Prognostic Autophagy-Related Genes of Gastric Cancer Patients on Chemotherapy

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Research

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Abstract

Background Gastric cancer(GC) treated with fluorouracil and cisplatin can cause chemotherapy resistance, which is one of the most common postoperative clinical complications and leads to in poor prognosis.

Methods The purpose of this study is to investigate the susceptibility of patients with GC after postoperative chemotherapy based on autophagy-related genes (ATGs). Under the background of TCGA database, for patients with GC undergoing and during chemotherapy,gene expression data was integrated and analyzed. Prognostic genes were screened based on univariate and various analysis regression models. Subjects were divided into two groups: high-risk group and low-risk group. Univariate and various analytical regression models were used to screen for prognostic genes. Median risk score was used for analysis. OS and DFS were evaluated by the product limit estimation method. Subject curve analysis is used to determine the accuracy of the forecast. We also have performed appropriate analysis and conducted some detailed assessments in our work. The differential expression of ATGs was mainly associated with chemotherapy resistance.

Results After chemotherapy administration, we have screened 9 ATGs outcomes in the subjects and DFS and OS were precisely predicted by the model of GEO and TCGA databases.

Conclusions 9 genes were established as prognostic markers to predict the relationship between ATGs and GC chemotherapy susceptibility, suggesting a better individualized treatment in clinical practice.

Introduction

Gastric cancer(GC) has become a principle health problem in the world and can lead to huge economical burdens. In East Asian countries, especially in China, GC has the highest incidence and mortality rates[1].Although overall survival has improved over the past few decades, the prognosis still remains remarkedly poor[2]. Drug resistance after long-term application of chemotherapy drugs is the main factor leading to poor prognosis of patients with GC. Because the traditional evaluation indicators cannot properly evaluate the chemotherapy prognosis, it is in sore need to have some clear understanding of patients with chemotherapy.

Autophagy is an important process for eukaryotic cells to transform intracellular structures and components [3]. Physiological imbalance in some processes of autophagy can lead to various diseases, such as cancer [4]. Some malignant tumors have some important pathophysiological processes [5-7]. For example, Beclin1 gene has a certain correlation with autophagy, which is highly expressed in gastric cancer tissues, but not expressed or low expressed in non-gastric tissues [8].In gastric cancer cells, glutamine decomposition provides energy and autophagy activation also contribute to abnormal glutamine decomposition to facilitate promotion and metastasis [9].LC3,a widely used biomarker for autophagosome, which is highly expressed in 58% of gastric cancer cells, but is not found in normal

gastric epithelial cells[10]. P62/SQSTM1, a characteristic substrate of ubiquitin-protein in autophagy, which is more significantly up-regulated in gastric cancer specimens than in normal gastric mucosa [11,12]. The results showed that autophagy-related genes can act as prognostic indicators in patients with GC.

Recent studies have demonstrated that many chemotherapy drugs can induce and enhance autophagy, which is a survival mechanism contributing to the acquired drug resistance development. Autophagy can inhibit the apoptosis of 5-FU-induced Sk-Hep1 in hepatocellular carcinoma cells [13]. Oxaliplatin can inhibit the apoptosis of MGC803 cells [14]. Aquaporin 3(AQP3) can promote the resistance of GC cells AGS to cisplatin through autophagy [15]. Presently, chloroquine（CQ）and hydroxychloroquine（HCQ）are only used for autophagy analysis[16]. CQ combined with chemoradiotherapy has been used in the treatment of glioblastoma, and median survival time has more than doubled compared with the control group[17.18]Preoperative treatment of pancreatic adenocarcinoma with HCQ and gemcitabine reduced serum levels, tumor markers, and CA19-9 combined antigens in 60% of patients [19].

Some related work shows that activation plays a huge role in drug resistance, and chemotherapy drugs combined with autophagy inhibitors are of great help to improve the resistance of tumor to chemotherapy drugs. On this basis, this study used bioinformatics methods to screen autophagy related genes to predict chemotherapy precursors in gastric cancer patients. This model can help clinicians make more personalized chemotherapy regimens and serve patients better and more effectively.

Materials And Methods

1.1 Data collection

Autophagy-related genes (ATGs) were downloaded and organized from the Human Autophagy Databases (http://autophagy.lu/clustering/index.html). Chemotherapy regimens based on cisplatin and fluorouracil have been widely used. Gene expression data and clinical information of 157 patients with gastric cancer who received cisplatin or fluorouracil after surgery were obtained from the TCGA Data portal(https://portal.gdc.cancer.gov/).The TCGA cohor was included to analyze the relationship between ATGs and chemotherapy sensitivity.The incomplete clinical information was excluded. The GSE26253 gene expression profile was downloaded from the GEO database, and 432 patients were treated with fluorouracil. R 4.0.2 software was used to process and analyze the original data.

1.2 Differential expression of ATGs and the Enrichment Analysis

ATGs differentially expressed genes (DEGs) in non-tumor specimens from chemotherapy group and TCGA database were calculated using LIMMA R software package. P value < 0.05,DEGs change at least doubled. Volcanoes were used to visualize the results. To investigate the main biological characteristics of chemotherapy-related ATGs. ATGs differentially expressed gene ontology (GO) and Kyoto Genomic

Encyclopedia (KEGG) pathway in the chemotherapy group were detected using the Cluster Profiler R package. P < 0.05 was considered statistically significant.

