Comprehensive analysis of the clinical significance and prospective molecular mechanisms of differentially expressed autophagy-related genes in thyroid cancer

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Abstract. Thyroid cancer (TC) is the most common endocrine malignancy, accounting for approximately 90% of all malignancies of the endocrine system. Despite the fact that patients with TC tend to have good prognoses, the high incidence rate and lymph node metastases remain unresolved issues. Autophagy is an indispensable process that maintains intracellular homeostasis; however, the role of autophagy in several steps of the initiation and progression of TC has not yet been elucidated. In this study, we first identified several autophagy-related genes (ARGs) that were provoked in the onset of TC. Subsequently, a bioinformatics analysis hinted that these genes were markedly disturbed in several proliferative signaling pathways. Moreover, we demonstrated that the differentially expressed ARGs were closely related to several aggressive clinical manifestations, including an advanced tumor stage and lymph node metastasis. Our study further selected prognostic ARGs and developed a prognostic signature based on three key genes (ATG9B, BID and BIDNAB1), which displayed a moderate ability to predict the prognosis of TC. On the whole, the findings of this study demonstrate that ARGs disrupt proliferation-related pathways and consequently lead to aggressive clinical manifestations. These findings provide insight into the potential molecular mechanisms of action of ARGs and their clinical significance, and also provide classification information of potential therapeutic significance.

Introduction

Thyroid cancer (TC) is the most common endocrine malignancy, accounting for approximately 90% of all malignancies of the endocrine system (1,2). The morbidity associated with TC is exceedingly high, and an increasing number of asymptomatic TC cases are detected due to the wide use of routine high-resolution ultrasonography (3,4). In the United States, it is estimated that 53,990 cases of TC will be diagnosed in 2018 (2). In fact, TC has become the fifth most common type of cancer affecting women. Despite the fact that TC exhibits an indolent and non-aggressive behavior in the majority of cases, the high incidence rate and several grievous clinical manifestations, such as lymph node metastases, remain unresolved issues (4-6). Based on the histopathology, TC is classified into four main tumor types, including papillary thyroid cancer (PTC), follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC) and anaplastic thyroid cancer (ATC) (7,8). The different subtypes of TC have enormous heterogeneity in terms of their morphological characteristics and prognoses. Although PTC is the predominant type, accounting for 80% of all TCs, it presents a favorable prognostic tendency overall; however, the specific situation of each patient with PTC is not the same (9,10). Therefore, there is an urgent need to further reveal the molecular pathogenesis of TC.

Autophagy is a highly conserved ‘self-devouring’ process that ensures the orderly degradation of the cytoplasmic contents and the recycling of the macromolecular constituents to maintain cellular homeostasis (11). Considering the fundamental roles of autophagy in regulating cellular biological processes, it is no surprise that it plays essential roles in a wide spectrum of physiological conditions and diseases, particularly cancer (12-15). Moreover, exploring the potential mechanisms of action of autophagy would not only expose the mysterious veil of tumorigenesis, but may also aid in the identification of novel therapeutic targets for autophagy in cancer (16). Several studies have reported the probability of harnessing autophagy in the development of cancer (16-18). As regards TC, autophagy-targeted therapy is now considered to be a valuable strategy, as the regulation of the autophagy level can
mediate the chemosensitivity and radiosensitivity of TC cells and can markedly affect the regulation of apoptosis (19). For example, Wang et al found that targeting autophagy sensitized BRAF-mutant TC to vemurafenib (20). In addition, it has been suggested that rapamycin can inhibit the invasive ability of TC cells (21). However, these previous studies only examined single oncogenes or suppressor genes in TCs, which reflect the partial functions of autophagy. There are very few systematic comprehensive analyses addressing the clinical significance and potential biological process of autophagy in TC (22,23).

The Human Autophagy Database (HADb) offers an exhaustive and up-to-date list of autophagy-related genes (ARGs) to satisfy the needs of researchers (24). Previously, Zhang et al calculated 74 differentially expressed ARGs, and two of these were associated with the prognosis of patients with glioma (25). However, the clinical values of 234 ARGs in TC remain unclarified. In this study, we described the expression profiles of 234 ARGs, and we identified a class of ARGs with disrupted expression statuses in the onset of TC based on the Cancer Genome Atlas (TCGA) database. Notably, we also identified the biological process pathways enriched by these genes, which may help in the development of more effective treatments. On the basis of the underlying proliferative functions of ARGs, we further emphasized the clinical significance of differentially expressed ARGs in providing novel insight into the clinical management of TC. The present study provides a deeper understanding of the role of autophagy in TC and may help to improve the clinical outcomes of patients with TC.

