Prognostic Autophagy-Related Genes of Gastric Cancer Patients on Chemotherapy

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Research

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Abstract

Background Chemotherapy resistance based on fluorouracil and cisplatin is one of the most encountered postoperative clinical problems in patients diagnosed with gastric cancer (GC), resulting in poor prognosis.

Methods This study aimed to combine autophagy-related genes (ATGs) to investigate the susceptibility of victims with gastric malignancy to postoperative chemotherapy. Based on the TCGA database, gene expression data for GC patients undergoing and during chemotherapy were integrated and analyzed. Prognostic genes were screened based on univariate and various analysis regression models. Subjects were divided into high-risk and low-risk groups and analyzed by the median risk score approach. The product limit estimator method was used to evaluate the OS and DFS. The accuracy of the prediction was resolved by the subject curve analysis. In addition, proper analysis carrying out was done in our work for some detailed assessments. The differential expression of ATGs is mainly related to chemotherapy resistance.

Results A total of 9 ATGs of chemotherapy administration outcomes in these suffers were screened. Based on GEO and TCGA databases, the model accurately predicted DFS and OS after chemotherapy administration.

Conclusions This study established prognostic markers based on 9 genes, predicting that ATGs are related to chemotherapy susceptibility of GC patients, which can provide better individualized treatment regimens for clinical practice.

Introduction

Gastric cancer(GC) is a major health problem worldwide, which is also a challenge resulted in huge economical burdens. In East Asian countries, especially in China, GC has the highest incidence and mortality rates world-wide[1].Although overall survival has improved over the past few decades, the prognosis still remains remarkedly poor[2]. Drug resistance of chemotherapeutic drugs is the main factor that causes a poor prognosis in patients. Conventional evaluation indexes cannot appropriately evaluate the prognosis of patients with chemotherapy, so it is necessary to have some explicit knowledge and explore victims undergoing chemotherapy.

Autophagy is an important process of eukaryotic transformation of intracellular structures and components [3]. Physiological imbalance problems in some processes of autophagy can lead to various diseases and ailments, such as cancer [4]. There are some significant pathophysiological processes with regard to some malignancies [5–7]. For instance, Beclin1 gene is associated with autophagy to some extent, which is highly expressed in gastric cancer tissues, but not or low expressed in non-gastric cancer tissues [8]. Glutamine decomposition provides energy for tumor cells, and autophagy activation also contribute to abnormal glutamine decomposition in gastric cancer cells, promoting promotion and

metastasis [9].LC3 has been widely used as a biomarker for autophagosome, with high expression of LC3 detected in 58% of gastric cancer cells, but not 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],„while the interpretation of P62/SQSTM1 has some adverse clinical outcomes of the ailment [12]. The research results have revealed that autophagy-related genes can be used as prognostic indicators in the analysis of gastric cancer patients.

Recent studies have shown that many chemotherapy drugs can induce and enhance autophagy. This induction of autophagy is a survival mechanism that contributes to the development of acquired drug resistance. Autophagy can inhibit the apoptosis of 5-FU-induced MGC803 in gastric carcinoma cells [13]. Oxaliplatin can inhibit the apoptosis of MGC803 cells [14]. Aquaporin 3(AQP3) promotes the resistance of gastric cancer 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 for glioblastoma management, and median survival time has more than doubled compared with the control group[17.18]Preoperative treatment with HCQ combined with gemcitabine resulted in 60% of patients with pancreatic adenocarcinoma with reduced serum levels, tumor markers and CA19-9 combined antigens [19].

Some relevant work has demonstrated that activation plays a huge role in drug resistance, and chemotherapeutic drugs combined with autophagy inhibitors are of great assistance to improve the resistance of tumor to chemotherapeutic drugs. On this basis, our study used bioinformatics methods to predict the progonisis of chemotherapy in gastric cancer patients by screening autophagy ralated genes.This model is helpful for clinicians to develop more individualized chemotherapy regimens and serve patients betterl and more efficiently.