1.3 Identification of prognostic gene signatures

To determine that ATGs were significantly correlated with disease-free survival (DFS) and total survival (OS) in the gastric cancer chemotherapy group, univariate Cox proportional hazard regression analysis was first performed in THE TCGA and GEO databases.The prognostic model of ATGs was established by multivariate Cox regression analysis. The risk score was calculated based on the expression level of ATGs. Optimal cutoff values were used to divide patients into low-risk and high-risk groups. In addition, Kaplan-Meier method was used to conduct survival analysis based on risk score. To investigate whether the autophagy-related risk index in the TCGA dataset could be used as an independent predictor of OS, univariate and multivariate Cox regression analyses were applied. Risk score, age, sex, tumor subtype, pathological stages, and histological grades were used as covariates. T test was used to calculate the correlation between risk score and clinicopathological variables. P < 0.05 was considered statistically significant.

1.4 Gene set enrichment analysis (GSEA)

GSEA studies the genetic characteristics of high-risk and low-risk populations.

The difference between GSEA-p < 0.05 and FDR < 0.25 with GSEA3.0(http://www.broad.mit.edu/gsea/).was considered statistically significant.

Results

2.1 Identification of the differentially Expressed ATGs in Chemotherapy Group and Non-tumor samples

Data from 407 STAD patients in TCGA database were analyzed. A total of 232 ATGs were obtained in this study. There were 221 ATGs expressed in the TCGA-STAD group. Results 157 patients received chemotherapy, 32 were normal. The basic clinical characteristics of these patients in TCGA database were compared, as shown in Table 1. With FDR 1 as the screening standard, a total of 24 CASES of ATGs were detected (Figure 1A,B). The ATGs were IFNG, ATIC, BIRC5, CASP8, VMP1, IL24, CDKN2A, HSP90AB1, VEGFA, CTSB and ERBB2. Down-regulated ATGs include :PRKN, CDKN1A, GRID2, HSPB8, NRG3, NRG2, FOS and NKX2-3.

Table 1
Clinical characteristics of GC patients with chemotherapy in TCGA cohort

Gene	Co-ef	HR	HR.95L	HR.95H
GABARAPL1	0.370661	1.448692	0.912786	2.299233
GRID2	2.358799	10.57824	0.898029	124.6053
CXCR4	0.302963	1.353864	1.034964	1.771025
NCKAP1	0.71455	2.043268	0.967303	4.316067
ITGA3	0.269185	1.308897	0.971892	1.762759
GABARAPL2	1.334027	3.796301	1.55472	9.26977
IRGM	2.963281	19.36138	1.362477	275.1335
BNIP3L	0.592749	1.808954	1.091792	2.997195
ERBB2	0.319098	1.375887	1.105664	1.712152

2.2 Enrichment of ATGs

We utilized some techniques to analyze and explore the possible signaling pathways in GC that may be associated with chemotherapy response. Based on GO analysis, the differences in the cellular morphology, neuron death, AGT regulation on cellular membranous surfaces, autophagy and other aspects were studied(Figure 2A). In the KEGG pathways, ATGs were elucidated with regard to different ailments and pathways(Figure 2B).

2.3 The construction of Prognostic Markers of ATGs for OS in TCGA GC Chemotherapy Group

221 ATGs were analyzed by some analytical methods. In TCGA-STAD cohort, 13 ATGs had prognostic measures of chemotherapy patients(Figure3). 9 ATGs were finally tabulated and pinpointed to Table 2.

Table 2
Multivariate Cox regression analysis of prognostic genes.

Characteristic	Variables	Total	Percentage (%)
Age	<=65	79	53.7
	> 65	67	46.3
Sex	Male	92	62.6
	Female	55	37.4
Grade	G1-2	49	33.3
	G3	93	63.3
	GX	4	3.4
Stage	I	10	6.8
	II	46	31.3
	III	73	49.7
	IV	17	11.6
T stage	T1	4	2.7
	T2	29	19.7
	T3	73	49.7
	T4	42	27.9
N stage	N0	28	19.0
	N1	49	33.3
	N2	31	21.1
	N3	38	26.6
M stage	M0	130	88.4
	M1	10	6.8
	Mx	7	4.8

2.4 ATGs and the OS of GC victims in chemotherapeutic group

Risk scores were calculated based on ATGs-related mRNA expression levels and risk factors. Patients were classified into related groups. The product limit estimation analysis tool was utilized for data representation. Five-year survival rates were analyzed(Figure 4A). ROC curves were drawn and plotted to

determine the ability of patients in chemotherapy group to predict ATGs (Figure 4B). The area under the curve was well interpreted. Genetic research, which has been well documented in advances in research (Figure 4C), increases the number of deaths (Figure 4D). Both groups have created heat maps (Figure 4E). These results suggest that risk scores accurately reflect patient survival.

To determine whether autophagy-related scoring features were independent prognostic factors in GC patients undergoing chemotherapy, we conducted a study. Similarly, by using the risk-ratio technique diagram (Figure 5A), there was a significant correlation between risk scores and clinical variables. In Cox regression analysis, several Cox regression factors affecting the prognosis of chemotherapy-treated gastric cancer patients were well mapped (Figure 5B).And draw the comparison results of the two groups (Figure 5C). Enrichment of tumor pathways suggests that autophagy is involved in the regulation of chemotherapy in high-risk gastric cancer patients.

