The Prognostic Value of an autophagy-related lncRNA Signature in Esophageal Cancer

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Abstract

Esophageal cancer is a clinically challenging disease, seriously affecting the quality of life and prognosis of patients. Autophagy is a basic intracellular event that can maintain cell homeostasis by degrading damaged or degenerate proteins and organelles. Long noncoding RNAs (lncRNAs) are a class of RNA transcripts with a length greater than 200 nucleotides that participate in fundamental biological processes and tumor progression. However, whether autophagy-related lncRNAs can be used to predict the survival of patients with esophageal cancer still needs in-depth research. The Human Autophagy Database¹⁹ (HADb) and The Cancer Genome Atlas (TCGA) database were used to identify autophagy-related lncRNAs. Univariate and multivariate COX analyses were performed to establish a prognostic risk score model. Kaplan–Meier curves and ROC curves were carried out to evaluate the prognostic value of the autophagy-related lncRNAs signature. 1757 autophagy-related lncRNAs in esophageal cancer were identified based on specific screening criteria and four key lncRNAs (ZFAS1, AC092687.3, AC083799.1, AL080317.1) were selected to construct a novel prognostic model. Particularly, the risk score for each patient could be calculated based on the expression levels of these four lncRNAs, and a high risk score was positively associated with shorter survival time and poor prognosis of esophageal cancer patients. The autophagy-related lncRNAs signature constructed in this study can be used as a novel risk score model to predict the survival and prognosis of patients with esophageal cancer.

1. Introduction

Esophageal cancer (EC) is one of the most aggressive gastrointestinal cancers and a public health concern worldwide, contributing 3.1% of newly diagnosed cancer cases and 5.5% of deaths annually[1]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) account for more than 95% of malignant esophageal tumors[2]. These two subtypes of esophageal cancer are distinct diseases and show striking differences in geographical distribution, which may be due to different risk factors exposure, such as smoking, alcohol, obesity, achalasia and gastroesophageal reflux disease[3–6]. Since patients with esophageal cancer are often asymptomatic in early stages, most patients are diagnosed during its advanced stages[7]. Although esophagectomy is a key curative treatment for locally advanced esophageal cancer, it still has a considerable risk of postoperative morbidity[8]. Besides, postoperative adverse side effects such as dysphagia, appetite loss, early satiety, and reflux also seriously affect the quality of life of patients[9,10]. At present, multidisciplinary approaches such as neoadjuvant chemoradiotherapy and surgery resection combined with perioperative chemotherapy have been increasingly applied in the treatment of esophageal cancer[8,11]. However, the overall 5-year survival rate ranges from 15 to 25% worldwide, despite advances in diagnosis and treatment of esophageal cancer, and the clinical efficacy remains limited[10].

Autophagy is a fundamental cellular process that can maintain cell homeostasis by participating in the clearance and turnover of cytoplasmic macromolecules, aggregated proteins and damaged organelles[12]. This autophagy-mediated lysosomal degradation mode is of great significance for cells to sustain metabolism and survival under starvation, and to maintain the quality control of intracellular

proteins and organelles[13]. Once cells are stimulated by a variety of intrinsic or extrinsic stimuli, such as hypoxia, starvation, pathogens and growth factor deprivation, autophagy can be induced by a series of cellular transduction signal pathways to exert physiological or pathological functions[14]. Many studies have shown that autophagy is involved in many disease processes, including autoimmune diseases, programmed cell death, neuron degeneration, infection, heart diseases and malignant tumors[15,16]. In the case of cancer, autophagy plays a dynamic tumor inhibition and tumor promotion role, depending on different contexts and stages of tumor development. Saxena *et al.* summarized current literature examining roles of autophagy in esophageal carcinogenesis and indicated that autophagy may promote the survival or death of esophageal cancer cells in a context-dependent manner[17]. Therefore, screening autophagy-related molecules is expected to provide potential targets for predicting the survival of patients with esophageal cancer.