Materials And Methods

1.1 Data collection

This study downloaded and organized autophagy-related genes (ATGs) from the Human Autophagy Databases (http://autophagy.lu/clustering/index.html). Chemotherapy regimens based on cisplatin and fluorouracil were widely used. Gene expression data and clinical information were obtained from TCGA data portal (https://portal.gdc.cancer.gov/) in 157 patients with GC who received cisplatin or fluorouracil post operatively. 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

The differentially expressed genes (DEGs) of ATGs between chemotherapy group and non-tumor samples from TCGA database were calculated using limma R package. P-value < 0.05 and DEGs at least double changes.. Volcanic were utilized to visualize the results. To explore the main biological characteristics of ATGs related to chemotherapy. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of differentially expressed ATGs in the chemotherapeutic group and were performed using Cluster Profiler R package. P < 0.05 was considered statistically significant.

1.3 Identification Of Prognostic Gene Signatures

To identify ATGs that were significantly correlated with disease-free survival (DFS) and overall survival (OS) in GC chemotherapy group, univariate Cox proportional hazard regression analysis was first performed in TCGA and GEO database. 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. The correlation between risk score and clinicopathological variables was calculated by using the T-test. P < 0.05 was considered statistically significant.

1.4 Gene Set Enrichment Analysis (gsea)

GSEA was conducted to explore the characteristics of gene Hallmarks in high-risk and low-risk populations. GSEA was performed using GSEA3.0 (http://www.broad.mit.edu/gsea/).The differences between nominal P < 0.05 and FDR < 0.25 were considered statistically enriched.

Results

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

Data from 407 subjects in the STAD cohort in TCGA database were analyzed. 232 ATGs were obtained in our study. A total of 221 ATGs were expressed in TCGA-STAD cohort. The results were 157 patients who received chemotherapy and 32 normal samples. The basic clinical characteristics of these patients in the TCGA database was also compared, as shoen in Table 1. With FDR < 0.05 and |log2 FC| >1 as the

screening criteria, 24 ATGs were presented(Figures 1A,B). The upregulated ATGs were IFNG, ATIC, BIRC5, CASP8, VMP1, IL24, CDKN2A, HSP90AB1, VEGFA, CTSB and ERBB2. The downregulated 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(Fig. 2A). In the KEGG pathways, ATGs were elucidated with regard to different ailments and pathways(Fig. 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(Fig. 3). 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(Fig. 4A). ROC curves were drawn and plotted to determine the ability of patients in chemotherapy group to predict ATGs (Fig. 4B). The area under the

curve was well interpreted. Genetic study, which was well pointed out during the study progression, (Fig. 4C), increases in the number of deaths(Fig. 4D). Heatmaps were created for both groups(Fig. 4E). These results suggested that risk scores accurately reflected patient survival.

To determine whether autophagy-related scoring features were independent prognostic factors in GC patients undergoing chemotherapy, we conducted a study. Similarly, the significant correlation between risk scores and clinical variables was achieved by the utilization of hazard ratio technique sketch diagrams(Fig. 5A). In Cox regression analysis, several Cox regressions factors affecting the prognosis of chemotherapeutic patients with gastric cancer were well plotted(Fig. 5B). Furthermore, the comparison results of the two groups were plotted(Fig. 5C). Cancer pathways were enriched, suggesting that autophagy is involved in the regulation of chemotherapy for 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(Fig. 7A). 7 ATGs were well obtained and a division was well established in the victims. Kaplan-Meier analysis (product limit estimator) revealed that, $P < 0.001$(Fig. 7B). Heatmaps were developed for both groups, (Fig. 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-drug resistanceisenhanced, 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 databases to accomplish our work. The prognosis of GC patients

receiving postoperative chemotherapy was analyzed. We also studied the biological function and role of ATGs in GC.

First and foremost, there was a dependent interaction between ATGs and normal stomach in the GC chemotherapeutic group, which was identified in our study. Furthermore, some analysis revealed that ATGs were enriched differentially in platinum resistance. Research has demonstrated that a combination of inhibitors in GC can improve cisplatin resistance[26–28], which is consistent and concurs with our results. ATGs can promote progress in GC disease progress through platinum resistance. Moreover, there were 13 genes associated with prognosis in the GC chemotherapy group. We used multivariate Cox regression to construct and compute data set for 9 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, affecting binding and de-binding of the autophagosome through TBK1- mediated phosphorylation[31]. In glioblastoma, BNIP3L is involved in temozolomide resistance[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 GC patients to chemotherapy and thus lead to 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 GC undergoing chemotherapy, which can independently predict the prognosis of GC patients and provide new therapeutic targets for GC. Our study is bound to have some limitations. Although internal verification has been conducted, 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.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Figures

