An Independent Prognostic Model Based on Ten Autophagy-Related Genes in Pancreatic Cancer

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Research Article

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Abstract

Purpose

Pancreatic cancer(PC) is a common cancer with high lethality and low survival rate. Autophagy is involved in the biological process of PC. Thus, we intended to explore the function of autophagy-related long noncoding RNA signature for survival assessment in PC.

Methods

Based on 10 autophagy-related lncRNAs, the prognostic model was attained through univariate and multivariate Cox regression analysis. Subsequently, the relationship network of 10 lncRNAs was crystallized in co-expression network and Sankey diagram. Survival analysis and ROC curve were used to evaluate the signature. GSEA was utilized to screen enriched gene sets.

Result

The OS has significant deference in low-risk group and high-risk group($P < 0.001$). The ROC curve further proved the potential utility of the signature($AUC = 0.815$). GSEA was significantly enriched in cancer-related gene sets.

Conclusion

The signature has potential to evaluate clinical prognosis in PC. The 10 autophagy-related lncRNAs may achieve great development for PC in target therapy field.

1. Introduction

Pancreatic cancer(PC) is a type of the highly malignant cancer of the digestive system with only 8% incidence rate in 5 years, leading the 11^{th} most common malignant tumor[1, 2] . Common detection methods leads to poor early diagnosis of pancreatic cancer due to its insidious onset[3]. Currently, surgery is the only treatment available for pancreatic cancer, However, because of the delayed diagnosis, patients are often characterized by poor prognosis[4, 5]. Although the striking advances of these technologies for screening and testing pancreatic cancer has been witnessed, it still has limitation for complete eradication of tumor. It is naturally imperatives to explore potent therapeutic targets and emerging prognostic biomarkers for better diagnose and treat PC[6].

Autophagy is a cellular catabolic mechanism to maintain cellular homeostasis through degrade cytoplasmic metabolites[7, 8]. Autophagy involves in the development of many cancers. Upon different stage and tumor type, autophagy may prevent or improve cancer growth[9, 10]. On the one hand,

autophagy may keep cells from harmful substances in the early stage of tumorigenesis. On the other hand, adaptive metabolic response of autophagy provides nutritional conditions for tumor progress under unfavorable environment[11].Recently, research have conducted on autophagy pathways for its promising application in targeted anti-cancer field[12].

Long non-coding RNAs (lncRNAs) are transcripts with a length of more than 200 nucleotides without protein coding function. LncRNAs have involved in biological processes as an important role, such as proliferation, differentiation, apoptosis, invasion, and metastasis of cancer[13, 14].Recently, growing evidence indicated that lncRNAs primarily participant in the occurrence and development of cancer, which may related to its potential of regulating autophagy[15, 16].Thus, developing prognostic biomarker related to autophagy for PC patients may be helpful in treatment and prognosis of PC.

Consequently, autophagy-related lncRNAs may be developed in the aspect of targeted therapy and novel diagnostic biomarkers for PC. Hence, this research devoted to construct an autophagy-related lncRNAs model of PC and to better the prognostic evaluate and targeted treatment in PC.

2. Results

2.1 Construction of a co-expression network for autophagy–lncRNAs

A total of 182 lncRNAs were download from TCGA, a total of 232 autophagy-related lncRNAs were download from the HADb (Human Autophagy Database, http://www.autophagy.lu/).

2.2 Establishment of prognostic model on autophagy-related lncRNAs

28 autophagy lncRNAs used for survival assessment were filtered through univariate Cox regression analysis (P<0.01, table1). Besides, we further screened out 10 prognostic autophagy lncRNAs on the basis of the above 28 autophagy lncRNAs by the analysis of multivariate cox, in which 4 lncRNAs were poor prognosis factors (AC245041.2, AC036176.1, LINC01089 and LINC02257) and 6 lncRNAs were beneficial prognosis factors (FLVCR1-DT, AC006504.7 , AC125494.2, AC012306.2 ,ST20-AS1 and AC005696.1)(Table2).

Accordingly, the co-expression network for the 10 lncRNAs were established to clarify the interaction between the autophagy genes and prognostic related lncRNAs (FIGURE 1). According to the result of Sankey diagram, the association with autophagy related gene, prognostic related lncRNAs and risk types were showed in FIGURE 2. Kaplan-Meier survival curve further indicated that 10 lncRNAs were closely related to the prognosis of PC (P<0.001, FIGURE 3).

2.3 Evaluation impact of the prognostic autophagy-related lncRNAs model

Risk model of prognostic autophagy-related lncRNAs was established based on the risk score. Patients were divided into two group including high-risk group and low-risk group. Patients with higher overall survival (OS) in the low-risk group were better illustrated through the risk curve and scatterplot (FIGURE

4A and 4B). The heat map of 10 differentially expressed prognosis lncRNAs were visualized in FIGURE 4C. Furthermore, KM survival analysis showed that the low-risk group had better prognostic impact than the high-risk group (P=2.527e-11, FIGURE 5A).

The ROC curve was demonstrated in FIGURE 5B to evaluate the diagnostic value of risk model,

The AUC value for autophagy-related lncRNAs was 0.815 showed that risk model had potential evaluation value in PC prognosis (FIGURE 5B).