Materials and methods

Collection of ARGs. Our researchers acquired the ARGs from the HADb. Subsequently, we downloaded the TC gene expression dataset from the TCGA database, from which we extracted the expression levels of the ARGs. TCGA provided 502 TC and 52 non-tumor cases with gene expression profiles. Accordingly, the clinicopathological data of the patients with TC were also downloaded for use in the current study.

Calculation of the differentially expressed ARGs and the functional analysis of their enriched pathways. The edgeR software package was applied in this study to filter and normalize the expression profiles found in the TCGA database, aiming to analyze the differentially expressed ARGs, not only in TC tumors, but also in the adjacent normal tissues. The standard was a fold change of >2 and P<0.05 after correction. We constructed the network of protein-protein interactions (PPIs) with the help of STRING (https://string-db.org/) (26), an online database available to all, and we then input the interacting data derived from STRING to Cytoscape 3.5.1 (27) for visualization. In addition, enrichment analyses were conducted for a better comprehension of the biological functions of the differentially expressed ARGs in TC, which included the analyses of the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Disease Ontology (DO). The enrichment analyses were carried out using the R clusterProfiler package (28) and the results were displayed with the GOplot package (29).

Prognosis index (PI) construction. A univariate Cox analysis was used to determine the genes associated with the survival time of the patients with TC. Subsequently, a univariate Cox analysis was performed to exclude the genes that failed to become independent prognostic biomarkers. Finally, a PI model was constructed with the eligible ARGs based on the formula in which the gene expression was multiplied by the regression coefficient. The Kaplan-Meier (KM) method was used to depict the differences in survival between the high-risk and low-risk groups, and a log-rank analysis was employed for the assessment. In order to investigate the mechanisms of action of ARGs that were consistent with the PI, we obtained the ARG expression, methylation, and copy number variations from the cBioPortal database (30), which provided the data of 508 patients with TC for analysis. We also assessed the ARG splicing events using the TCGA SpliceSeq database (http://bioinformatics.mdanderson.org/TCGASpliceSeq/).

Statistical analysis. SPSS Statistics for Windows version 24.0 (SPSS Inc., Chicago, IL, USA) and R 3.3.1 (https://www.r-project.org/) were utilized for the statistical analyses. R, GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) and OriginPro 2017 (OriginLab Corp., Northampton, MA, USA) were used for the plotting. Following normalization, all the expression data were transformed to $\log_2$ (value +1). We employed an independent t-test to compare the differential expression levels of the ARGs in the TC and adjacent tissues, and to determine the associations between these genes and the clinicopathological characteristics of the patients with TC. Scatter diagrams and boxplots were used to display gene expression. In order to examine the ability of the differentially expressed ARGs to identify tumors, we applied GraphPad software for plotting and calculating the receiver operating characteristic (ROC) curves of each dataset and determining the area under the curve (AUC). The prognostic values of the ARGs were estimated using a univariate regression analysis; subsequently, the prognostic potentials of the ARGs were obtained using a multivariate Cox regression analysis for the construction of the PI model. Correlations between gene mRNA expression levels and methylation degree were examined by Pearson's correlation analysis. A value of P<0.05 was considered to indicate a statistically significant difference.

Results

Differentially expressed ARGs. A total of 234 ARGs were obtained from the HADb database, and 502 TC cases with gene expression profiles were acquired from the TCGA database (Table I). In addition, 52 cases with adjacent normal tissue data were obtained from the TCGA database. After extracting the gene expression levels of 234 cases, we conducted the differential analysis. Conforming to the standards mentioned above, we eventually acquired 31 differentially expressed ARGs, among which 17 were upregulated, while 14 were downregulated (Figs. 1-3). All of the differentially expressed genes are listed in Table II.

