of glioma patients. Accurate prognosis is the key to personalized medicine. Thus, we combined autophagy-related prognostic markers with pathological features to construct a visual prognostic prediction model for glioma patients.

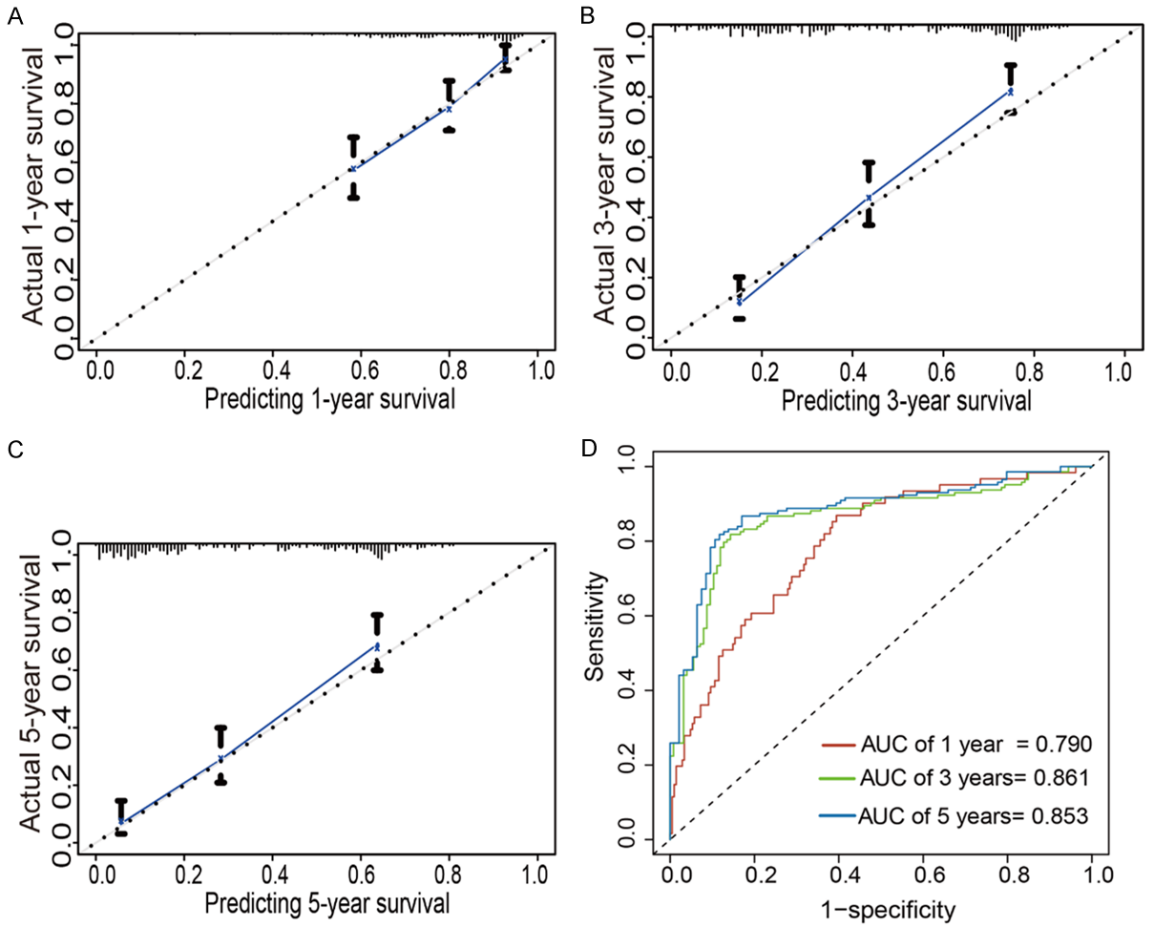

Figure 6. Calibration curves and time-dependent ROC curves for validation of the nomogram. A-C. Calibration curves for predicting OS at 1, 3, and 5 years show the plots for each model in terms of the agreement between predicted (blue line) and observed outcomes (the 45-degree line). D. Time-dependent ROC curves for the ability of the nomogram to predict the 1-, 3-, and 5-year survival rates in glioma patients.