2.4 Correlation analysis of clinical characteristics and risk models on PC

To determine whether the risk model was an independent prognostic factor for PC survival analysis, the univariate and multivariate Cox regression analyses were showed in FIGURE 6, both were revealed that risk score could be an effective prognostic factor (univariate regression:

HR=1.406,95% CI=1.295-1.526, P < 0.001, multivariate regression: HR=1.422,95% CI=1.298-1.558, P<0.001). Following that, the detail of clinical factors was showed in Table 3, including age, Gender, Stage, and tumor-node-metastasis status. There existed significantly difference in risk score.

2.5 Gene Set Enrichment Analysis

According to the analysis of GSEA, the deferentially expressed genes were screened out. There were 7 lncRNAs up-regulated in high-risk groups at FDR< 0.05 and nominal P-value < 0.01.Several sets including cell migration,ZEB1 targets, EGFR signaling, lin silenced by tumor microenvironment,CDH1 targets and et.al., which are all closely linked to cancer. Moreover, it also provides potential possibility in diagnosis and treatment of PC.

3. Discussion

Pancreatic cancer(PC) is an aggressive cancer worldwide with leading morality approaching to its morbidity rate despite advanced treatment techniques got development[17, 18]. Recent study mainly focused on biomarkers used for targeted therapies in PC, such as lncRNAs, which may drive customized treatment and optimize the therapeutic effect[19-21]. Autophagy has positive and negative regulatory effects on PC based on setting and stage [22].Emerging evidence proved that lncRNAs may serve as prognostic and diagnostic biomarkers during the initiation and development of PC[21, 23]. Chandra et al. discovered that lncRNA HULC participate in the Wnt/β-catenin signaling pathway in pancreatic cancer cells and could be qualified as an effective biomarkers for the diagnose of PC[24]. However, the selection of autophagy-related lncRNAs to assess the prognosis in PC has little reported. Consequently, we develop a risk model to evaluate survival of PC patient through screening autophagy-related lncRNAs.

Based on the formation of co-expression network, autophagy-related lncRNAs for survival assessment in PC were subsequently selected. Secondly, 10 autophagy-related lncRNAs including AC245041.2, AC036176.1, LINC01089, LINC02257, FLVCR1-DT, AC006504.7, AC125494.2, AC012306.2, ST20-AS1 and

AC005696.1 were obtained through Cox regression. Whether the 10 risk factors can be used for prognostic assessment with PC awaits further exploration.

Eight autophagy-related lncRNAs were involved in the prognosis of cancer, including AC245041.2, AC036176.1, LINC01089, LINC02257, FLVCR1-DT, AC125494.2, AC012306.2 and ST20-AS1. Regarding the remaining AC006504.7 and AC005696.1, there were no detailed reports on their risk assessment role played in cancer. (1) Strongly correlated lncRNA AC245041.2 and mRNA LAMA3 are relevant to KRAS mutation which may indicate poor prognosis of PC[25]. Cao et al. screened out multiple autophagy-associated lncRNAs including AC245041.2 based on bioinformatic analysis to develop risk model for prognostic analysis in PC[26]. (2) AC036176.1 as ferroptosis-related lncRNA may be a prognostic biomarker in PC[27]. (3) LINC01089 was proved to serve an influential factors in cancer[28]. (4) LINC02257 were highly expressed in colorectal cancer and seem to perform as hazard Factor [29]. Xu et al. spotted that LINC02257 as adverse lncRNA and FLVCR1-DT as favorable lncRNA a were utilized to monitor prognosis in PC after model-based construction [30]. (5) AC125494.2 were also reported as beneficial factors for PC survival assessment[26]. (6) Shen et al. predicted that AC012306.2 a is positively correlated with the occurrence of cervical cancer[31]. (7) The immune-related lncRNA ST20-AS1 model was established in patients with anaplastic gliomas[32].

Ten autophagy-related lncRNAs were filtered to develop risk signature for prognosis identification in PC. Univariate Cox and multivariate cox were further illustrated the reliablity that the ten autophagy-related lncRNAs perform as prognosis factors to predict overall survival in the model. Subsequently, on the basis of co-expression network and sankey diagram, the relationship between mRNA and lncRNA were visualized. The AUC value was 0.815 which illustrate the feasibility of model prediction in PC.

GSEA analysis suggested that the gene set was primarily centered on pathways associated with tumor progression, metastasis and cell migration. Take two crucial gene sets Zeb1 and EGFR for illustration.Zeb1 is identified as one of the key EMT genes and its overexpression is linked to tumor metastasis[33].EGFR is receiving extensive research in the field of targeted anti-cancer therapy. EGFR signaling downregulation via CDF compounds upregulating miR-146a may provide a new therapeutic option against PC[34]. EGFR associated with resistance to conventional cancer therapy, drug resistance from EGFR-targeted therapy can be attenuated by autophagy inhibition and thus become a new tumor treatment [35].

There are some inadequacies in this study. (1) The database is single-source and the study data is limited to 182 cases, and the clinical features are also incomplete. (2) Additional validation research is required to ensure the assessment potential of the prognostic model, such as independent cohort. (3) This study only performed data analysis and lacked basic experiments to validate the explicit function of autophagy-related lncRNAs in disease.