2.5 ATG's progression in gastric cancer

The direct effects of ATGs and their correlation with gastric cancer progression, OS,genes and clinicopathological variables was evaluated.Figure 6 showed that BNIP3L, CXCR4, ERBB2, GABRAPL, ITGA3 and NCKAP1 significantly correlated with the pathological classification of GC.On the one hand,BNIP3L, CXCR4, ERBB2, GABRAPL and NCKAP1 were significantly correlated with Lauren typing. ERBB2 and GABRAPL were also significantly correlated with tumor grade. On the other hand, BNIP3L, ERBB2, ITGA3 and NCKAP1 were significantly correlated with TNM staging.

2.6 Prognostic ATGs for DFS of GC Patients in the Chemotherapy Group

Data have been obtained on certain types of biomarkers in GC patients undergoing chemotherapy. GSE26253 dataset was incorporated. According to univariate Cox regression analysis, there was a certain significant correlation among the 9 ATGs(Figure 7A). 7 ATGs were well obtained and a division was well established in the victims.Kaplan-Meier analysis (product limit estimator) revealed that, $P<0.001$(Figure 7B). Heatmaps were developed for both groups(Figure 7C). Results about the chemotherapy of GC patients were summarized.

Discussion

GC is a challenge in terms of its treatment costs and imposes considerable financial burdens worldwide. Cisplatin as well as fluorouracil - based drug resistance are the mean causes of poor prognosis[20]. The process of autophagy in particular in GC is ancient, regulating cellular mechanisms and homeostasis[21].Several researches have demonstrated that this process is related to some proteomics and chemotherapeutic resistance in GC victims[22, 23]. Studies have found that the autophagy of gastric cancer cells with enhanced chemotherapy resistance is enhanced, and inhibition of autophagy can eliminate chemotherapy-resistance[24, 25].Considering the importance of autophagy in chemotherapy resistance of GC, we can further explore the prognostic value of autophagy in the treatment of GC. In this study, we combined TCGA and GEO database to complete our work. To analyze the prognosis of

postoperative chemotherapy for gastric cancer patients. We also investigate the biological function and role of ATGs in gas chromatography.

First and foremost, ATGs had a dependent interaction with normal stomach in the gastric cancer chemotherapy group, which was confirmed in our study. In addition, some analyses showed differential enrichment of PLATINUM resistance in ATGs. Studies have shown that combined use of GC inhibitors can improve cisplatin resistance [26-28], which is consistent with our research results. ATGs can promote gastric cancer progression through platinum resistance. The gastric cancer chemotherapy group had 13 genes associated with prognosis. We used multivariate Cox regression to construct and compute data sets for nine genes.

It has been found that in pancreatic cancer, by inhibiting the CXCL12/CXCR4 signaling pathway in combination with the autophagy inhibitor chloroquine, ERK and STAT3 phosphorylation levels can be reduced, thus improving the poor prognosis of pancreatic cancer[29]. In colorectal cancer, Mir-125b induces the CXCL12/CXCR4 signaling pathway to enhance autophagy, thereby promoting tumor infiltration and the effect of colorectal cancer on chemotherapy resistance. The GABARAP subfamily plays a role in the late stage of auto phagosomal-closure and auto phagosom-lysosomal fusion[30]. IRGM and GABARAP can participate in autophagy and regulation. GABARAP-L2 has been shown to be involved in the autophagy regulatory mechanism, influencing the binding and de-binding of autophagosomes through TBK1- mediated phosphorylation [31]. BNIP3L is involved in temozolomide resistance in glioblastoma [32,33]. GSEA results showed that autophagy regulation was mainly concentrated in the high-risk group, suggesting that autophagy in the high-risk group may regulate the tolerance of gastric cancer patients to chemotherapy, thus affecting the prognosis [34,35]. In addition, the predictive characteristics of DFS were established based on GEO database.

In conclusion, we constructed autophagy related markers for OS and DFS in patients with gastric cancer undergoing chemotherapy, which can independently predict the prognosis of patients with gastric cancer and provide new therapeutic targets for gastric cancer. Our research is bound to have some limitations. Although internal verification has been carried out, further experiments are needed for verification and confirmation.

Declarations

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Not applicable

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Data Availability Statement

All data used in this study were included in the manuscript and supplementary materials.

Authors' contributions

CH and MZ conceived of the study and participated in design and coordination, drafted and revised the manuscript. LXLand YY performed gene differential analysis and survival analysis using GEO and TCGA data. MYL and ZL Collected and analyze immune related information. LYF and Paul revised manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

