

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 the use of fluorouracil and cisplatin is one of the most encountered clinical problems resulting from post operation and gives rise to poor prognosis in patients diagnosed with gastric cancer (GC).

Methods, this study aims to combine autophagy-related genes (ATGs) to provide an investigation of the susceptibility of victims with gastric malignancy to postoperative chemotherapy. Based on the TCGA database, gene expression data for GC patients undergoing and are using chemotherapy were integrated and analyzed. Prognostic genes were screened based on univariate and various analysis regression models. Subjects were divided into those with high and low-risk groups. This was analyzed by the utilization of the median risk score approach. The product limit estimator method had to be used to evaluate the OS and DFS. The accuracy of the prediction was resolved by the subject curve analysis. In addition, the carrying out of proper analysis 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, which can predict ATGs related to chemotherapy susceptibility of GC patients, and provide better individualized treatment regimens for clinical practice.

Introduction

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

Autophagy is an important process of eukaryotic turnover of intracellular structures and components ^[3]. Problems in a physiological imbalance in some processes of autophagy can lead to various diseases and ailments, such as cancer ^[4]. It has some significant pathophysiological processes with regard to some malignancies ^[5-7]. For instance, Beclin1 gene has some association with autophagy, and its expression is high in gastric cancer tissues, but not expressed or low in non-gastric cancer tissues ^[8]. Glutamine decomposition provides energy for tumor cells, autophagy activation can also help in abnormal glutamine decomposition, growth promotion and metastasis of gastric cancer cells^[9].LC3 is widely used

as a biomarker for autophagosome, high expression of LC3 was detected in 58% gastric cancer cells, but no expression was observed in normal gastric epithelial cells^[10]. P62/SQSTM1, a characteristic substrate of ubiquitin-protein in autophagy, was upregulated more significantly in gastric cancer specimens than in normal gastric mucosa^[11] and the interpretation of P62/SQSTM1 has some adverse clinical outcomes of the ailment^[12]. 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 induce and enhance autophagy. This induction of autophagy is a survival mechanism and contributes to the development of acquired drug resistance. Autophagy can inhibit the apoptosis of 5-FU-induced MGC803 in gastric carcinoma cells^[13]. The apoptosis of MGC803 induced by oxaliplatin can be inhibited^[14]. Aquaporin 3(AQP3) promotes the resistance of gastric cancer cells AGS to cisplatin through autophagy^[15]. Presently, chloroquine(CQ) and hydroxychloroquine [HCQ] are the only drugs used for autophagy analysis^[16]. A combination of CQ and chemoradiotherapy has been used for glioblastoma management, and it has been found that the median survival time was more than double compared with the control group^[17,18]. Preoperative treatment with HCQ combined with gemcitabine resulted in serum reduction, tumor markers and oncoantigen CA19-9 in 60% of patients with pancreatic adenocarcinoma^[19].

Some relevant work has demonstrated that activation has 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. Based on this, our study screened genes related to autophagy to predict the prognosis of chemotherapy in gastric cancer patients by means of bioinformatics. This model is helpful for clinicians to develop chemotherapy regimens with more individualized characteristics and serve patients well 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. To analyze the relationship between ATGs and chemotherapy sensitivity in the TCGA cohort was incorporated. The incomplete clinical information was excluded. The GSE26253 gene expression profile was downloaded from the GEO database, of which 432 patients were treated with fluorouracil. R 4.0.2 software was used to process and analyze the raw 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 in TCGA database were calculated using limma R package. P-value < 0.05 and at least a twofold change was identified as DEGs. Volcanic were utilized to visualize the results. To explore the main biological characteristics of ATGs related with chemotherapy. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of ATGs which were differentially expressed in the chemotherapeutic group and were performed using a 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. Multivariate Cox regression analysis was then performed to establish a prognostic model of ATGs. 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, this study performed survival analysis based on risk score using Kaplan-Meier method. To investigate whether the autophagy-related risk index in the TCGA dataset could be 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/>). Differences for which the nominal $P < 0.05$ and the FDR < 0.25 were considered statistically enriched.

Results

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

Data of 407 subjects in the STAD cohort from the TCGA database was analyzed. Two hundred and thirty-two ATGs was obtained for our study. A total of 221 ATGs were expressed in TCGA-STAD cohort. We ultimately obtained 157 patients who received chemotherapy and 32 normal samples. The basic clinical

characteristics of these patients in the TCGA database was also compared, 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 following ATGs were downregulated: PRKN, CDKN1A, GRID2, HSPB8, NRG3, NRG2, FOS and NKX2-3.

