

Construction and validation of prognostic model based on autophagy-related lncRNAs in gastric cancer

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Abstract: Gastric cancer (GC) is one of the most common cancer worldwide. Although emerging evidence indicates that autophagy-related long non-coding RNA (lncRNA) plays an important role in the progression of GC, the prognosis of GC based on autophagy is still deficient. The Cancer Genome of Atlas stomach adenocarcinoma (TCGA-STAD) dataset was downloaded and separated into a training set and a testing set randomly. Then, 24 autophagy-related lncRNAs were found strongly associated with the survival of the TCGA-STAD dataset. 11 lncRNAs were selected to build the risk score model through the least absolute shrinkage and selection operator (LASSO) regression. Every patient got a risk score (RS), and patients were separated into a high-risk group and a low-risk group due to the median RS. The multivariate Cox analysis showed that the RS could be an independent prognosis predictor. The Kaplan-Meier survival analysis and the Receiver Operating Characteristic (ROC) curve indicated the model had an excellent prediction effect. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that the mRNAs in the prognostic network were mainly involved in the autophagy and ubiquitin-like protein ligase binding. Gene Set Enrichment Analysis (GSEA) analysis uncovered that the differentially expressed genes (DEGs) in the high-risk group partially participated in the ECM receptor interaction and other signaling pathways. Our results indicated that the risk score model based on the autophagy-related lncRNAs performed well in the prediction of prognosis for patients with GC.

Introduction

Gastric cancer (GC) is the fifth most prevalent tumor and one of the deadliest carcinomas worldwide (Bray *et al.*, 2018), among which 90% are adenocarcinomas (Crew and Neugut, 2006). Although there has been a stable decrease in GC occurrence and mortality rates globally over several decades after the beginning of the 19th century (Bosetti *et al.*, 2013; Kamangar *et al.*, 2006), GC remains a high case fatality rate of 75% throughout the world (Fock, 2014). Due to the insufficient understanding of the molecular mechanism (Rocken, 2017) and the lack of relevant clinical prediction systems (Jemal *et al.*, 2011), most patients with GC have already been in the advanced stage when they are diagnosed (Schmidt *et al.*, 2005), which brings great trouble to the patient's survival (Verdecchia *et al.*, 2007) and clinical therapy. Therefore, it is critical to construct an accurate prediction system for GC, which has the ability for early

diagnosis and to prevent the disease before premalignant lesions have developed.

Autophagy is a highly conserved and evolutionarily ancient catabolic process that can degrade the misfolded proteins and damaged organelles (Mizushima, 2007). For the past few years, knowledge about the function of autophagy has developed. It has been found that autophagy participated in plenty of physiological processes in mammals, including quality control of proteins and organelles, immunity, nutrient deprivation, hypoxia, drug stimuli, stress, and prevention of neurodegeneration (Mizushima and Komatsu, 2011). Autophagy can also regulate biological process including apoptosis, protein synthesis, cell growth, and proliferation through the AMPK/mTOR pathway, PI3K/Akt/mTOR pathway, P53 pathway, and other signaling pathways (Chang *et al.*, 2017; Ren *et al.*, 2016; Zhao *et al.*, 2015). Furthermore, the role of autophagy in the progression of carcinoma also has several breakthroughs.

The effect of autophagy is considered controversial in tumorigenesis, which can both promote and suppress cancer development under different cell types or stress modes (Glick *et al.*, 2010). It is thought that autophagy prevented

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carcinogenesis (Aita *et al.*, 1999; Liang *et al.*, 1999). Nevertheless, once the tumor is established, increased autophagic flux often promotes tumor cell growth by providing energy and vital compounds upon various stress stimuli (White, 2012). Similarly, growing studies have also proved that autophagy had a significant effect on the GC.

The role of autophagy in GC is also complex and contradictory. Some research supported that autophagy was a tumor promoter in GC. Cause autophagy inhibitor will destroy the protective mechanism of GC cell and promotes cell death induced by therapeutic drugs (Li *et al.*, 2017b; Maes *et al.*, 2014; Maes *et al.*, 2013). However, PD-L1 expression was also found enhanced by autophagy inhibition in GC (Wang *et al.*, 2019b). Moreover, a study has shown that autophagy inducers such as AMPK inducers could be used in the GC treatment, which will lead to autophagic cell death of tumor cells (Shen *et al.*, 2014).

In recent years, the relationship between long non-coding RNA (lncRNA) and autophagy has several breaks in the diagnosis, treatment, and prognosis of GC. Pieces of evidence showed that lncRNA is vital for the occurrence, prognosis, and chemoresistance of GC by regulating autophagy-related mRNA (Hu *et al.*, 2017; Zhao *et al.*, 2014; Zhu *et al.*, 2019). Some studies have demonstrated that silencing of LINC01419 and CCAT2 promotes autophagy through constraining the PI3K/Akt1/mTOR pathway, thus inhibiting the invasion and migration of GC cells (Li *et al.*, 2019; Yu *et al.*, 2018). Another study also revealed that autophagy was associated with the proliferation of GC cells, partially due to the MALAT1 promoted by downregulating miR-204 (Shao *et al.*, 2020). However, most of these experiments only explored the role of one or a few lncRNAs in the autophagy of GC and could not fully explain the relevant mechanisms.

To be concluded, the role of autophagy-related lncRNA in GC is complicated and controversial, which involves hundreds of molecules in this process. Therefore, a model consisted of multiple autophagy-related lncRNAs will have a better prognosis predicting accuracy than the single. For this purpose, we built a risk score model based on the multiple autophagy-related lncRNAs, which also performed well in the prediction of prognosis for the GC patients in the clinic.

Materials and Methods

Screening for the autophagy-associated lncRNAs

Autophagy-associated genes were obtained through the Human Autophagy Database (HADb, <http://www.autophagy.lu/>). Pearson correlation coefficient was calculated using the “limma” package from R (<http://www.bioconductor.org>) to determine the correlation between the expression of the lncRNAs and the corresponding autophagy-related genes. Then, lncRNAs with Pearson correlation coefficient > 0.3 and $p < 0.001$ were filtered out to be the autophagy-related lncRNAs.

Data acquisition

All 350 patients' data of STAD were downloaded from The Cancer Genome Atlas (TCGA) website (<https://portal.gdc.cancer.gov/>). All the data was submitted to R software and randomly separated into the training set and testing set.

Identification of the prognostic autophagy-related lncRNAs

Kaplan-Meier and univariate Cox regression analysis was applied to select the prognostic autophagy-related lncRNA in the TCGA-STAD dataset ($P < 0.05$).