Enrichment analyses of the differentially expressed ARGs. Our researchers carried out a series of gene enrichment and pathway analyses, hoping to further investigate the biological functions of autophagy in the development of TC. Using STRING and Cytoscape, we set up the PPI network for these genes (Fig. 4),
and discovered the core genes, such as BCL2, JUN and CDKN1A, which cast light on the functions of autophagy in TC and laid the foundation for multi-targeted therapies. The GOplot analysis revealed that in the biological processes, these genes were related to autophagy, as well as to the intrinsic

| Variables                  | Number of patients | Variables                  | Number of patients |
|---------------------------|--------------------|---------------------------|--------------------|
| Age ≥60                   | 120                | Pathological stage III-IV | 167                |
| Age <60                   | 382                | I-II                      | 333                |
| Sex Male                  | 135                | T stage T3-T4             | 193                |
| Sex Female                | 367                | T1-T2                     | 309                |
| Tumor status              |                    | N stage N1-3              | 223                |
| With tumor                | 44                 | N0                        | 229                |
| Tumor free                | 446                | M stage M1                | 9                  |
| Primary neoplasm focus type | Multifocal | M0                        | 282                |
|                          | 226                |                           |                    |
|                          | Unifocal           |                           |                    |
|                          | 266                |                           |                    |

T stage, size or direct extent of the primary tumor; N stage, degree of spread to regional lymph nodes; M stage, presence of distant metastasis.

Figure 1. Volcano plot showing the differentially expressed genes. Red color represents upregulation and blue represents downregulation. Black color represents no differentially expressed genes.

Figure 2. Heatmap of the 234 autophagy-related genes. Blue and red color represent the intensity of the expression level of differentially expressed genes. Blue represents a low intensity of either a low or high expression and red represents a high intensity of either a low or high expression.
apoptotic signaling pathway, neuron apoptotic process and the response to gamma radiation (Fig. 5A). In terms of the cellular components, these genes participated in the mitochondrial outer membrane, autophagosome and organelle outer membrane functions (Fig. 5B). With regard to the molecular function, these genes played indispensable roles in certain key functions, such as ubiquitin protein ligase binding (Fig. 5C). In addition, KEGG pathway enrichment analysis indicated that these genes were mainly enriched in the pathways relevant to the cell cycle, including autophagy and apoptosis (Fig. 6). Notably, DO analysis (Fig. 7) suggested that these genes were associated with multiple tumors, other than TC, which manifested the fundamental roles of these genes in tumorigenesis and development.

ARG expression in TC and the clinical significance. As the differentially expressed ARGs were largely enriched in tumor-related pathways, it is most likely that these genes could accelerate the development of TC. Therefore, we analyzed the expression of these genes in various clinical parameters and enquired into their associations with the clinical progress. Initially, we verified the expression of these genes in TC. The independent samples t-test and the analysis of the TCGA database indicated that the expression levels of 17 genes were markedly upregulated in the tumor tissues, including CTSB, CDKN1A, BAX, ATG9B, BCL2L1, SPHK1, CX3CL1, DRAM1, DAPK2, ITGA3, BID, ITGB4, DIRAS3, TP63, EVA1A, CDKN2A and SERPINA1. Conversely, 12 genes displayed markedly lower expression levels in the tumor tissues, including ITPR1, JUN, FOS, BCL2, NAJB1, PRKCQ, MAP1LC3C, CCL2, PPP1R15A, GRID2, HSPB8 and TP53INP2 (Fig. 8). Due to the differences between the t-test and edgeR, the IL24 and CXCR4 genes did not show statistical significance. Subsequently, we performed a ROC analysis to determine the ability to differentiate the cancerous and non-cancerous tissues. A total of 28 genes had AUC values >0.7 (Fig. 9), which suggested

Table II. Characteristics of the differentially expressed autophagy-related genes by using edgeR (tumor vs. normal).