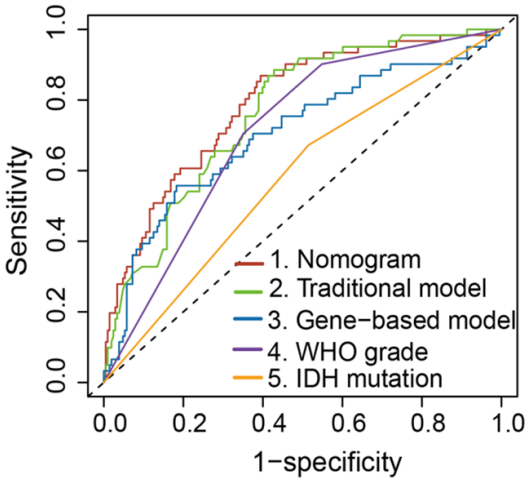

Figure 7. Comparison of the predictive efficiencies of different prognostic models and common markers by ROC curve analysis. We evaluated and compared the predictive performances of different models by calculating the AUC values.

The presented nomogram is a graphical prediction model based on statistical algorithms, which confer points to each variable (i.e., age, histopathology, recurrence, radiotherapy, and ARS). By summarizing all the points, it allows for the survival probability of each patient to be read from the 1-, 3-, and 5-year survival rates. Through a comparison of ROC curves, we found that the developed nomogram is more accurate than the traditional clinical model and autophagy gene model. Thus, we proposed that the ARS derived from autophagy-related gene expression could be incorporated into a predictive nomogram model to better predict clinical outcomes. This model supports the value of integrating gene signature and traditional prognostic factors to more accurately predict the prognosis of glioma patients.

There are some limitations in this study. The nomogram included 10 autophagy-related ge-

nes, and thus, could likely be further optimized and made more suitable for clinical practice. Additionally, future research should seek to elucidate the underlying mechanisms of the genes linked to poor prognosis in glioma patients in vivo and in vitro. The next steps include studying the effects of each gene on the occurrence and development of glioma disease, to determine the potential role of each gene as an oncogene. Such research will provide insight into the underlying mechanisms for the poor prognosis of glioma patients and further provide a theoretical basis for the development of targeted therapies.

In conclusion, we identified 10 autophagy-related genes with prognostic value in glioma cohorts. These genes were independent indicators of prognosis, and a nomogram based on the 10 autophagy-related genes as well as clinicopathological features showed good efficacy for predicting the 1-, 3-, and 5-year survival probabilities for glioma patients. Our prediction model accurately predicted survival outcomes in the included patients, indicating its potential utility in clinical practice.

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Disclosure of conflict of interest

None.

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Table S1. Clinicopathological characteristics of the 269 glioma patients

Characteristics	Number of cases (%)
Age, years	
≥42	139 (51.7)
<42	130 (48.3)
Gender	
Male	154 (57.2)
Female	115 (42.8)
WHO grade	
II	100 (37.2)
III	53 (19.7)
IV	116 (43.1)
Histopathology	
O	16 (5.9)
OA	34 (12.6)
A	50 (18.6)
AO	13 (4.8)
AOA	26 (9.7)
AA	14 (5.2)
GBM	116 (43.1)
IDH	
Mutation	121 (45.0)
Wild-type	148 (55.0)
Radiotherapy	
Yes	241 (89.6)
No	28 (10.4)
Chemotherapy	
Yes	146 (54.3)
No	123 (45.7)
Recurrence	
Yes	20 (7.4)
No	249 (92.6)
ARS	
Low	134 (49.8)
High	135 (50.2)

WHO, World Health Organization; O, oligodendroglioma; OA, oligoastrocytoma; A, astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma; IDH, isocitrate dehydrogenase; ARS, autophagy risk score.