4. Methods

4.1. Collection of datasets and clinical information with PC

The 470 RNA-seq and 472 clinical data of patients from the TCGA website (https://portal.gdc.cancer.gov/) were acquired. Thus, 176 patients of PC with complete clinicopathological information were screened.

4.2. Selection of autophagy-Related Genes in PC

The autophagy genes(ATGs) were obtained from the Human Autophagy Database(http://www.autophagy.lu/index.html).The lncRNAs expression were normalized by log2 transformation using the edgeR package. Pearson's correlation test was conducted to screen lncRNAs that related to autophagy genes. The correlation coefficient $|R^2|$ >0.4 and P < 0.001 as the criteria to identify the autophagy-related lncRNAs. Finally, use Cytoscape software(version 3.8.2) to construct the co-expression networks.

4.3. Construction of the prognostic model

Single variable Cox was used to identify autophagy-related lncRNAs with prognostic value(P<0.01). Then, multivariate Cox was performed to optimize the risk model, nine lncRNAs were included into the formula to assess the prognosis of PC patients.

Risk score= $\sum$β*(expression of lncRNAs)

“β” is the regression coefficient for each gene.

Patients were split into two subgroups (low-risk groups & high-risk groups) based on the median risk score. Kaplan-Meier survival curve were analysis to compare survival differences between subgroups.

4.4. Feasibility of the risk model used for clinical evaluation

Firstly, to examine independent predictive ability of the risk score and clinical factors, including univariate and multivariate Cox regression analyses were conducted. Subsequently, receiver operating characteristic (ROC) curves were applied to detect the validity of risk system by comparing the area under the curve(AUC).

4.5. Functional Analysis

Gene set enrichment analysis(GSEA, http://www.broadinstitute.org/gsea/index.jsp) was used to screen deferentially expressed genes. The purpose of the method is to analyze whether gene sets are significantly different and enriched between high and low expression groups in the process of autophagy.

5. Conclusion

In conclusion, ten autophagy-related lncRNAs were included to determine a prognostic signature for PC patient. These prognostic factors in the signature may provide new prospects for targeted therapy and clinical evaluation of PC.

Declarations

Acknowledgements

We express our sincere gratitude to all contributor who provided data, we appreciate that the TCGA database is available for free download and use.

AUTHOR CONTRIBUTIONS

TJ: Experimental design, data process, manuscript preparation. ZZ: Data analysis and revision. FX and FC: Format modification. WY: Article revision and research design.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Tables

Table 1 .uniCox analysis of 28 autotroph-related lncRNAs.

gene	KM	B	SE	HR	HR.95L	HR.95H	pvalue
FLVCR1-DT	<0.001	-1.049	0.273	0.350	0.205	0.598	<0.001
AC064836.2	<0.001	-0.512	0.141	0.599	0.454	0.790	<0.001
LINC01004	<0.001	-0.401	0.105	0.670	0.546	0.823	<0.001
AC245041.2	<0.001	0.201	0.051	1.222	1.106	1.350	<0.001
AC142472.1	<0.001	-0.983	0.265	0.374	0.223	0.629	<0.001
AC006504.7	<0.001	-0.816	0.196	0.442	0.301	0.649	<0.001
AC125494.2	0.001	-1.299	0.327	0.273	0.144	0.518	<0.001
AC012306.2	<0.001	-0.666	0.155	0.514	0.379	0.696	<0.001
ST20-AS1	<0.001	-1.629	0.412	0.196	0.087	0.440	<0.001
PTOV1-AS2	<0.001	-0.196	0.055	0.822	0.739	0.915	<0.001
AC036176.1	<0.001	-0.677	0.191	0.508	0.349	0.739	<0.001
U62317.1	<0.001	0.112	0.033	1.119	1.048	1.194	0.001
AC005332.3	<0.001	-0.307	0.069	0.736	0.643	0.842	<0.001
AC127024.5	<0.001	-0.661	0.156	0.516	0.380	0.701	<0.001
AL513165.1	<0.001	-0.232	0.066	0.793	0.697	0.902	<0.001
AL022328.1	<0.001	-0.556	0.161	0.574	0.418	0.787	0.001
AL358472.2	<0.001	-1.245	0.299	0.288	0.160	0.518	<0.001
LINC01089	<0.001	-0.270	0.075	0.764	0.660	0.884	<0.001
AC005332.6	<0.001	-0.162	0.044	0.851	0.781	0.927	<0.001
AC005696.1	<0.001	-1.048	0.260	0.350	0.211	0.583	<0.001
AL122010.1	0.001	-0.619	0.149	0.538	0.402	0.721	<0.001
AC020765.2	0.001	-0.923	0.276	0.397	0.232	0.682	0.001
LINC02257	0.001	0.423	0.093	1.526	1.271	1.832	<0.001
AC005332.5	<0.001	-0.559	0.151	0.572	0.425	0.769	<0.001
AC090114.2	<0.001	-0.865	0.215	0.421	0.276	0.642	<0.001
LINC01705	0.001	0.103	0.026	1.108	1.053	1.167	<0.001
AC145207.5	<0.001	-1.057	0.269	0.347	0.205	0.589	<0.001
AL022328.4	<0.001	-1.150	0.293	0.317	0.178	0.562	<0.001