| Gene symbol | Entrez ID | logFC | Regulation | P-value | FDR |
|-------------|-----------|-------|------------|---------|-----|
| PRKCQ       | 5588      | -1.23797 | Down | 6.81E-51 | 1.59E-48 |
| BID         | 637       | 1.879533 | Up    | 1.39E-49 | 1.62E-47 |
| DNAJB1      | 3337      | -1.3017 | Down | 2.27E-49 | 1.76E-47 |
| SERPINA1    | 5265      | 4.544121 | Up    | 3.44E-46 | 2.00E-44 |
| JUN         | 3725      | -1.74985 | Down | 2.82E-41 | 1.31E-39 |
| BCL2        | 596       | -1.48235 | Down | 8.73E-40 | 3.39E-38 |
| ITGA3       | 3675      | 1.639519 | Up    | 5.04E-39 | 1.68E-37 |
| CX3CL1      | 6376      | 1.314813 | Up    | 8.86E-36 | 2.58E-34 |
| ITPR1       | 3708      | -1.85687 | Down | 4.25E-35 | 1.10E-33 |
| BCL2L1      | 598       | 1.139524 | Up    | 1.20E-34 | 2.80E-33 |
| BAX         | 581       | 1.043169 | Up    | 2.69E-33 | 5.22E-32 |
| EVA1A       | 84141     | 2.717545 | Up    | 5.52E-31 | 9.19E-30 |
| TP53INP2    | 58476     | -1.02698 | Down | 6.57E-28 | 1.02E-26 |
| CDKN2A      | 1029      | 3.02178 | Up    | 1.16E-27 | 1.59E-26 |
| PPP1R15A    | 23645     | -1.14889 | Down | 2.55E-27 | 3.30E-26 |
| DRAM1       | 55332     | 1.470791 | Up    | 6.77E-27 | 8.30E-26 |
| DIRAS3      | 9077      | 2.089879 | Up    | 1.54E-26 | 1.80E-25 |
| ITGB4       | 3691      | 1.972199 | Up    | 8.83E-26 | 9.80E-25 |
| FOS         | 2353      | -1.53364 | Down | 1.92E-20 | 1.60E-19 |
| DAPK2       | 23604     | 1.544573 | Up    | 2.12E-20 | 1.71E-19 |
| SPHK1       | 8877      | 1.226448 | Up    | 4.12E-20 | 3.10E-19 |
| CTSB        | 1508      | 1.020784 | Up    | 1.17E-18 | 7.56E-18 |
| IL24        | 11009     | -1.41312 | Down | 2.78E-18 | 1.75E-17 |
| CDKN1A      | 1026      | 1.033733 | Up    | 6.07E-15 | 2.89E-14 |
| TP63        | 8626      | 2.410479 | Up    | 3.43E-14 | 1.40E-13 |
| MAP1LC3C    | 440738    | -1.22583 | Down | 3.57E-13 | 1.41E-12 |
| CCL2        | 6347      | -1.21423 | Down | 9.06E-13 | 3.35E-12 |
| CXCR4       | 7852      | -1.10962 | Down | 1.51E-12 | 5.51E-12 |
| HSPB8       | 26353     | -1.03221 | Down | 2.29E-10 | 7.50E-10 |
| ATG9B       | 285973    | 1.099005 | Up    | 5.72E-08 | 1.42E-07 |
| GRID2       | 2895      | -1.04415 | Down | 7.09E-06 | 1.33E-05 |

logFC, log (Fold change); FDR, false discovery rate.
that these genes were useful in differentiating between cancerous and non-cancerous tissues. Based on the fact that these genes were expressed differentially in thyroid cancer, we further explored the associations between these 31 genes and some of the major clinical parameters: Age (over or under 60 years), sex (male/female), tumor recurrence (yes/no), primary tumor type (multifocal/unifocal), pathological stage (stage III, IV/stage I, II), T stage (stage III, IV/stage I, II), N stage (presence of lymphatic metastasis or not) and M stage (presence of distant metastasis or not). It was revealed that these 31 genes were closely connected with the majority of the parameters, particularly those linked with tumor
Table III. Associations between the expression levels of the differentially expressed autophagy-related genes and the clinicopathological characteristics of the patients with thyroid cancer.