Table S2. Univariate analysis of autophagy-related genes affecting the prognosis of patients with glioma

Characteristics	Hazard Ratio	95% Confidence Interval	*P-Value*
VMA21	1.52	1.19-1.94	0.001
EPG5	0.67	0.5-0.91	0.009
ATG16L1	1.52	1.08-2.13	0.016
ULK1	0.67	0.52-0.87	0.002
ATG13	0.42	0.33-0.53	<0.001
ATG4B	0.57	0.41-0.79	0.001
PIK3C3	0.53	0.41-0.69	<0.001
ATG9A	0.45	0.35-0.59	<0.001
ATG10	0.51	0.34-0.77	0.001
ATG9B	1.39	1.16-1.67	<0.001
MAP1LC3B	0.66	0.51-0.85	0.002
TFEB	0.67	0.53-0.86	0.001
ULK2	0.65	0.52-0.82	<0.001
GABARAPL2	0.55	0.46-0.65	<0.001
PIK3R4	0.54	0.44-0.67	<0.001
DRAM1	1.31	1.13-1.52	<0.001
GABARAPL1	0.7	0.61-0.81	<0.001
ATG16L2	0.57	0.45-0.73	<0.001
ATG2A	0.46	0.37-0.57	<0.001
RB1CC1	0.65	0.53-0.79	<0.001
BCL2	0.79	0.67-0.92	0.003
GABARAP	0.71	0.56-0.91	0.006
DRAM2	1.38	1.1-1.73	0.005
WIPI1	1.49	1.29-1.72	<0.001
MAP1LC3C	1.37	1.24-1.51	<0.001
UVRAG	0.6	0.45-0.8	<0.001
RUBCN	0.39	0.25-0.62	<0.001
PRKAA1	1.59	1.24-2.03	<0.001
NBR1	0.58	0.46-0.74	<0.001
SH3GLB1	1.73	1.5-2	<0.001
RUBCNL	1.12	1.01-1.24	0.038
TP53INP2	0.81	0.72-0.91	0.001
SIRT1	0.6	0.49-0.72	<0.001
TECPR2	0.55	0.43-0.7	<0.001
SOGA1.	1.25	1.01-1.54	0.042
BNIP3	0.71	0.59-0.86	<0.001
FOXO3	0.42	0.32-0.54	<0.001
VMP1	1.53	1.32-1.78	<0.001
DAPK1	0.66	0.57-0.77	<0.001
PRKN	0.46	0.34-0.62	<0.001
WDFY3	0.42	0.33-0.53	<0.001
GSK3B	0.49	0.35-0.69	<0.001
LRRK2	1.2	1.09-1.32	<0.001
TP53INP1	1.36	1.09-1.69	0.006
CCDC115	0.34	0.26-0.45	<0.001
TMEM199	1.67	1.16-2.41	0.006
CISD2	2.03	1.54-2.68	<0.001

NRBF2	0.77	0.61-0.98	0.03
MAPK8	0.53	0.44-0.65	<0.001
CASP3	1.6	1.34-1.92	<0.001
PINK1	0.64	0.55-0.74	<0.001
USP10	0.76	0.59-0.98	0.032
RPTOR	0.75	0.58-0.97	0.028
TBC1D5	0.3	0.23-0.41	<0.001
STK11	0.64	0.43-0.95	0.027
SNCA	0.9	0.84-0.96	0.002
TECPR1	0.67	0.51-0.89	0.005
WDR41	2.35	1.82-3.03	<0.001
STX17	1.5	1.03-2.18	0.033
FIG4	0.68	0.55-0.84	<0.001
VAC14	0.57	0.42-0.77	<0.001
DEPP1	1.33	1.21-1.47	<0.001
SIRT2	0.72	0.59-0.88	0.001
DAP	1.41	1.12-1.77	0.003
PIK3CB	0.7	0.6-0.82	<0.001
DAPK3	1.27	1.01-1.59	0.038
ULK3	0.54	0.4-0.71	<0.001
TMEM59	1.6	1.22-2.11	0.001
STING1	1.37	1.17-1.59	<0.001
CTSD	1.22	1.01-1.47	0.037
NOD2	1.15	1.02-1.3	0.019
MCL1	1.37	1.17-1.61	<0.001
DAPK2	0.8	0.68-0.95	0.009
KRAS	0.64	0.52-0.8	<0.001
PTEN	0.69	0.51-0.93	0.016
UBQLN1	1.75	1.3-2.37	<0.001
RAB33B	1.4	1.19-1.64	<0.001
IFNG	1.3	1.12-1.51	0.001
TRIM5	1.45	1.21-1.74	<0.001
TRAF6	1.96	1.4-2.74	<0.001
STAT3	1.33	1.06-1.67	0.013
CTSB	1.36	1.17-1.57	<0.001
TSC2	0.44	0.35-0.55	<0.001
HSPA8	0.75	0.58-0.97	0.026
RPS6KB1	1.5	1.13-2	0.005
SBF2	0.75	0.63-0.88	0.001