Table 2. multivariate cox regression analysis of 10 lncRNAs

id	coef	HR
FLVCR1-DT	-0.512	0.599
AC245041.2	0.261	1.298
AC006504.7	-0.568	0.566
AC125494.2	-1.199	0.301
AC012306.2	-0.540	0.583
ST20-AS1	-0.694	0.499
AC036176.1	0.406	1.501
LINC01089	0.258	1.294
AC005696.1	-0.578	0.561
LINC02257	0.277	1.319

Table 3: Correlation analysis of clinical characteristics and risk models on PC

Clinical	Group	*n*	Mean	SD	*t*	P
Age	<=65	87	1.674	1.651	-0.553	0.581
	>65	81	1.835	2.081		
Gender	Female	76	1.694	1.787	-0.364	0.716
	Male	92	1.799	1.939		
Grade	G1-2	118	1.614	1.599	-1.265	0.21
	G3-4	50	2.077	2.37		
Stage	Stage I-II	161	1.755	1.89	0.169	0.87
	Stage III-IV	7	1.667	1.317		
T	T1-2	28	1.186	1.439	-2.141	0.037
	T3-4	140	1.865	1.926		
N	N0	47	1.683	2.274	-0.259	0.797
	N1	121	1.778	1.693		

Figures

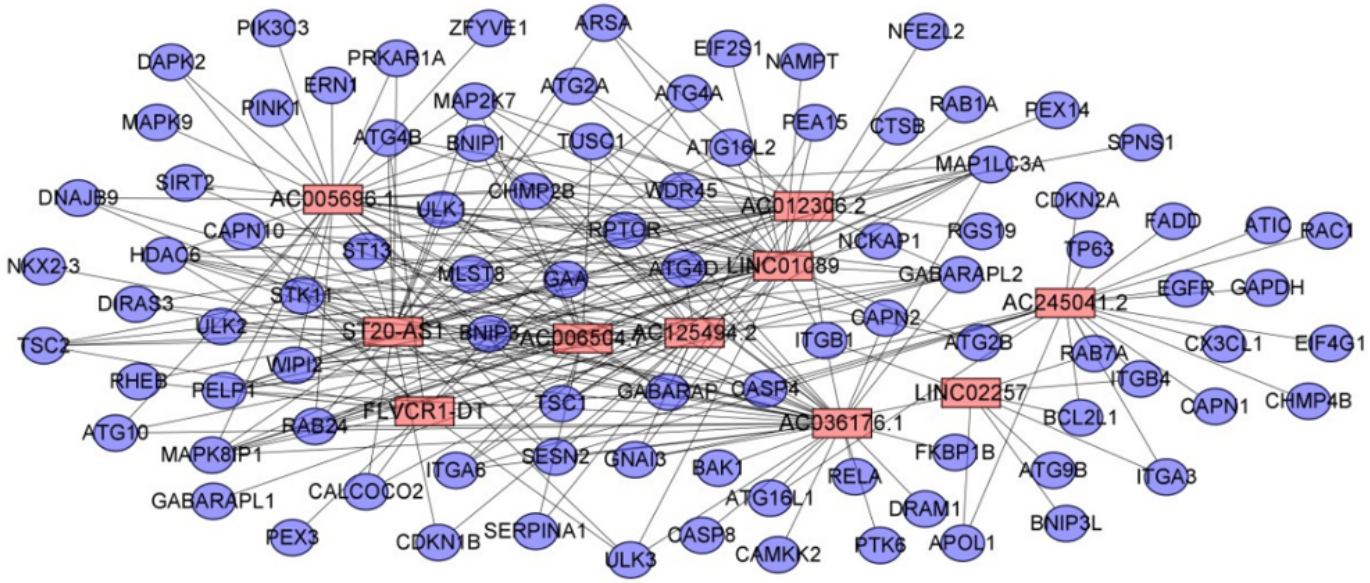

Figure 1

Co-expression network for autophagy related genes and ten independently diagnosed lncRNAs.The red rectangular nodes represent independently diagnosed lncRNAs. And the blue-purple round nodes represent autophagy related genes.