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

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

2.2 Enrichment of ATGs

We utilized some techniques to analyze and to explore the possible signaling pathways associated with chemotherapy response in GC. Based on GO analysis, the difference in the cellular morphology, neuron death, AGT's regulation to cellular membranous surfaces was studied, autophagy, (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

There were 221 ATGs and were analyzed by some analytical methods. Thirteen ATGs had prognostic measures of subjects with chemotherapy in TCGA-STAD cohort (Figure3). 9 ATGs were finally also tabulated and pinpointed, Table 2.

Table 2
Multivariate Cox regression analysis of prognostic genes.

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

2.4 ATGs and the OS of GC victims in chemotherapeutic group

A risk score was calculated based on ATGs associated mRNA expression levels and risk factors. Patients were classified according to their relevant groups. The product limit estimator analysis tool was utilized for data presentation. Survival rates over a five-year period were analyzed, (Figure 4A). A ROC curve was drawn and plotted to determine the ability of ATGs prediction for patients in chemotherapy group (Figure 4B). The area under the curve was well interpreted. Gene study, was well pointed out during the study progression, (Figure 4C), with an increment in the number of deaths, (Figure 4D). Heatmaps were created for both groups, (Figure 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, was carried out. Similarly, a significant correlation between risk scores and clinical variables was by the utilization of hazard ratio technique sketch diagrams, (Figure 5A). Several Cox regressions factors affecting the prognosis of gastric cancer victims undergoing chemotherapeutic administrations, were well plotted, ((Figure 5B). Furthermore, results showing a comparison un the two groups were plotted, (Figure 5C). Cancer pathways were enriched, suggesting autophagy is involved in the regulation of chemotherapy for high-risk gastric cancer patients.

2.5 ATG's progression in gastric cancer

ATGs immediate affection and its the progression in gastric cancer, correlation between 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. BNIP3L, CXCR4, ERBB2, GABRAPL and NCKAP1 significantly correlated with Lauren typing. ERBB2 and GABRAPL also significantly correlated with tumor grade. BNIP3L, ERBB2, ITGA3 and NCKAP1 on the other hand significantly correlated with TNM staging.

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

GC patients undergoing chemotherapy, with regard to certain types of biomarkers had their data obtained. GSE26253 dataset was incorporated. . After univariate Cox regression analysis, 9 ATGs had some significant relation, (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 summarized GC patient's situation undergoing chemotherapy.

Discussion

GC is a challenge and has brought about some well significant economic burdens world-world as far as its treatment costs are concerned. Cisplatin as well as fluorouracil - based drug resistance is the leading cause which leads to poor prognosis^[20]. The processes in GC particularly autophagy is ancient, and

regulates cellular mechanisms and homeostasis^[21]. Several researches demonstrated this process is related to some proteomics and chemotherapeutic resistance in GC victims^[22, 23]. Studies have found that gastric cancer cells with enhanced resistance to chemotherapy have enhanced autophagy, 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 chemotherapy after surgery was analyzed. We also studied the biological function and role of ATGs in GC.

First and foremost, ATGs in the GC chemotherapeutic group and the normal stomach, have dependent interaction which was identified in our study. Furthermore, some analysis revealed ATGs were differentially enriched in platinum resistance. Research has demonstrated that a combination of inhibitors in GC improves 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 a data set of 9 genes and calculations were done accordingly.

It has been found that in pancreatic cancer, combined with the autophagy inhibitor chloroquine, the phosphorylation levels of ERK and STAT3 can be reduced by inhibiting the CXCL12/CXCR4 signaling pathway, 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 phagosome-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, prognostic characteristics of DFS were established based on GEO database.

In summary, 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 inevitably has some limitations. Although internal verification has been conducted, further experiments are needed for verification and confirmation.