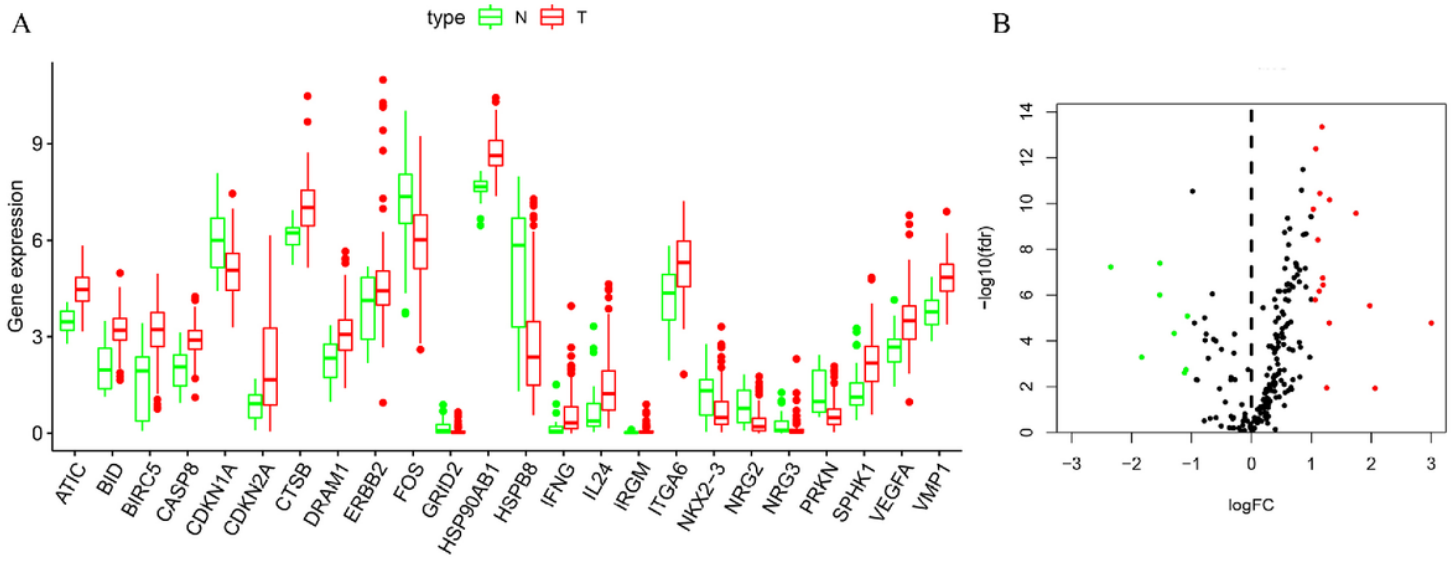
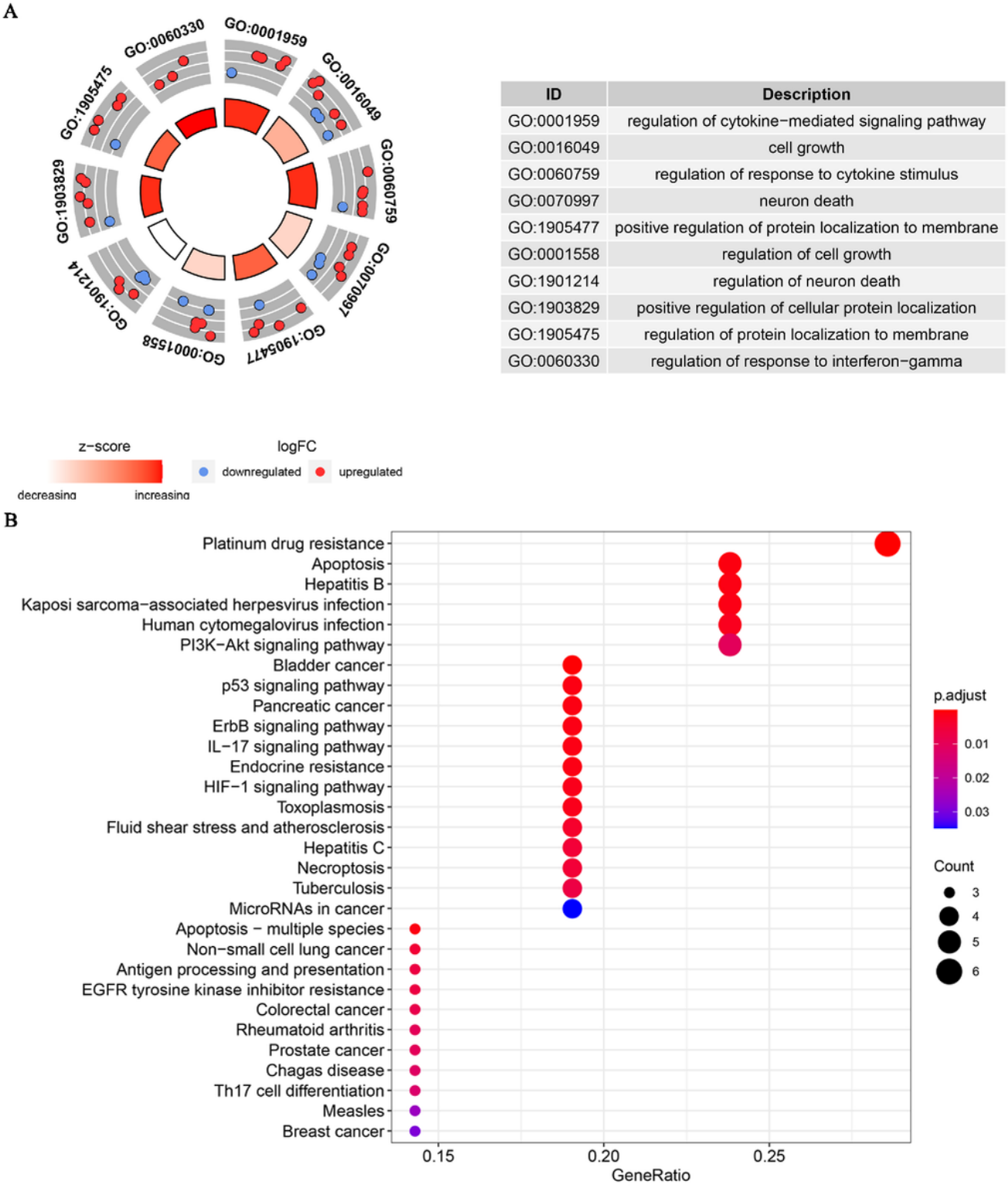


Figure 1

The differentially expressed autophagy-related genes in a chemotherapeutic group and normal tissues. (A) Visualization of the expression levels of the 24 differentially expressed autophagy-related genes. N normal; T tumor; (B)Volcano plot of 221 autophagy-related genes. Red upregulation; Green downregulation.