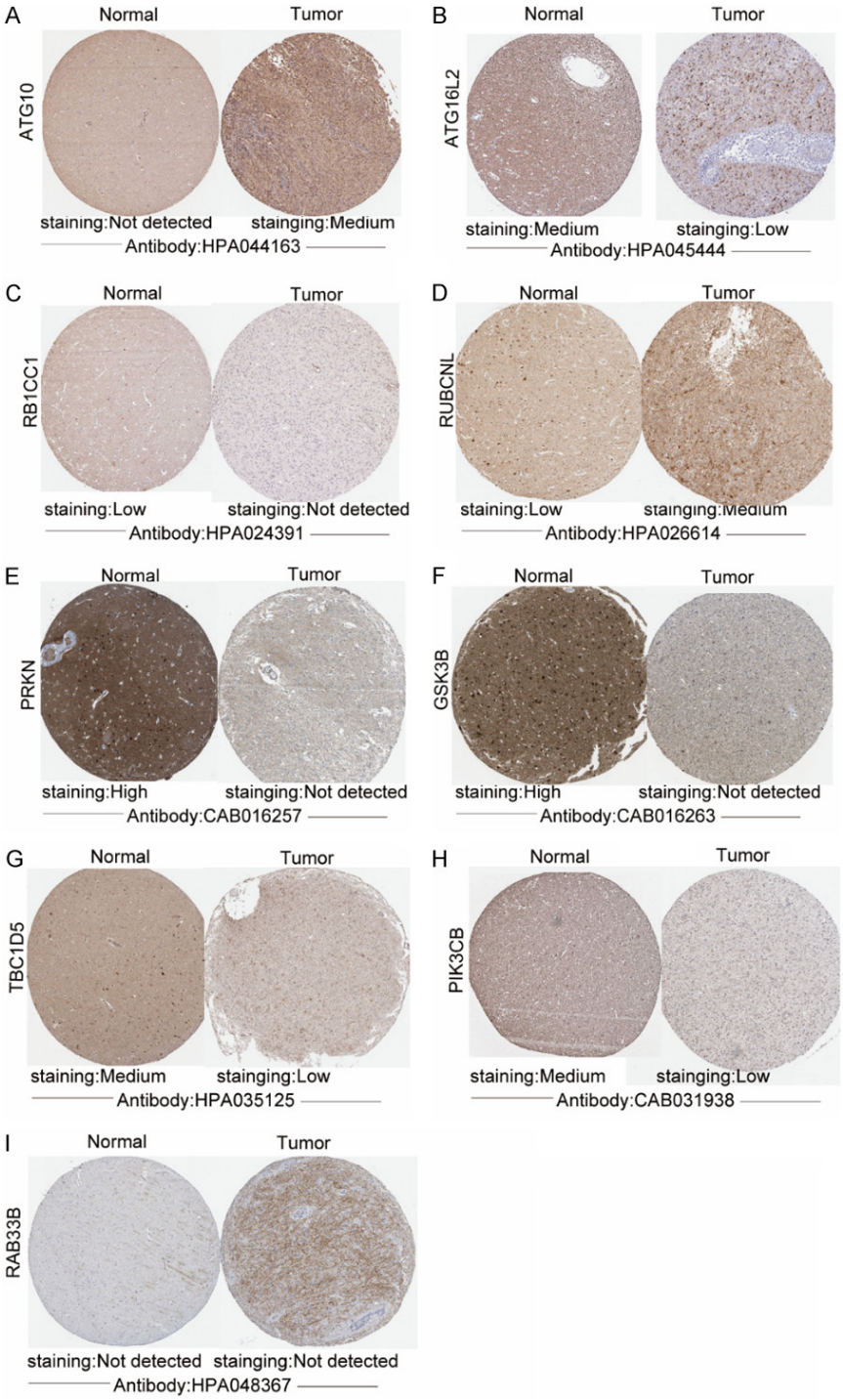

Figure S1. Protein expression of autophagy-related genes in glioma and normal brain tissues from the HPA database.