Declarations

Acknowledgements

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. LXL, Paul and YY performed gene differential analysis and survival analysis using GEO and TCGA data. MYL and BYP Collected and analyze immune related information. LXM, GBH and QWB 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

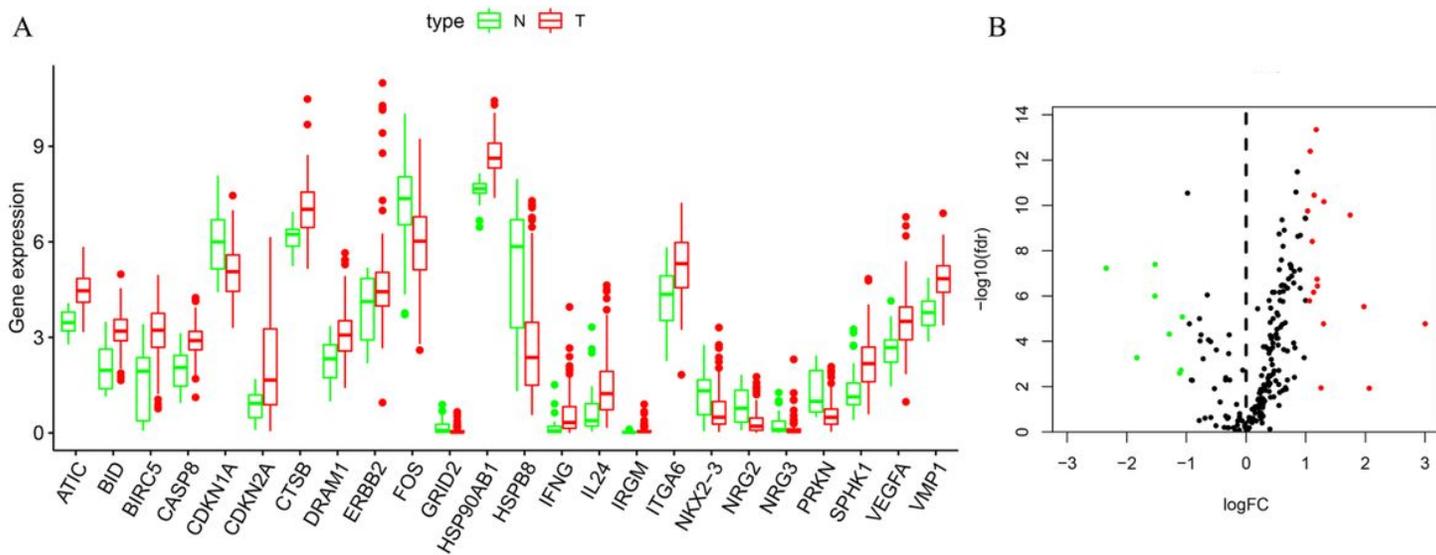


Figure 1

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.