Survival analysis

Least absolute shrinkage and selection operator (LASSO) regression was adopted to build the model using the R package “glmnet” with prognostic autophagy-related lncRNAs selected before. In this process, only the most potent prognostic markers were chosen and constituted the optimal panel of prognosis that achieved the best performance. Each patient got a risk score (RS) incorporating the expression of the lncRNAs (Exp_i) and the corresponding LASSO coefficients ($Coef_i$), Risk Score = $\sum_{i=1}^n Exp_i * Coef_i$. The median RS was chosen to divide patients into a high-risk group and a low-risk group in the training set, testing set and TCGA-STAD dataset, respectively. Multivariate Cox regression was used to assess whether the RS could be an independent predictor. Kaplan-Meier curves were used to check the significant difference in survival probability for patients between two types of risk groups. An area under the Receiver Operating Characteristic (ROC) curve (AUC) was used to examine the accuracy. All statistics were processed through R version 3.6.3 software (<https://www.r-project.org/>) ($p < 0.05$).

Network construction and pathway analysis

The mRNA expression data of these GC patients were downloaded from the TCGA database. The mRNAs co-expressed with the lncRNAs in the risk score model were selected from the mRNA expression data by using the correlation test through the “limma” package (<http://www.bioconductor.org>) with R software (Pearson correlation coefficient > 0.3 , $p < 0.001$). The network was constructed and visualized through Cytoscape software (<https://cytoscape.org/>). The Sankey diagram was built using the R software. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed using the “clusterProfiler” package of R software (Gene ratio > 0.05 , $p < 0.05$). The signaling pathways that the differentially expressed genes (DEGs) mainly participated in between two types of risk groups were distinguished through Gene Set Enrichment Analysis (GSEA).

Development of nomogram

A nomogram was constructed through the “rms” package for R (<https://cran.r-project.org/web/packages/rms/>), combining the risk type and other clinical features including age, gender, stage, and TNM stage to predict the individual's survival probability.

Results

Characteristics of patients

TCGA-STAD dataset was patients diagnosed with stomach adenocarcinoma, which consisted of a total of 350 patients. Kaplan-Meier survival curves were plotted for the TCGA-STAD dataset regarding major clinical features (Suppl. Fig. S1).

Selecting of prognostic autophagy-related LncRNA for TCGA-STAD dataset

The autophagy-related lncRNAs were obtained by using the correlation test of autophagy-related genes (Pearson correlation coefficient > 0.3 , $p < 0.001$). A total of 24 autophagy-related lncRNAs were significantly correlated with the survival for TCGA-STAD through Kaplan-Meier and univariate Cox regression analysis ($p < 0.05$) (Fig. 1).

Construction of prognostic risk score model based on autophagy-related lncRNAs

All the patients from TCGA-STAD cohort were randomly separated into a training set and testing set ($p > 0.05$) (Tab. 1). Then all these 24 identified prognostic lncRNAs were analyzed with the LASSO regression analysis (Fig. 2A) and 3-fold positive cross-validation (Fig. 2B) in the training set. The regression coefficient was computed to select the optimal panel of prognosis. The model achieved the best performance while 11 autophagy-related lncRNAs were included. Combining the expression of the autophagy-related lncRNAs and LASSO coefficients (Tab. 2), each patient got an RS as a measure of survival risk.

Validation of the risk score model for survival prediction in the training set, testing set and TCGA-STAD dataset

Based on the median RS, patients were divided into a high-risk group and a low-risk group. lncRNA AC005586.1, AL353804.1, IPO5P1, AP003392.1, AL355574.1 and AC092574.1 were

considered as protective lncRNA ($HR < 1$, $B < 0$, LASSO coefficient < 0) while LINC01705, AP001528.2, AC009948.1, HAGLR and AP001033.2 were risk lncRNA ($HR > 1$, $B > 0$, LASSO coefficient > 0). The heatmap showed the lncRNA expression levels in the high-risk group and low-risk group in the training set (Fig. 3A), testing set (Fig. 3B), and TCGA-STAD dataset (Fig. 3C). As illustrated, the low-risk group tended to express protective lncRNA while the high-risk group was more likely to express risk lncRNA.

The correlation between RS and survival time and status in STAD patients was also plotted in the training set (Fig. 3D), testing set (Fig. 3E), and TCGA-STAD dataset (Fig. 3F). In all these datasets, patients with higher RS tended to face a shorter survival time and worse survival status.

Multivariate Cox regression analysis was used to evaluate the independent prognostic indicators. The results of the multivariable analysis showed the RS was a robust and independent prognostic indicator in the training set (Fig. 4A), testing set (Fig. 4B), and TCGA-STAD dataset (Fig. 4C) ($p < 0.05$). Kaplan-Meier analysis was also conducted on the training set (Fig. 4D), testing set (Fig. 4E), and TCGA-STAD dataset (Fig. 4F). Distinctly, there is a strong and significant difference between the two types of risk groups ($p < 0.0005$). Furthermore, a ROC curve was adopted to appraise the accuracy of the model in the training set (Fig. 4G), testing set (Fig. 4H), and TCGA-STAD dataset (Fig. 4I). The area under the curve (AUC) was applied to evaluate the accuracy of the model's