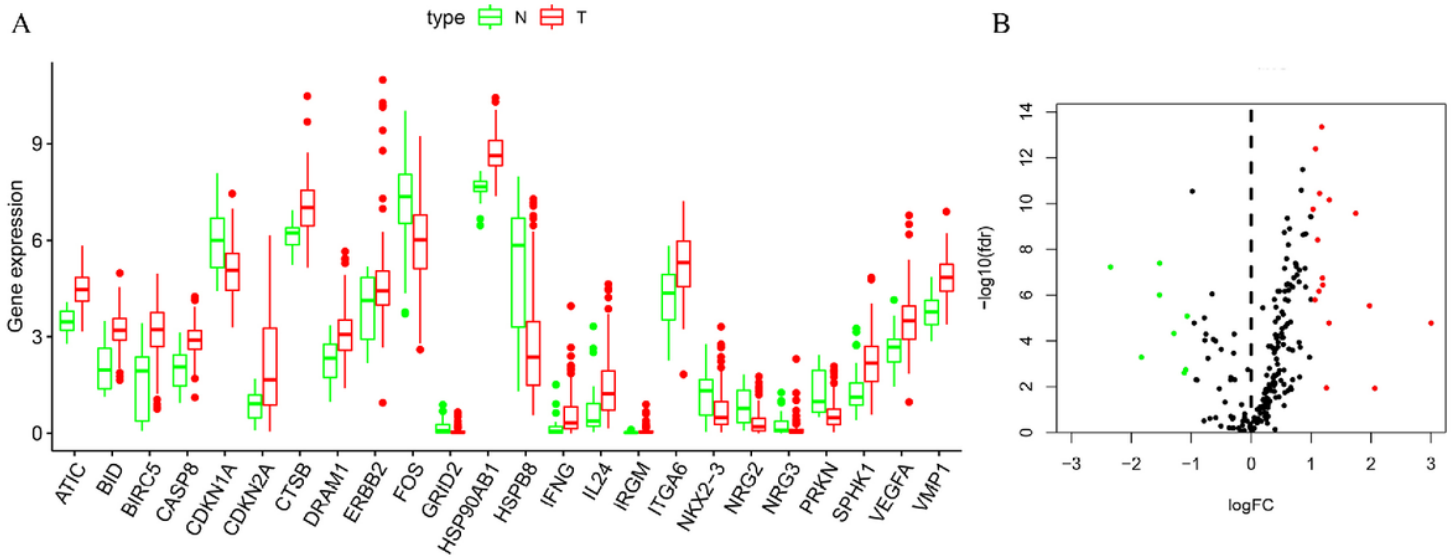
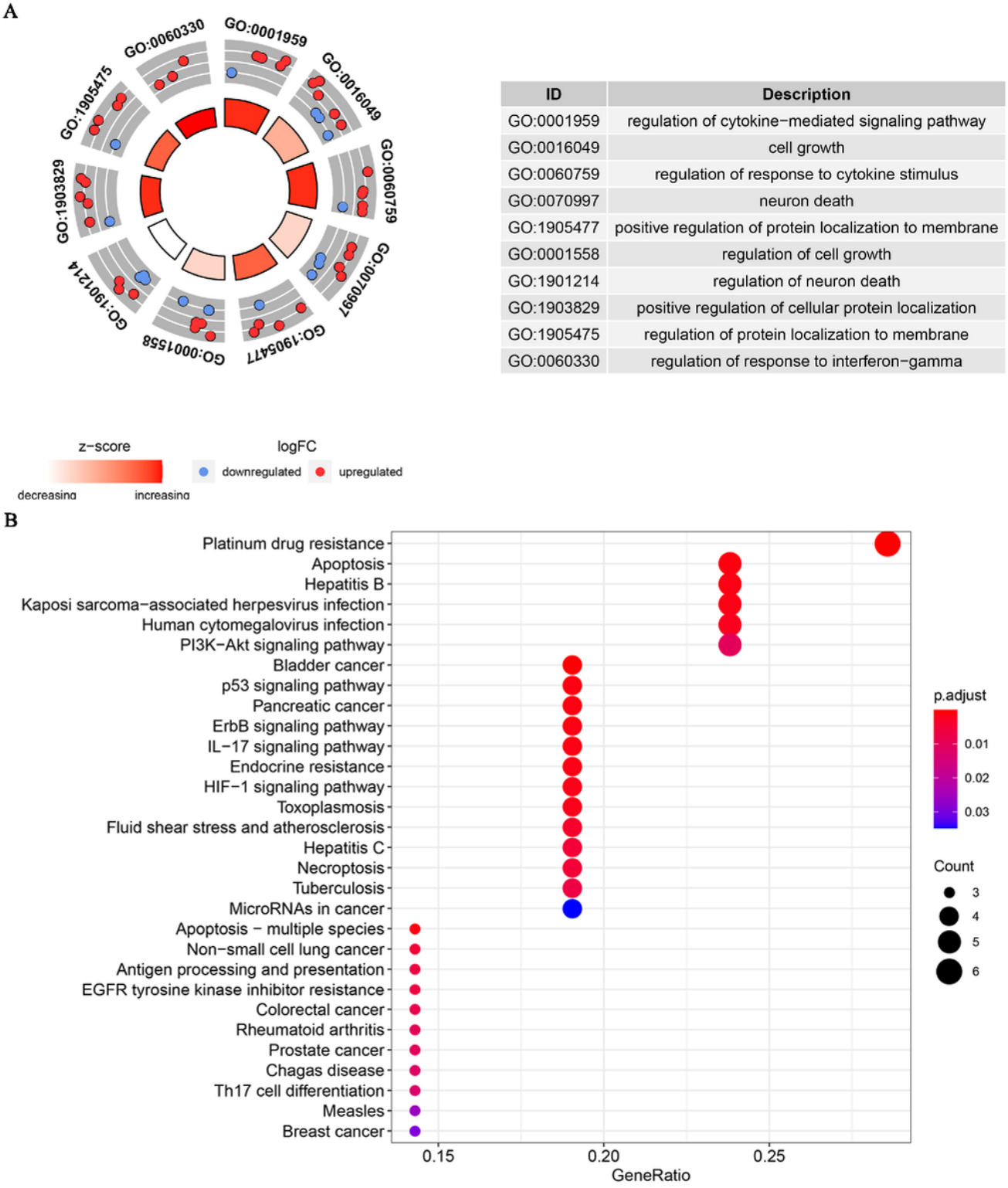