Long non-coding RNAs (lncRNAs) are defined as endogenous cellular RNAs with a length larger than 200 nucleotides that generally cannot be translated into proteins[18]. lncRNAs have been shown to regulate several biological processes by interacting with DNA, RNA and proteins, including chromatin reconstruction and RNA transcription[19,20]. Although the number of lncRNAs whose functions have been annotated is relatively rare, accumulated evidence has shown that the dysregulated lncRNA profile is associated with oncogenesis and involved in the pathogenesis of tumors[21,22]. Yang *et al.* summarized recent progress in autophagy-related lncRNAs and their specific autophagic targets, indicating that lncRNAs related to autophagy initiation, phagophore nucleation, autophagosome elongation/closure and autolysosome fusion[23]. Recently, Meng *et al.* identified a series of dysfunctional lncRNAs based on RNA sequencing data and autophagy datasets, providing potentially useful biomarkers for the diagnosis and prognosis of ovarian cancer[24]. However, there is still a lack of systematic research to explore whether autophagy-related lncRNAs can predict the overall survival of patients with esophageal cancer.

In the present study, we first obtained a series of autophagy-related genes based on the Human Autophagy Database19 (HADb) and further identified a large number of lncRNAs associated with autophagy-related mRNA expression in esophageal cancer from The Cancer Genome Atlas (TCGA) database. Then, we performed univariate and multivariate COX analyses and screened four key lncRNAs (ZFAS1, AC092687.3, AC083799.1, AL080317.1), which can be used as a novel four-gene signature to predict survival and prognosis in patients with esophageal cancer.

2. Materials And Methods

Data collection

The mRNA expression profiles and the clinical data of esophageal carcinoma (TCGA-ESCA) were obtained from The Cancer Genome Atlas, including 160 esophageal carcinoma tissues and 11 adjacent nontumorous tissues (<https://portal.gdc.cancer.gov/>). The collected clinical pathological data included gender, stage, TMN classification, survival status and number of days of survival. The list of autophagy-

related genes was obtained from the Human Autophagy Database¹⁹ (HADb), a web-based resource, providing a comprehensive and up-to-date list of human genes and proteins related to autophagy. HADb included 232 autophagy-related genes. Pearson correlation was used to calculate the correlations between the autophagy-related genes and lncRNAs. An lncRNA with a correlation coefficient $|R^2| > 0.3$ and $P < 0.0001$ was considered to be an autophagy-related lncRNA.

KEGG and GO enrichment analyses

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a resource for exploring high-level gene functions and associating genomic data from large-scale molecular datasets. Gene ontology (GO) functional analysis (biological processes (BP), cellular components (CC) and molecular functions (MF)) is a powerful bioinformatics tool to analyze biological processes and annotate genes. To explore the function of the identified 232 autophagy-related genes, biological analyses were carried out using GO and KEGG pathway analysis via the R language ggplot2 package to generate figures.

Construction of a prognostic autophagy-related lncRNA signature

The autophagy-related lncRNAs were used to construct prognostic signature. Univariate and multivariate Cox analysis were used to screen autophagy-related lncRNA with prognostic values. The lncRNA levels that were significant in both univariate and multivariate Cox analysis were chosen as autophagy-related lncRNAs. The prognostic gene signature was calculated as risk score = (Coefficient of lncRNA1 × expression of lncRNA1) + (Coefficient of lncRNA2 × expression of lncRNA2) + ... + (Coefficient of lncRNA_n × expression lncRNA_n). The “survival” and “survminer” R packages were used to explore the optimal cut-off of risk score and analyze the Kaplan-Meier survival curve. The “survivalROC” R package was performed to explore the time-dependent prognostic value of the gene signature. The $P < 0.05$ was considered statistically significant for prognostic analysis.

Statistical analysis

Student's *t*-test was used to analyze gene expression between tumor tissues and adjacent nontumorous tissues. Univariate and multivariate Cox regression analyses were implemented to identify independent predictors of overall survival. All statistical analyses were performed with R software (version 3.6.1) or SPSS (version 23.0). $P < 0.05$ was considered as statistically significant.