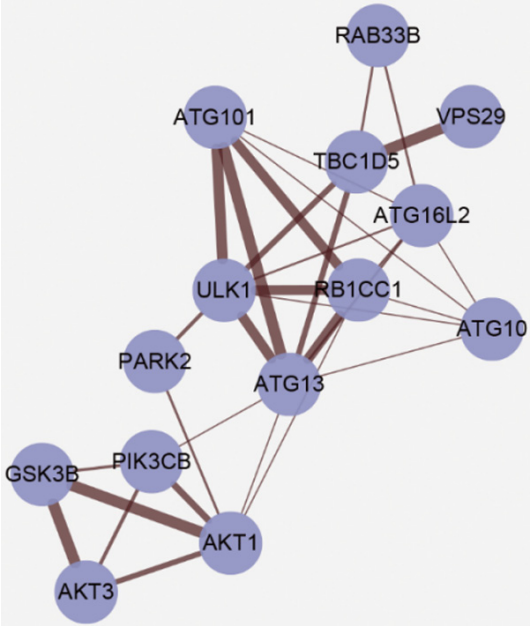

Figure S2. Protein-protein interaction (PPI) network analysis of proteins encoded by prognostic autophagy-related genes. The nodes represent the proteins encoded by autophagy-related genes, and the thickness of the line between any two nodes represents the strength of the connection.