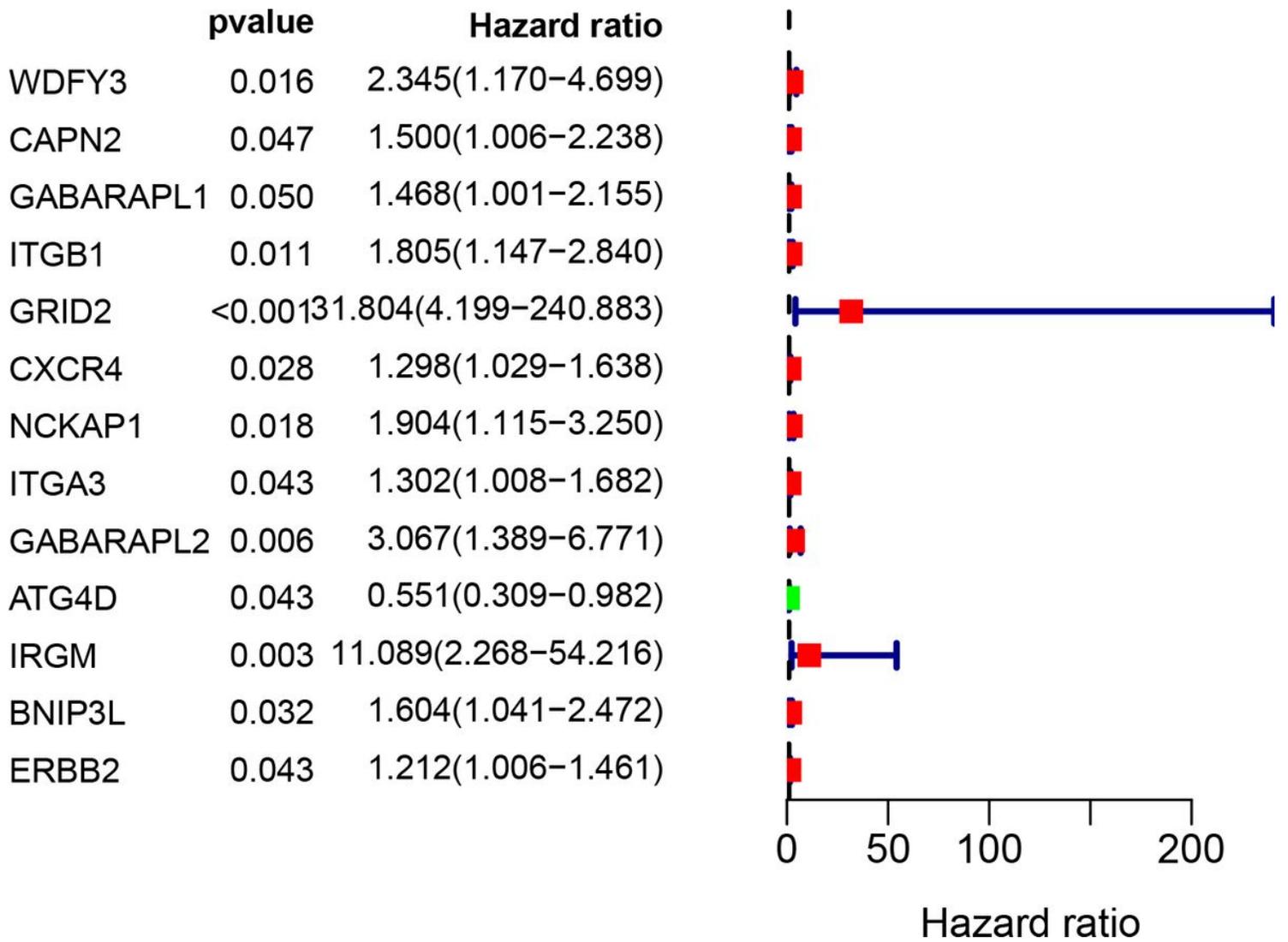


Figure 3

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