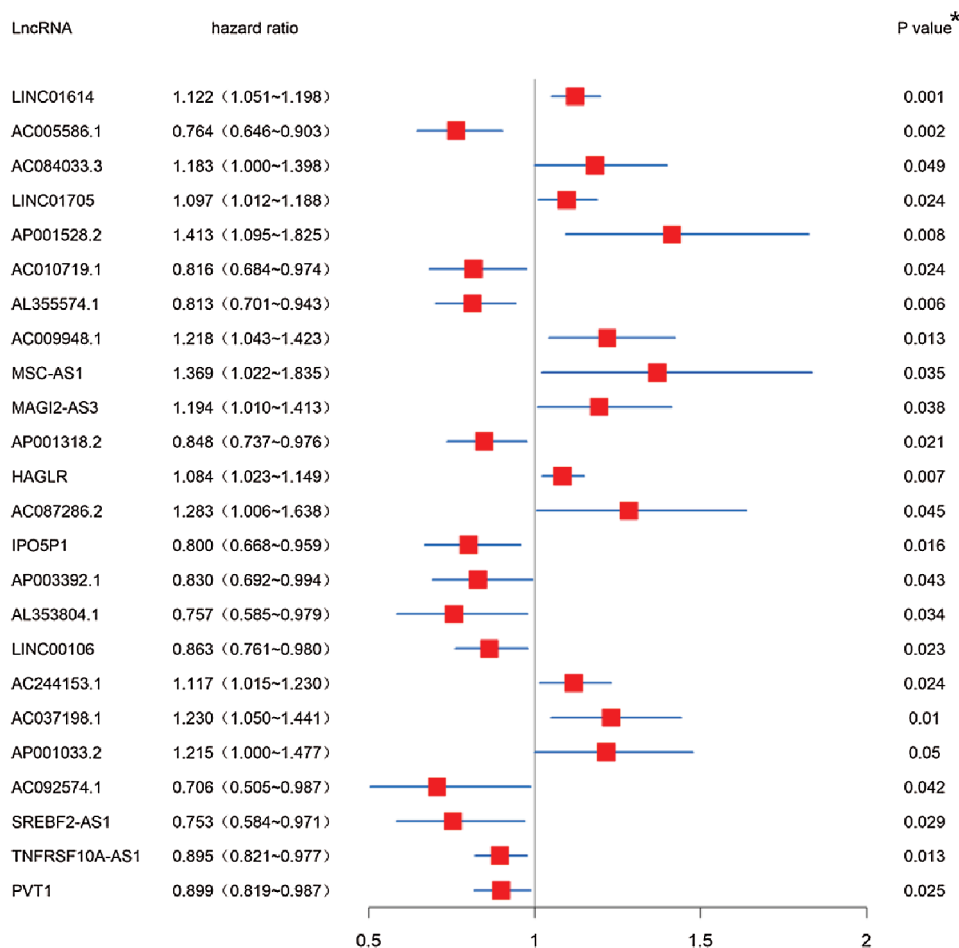


FIGURE 1. Screening of autophagy-related lncRNAs linked with the survival in GC. Forest plot of prognostic autophagy-related lncRNAs in TCGA-STAD dataset based on Kaplan-Meier and univariate Cox regression analysis. * $p < 0.05$.

TABLE 1

Major clinical characteristics for the STAD patients in the training set, testing set and TCGA-STAD dataset

Characteristics	TCGA-STAD cohort		<i>p</i> -value
	Training set N = 175(%)	Testing set N = 175(%)	
Age			
30–50	19(10.86%)	10(5.71%)	0.1918
50–70	104(59.43%)	96(54.86%)	
70–90	52(29.71%)	69(39.43%)	
Gender			
female	62(35.43%)	62(35.43%)	0.3265
male	113(64.57%)	113(64.57%)	
T stage			
T1–T2	37(21.14%)	53(30.29%)	0.909
T3–T4	138(78.86%)	122(69.71%)	
N stage			
N1	49(28.00%)	54(30.86%)	0.6206
N2	44(25.14%)	49(28.00%)	
N3	35(20.00%)	37(21.14%)	
N4	47(26.86%)	35(20.00%)	
M stage			
M0	156(89.14%)	154(88.00%)	0.2374
M1	19(10.86%)	21(12.00%)	
Clinical Stage			
Stage I	21(12.00%)	26(14.86%)	0.6475
Stage II	50(28.57%)	61(34.86%)	
Stage III	83(47.43%)	63(36.00%)	
Stage IV	21(12.00%)	25(14.29%)	
Pathological Grade			
Grade 1	4(2.29%)	5(2.86%)	0.3813
Grade 2	54(30.86%)	71(40.57%)	
Grade 3	117(66.86%)	99(56.57%)	

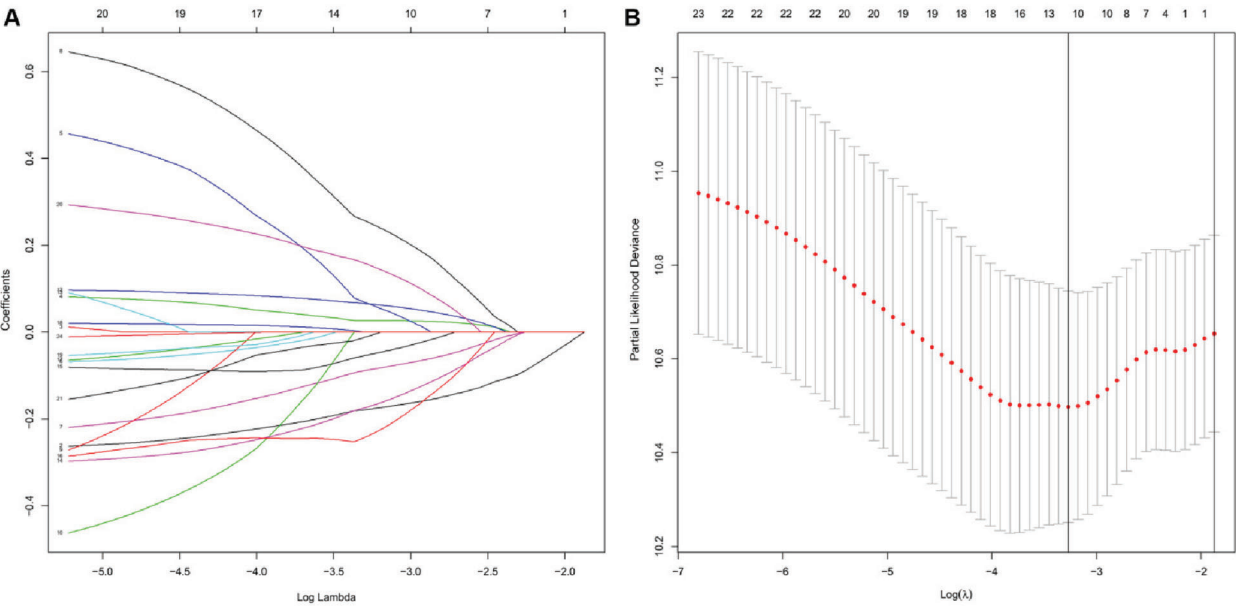


FIGURE 2. Construction of prognostic risk score model based on LASSO regression analysis. (A) Coefficient profile of each 24 prognostic autophagy-related lncRNA in the training set. (B) Selection of the optimal panel (lambda) for the model in the training set.