Figure 1

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.

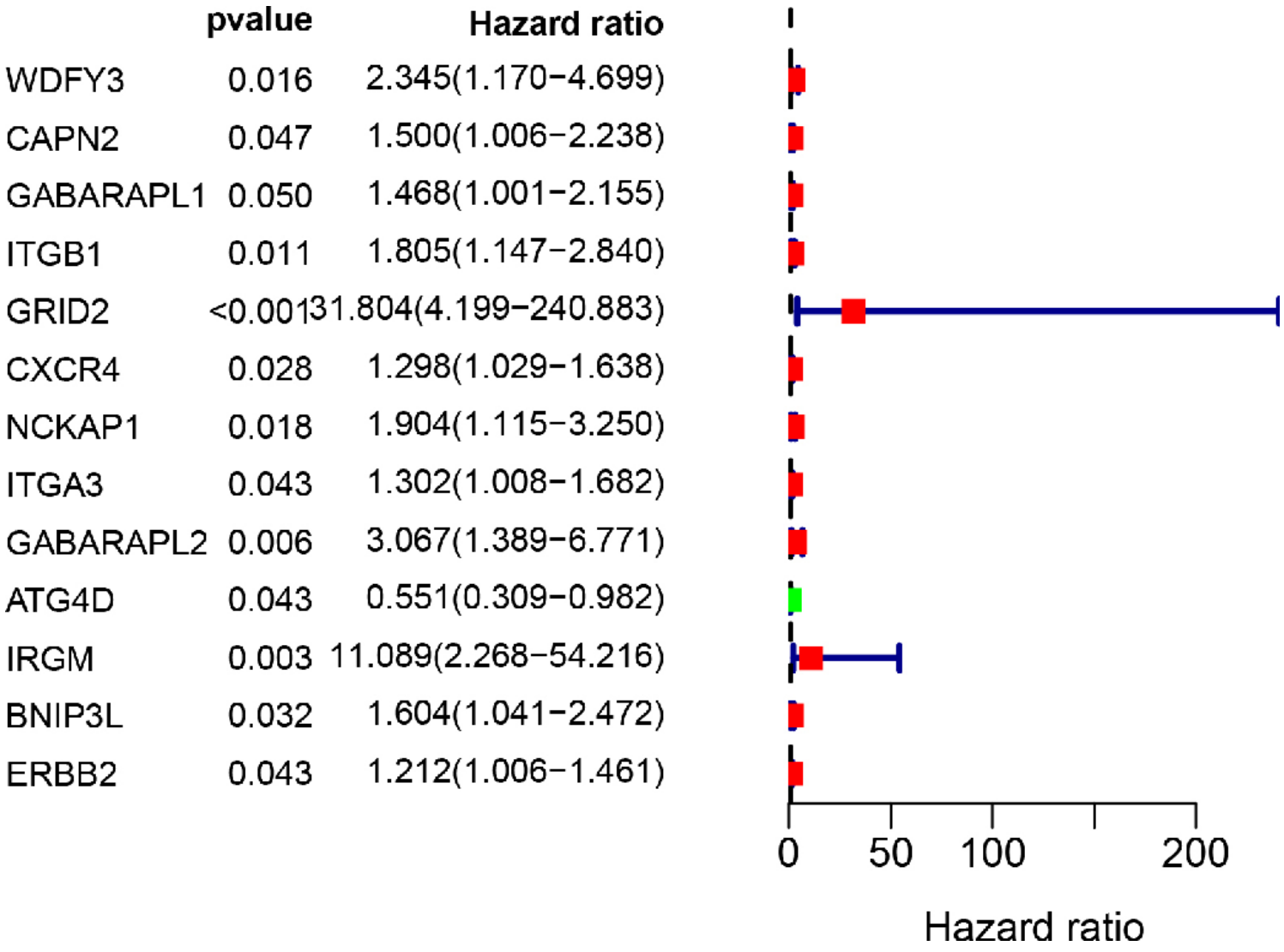


Figure 3