ID	Description
GO:0001959	regulation of cytokine-mediated signaling pathway
GO:0016049	cell growth
GO:0060759	regulation of response to cytokine stimulus
GO:0070997	neuron death
GO:1905477	positive regulation of protein localization to membrane
GO:0001558	regulation of cell growth
GO:1901214	regulation of neuron death
GO:1903829	positive regulation of cellular protein localization
GO:1905475	regulation of protein localization to membrane
GO:0060330	regulation of response to interferon-gamma

Figure 2

GO and KEGG enrichment analysis. (A) GO analysis of 24 differentially expressed autophagy-related genes. Red indicates upregulated autophagy-related genes, and blue indicates downregulated autophagy-related genes. (B) Bubble diagram of KEGG enrichment analysis.

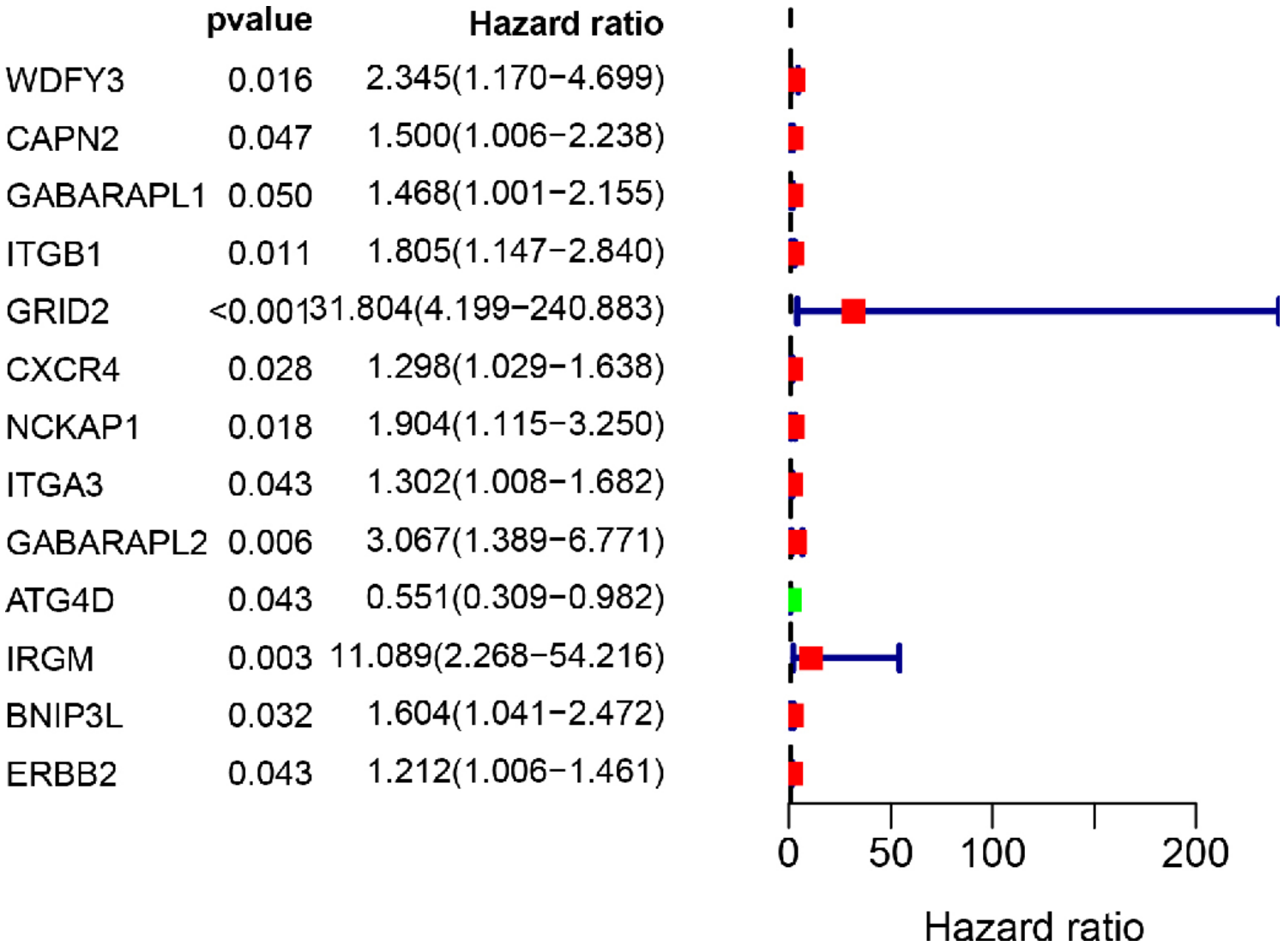


Figure 3

Univariate Cox regression analysis of autophagy genes related to overall survival of GC patients with chemotherapy.