TABLE 2

The lncRNAs in the risk score model were strongly associated with the survival in TCGA-STAD dataset

lncRNA	Kaplan-Meier analysis			Univariate Cox regression analysis				LASSO coefficient
	B	SE	p-value*	HR	HR.95L	HR.95H	p-value	
AC092574.1	-0.348	0.171	0.006	0.706	0.505	0.987	0.042	-0.009
AL353804.1	-0.279	0.131	0.002	0.757	0.585	0.979	0.034	-0.238
AC005586.1	-0.270	0.085	0.000	0.764	0.646	0.903	0.002	-0.178
IPO5P1	-0.223	0.092	0.022	0.800	0.668	0.959	0.016	-0.171
AL355574.1	-0.207	0.076	0.033	0.813	0.701	0.943	0.006	-0.087
AP003392.1	-0.187	0.092	0.017	0.830	0.692	0.994	0.043	-0.052
HAGLR	0.081	0.030	0.001	1.084	1.023	1.149	0.007	0.065
LINC01705	0.092	0.041	0.039	1.097	1.012	1.188	0.024	0.027
AP001033.2	0.195	0.099	0.049	1.215	1.000	1.477	0.050	0.154
AC009948.1	0.197	0.079	0.041	1.218	1.043	1.423	0.013	0.252
AP001528.2	0.346	0.130	0.026	1.413	1.095	1.825	0.008	0.065

prediction in 1-year, 3-year, and 5-year. The value of AUC prompts the model to predict well in prognostic prediction.

Construction of the prognostic mRNA-lncRNA network and identification of involved signaling pathways

The mRNAs co-expressed with the lncRNAs in the model were obtained by using the correlation test with R (Suppl. Tab. S1). Then all these mRNAs and lncRNAs were used to construct the prognostic mRNA-lncRNA interaction network (Figs. 5A–5B). The most significant GO terms for biological process (BP) (Fig. 5C), cellular component (CC) (Fig. 5D), and molecular function (MF) (Fig. 5E), as well as KEGG pathways (Fig. 5F), were analyzed to reveal potential biological functions of the mRNAs in the network (Gene ratio >0.05, $p < 0.05$). The results showed that the mRNAs in the network were mainly involved in autophagy both in GO and KEGG analysis. GSEA analysis uncovered that the DEGs were significantly enriched in several common signaling pathways (Tab. 3).

Nomograms for personalized prognostic prediction in STAD patients

A nomogram incorporating the risk type and clinical characters was built to estimate individual survival probability quantitatively of STAD patients. The concordance index (C-index) of the nomogram was 0.682. The plot showed the 1-year, 3-year, and 5-year survival probabilities in the TCGA-STAD dataset (Fig. 6).

Discussion

Autophagy is a self-degradative process that plays an essential role in equilibrating energy, eliminating misfolded or aggregated proteins, and reacting to stimuli (Ravanan *et al.*, 2017). To date, three types of autophagy have been found: macroautophagy, microautophagy, and chaperone-mediated autophagy (Mizushima and Komatsu, 2011). Macroautophagy is considered the main form of autophagy, which is extensively studied compared to the other two

types (Munz, 2017; Nishida *et al.*, 2016; Wen and Klionsky, 2016). The process of autophagy is also classified into four critical steps: initiation, nucleation, maturation, and degradation (Feng *et al.*, 2014). In the past decades, researchers have discovered 32 autophagy-related genes (Atgs) in yeast, most of which are also highly conserved in mammals significantly (Nakatogawa *et al.*, 2009).

In recent years, some studies have reported that autophagy has a strong relationship between the prognosis and survival in GC. Qu *et al.* (2017) found that Beclin1 (protein homolog of the yeast ATG6) was much overexpressed in malignant tissues than the nonmalignant tissues in GC. Moreover, they also found that overexpression of Beclin1 was related to a poor prognosis for GC. Liao *et al.* (2014) discovered that the “stone-like” structure pattern of LC3A (an autophagosomal biomarker) was correlated with increased recurrence and worse survival possibilities in gastric carcinoma.

As recognized previously, lncRNAs such as H19 and HOTAIR played a key role as primary regulators in the carcinogenesis of GC (Cheng *et al.*, 2018; Endo *et al.*, 2013; Okugawa *et al.*, 2014). Until now, numerous studies have proved that lncRNAs were highly active in plenty of pathological processes of GC, such as proliferation and metastasis. Among them, some lncRNAs were defined as protective factors while others were risk factors (Fanelli *et al.*, 2018; Guo *et al.*, 2014; Li *et al.*, 2017a; Ma *et al.*, 2016; Zhu *et al.*, 2019). Furthermore, several studies have proved that lncRNAs participated in the progression, especially malignant progression of GC through regulating autophagy-related mRNAs (Chen *et al.*, 2018; Shao *et al.*, 2020; Wang *et al.*, 2019a; Hu *et al.*, 2017; Yu *et al.*, 2018; Zhao *et al.*, 2014; Zhu *et al.*, 2019).

Although numerous studies have been performed and much is known about lncRNAs and autophagy in GC, previous studies mainly focused on the single lncRNA. The prognostic system that relied on the multiple autophagy-related lncRNAs is still not clear. More importantly, as one