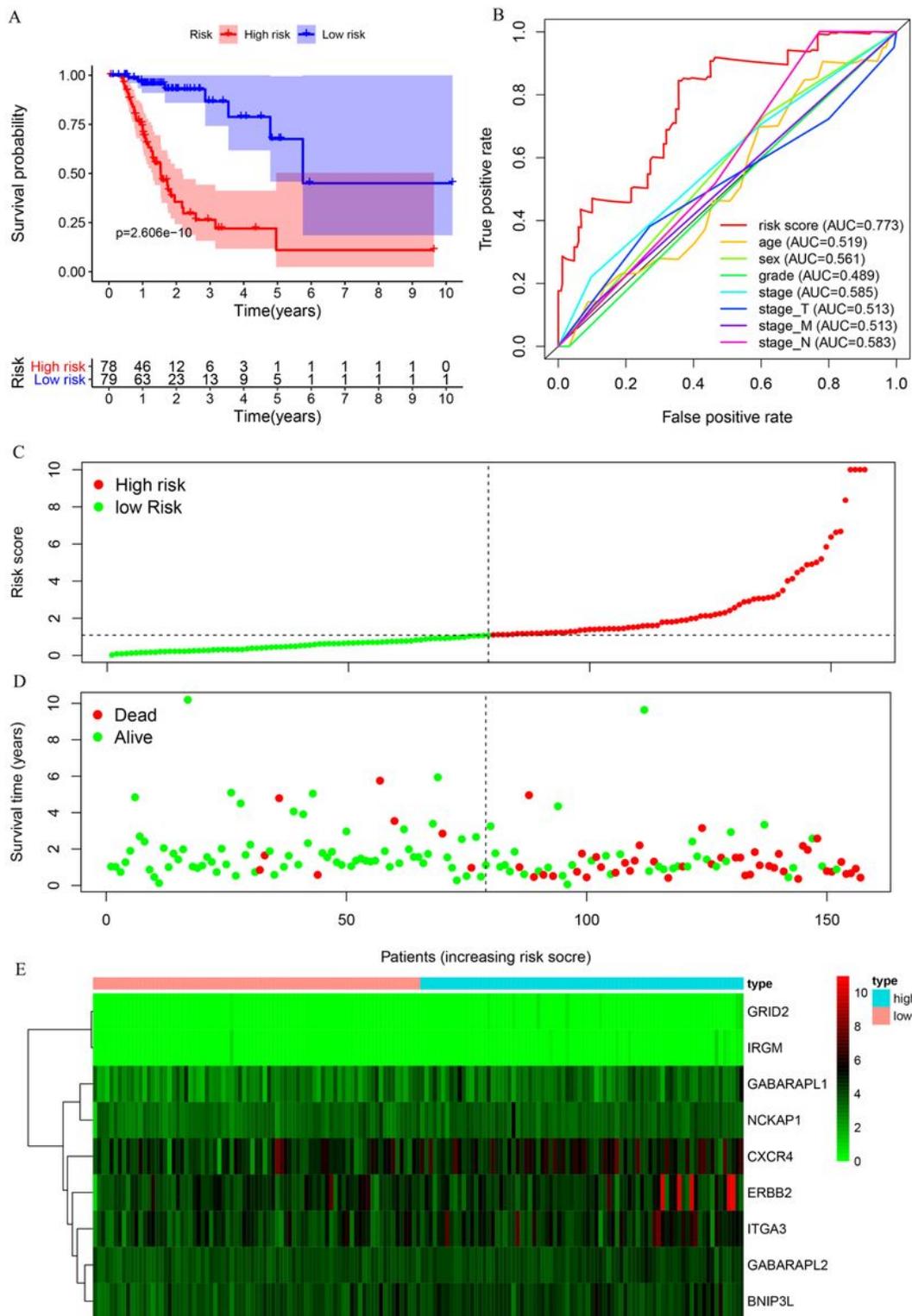


Figure 4

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.