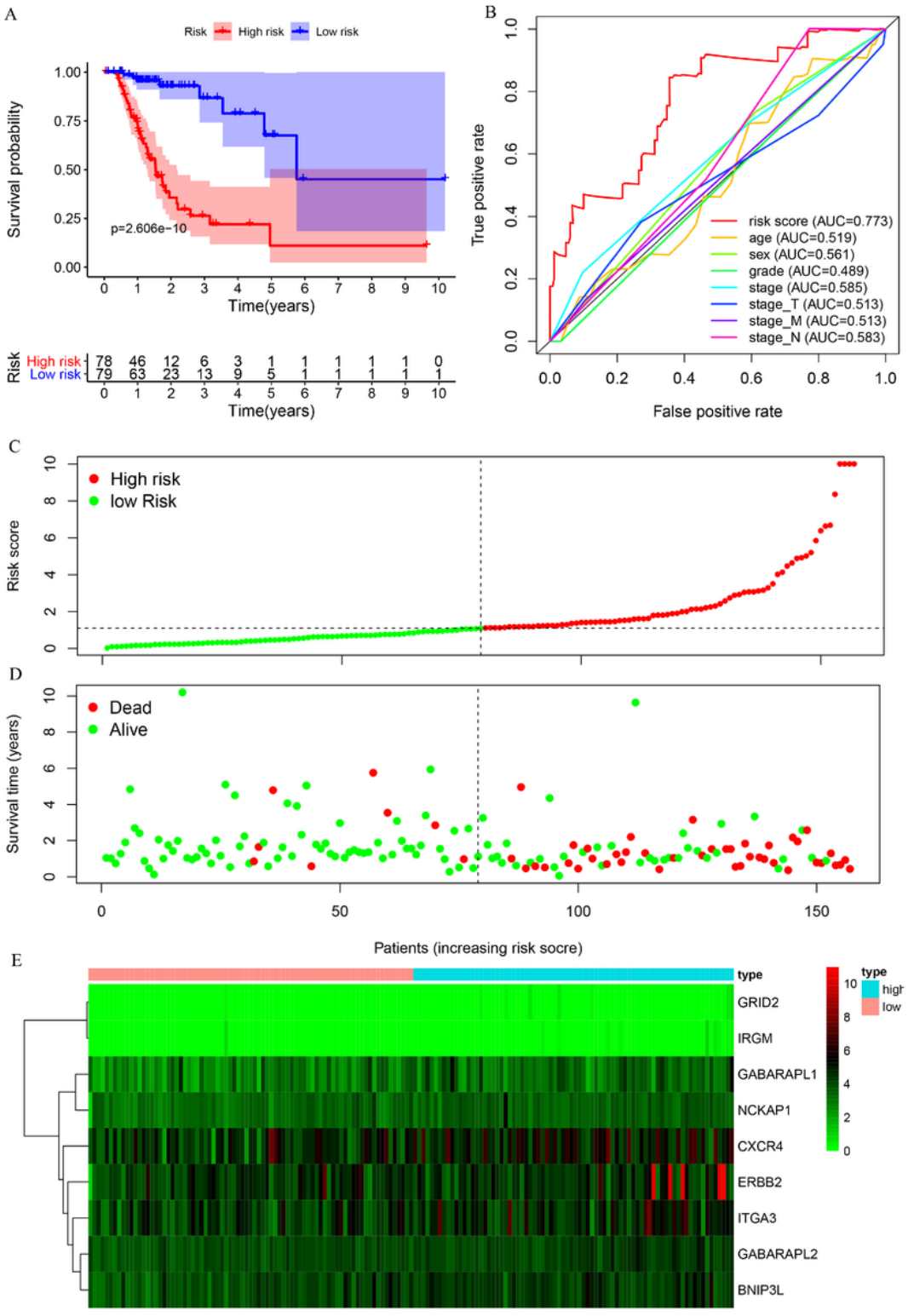


Figure 4

The correlation between the nine-gene autophagy-related signature for the OS of patients with GC. (A) Kaplan-Meier OS curves for TCGA gastric cancer patients treated with chemotherapy by median risk. (B) Multi-index ROC curve of risk score and other indicators. (C) Distribution of the risk scores of GC patients. (D) The number of survivors and non-survivors with different risk scores; red represents the number of

non-survivors, and color green represents the number of survivors. (E) The expression of nine autophagy-related genes in the high- and low-risk groups.