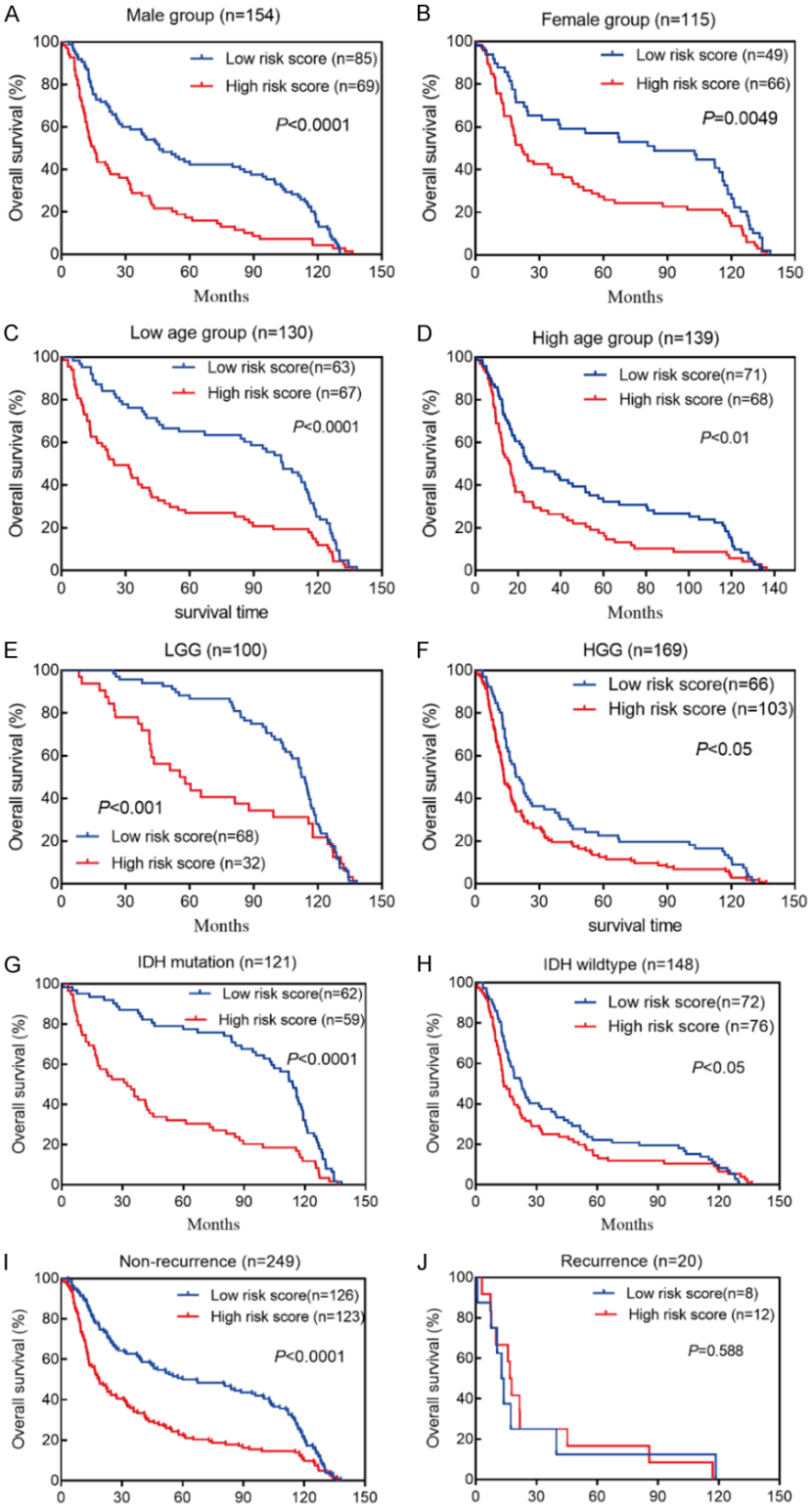

Figure S3. Kaplan-Meier survival curves from subgroup analyses of different clinical factors. A and B. Male subgroup (HR=0.58, 95% CI=0.41-0.81, *P*<0.0001) and female subgroup (HR=0.62, 95% CI=0.43-0.90, *P*=0.0049). C and D. Low age subgroup (HR=0.56, 95% CI=0.40-0.80, *P*<0.0001) and high age subgroup (HR=0.70, 95% CI=0.50-0.99, *P*<0.01). E and F. Low-grade glioma subgroup (HR=0.50, 95% CI=0.32-0.76, *P*<0.001) and high-grade glioma subgroup (HR=0.71, 95% CI=0.52-0.96, *P*<0.05). G and H. IDH mutation subgroup (HR=0.48, 95% CI=0.32-0.70, *P*<0.0001) and IDH wild-type subgroup (HR=0.61, 95% CI=0.44-0.84, *P*<0.05). I and J. Non-recurrence subgroup (HR=0.62, 95% CI=0.48-0.80, *P*<0.0001) and recurrence subgroup (HR=1.06, 95% CI=0.41-2.74, *P*=0.59).