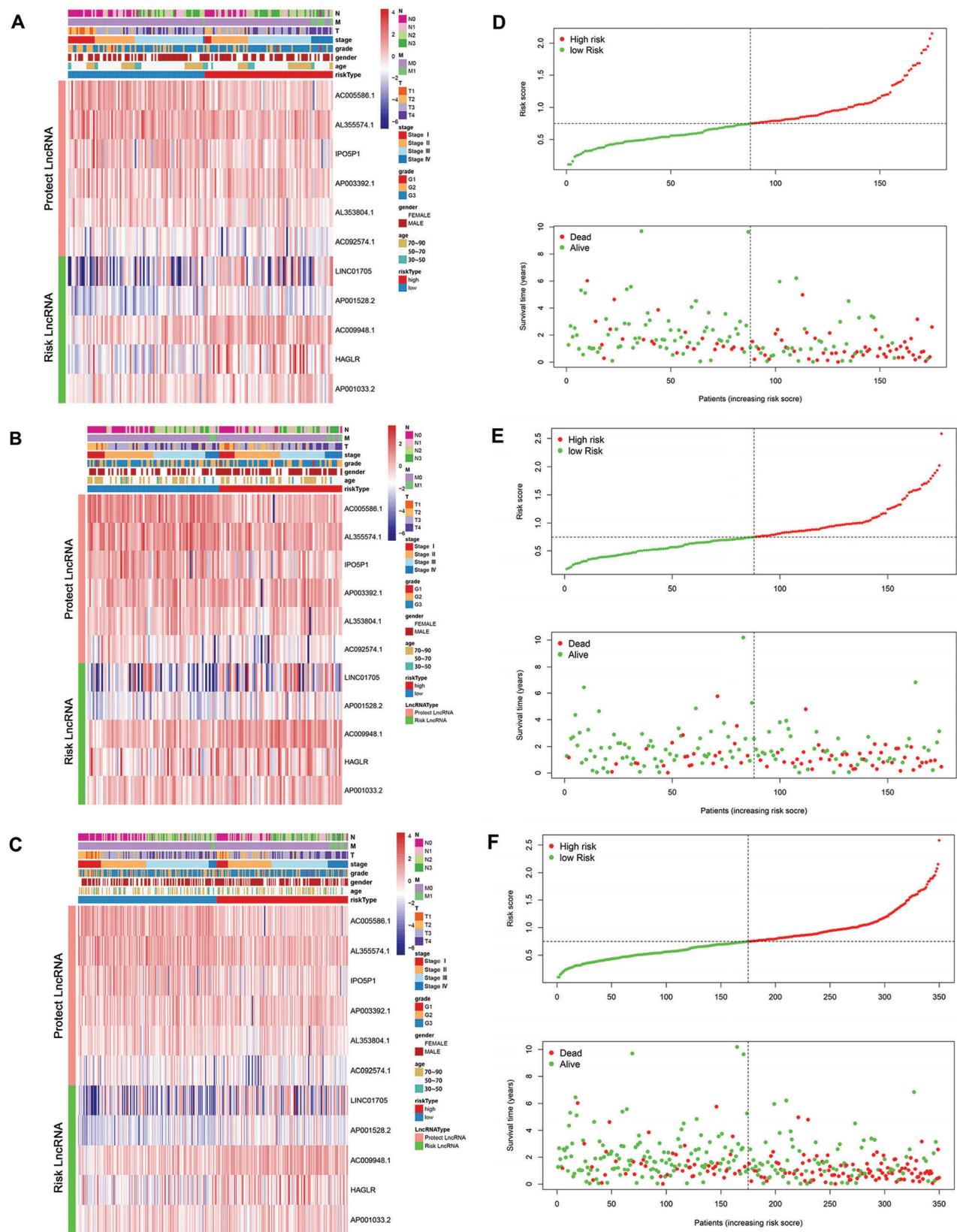


FIGURE 3. The link between clinical data and risk score. (A–C) Heatmap plot for the lncRNAs in the model in the training set, testing set, and TCGA-STAD dataset. (D–F) The relationship between the RS and survival time as well as status in the training set, testing set, and TCGA-STAD dataset.

of the deadliest cancers all over the world, prognosis evaluation of patients with GC still depends too much on the pathological analysis currently, which also facing many challenges and inconvenience in the clinic.

In this study, we systematically analyzed the prognostic value of autophagy-related lncRNAs in GC using bioinformatics and statistical tools with R software. A total of 24 lncRNAs was found strongly linked with the survival

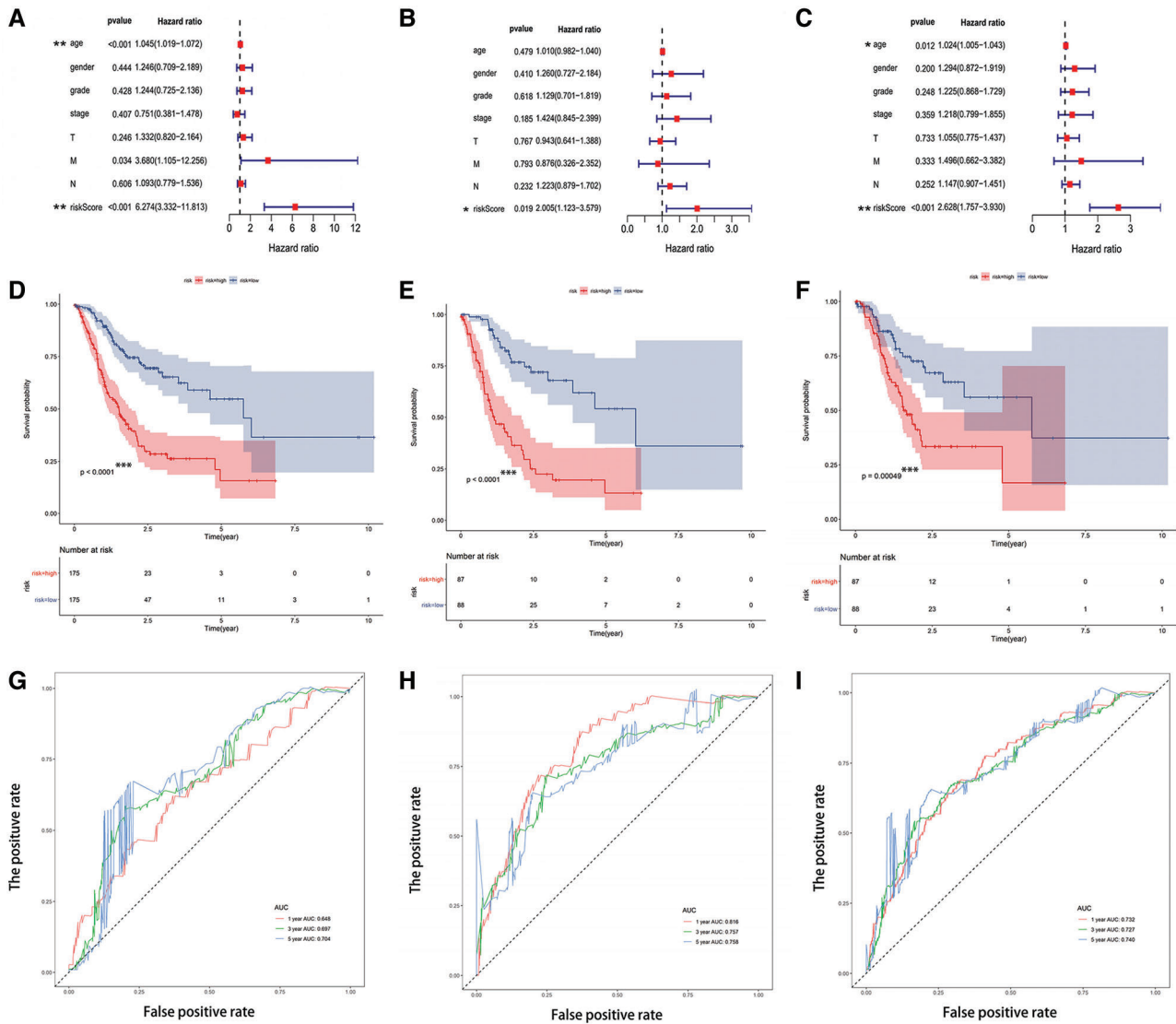


FIGURE 4. The risk score model performed well for survival prediction. (A–C) The RS was an independent prognostic predictor in the training set, testing set, and TCGA-STAD dataset. (D–F) Kaplan-Meier analysis based on risk type in the training set, testing set, and TCGA-STAD dataset. (G–I) The ROC curve and the value of AUC in 1-year, 3-year, and 5-year in the training set, testing set, and TCGA-STAD dataset. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$.