3. Results

KEGG and GO enrichment analyses

The 232 autophagy-related genes were performed functional enrichment analysis. The result of GO analysis (Fig. 1A) suggested that changes in biological processes were significantly enriched autophagy, process utilizing autophagic mechanism, macroautophagy and autophagosome assembly. Changes in cellular components were mainly enriched in the autophagosome, phagophore assembly site and

vacuolar membrane. Changes in molecular functions were mainly enriched in ubiquitin-like protein ligase binding, ubiquitin protein ligase binding and protein serine/threonine kinase activity. KEGG pathway analysis (Fig. 1B) suggested enrichment mainly in autophagy, cancer, virus infection, NOD-like receptor and FoxO/ PI3K-Akt signaling pathway. These results may provide important information for the further related functional analysis of autophagy-related genes.

Prognostic autophagy-related lncRNAs in esophageal carcinoma

According to the screening criteria, 1757 autophagy-related lncRNAs were identified.

The correlation analysis of autophagy genes and autophagy-related lncRNAs were showed in Supplementary Table 1. The lncRNA, AC025857.2 had the largest correlation coefficient and the target gene was CTSB ($R = 0.8982$, $P = 2.90E-58$). To screen the prognostic lncRNAs, all related lncRNAs were examined with univariate COX analysis. The univariate results were shown in Table 1. The lncRNAs of great significance ($P < 0.05$) in univariate COX analysis were examined with multivariate COX analysis (Table 2). As a result, four autophagy-related lncRNAs (ZFAS1, AC092687.3, AC083799.1, AL080317.1) were selected as independent prognostic factors of esophageal carcinoma patients. The risk score of individual patients in our study cohort was calculated. The cohort was divided into two groups (high- and low-risk) in relation to the median risk score value. The risk score = $0.0111 \times \text{Expression of ZFAS1} + 0.3525 \times \text{Expression of AC092687.3} + 0.0756 \times \text{Expression of AC083799.1} + 0.2383 \times \text{Expression of AL080317.1}$ (Table 2)

Table 1
Detailed information for 12 autophagy-related long non-coding RNAs (lncRNAs) significantly associated with OS in esophageal carcinoma.

LncRNA	B	SE	HR	HR.95L	HR.95H	pvalue
AC139100.2	0.394943	0.126845	1.484299	1.15758	1.903232	0.001848
ZFAS1	0.012133	0.005281	1.012206	1.001784	1.022737	0.021587
AC092687.3	0.379612	0.121845	1.461717	1.151194	1.856001	0.001836
AC079684.1	0.632091	0.198055	1.881541	1.276231	2.773947	0.001415
AL023284.4	0.060003	0.020965	1.06184	1.019092	1.106381	0.004209
TBILA	0.208059	0.070351	1.231285	1.072693	1.413324	0.003102
AL031673.1	0.202723	0.066115	1.224734	1.075881	1.394181	0.002168
UBR5-AS1	0.22969	0.072903	1.25821	1.090682	1.451471	0.001629
AC108673.2	0.395533	0.141621	1.485176	1.1252	1.960316	0.005224
AC083799.1	0.080767	0.037145	1.084118	1.007996	1.165989	0.029676
AL080317.1	0.263987	0.071428	1.302112	1.132006	1.497779	0.000219
IPO5P1	0.133708	0.054602	1.143059	1.02705	1.272172	0.014334

Table 2
The multivariate COX analysis and correlation coefficient of lncRNAs for constructing prediction model.

id	coef	HR
ZFAS1	0.011089921	1.011152
AC092687.3	0.352476019	1.422586
AC083799.1	0.075559570	1.078487
AL080317.1	0.238292575	1.269080

Survival results of prognostic autophagy-related lncRNAs

The effect of four lncRNAs on patient survival was analyzed through Kaplan-Meier curves. The results showed that all four lncRNAs significantly affected esophageal carcinoma patient (Fig. 2). As shown in Fig. 3A, the Kaplan-Meier curves of the esophageal carcinoma cohorts showed the predictive overall survival (OS) value of the risk signature based on the four lncRNAs. Patients with high risk have poorer survival compared with the low-risk group ($P = 2.169 \times 10^{-3}$). We also established a patient risk survival status plot, which suggested that as the patient's risk score increases, the number of deceased patients also increases (Fig. 3B). We also used the ROC curves to investigate if the expression patterns of survival-

related lncRNAs could serve as biomarkers for early prediction esophageal carcinoma occurrence. Here, found an area under the curve (AUC) of 0.724 in our dataset, meaning that the sensitivity and specificity of this prognostic model are moderate (Fig. 3C).