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

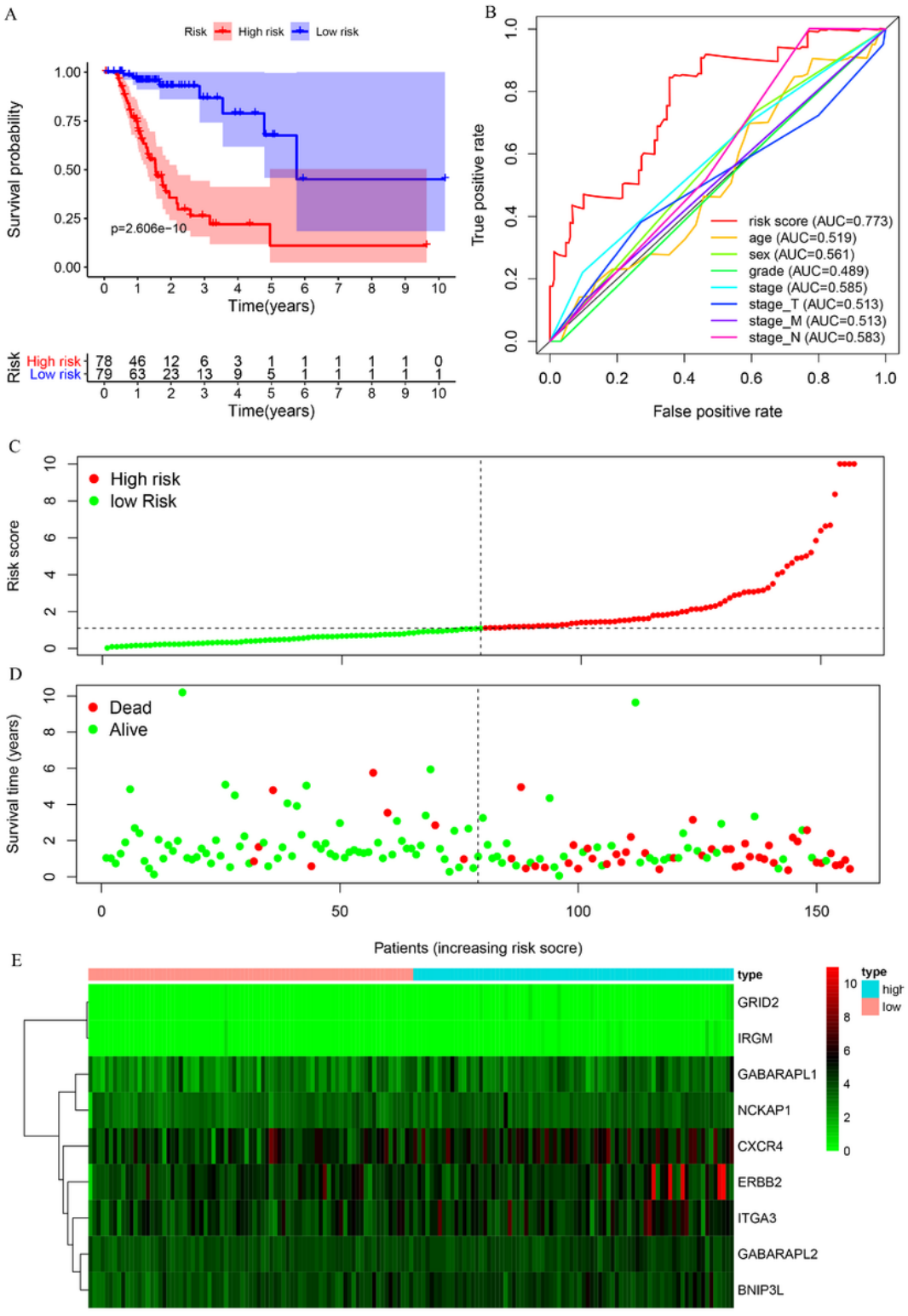


Figure 4

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.