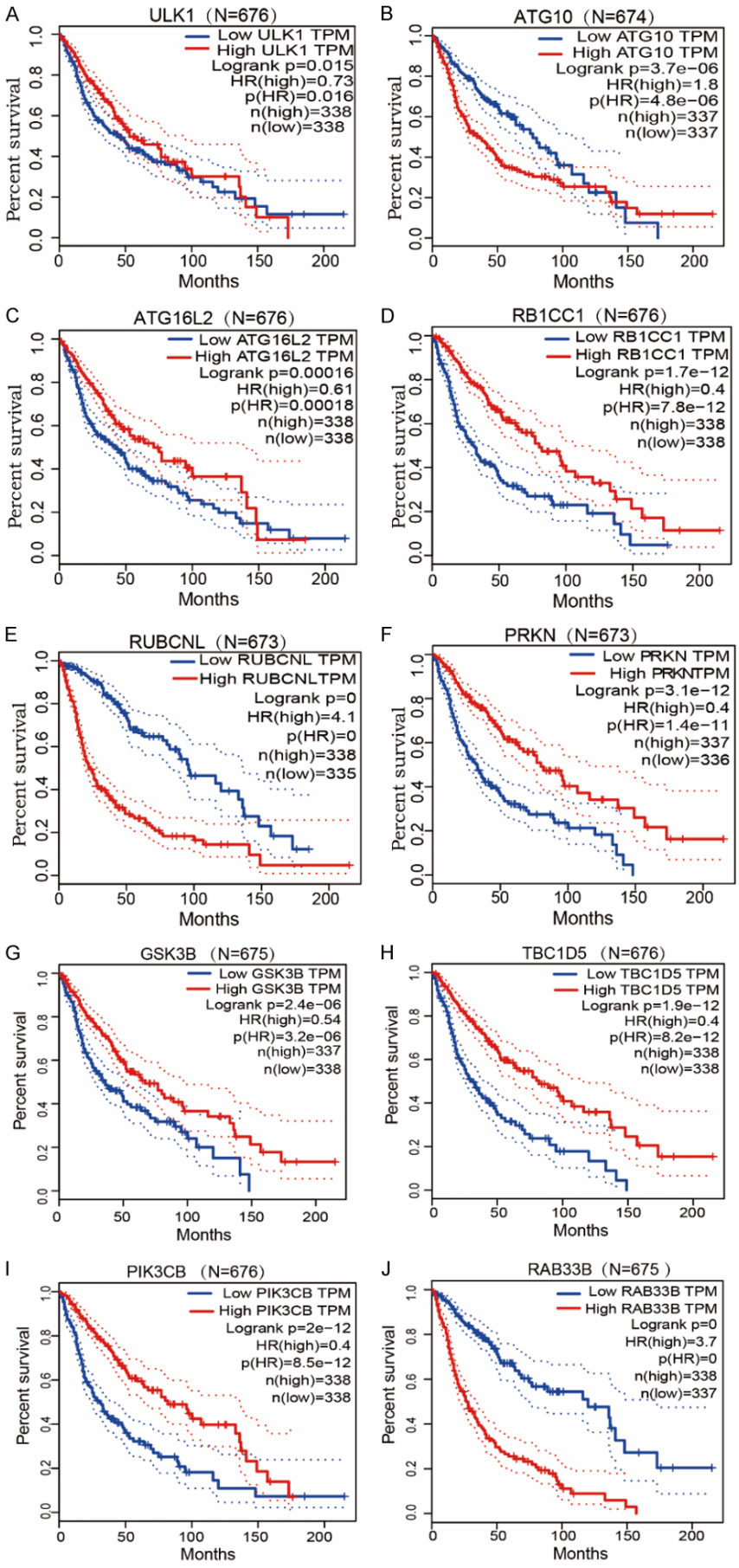
A
ULK1 (N=676)
Low ULK1 TPM
High ULK1 TPM
Logrank p=0.015
HR(high)=0.73
p(HR)=0.016
n(high)=338
n(low)=338
B
ATG10 (N=674)
Low ATG10 TPM
High ATG10 TPM
Logrank p=3.7e−06
HR(high)=1.8
p(HR)=4.8e−06
n(high)=337
n(low)=337
C
ATG16L2 (N=676)
Low ATG16L2 TPM
High ATG16L2 TPM
Logrank p=0.00016
HR(high)=0.61
p(HR)=0.00018
n(high)=338
n(low)=338
D
RB1CC1 (N=676)
Low RB1CC1 TPM
High RB1CC1 TPM
Logrank p=1.7e−12
HR(high)=0.4
p(HR)=7.8e−12
n(high)=338
n(low)=338
E
RUBCNL (N=673)
Low RUBCNL TPM
High RUBCNLTPM
Logrank p=0
HR(high)=4.1
p(HR)=0
n(high)=338
n(low)=335
F
PRKN (N=673)
Low PRKN TPM
High PRKNTPM
Logrank p=3.1e−12
HR(high)=0.4
p(HR)=1.4e−11
n(high)=337
n(low)=336
G
GSK3B (N=675)
Low GSK3B TPM
High GSK3B TPM
Logrank p=2.4e−06
HR(high)=0.54
p(HR)=3.2e−06
n(high)=337
n(low)=338
H
TBC1D5 (N=676)
Low TBC1D5 TPM
High TBC1D5 TPM
Logrank p=1.9e−12
HR(high)=0.4
p(HR)=8.2e−12
n(high)=338
n(low)=338
I
PIK3CB (N=676)
Low PIK3CB TPM
High PIK3CB TPM
Logrank p=2e−12
HR(high)=0.4
p(HR)=8.5e−12
n(high)=338
n(low)=338
J
RAB33B (N=675)
Low RAB33B TPM
High RAB33B TPM
Logrank p=0
HR(high)=3.7
p(HR)=0
n(high)=338
n(low)=337
Percent survival
Months

Figure S4. Kaplan-Meier survival curves for 10 hub autophagy-related genes associated with the prognosis of glioma patients in the GEPIA database. Red plot shows overexpression, while the blue plot shows low expression.