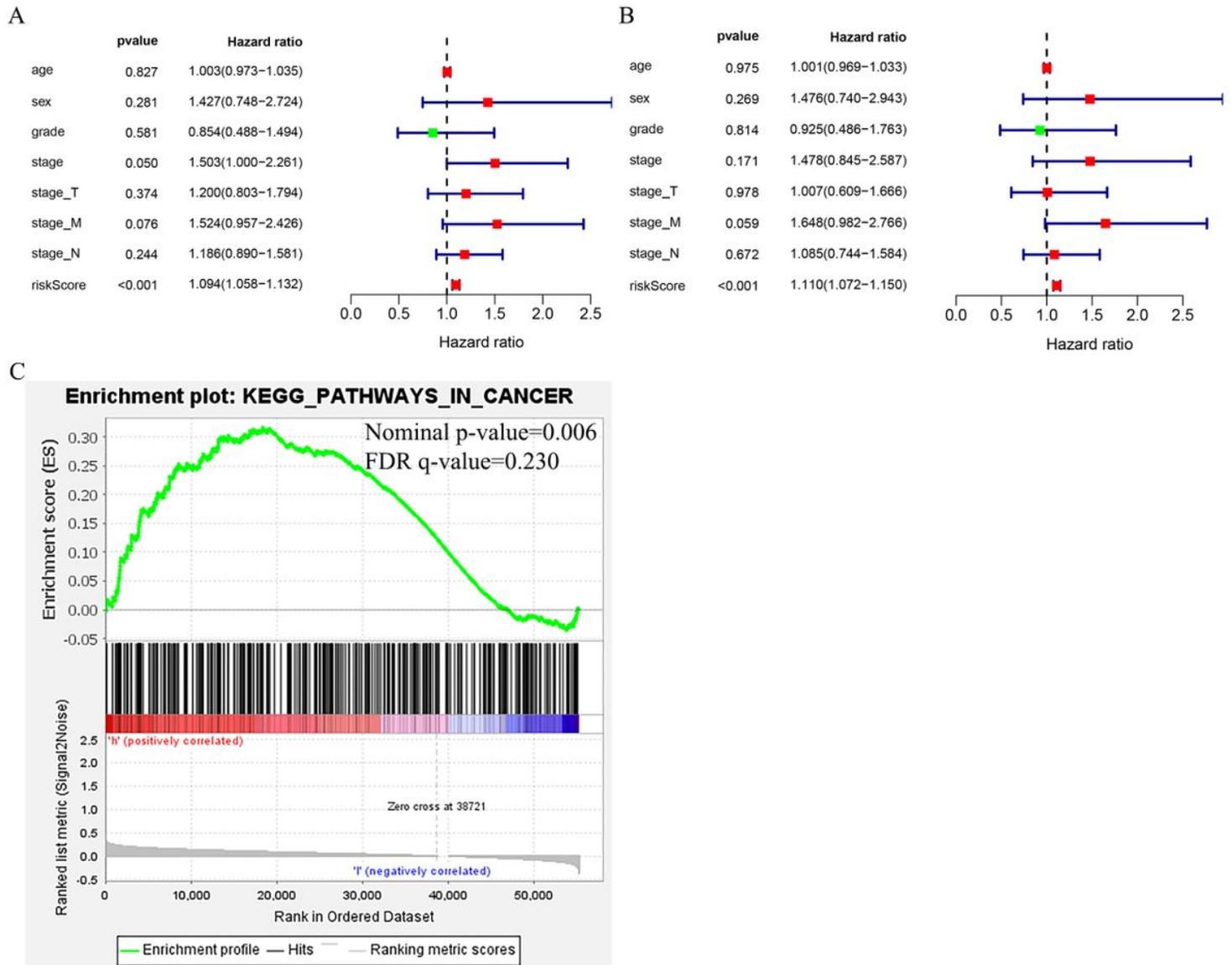


Figure 5

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.

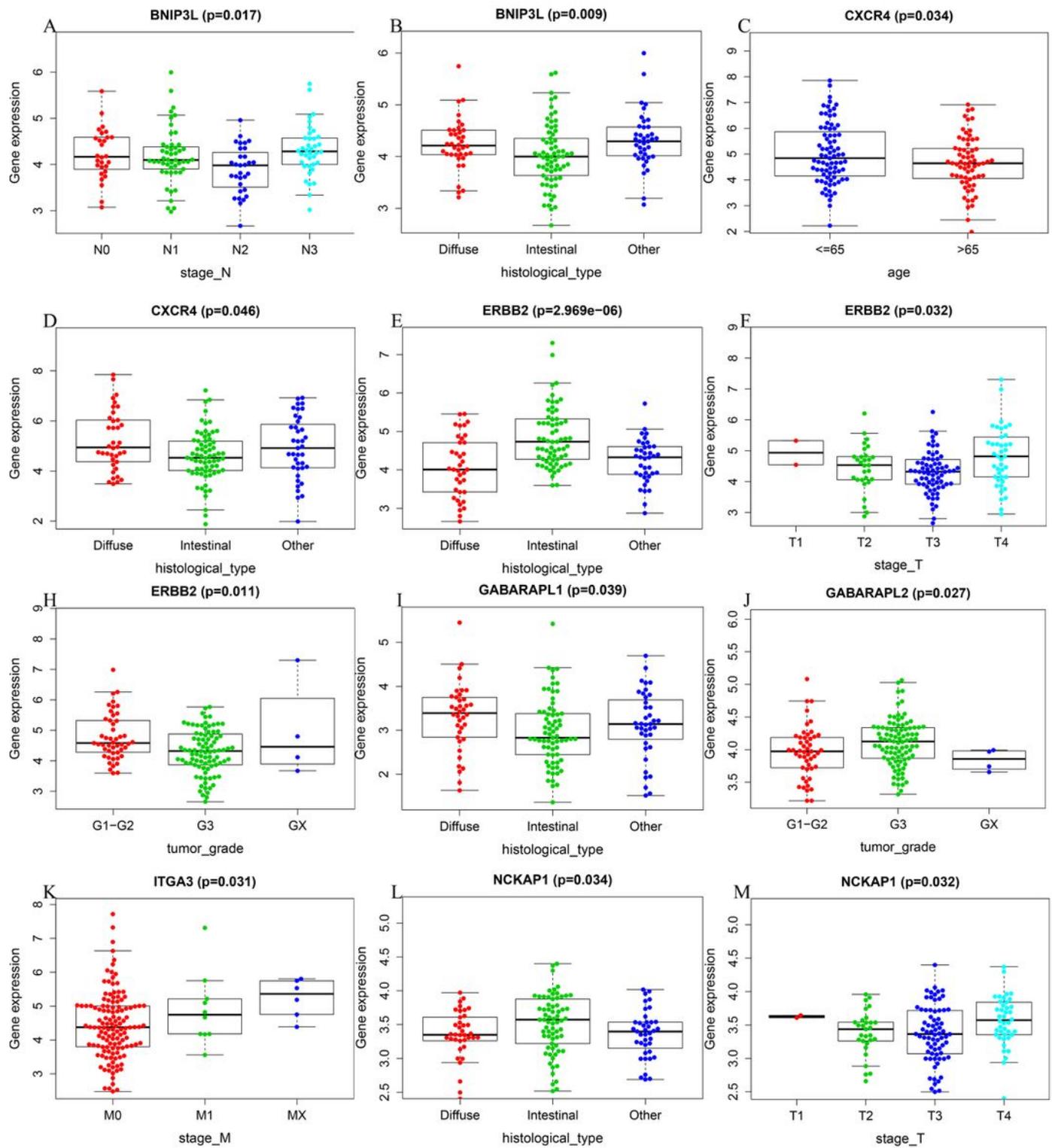


Figure 6

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.