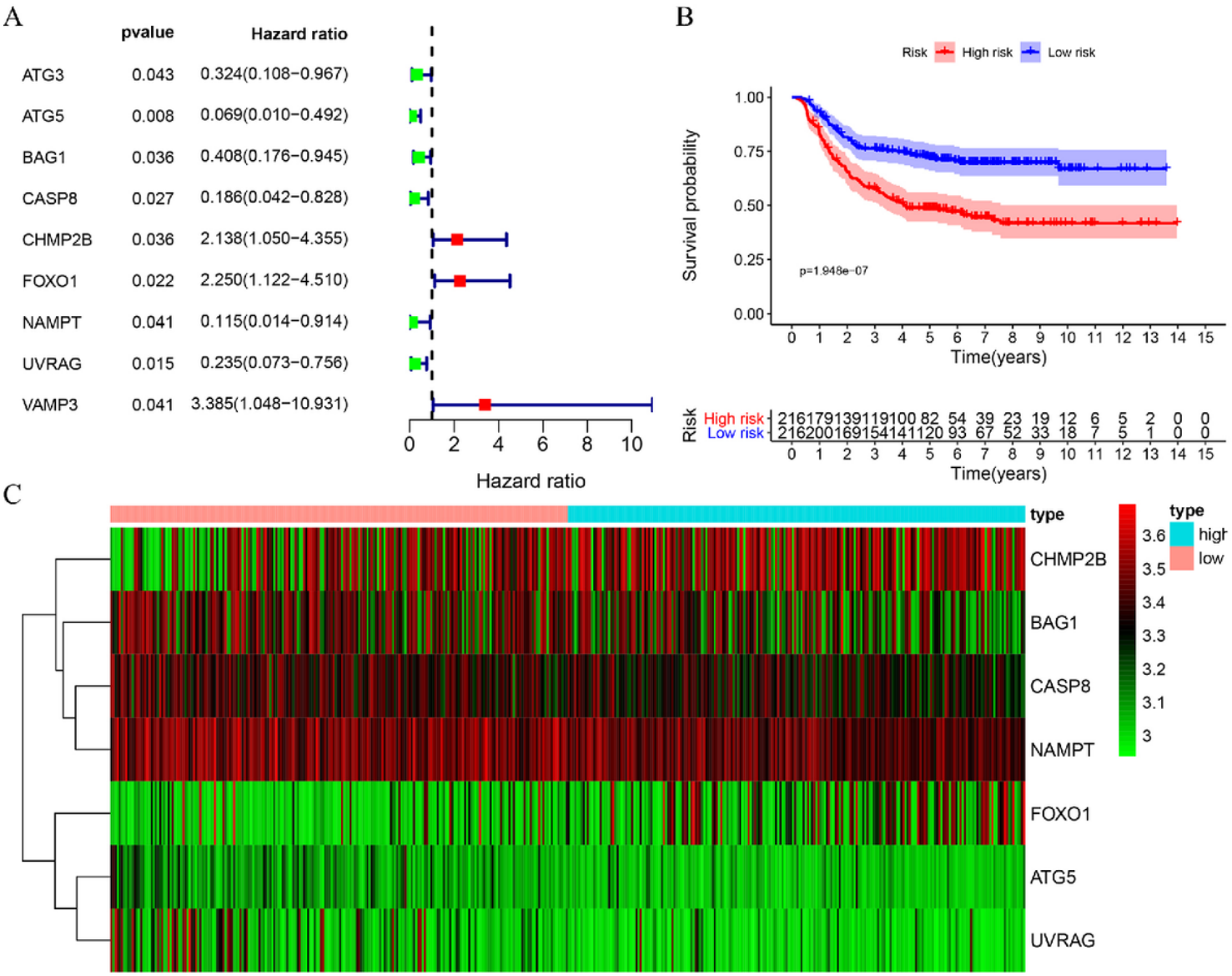


Figure 5

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.