Independent prognostic value of the four-gene signature

Univariate and multivariate Cox regression analyses were carried out among the available variables to determine whether the risk score was an independent prognostic predictor for overall survival. In univariate Cox regression analyses, the risk score was significantly associated with OS in the TCGA cohort (HR = 1.224, 95% CI = 1.123–1.335, $P < 0.001$) (Fig. 4A). After correction for other confounding factors, the risk score still proved to be an independent predictor for OS in the multivariate Cox regression analysis (HR = 1.189, 95% CI = 1.086–1.302, $P < 0.001$) (Fig. 4B).

Cytoscape was used to explore the potential link between the lncRNAs and target genes. As shown in Fig. 5, AL080317.1 was the largest node in the network, while AC092687.3 had the smallest matched target genes.

Then patients included in TCGA-ESCA cohort were stratified according to the gender, stage and TNM stage. Two cohort of patients in the high-risk group showed significantly poorer overall survival OS than those in the low-risk group both in stage G3-4 but not in G1-2 (Fig. 6). The TCGA-ESCA cohort of patients in the high-risk group only showed significantly poorer overall survival OS than those in the low-risk group both in TNM stage T3-4 (Fig. 6). The TCGA-ESCA cohort of patients in the high-risk group also showed significantly poorer overall survival OS than those in the low-risk group in male group (Fig. 6). Overall, these results provide useful information for survival and prognosis analysis regarding autophagy-related lncRNAs.

4. Discussion

Esophageal cancer is a clinically challenging disease, which is closely associated with the considerable decline in health-related quality of life and the poor clinical prognosis[11]. Endoscopy combined with tissue biopsy is usually used to confirm the diagnosis of esophageal cancer. Follow-up CT of the head, chest and abdomen is used to assess whether the patient has distant metastasis, thus guiding clinical treatment options[11]. Clinical strategies such as endoscopic mucosal resection, endoscopic submucosal dissection, chemotherapy or chemoradiotherapy are often used as a single or combined curative method for the treatment of esophageal cancer at early or locally advanced stages[25]. For advanced patients with esophageal cancer who are not eligible for curative treatment, or still have a higher risk of recurrence despite curatively intended treatment, they can only receive palliative strategies with the goal of controlling disease-related symptoms and prolonging survival[11]. Therefore, screening and identifying a series of biomarkers for predicting the overall survival and prognosis of patients with esophageal cancer is of great significance for guiding clinicians to select appropriate treatment strategies.

As a major self-degradative intracellular event, autophagy plays a key role in a variety of biological processes, such as regulation of cell metabolism and homeostasis, controlling of cell growth and death[26]. Dysfunctional autophagy in multiple tumor types is closely related to epigenetic changes, including DNA methylation-related enzymes, histone-modifying enzymes and non-coding RNAs[27–29]. Accumulated evidence has showed that lncRNAs participate in the process of autophagy by regulating ATG gene expression or acting as competing endogenous RNAs to regulate the miRNAs[23]. In our study, we obtained 232 autophagy-related genes from the Human Autophagy Database[19] and identified 1757 autophagy-related lncRNAs based on TCGA database in the esophageal cancer. Further, we performed univariate and multivariate COX analyses and screened four key autophagy-related lncRNAs (ZFAS1, AC092687.3, AC083799.1, AL080317.1) for the construction of a risk score signature, which can be used to predict survival and prognosis in patients with esophageal cancer.