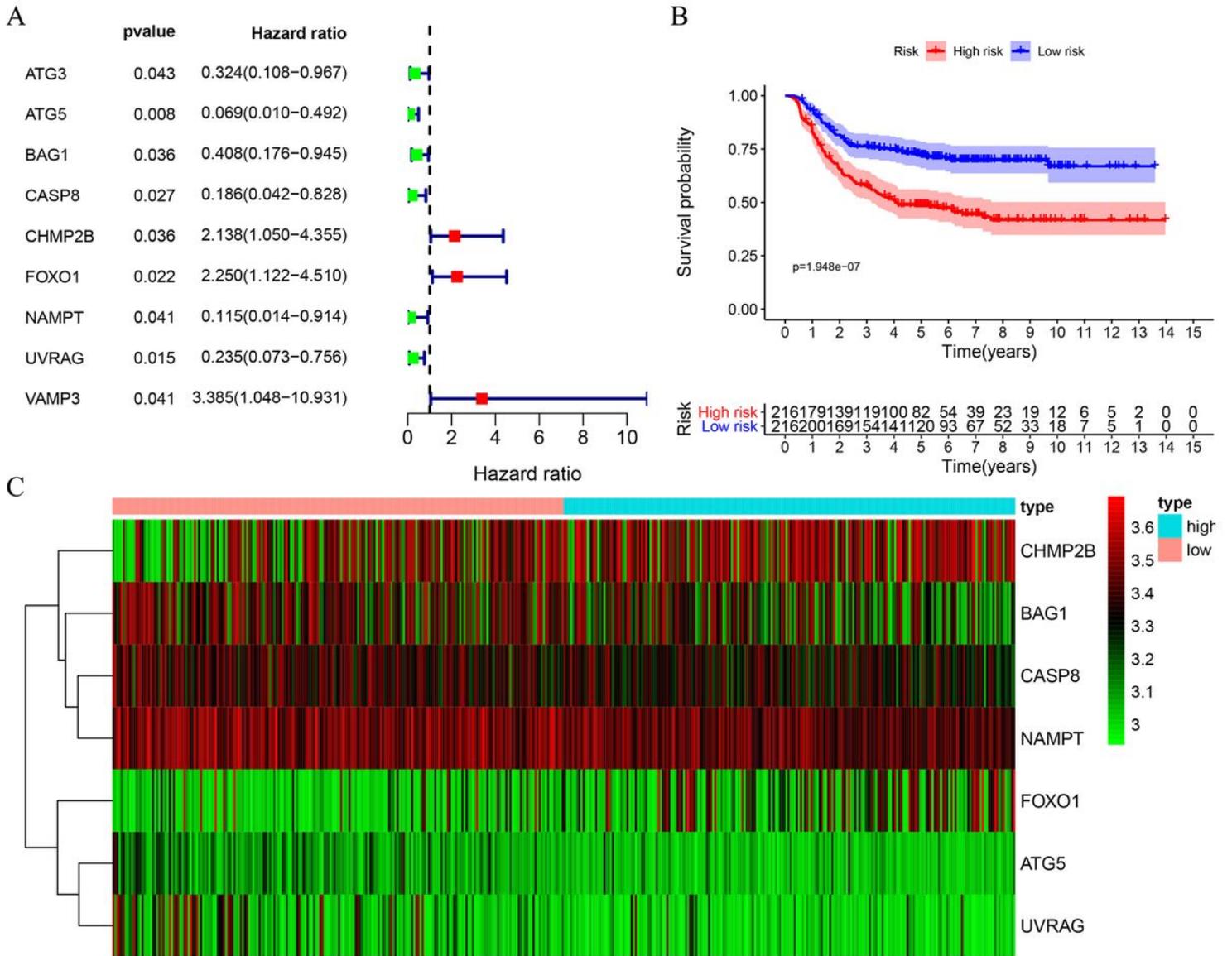


Figure 7

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.