of TCGA-STAD based on Kaplan-Meier and univariate Cox regression analysis. We used the LASSO regression analysis to build the model in the training set and found 11 prognostic signatures of lncRNAs. The RS was calculated by integrating lncRNAs expression levels and corresponding LASSO coefficients for each patient. AC005586.1, AL353804.1, IPO5P1, AP003392.1, AL355574.1 and AC092574.1 were considered as protective lncRNA while LINC01705, AP001528.2, AC009948.1, HAGLR, and AP001033.2 were risk lncRNA. The accuracy of the model was tested in the testing set, and the TCGA-STAD dataset and the RS were found to significantly correspond with patient outcomes in both the testing set and TCGA-STAD dataset.

GO analysis revealed that the mRNAs in the prognostic network were mainly involved in autophagy, which is consistent with the expected results. The MF of GO analysis uncovered that these mRNAs also had a link with ubiquitin or ubiquitin-like protein ligase binding. Ubiquitin (Ub) is a protein highly conserved in all eukaryotes and bears many

potential sites for additional post-translational modifications (Komander and Rape, 2012). Ub was one of the most prominent factors in modifying protein substrates and degradation (Zheng and Shabek, 2017). The proteolytic system based on ubiquitin and autophagy is two prime systems in eukaryotic cells (Ciechanover, 2015; Ciechanover and Kwon, 2017). Studies have shown that ubiquitin, as a capital regulator, has participated in all processes in the autophagy flux (McEwan and Dikic, 2011). Atg8 was a ubiquitin-like protein, which was also found crucial for the autophagosome formation and consisted of the lipid conjugation system in autophagy (Nakatogawa et al., 2007).

KEGG analysis uncovered that the mRNAs were primarily involved in the apoptosis, mTOR pathway, p53 pathway, and PI3K-Akt pathway. There are two types of autophagy-related signaling pathways. One is the mTOR-dependent pathway, such as the AMPK/mTOR and PI3K/Akt/mTOR pathways, and the other is the non-mTOR-dependent pathway, such as the p53 pathway (Cao et al., 2019). The PI3K/AKT/mTOR

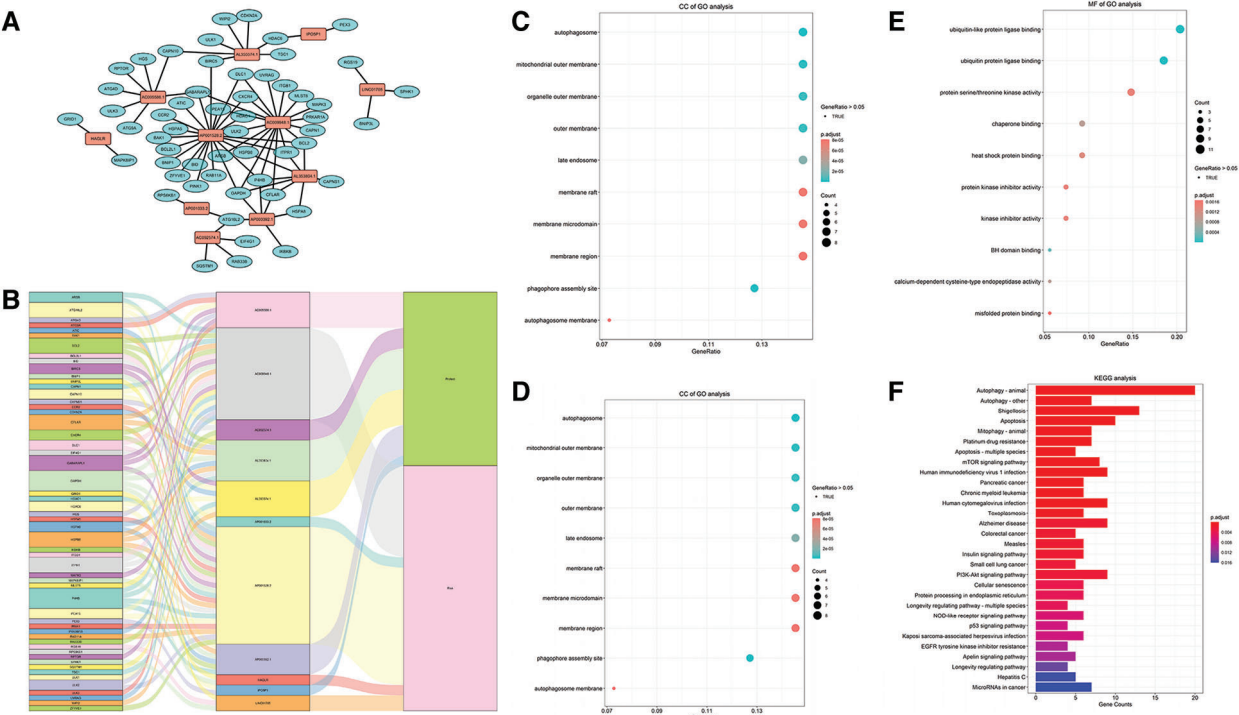


FIGURE 5. Construction of the prognostic mRNA-LncRNA interaction network and identification of involved signaling pathways. (A) The prognostic network based on the lncRNAs in the risk score model and the co-expressed mRNAs. (B) The Sankey diagram based on the prognostic network. (C–E) BP, CC, and MF of GO analysis for the mRNAs in the network. (F) KEGG analysis of the mRNAs in the network.

TABLE 3

The results of the GSEA analysis

Signaling pathway	NES	NOM <i>P</i> -value	FDR <i>q</i> -value
ECM receptor interaction	2.05	0.00	0.05
Neuroactive ligand receptor interaction	2.02	0.00	0.03
Complement and coagulation cascades	1.97	0.00	0.04
Focal adhesion	1.89	0.01	0.04
Calcium signaling pathway	1.88	0.00	0.04
Peroxisome	−2.26	0.00	0.00
Base excision repair	−2.19	0.00	0.00
Spliceosome	−2.15	0.00	0.00
Nucleotide excision repair	−2.11	0.00	0.01
RNA degradation	−2.11	0.00	0.01

pathway regulates many biological processes, including autophagy, and is frequently activated in various human cancers (Aoki and Fujishita, 2017). The molecular changes in the PI3K/Akt/mTOR signaling pathway were also found could increase the clinical stage and promote the recurrence in carcinoma (Aoki and Fujishita, 2017; Morgan et al., 2009). In GC, PI3K/Akt/mTOR activation was found significantly upregulated in GC cells, which results in the inhibition of autophagy of GC cells (Morgan et al., 2009). Additionally, inhibition of the PI3K/Akt/mTOR pathway increased the autophagic flux and promoted the apoptosis of cancer cells (Saiki et al., 2011).