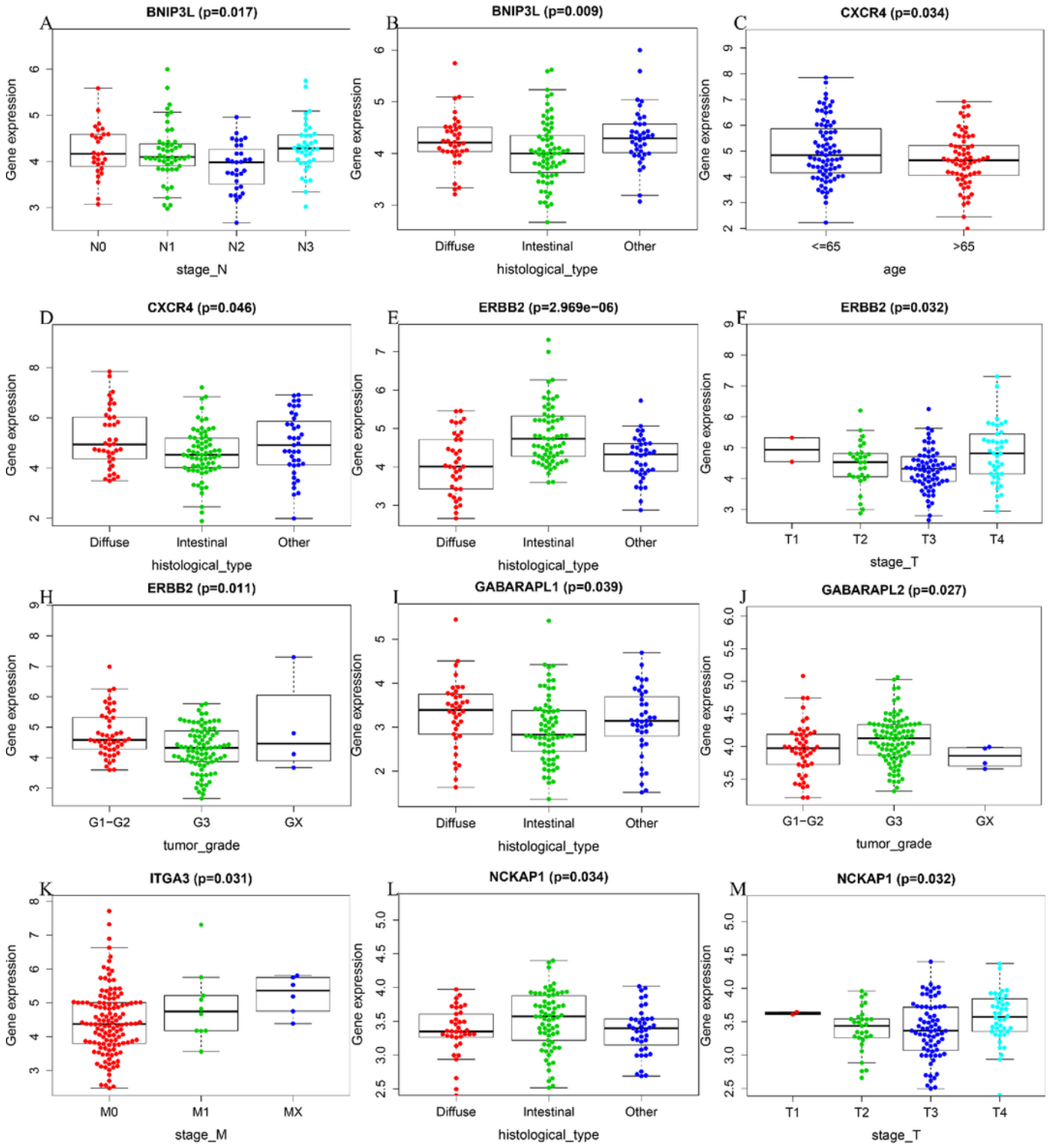


Figure 6

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.