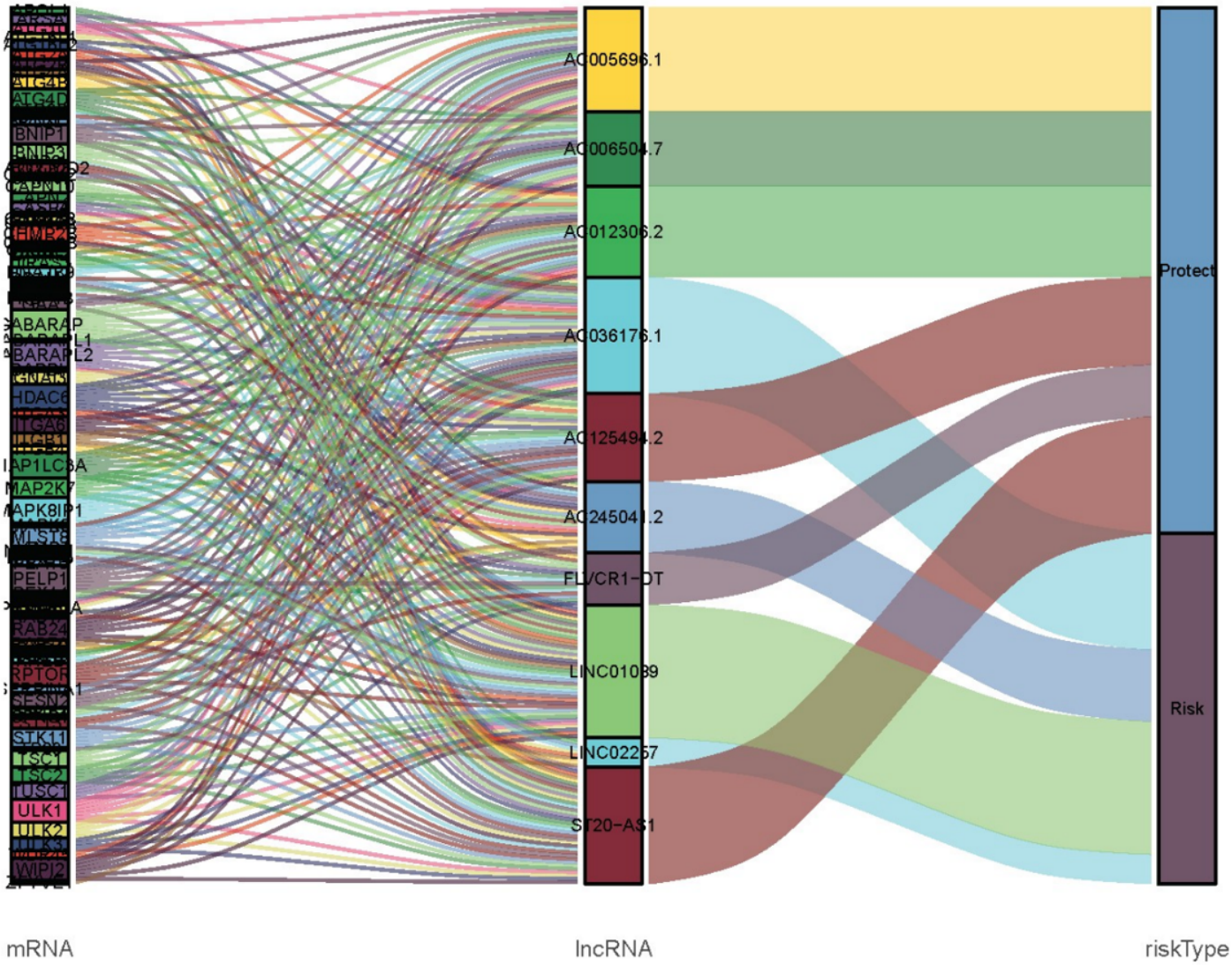

Figure 2

The relationship between autophagy related genes, independently diagnosed lncRNAs and risk types were demonstrated in Sankey diagram.