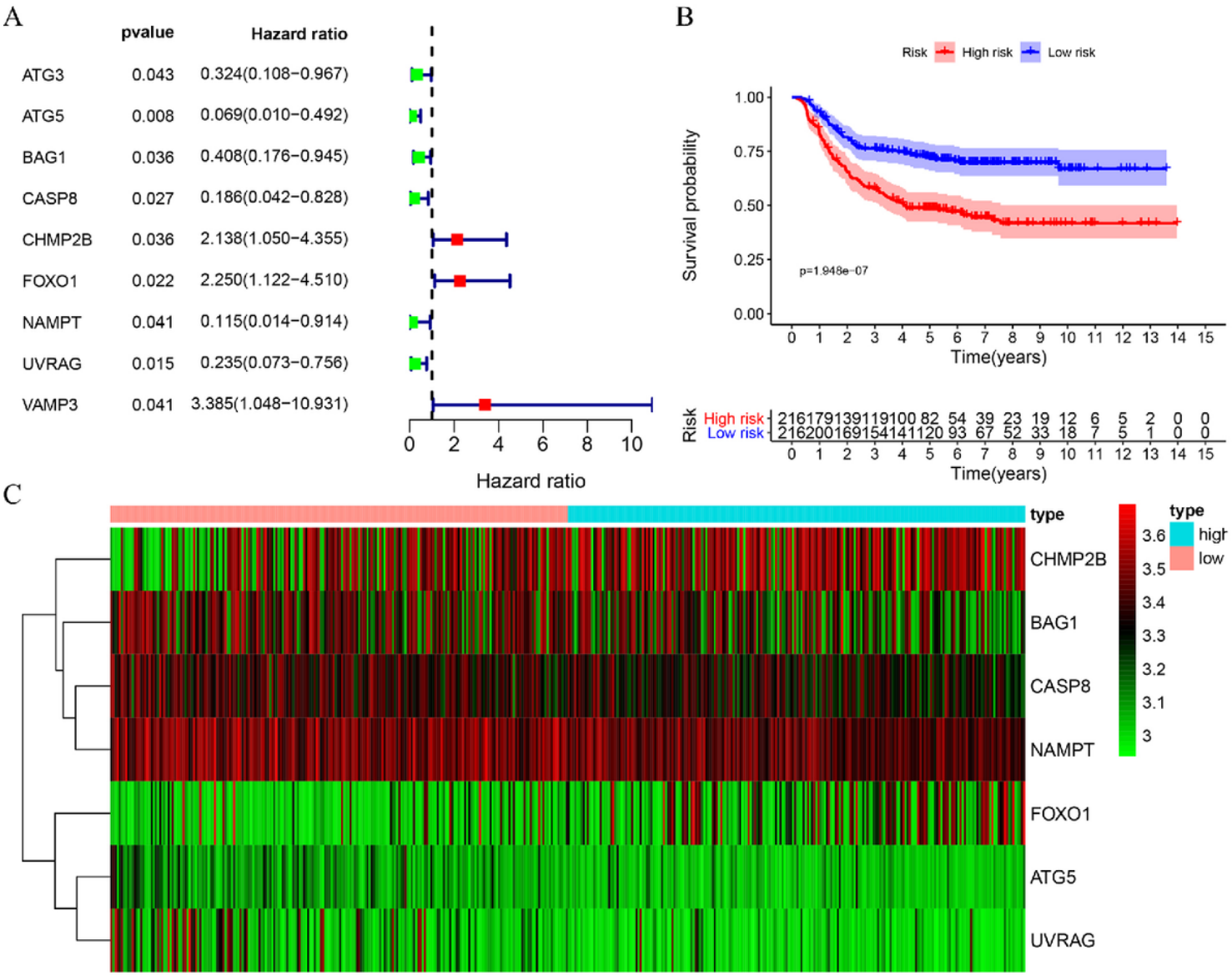


Figure 5

The ATGs for OS is an independent prognostic factor for GC. (A) Univariate Cox regression analysis of correlations between the risk score for OS and clinical variables. (B) Multivariate Cox regression analysis of correlations between the risk score for OS and clinical variables. (C) Gene set enrichment analysis comparing the high- and low-risk groups.