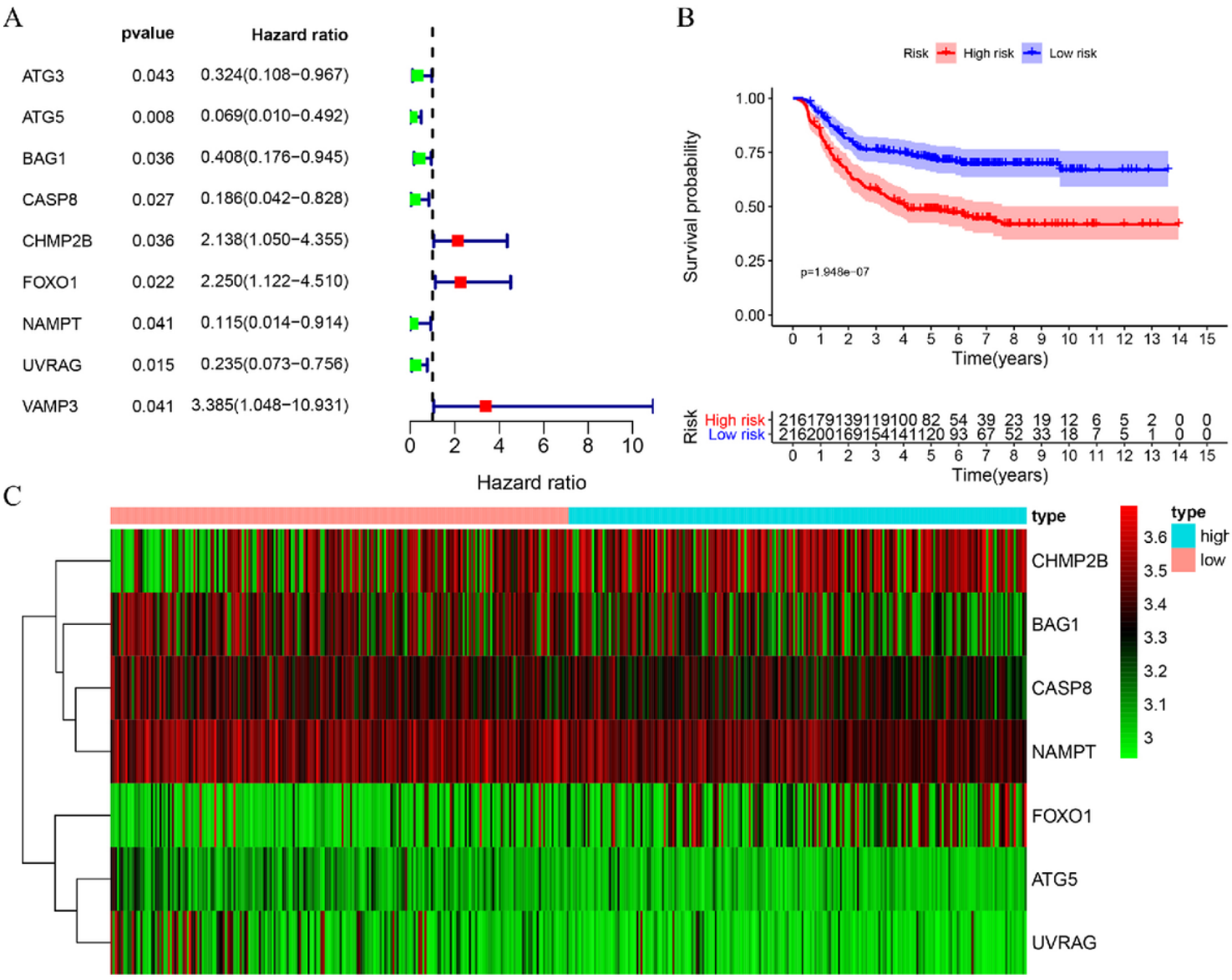


Figure 7

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.