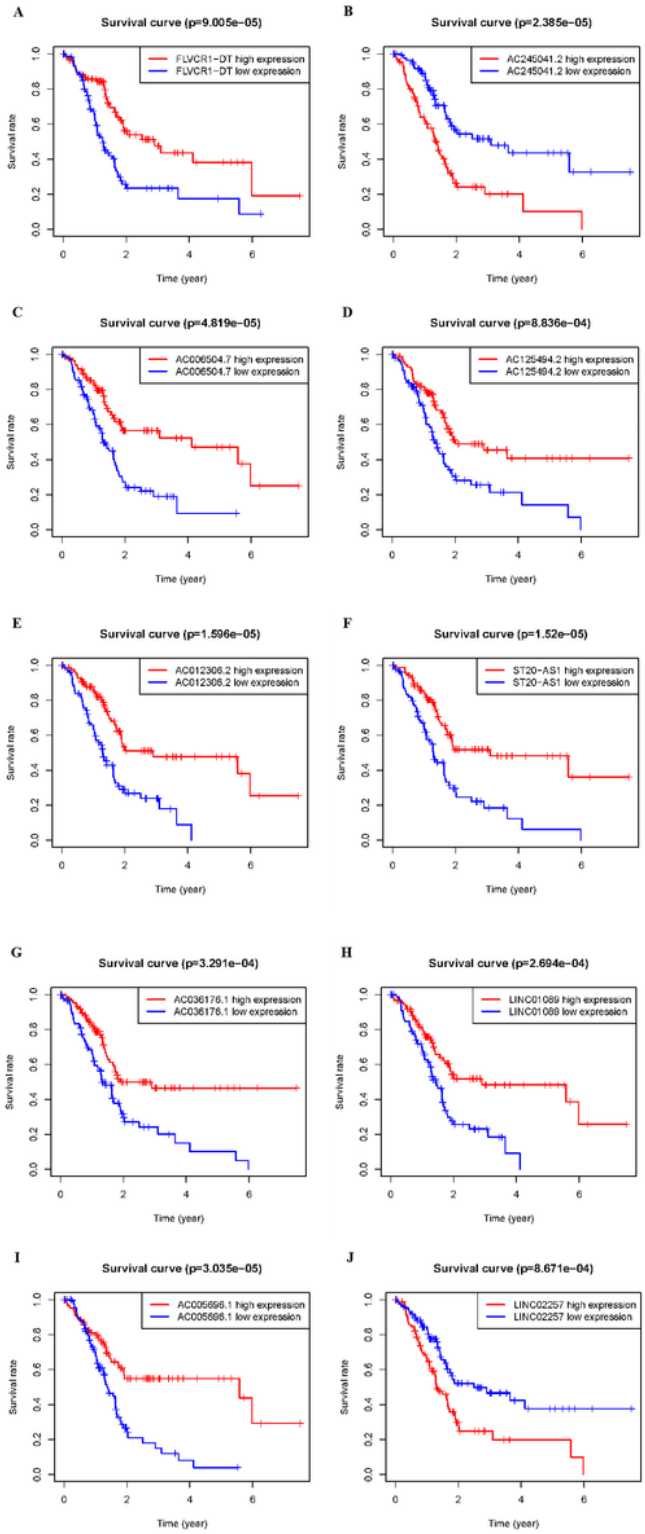

Figure 3

Kaplan-Meier survival curve of nine independently diagnosed lncRNAs in PC. 4 lncRNAs were poor prognosis factors (AC245041.2, AC036176.1, LINC01089 and LINC02257). 6 lncRNAs were beneficial prognosis factors (FLVCR1-DT, AC006504.7 ,AC125494.2, AC012306.2 ,ST20-AS1 and AC005696.1).

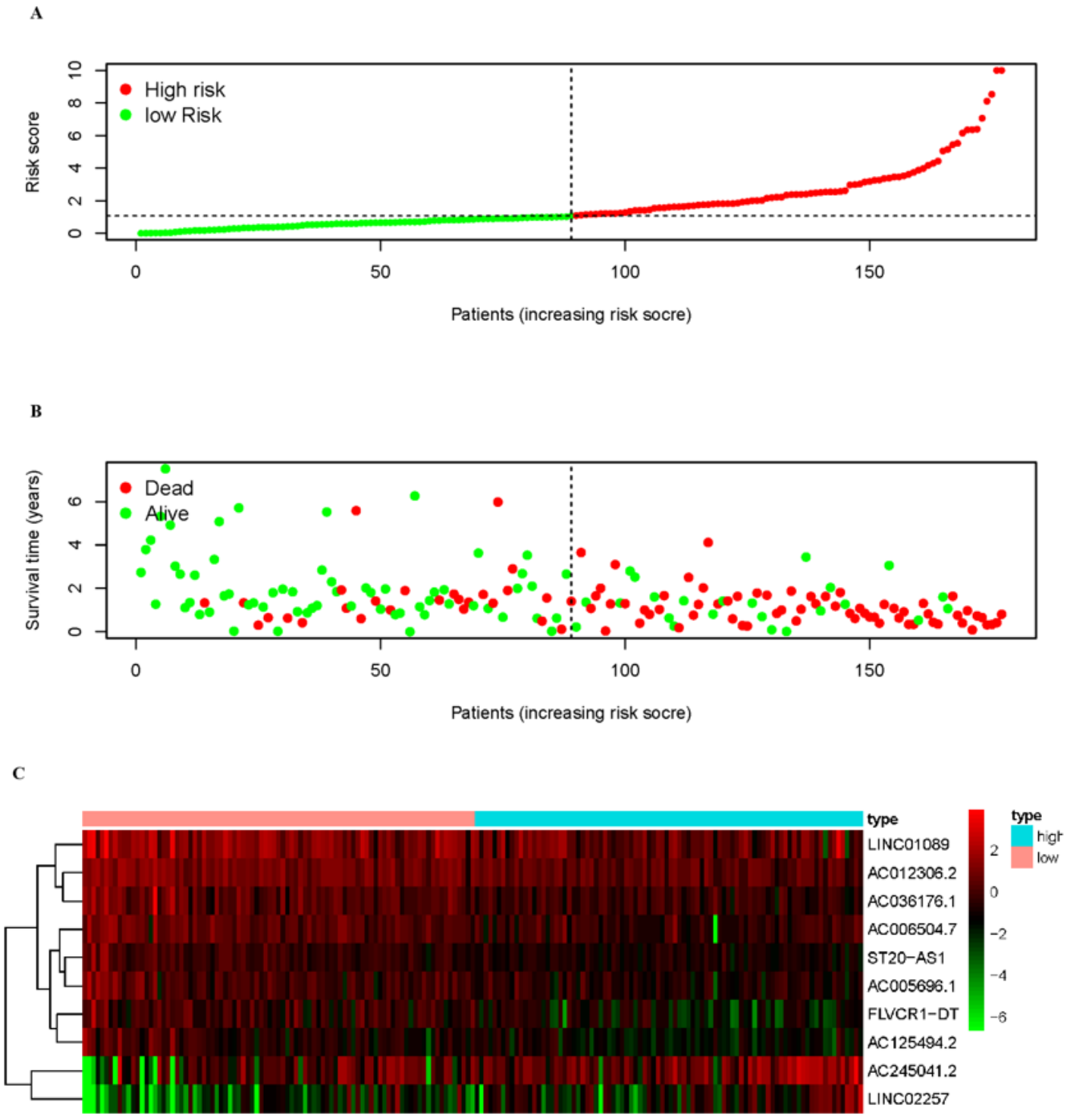

Figure 4

Risk score evaluation of prognostic autophagy-related lncRNAs model in PC. (A) Risk score of risk model. (B) The scatterplot reflected survival time of PC patients. (C) Heat map of 10 differentially expressed prognosis lncRNAs were exhibited.