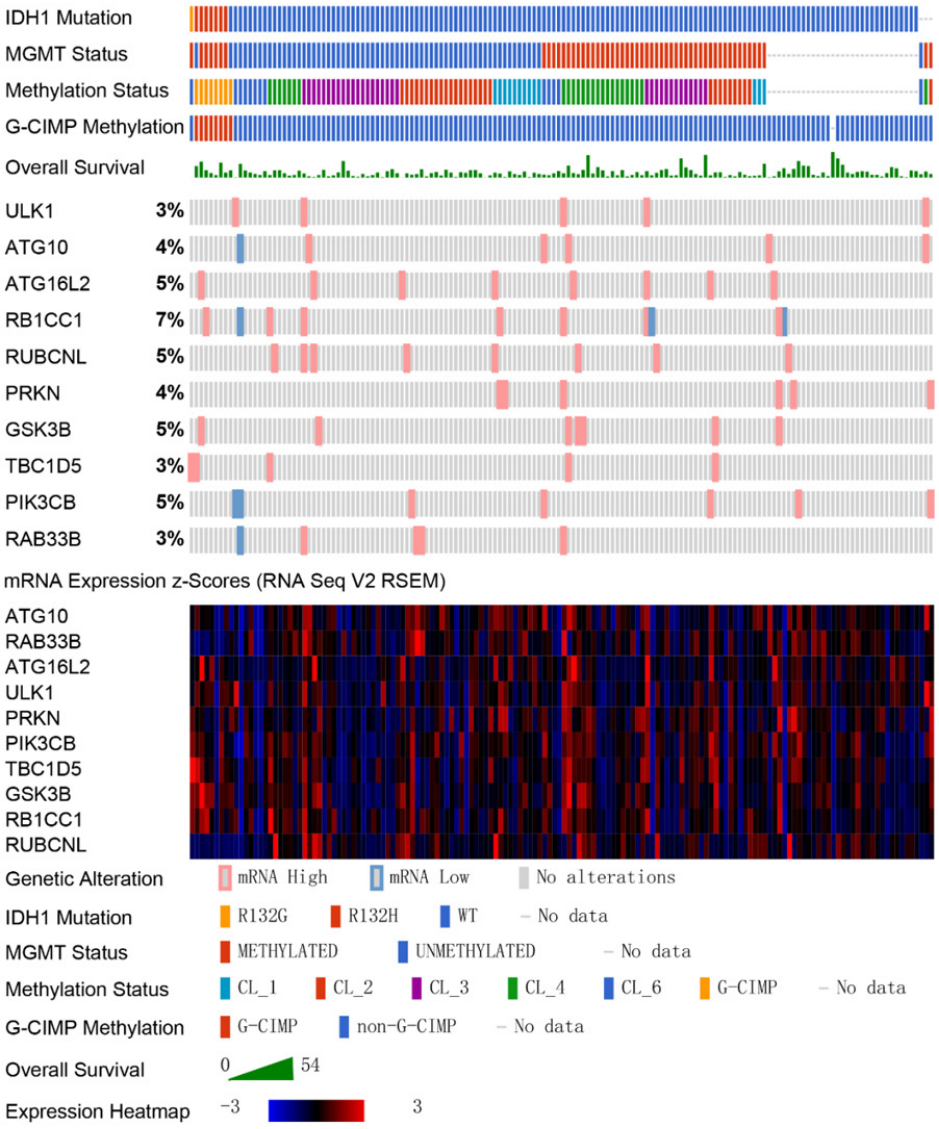

Figure S5. CBioPortal Oncoprint of 10 prognostic autophagy-related genes in samples from The Cancer Genome Atlas (TCGA) Glioblastoma Project. Genes are listed on the left. Percentage of gene alterations noted in the 152 samples of glioblastoma included in TCGA provisional sample are also seen on the left. The expression heatmap of the 10 prognostic autophagy-related genes is shown at the bottom of the chart.