ZNF1 antisense RNA 1 (ZFAS1) is an lncRNA that transcribes from the antisense strand near the 5' end of the protein-coding gene Znf1[30,31]. ZFAS1 was originally identified as a regulatory factor involved in mammary gland development and mammary epithelial cell differentiation[31]. A large number of studies have shown that the expression of ZFAS1 is relatively high in various cancer tissues and cells, including head and neck squamous cell carcinomas, colorectal cancer, prostate cancer, and gastric cancer[32–35]. However, accumulated studies have demonstrated that the expression of ZFAS1 was down-regulated in breast cancer and ZFAS1 overexpression significantly inhibited the proliferation, migration and invasion of breast cancer cells[36,37]. These data indicates that ZFAS1 may play a tumor-promoting or tumor-suppressing effect depending on different tumor types. In particular, Shi *et al.* reported that the expression of ZFAS1 in esophageal squamous cell carcinoma tissues was significantly higher than that in adjacent tissues, and high ZFAS1 expression was positively correlated with the poorer overall survival of esophageal cancer patients[38]. In addition, Li *et al.* also found that ZFAS1 expression was up-regulated in esophageal squamous cell carcinoma tissues, and their study showed that exosomal ZFAS1 could promote tumor progression in esophageal squamous cell carcinoma by up-regulating STAT3 and down-regulating miR-124[39]. Since these results are consistent with our research conclusions, this further deepens the credibility of ZFAS1, an autophagy-related lncRNA which we have screened by the biological informatics method, as a biomarker for predicting the overall survival of esophageal cancer.

AC092687.3 was reported to construct a six-lncRNA signature together with five other lncRNAs (AC016722.3, AL589986.2, AC006007.1, GS1-124K5.4, and AC007126.1), which could be used to diagnose dilated cardiomyopathy more specifically and sensitively[40]. However, the biological function, molecular mechanism and clinical significance of AC092687.3 in various tumors including esophageal cancer have not been reported yet. In addition, the functions and characteristics of the other two lncRNAs, AC083799.1 and AL080317.1, are still unknown. In our study, based on the expression level of lncRNAs and the clinical information of patients with esophageal cancer in the TCGA database, we found for the first time that high expression of AC092687.3, AC083799.1, or AL080317.1 was positively correlated with poor overall survival of patients with esophageal cancer. Although our research provides some potential novel biomarkers for predicting the prognosis and survival of patients with esophageal cancer, the

expression, distribution, function and mechanism of these lncRNAs still need to be further explored through experiments.

In addition, the four-lncRNA signature we constructed in this study can be used to calculate the risk score based their expression level to predict the survival and prognosis of patients with esophageal cancer, which has better sensitivity and credibility than the single-gene prediction model. However, considering that lncRNAs can interact with miRNAs through the mechanism of competing endogenous RNAs to regulate the expression of other RNAs and proteins and thus affect the disease process, how to further optimize the algorithm and screening mode to construct a composite signature containing lncRNAs and miRNAs to more accurately predict the survival and prognosis of patients with esophageal cancer still needs further research.

5. Conclusion

In conclusion, we identified a novel four autophagy-related lncRNAs signature based on the HADB and TCGA database, which could be used to predict survival and prognosis of esophageal cancer patients, providing valuable guidance for the selection of effective treatment strategies for patients with esophageal cancer in clinical practice.

Abbreviations

EC
Esophageal cancer
lncRNAs
Long non-coding RNAs
TCGA
The Cancer Genome Atlas
KEGG
Kyoto Encyclopedia of Genes and Genomes
GO
Gene ontology
AUC
Area under the curve
OS
Overall survival

Declarations

Data Availability

RNA-seq data and corresponding clinical data were acquired from the TCGA database (<https://portal.gdc.cancer.gov/>).

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Figures

Figure 1
A

B

Figure 1

The results of functional enrichment analysis based on 232 autophagy-related genes. (A) GO enrichment analysis; (B) KEGG enrichment analysis.

Figure 2

Figure 2

Kaplan–Meier curve results demonstrated that lncRNA expression significantly affects esophageal carcinoma patient overall survival.

Figure 3

Figure 3

Survival results and multivariate analysis. (A) Survival results, (B) risk survival status plot and (C) receiver operating characteristic (ROC) results.

Figure 4

Figure 4

Results of the univariate (A) and multivariate (B) Cox regression analyses regarding esophageal carcinoma in the TCGA cohort.

Figure 5

Figure 5

Cytoscape software was used to explore the potential link between long non-coding RNAs (lncRNAs) and target genes. The red nodes indicate lncRNAs and the orange indicates autophagy genes.

Figure 6

Figure 6

The survival rates of high and low risk esophageal carcinoma patients stratified by different clinicopathological characteristics.