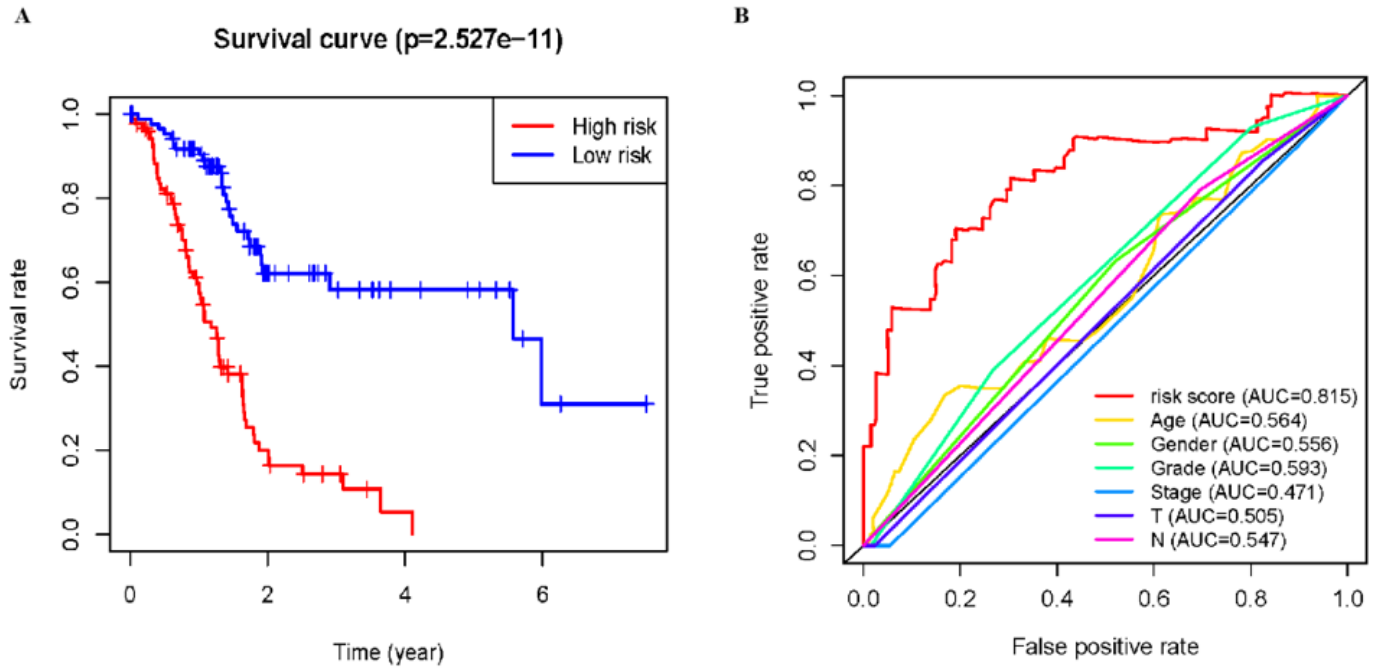

Figure 5

Prognostic impact of the risk signature. (A) Kaplan-Meier survival curve of risk model. (B) ROC curves risk score and other clinical factors based on AUC. AUC, acute area under the curve.