The results of the GSEA analysis showed that the high-risk group was much active in the ECM receptor

interaction. Some studies indicated that autophagy affected the extracellular matrix (ECM), thus participating in the invasiveness and metastasis of cancer cells. In a rapidly growing and aggressive tumor, biosynthesis is obviously highly demanded. And in this process, detachment-induced autophagy will help the cancer cells get rid of ECM contact and promoting the metastasis subsequently during the advanced cancer stage (Guadamillas et al., 2011; Lock and Debnath, 2008). Autophagy will also induce metastatic cancer cells to hibernation if a steady connection was not established between the new ECM microenvironment and the cancer cells (Lu et al., 2008; Su et al., 2015).

At last, we constructed a nomogram combining the risk type and other clinical features, including age, gender, stage,

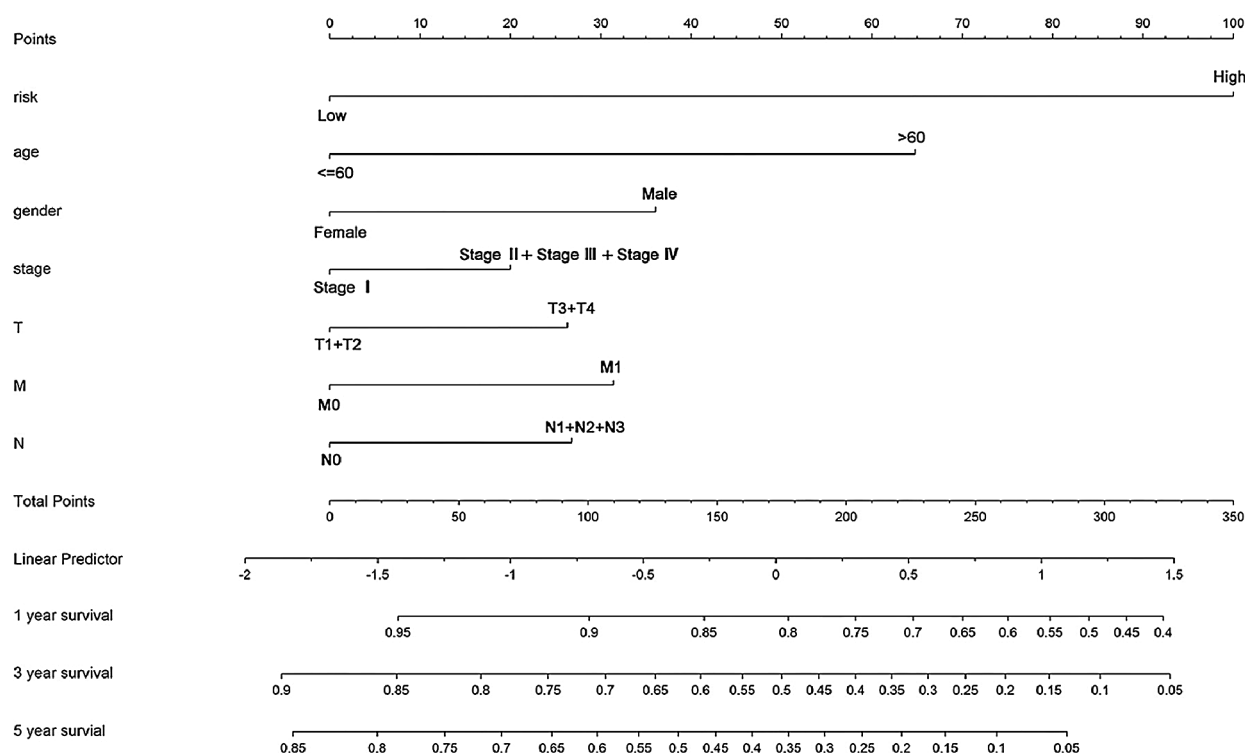


FIGURE 6. The nomogram for predicting an individual's survival rates in the TCGA-STAD dataset. The nomogram is incorporated with the risk type and clinical features to predict personalized survival probabilities in 1-year, 3-year, and 5-year.

and TNM stage, which can predict an individual's clinical outcome quantitatively. By using the nomogram, every patient will get total points based on his or her various indicators, respectively. The total points will predict the patient's survival probability in 1, 3-, and 5-year. Obviously, the higher the total points are, the lower the survival probability is.

Conclusion

In conclusion, we identified a prognostic risk score model consisted of 11 autophagy-related lncRNAs based on the TCGA-STAD dataset. This model was an independent predictor and performed well for prognosis in the training set, testing set, and TCGA-STAD dataset. A nomogram incorporating the model and clinical features could accurately predict the survival rate for individual GC patients. Our finding suggests that the 11-autophagy lncRNA risk score model may help facilitate individual prediction of a patient's prognosis with GC in the clinic.

Availability of Data and Materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contribution: The authors confirm contribution to the paper as follows: study conception and design: Bo Chen, Xin Xu and Mengqiu Cheng; data collection: Xin Xu, Wei Cao; analysis and interpretation of results: Mengqiu Cheng, Bo Chen, Guodong Cao; draft manuscript preparation: Mengqiu Cheng, Xin Xu. All authors reviewed the results and approved the final version of the manuscript.