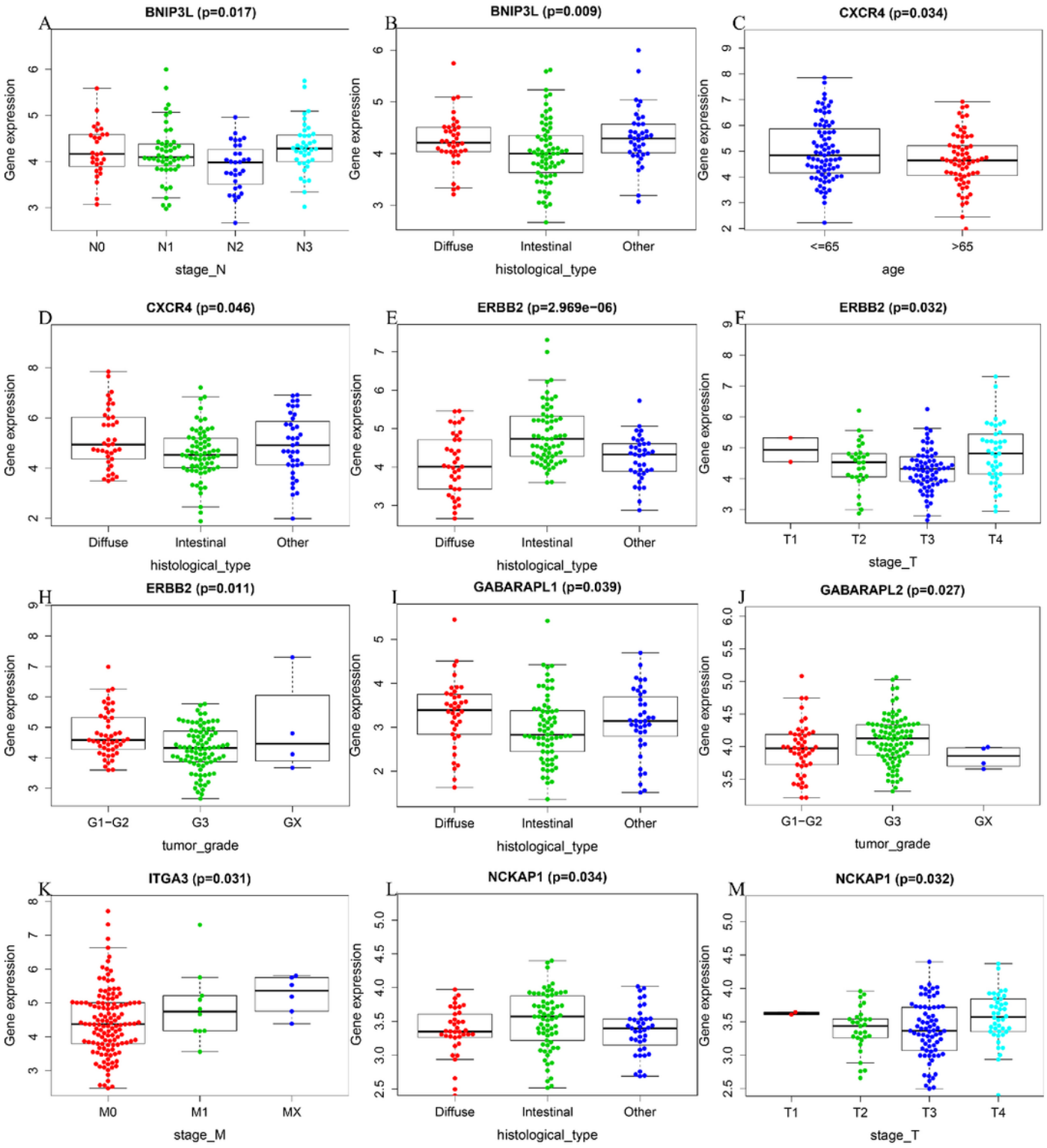


Figure 6

The relationships between the ATGs and clinicopathological variables. (A□B) BNIP3L. (C, D) CXCR4. (E, F, H) ERBB2. (I, J) GABRAPL. (K) ITGA3. (L, M) NCKAP1.

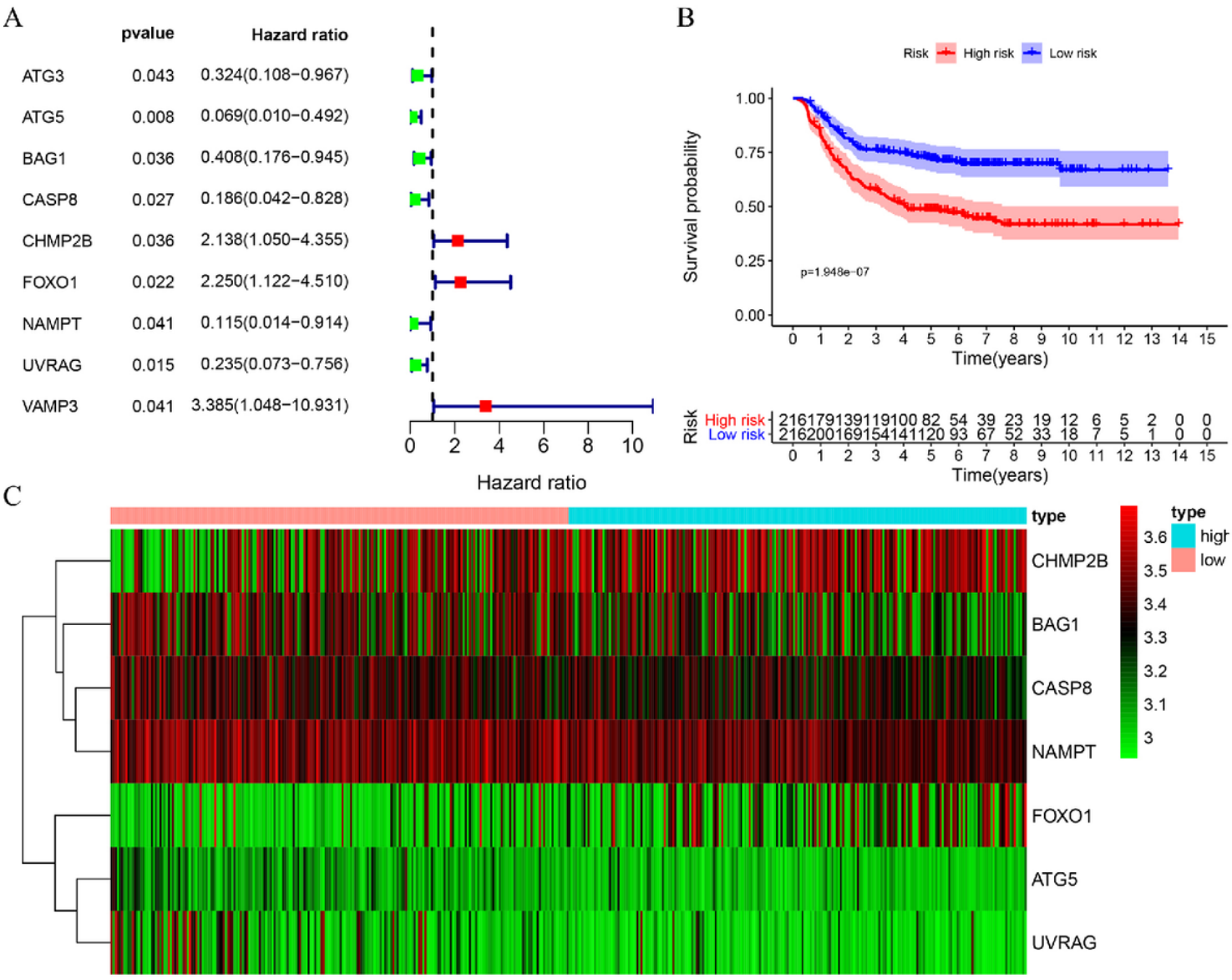


Figure 7

The ATGs for DFS is an independent prognostic factor for GC. (A) Univariate Cox regression analysis of autophagy genes related to DFS of GC patients with chemotherapy. (B) Kaplan-Meier DFS curves for high and low-risk groups; (C) The expression of nine autophagy-related genes in the high and low-risk groups.