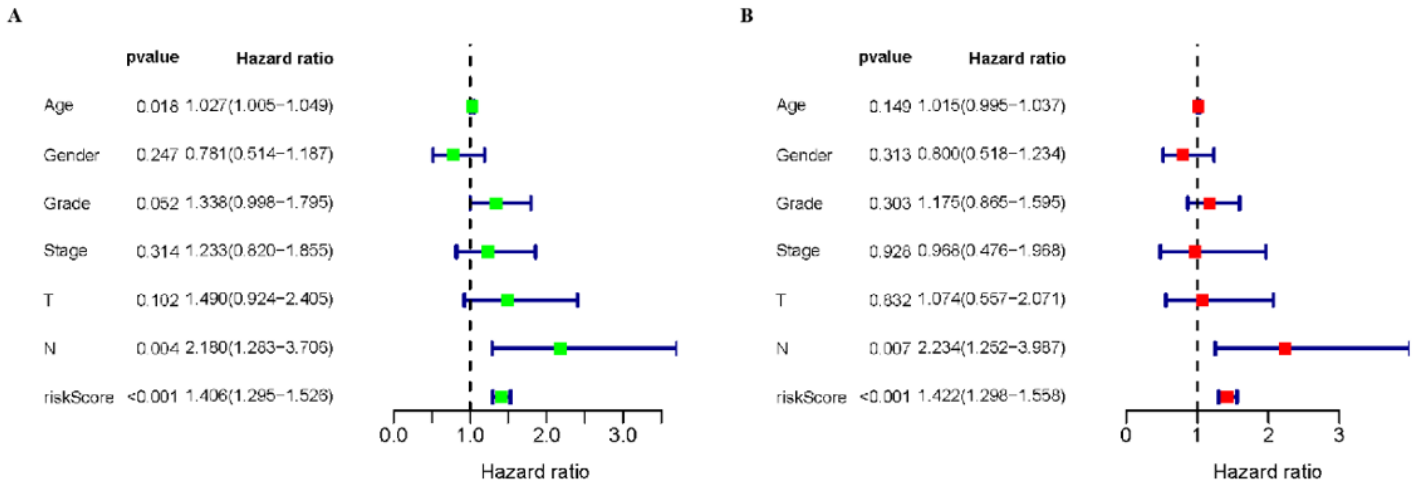

Figure 6

Predictive performance evaluation of the risk model on risk score and clinical factors through (A) univariate and (B) multivariate cox regression

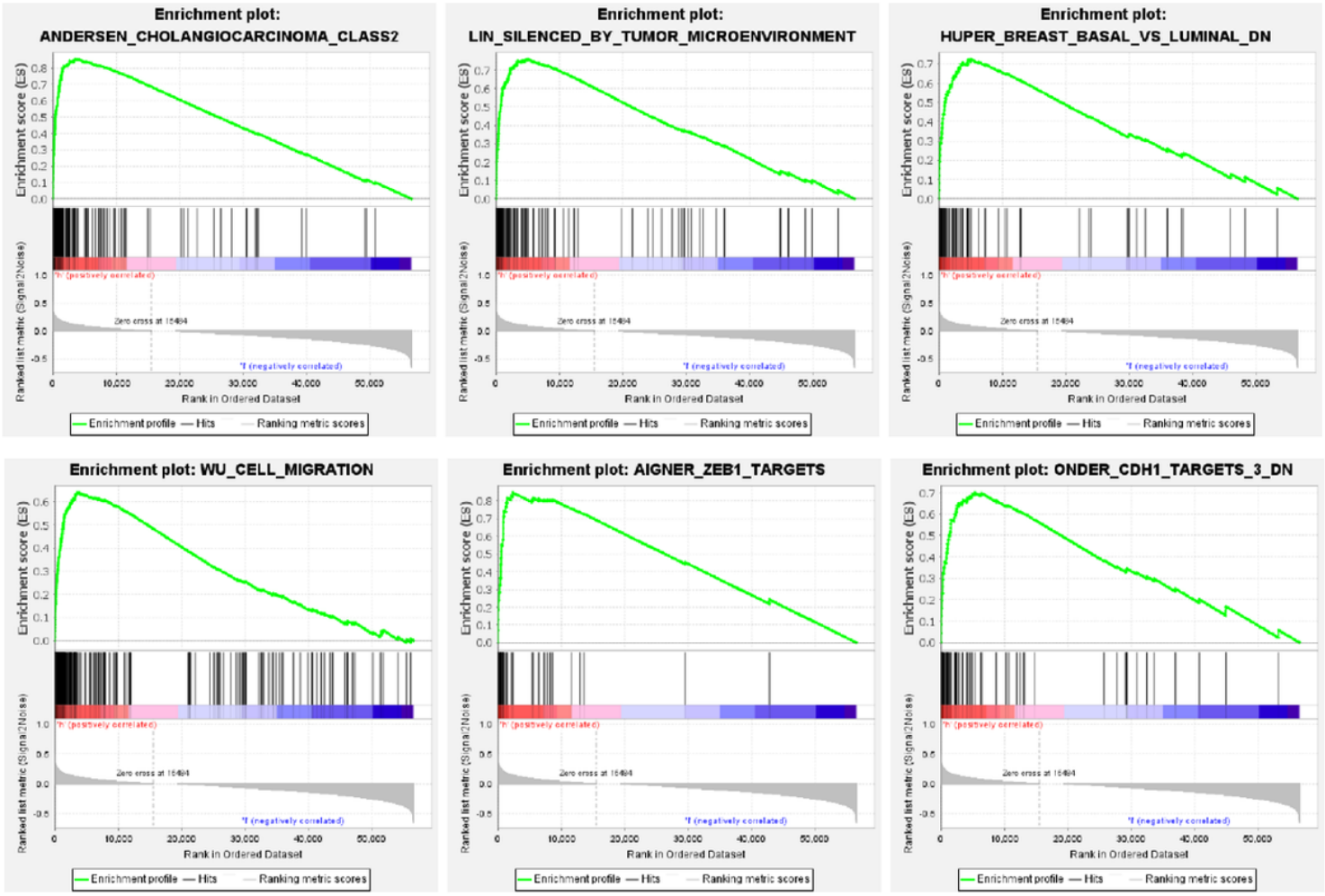

Figure 7

GSEA analysis of deferentially expressed genes