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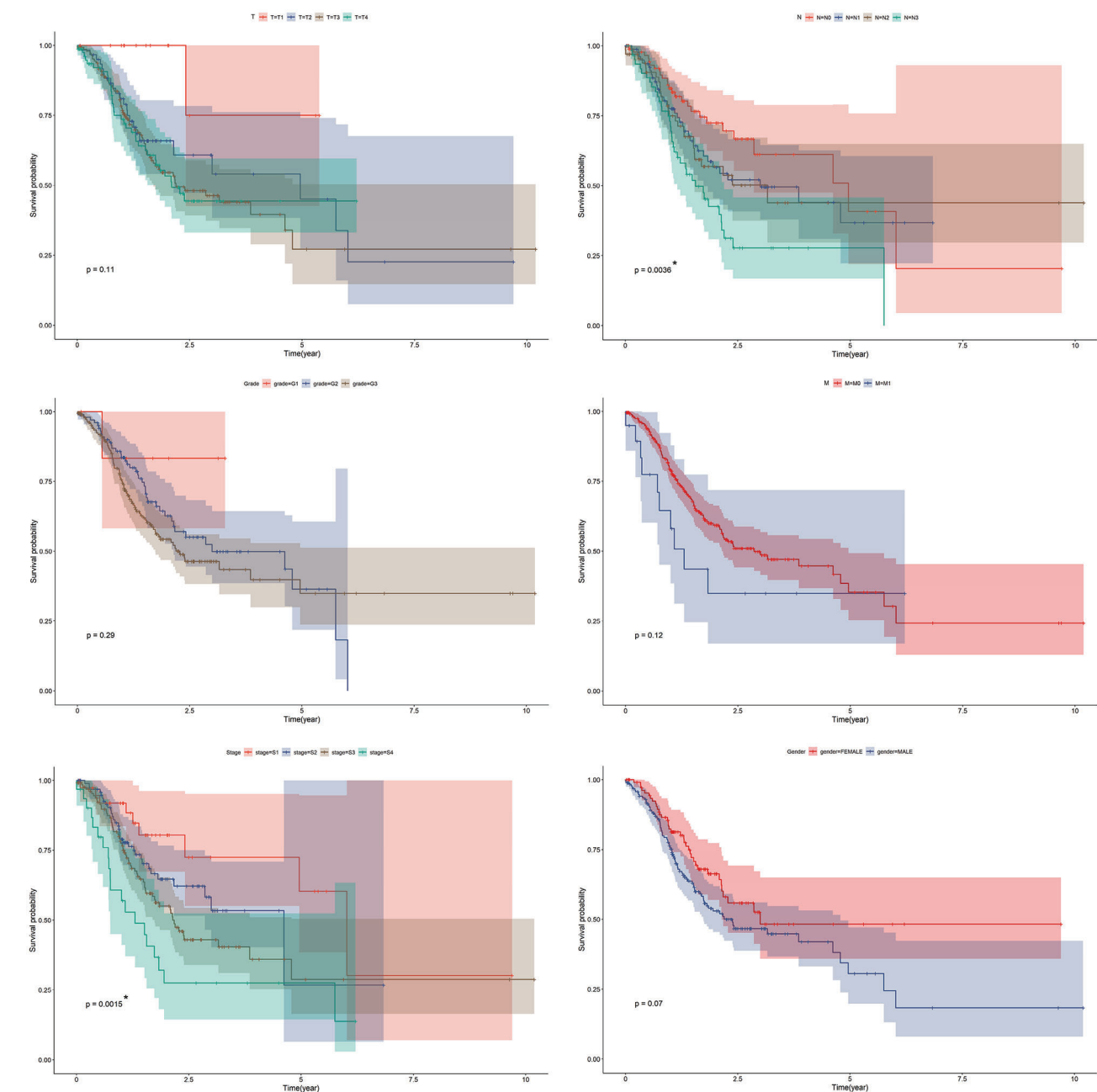
Conflicts of Interest: The authors declare that they have no conflict of interest.

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SUPPLEMENTARY FIGURE S1. Kaplan-Meier survival curves for TCGA-STAD dataset. Kaplan-Meier survival analysis regarding prime clinical features in GC. * $p < 0.05$.

SUPPLEMENTARY TABLE S1

The list of co-expressed mRNAs with the autophagy-related lncRNAs in the risk score model

Co-expressed mRNAs*	Full name
ARSB	Arylsulfatase B
ATG16L2	Autophagy related 16-like 2
ATG4D	Autophagy related 4D
ATG9A	Autophagy related 9A
ATIC	5-Aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
BAK1	BCL2-antagonist/killer 1
BCL2	B-cell CLL/lymphoma 2
BCL2L1	BCL2-like 1
BID	BH3 interacting domain death agonist

(Continued)

SUPPLEMENTARY TABLE S1 (continued).

Co-expressed mRNAs*	Full name
BIRC5	Baculoviral IAP repeat containing 5
BNIP1	BCL2 interacting protein 1
BNIP3L	BCL2 interacting protein 3 Like
CAPN1	Calpain 1
CAPN10	Calpain 10
CAPNS1	Calpain small subunit 1
CCR2	Chemokine (C-C motif) receptor 2
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CFLAR	CASP8 and FADD-like apoptosis regulator
CXCR4	Chemokine (C-X-C motif) receptor 4
DLC1	DLC1 Rho GTPase activating protein
EIF4G1	Eukaryotic translation initiation factor 4 gamma 1
GABARAPL1	GABA(A) receptor-associated protein like 1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GRID1	Glutamate receptor, ionotropic, delta 1
HDAC1	Histone deacetylase 1
HDAC6	Histone deacetylase 6
HGS	Hepatocyte growth factor-regulated tyrosine kinase substrate
HSPA5	Heat shock protein 5
HSPA8	Heat shock protein 8
HSPB8	Heat shock protein B8
IKBKB	Inhibitor of kappaB kinase beta
ITGB1	Integrin Subunit Beta 1
ITPR1	Inositol 1,4,5-trisphosphate receptor type 1
MAPK3	Mitogen-activated protein kinase 3
MAPK8IP1	Mitogen-activated protein kinase 8 interacting protein 1
MLST8	MTOR associated protein, LST8 homolog
P4HB	Prolyl 4-hydroxylase, beta polypeptide
PEA15	Phosphoprotein enriched in astrocytes 15
PEX3	Peroxisomal biogenesis factor 3
PINK1	PTEN induced putative kinase 1
PRKAR1A	Protein kinase CAMP-dependent type I regulatory subunit alpha
RAB11A	RAB11A, member RAS oncogene family
RAB33B	RAB33B, member RAS oncogene family
RGS19	Regulator of G-protein signaling 19
RPS6KB1	Ribosomal protein S6 kinase, 70kDa, polypeptide 1
RPTOR	Regulatory associated protein of MTOR, complex 1
SPHK1	Sphingosine kinase 1
SQSTM1	Sequestosome 1
TSC1	Tuberous sclerosis 1
ULK1	Unc-51 like autophagy activating kinase 1
ULK2	Unc-51 like autophagy activating kinase 2
ULK3	Unc-51 like autophagy activating kinase 3
UVRAG	UV radiation resistance associated
WIP1	WD repeat domain, phosphoinositide interacting 2
ZFYVE1	Zinc finger, FYVE domain containing 1

Note: Pearson correlation coefficient > 0.3, * $p < 0.001$.