| Genes   | Age (≥60/<60) | Sex (male/female) | Tumor status (with tumor/tumor free) | Primary neoplasm focus type (multifocal/unifocal) | Pathological stage (III-IV/I-II) | T stage (T3-T4/T1-T2) | N stage (N1-3/N0) | M stage (M1/M0) |
|---------|---------------|-------------------|-------------------------------------|-----------------------------------------------|---------------------------------|----------------------|------------------|-----------------|
| PRKCQ   | 0.738         | 0.461             | 1.769                               | 0.586                                         | -0.561                         | 2.177                | -4.205           | -4.422          |
| BID     | -2.118        | 0.036             | 0.116                               | 0.908                                         | -0.224                         | 2.455                | 2.939            | 7.215           |
| DNAJB1  | -0.404        | 0.686             | -2.022                              | 0.544                                         | -1.113                         | 1.602                | 1.623            | 0.722           |
| SERPNA1 | -1.395        | 0.165             | -0.159                              | 0.874                                         | -0.554                         | 0.800                | 4.473            | 4.128           |
| JUN     | -0.803        | 0.422             | -1.160                              | 0.414                                         | -1.061                         | 1.977                | -1.201           | -0.869          |
| BCL2    | -0.429        | 0.668             | -0.140                              | 0.888                                         | -0.851                         | 1.360                | -5.356           | -5.008          |
| ITGA3   | -1.303        | 0.194             | -0.474                              | 0.635                                         | 0.604                          | 0.949                | 4.597            | 4.925           |
| DNAJB1  | -0.404        | 0.686             | -2.022                              | 0.544                                         | -1.113                         | 1.602                | -5.356           | -5.008          |
| JUN     | -0.803        | 0.422             | -1.160                              | 0.414                                         | -1.061                         | 1.977                | -1.201           | -0.869          |
| BCL2    | -0.429        | 0.668             | -0.140                              | 0.888                                         | -0.851                         | 1.360                | -5.356           | -5.008          |
| DNAJB1  | -0.404        | 0.686             | -2.022                              | 0.544                                         | -1.113                         | 1.602                | -5.356           | -5.008          |
| JUN     | -0.803        | 0.422             | -1.160                              | 0.414                                         | -1.061                         | 1.977                | -1.201           | -0.869          |
| BCL2    | -0.429        | 0.668             | -0.140                              | 0.888                                         | -0.851                         | 1.360                | -5.356           | -5.008          |
| DNAJB1  | -0.404        | 0.686             | -2.022                              | 0.544                                         | -1.113                         | 1.602                | -5.356           | -5.008          |
| JUN     | -0.803        | 0.422             | -1.160                              | 0.414                                         | -1.061                         | 1.977                | -1.201           | -0.869          |
Of these 31 genes, 25 were expressed differentially in the patients with lymphatic metastasis, and 17 displayed marked differences in expression in the T stage. However, we failed to discover the genes closely associated with distant metastasis as these samples were not sufficient for valid research (n=9).

**Cox regression analysis** and ultimately acquired 3 genes: ATG9B, BID and DNAJB1. Accordingly, a PI model was constructed based on these 3 genes: PI=0.469*ATG9B expression - 0.796*BID expression + 0.782*DNAJB1 expression (Fig. 13). The median PI value was applied to divide the patients with TC into high- or low-risk groups. The KM test was employed in the current study for statistical analysis. The survival analysis revealed that the hazard ratio (HR) of the overall survival (OS) predicted by PI was 4.706 (95% CI, 1.742-12.710; log-rank, P=0.0023), which indicated that the high-risk patients had markedly shorter OS times than their low-risk counterparts.

**ARG-based PI construction**. The results of univariate analysis revealed that 6 ARGs were associated with the prognosis of patients with TC (Figs. 11 and 12). To improve the accuracy and validity of the conclusion, we performed a multivariate Cox regression analysis and ultimately acquired 3 genes: ATG9B, BID and DNAJB1. Accordingly, a PI model was constructed based on these 3 genes: PI=0.469*ATG9B expression - 0.796*BID expression + 0.782*DNAJB1 expression (Fig. 13). The median PI value was applied to divide the patients with TC into high- or low-risk groups. The KM test was employed in the current study for statistical analysis. The survival analysis revealed that the hazard ratio (HR) of the overall survival (OS) predicted by PI was 4.706 (95% CI, 1.742-12.710; log-rank, P=0.0023), which indicated that the high-risk patients had markedly shorter OS times than their low-risk counterparts.
low-risk counterparts (Fig. 14). Additionally, in the high-risk group, the ATG9B and DNAJB1 genes exhibited notably higher expression levels, whereas the BID gene exhibited a significantly lower expression (Fig. 15).

**Molecular mechanism of PI.** In order to investigate the molecular mechanism of the PI, we analyzed the alternative splicing, methylation, copy number variation and amplification of the ATG9B, BID and DNAJB1 genes. Using the cBioPortal
Figure 8. The expression patterns of the 31 differentially expressed autophagy-related genes.
Figure 9. The receiver operating characteristic (ROC) curves of the 31 differentially expressed autophagy-related genes. AUC, area under the curve.
program, we obtained the associations of the expression of these 3 genes with the methylation and copy number variations (Fig. 16). As shown in Fig. 17A, of the 508 TC cases, genetic alterations of these 3 genes were detected in 33 cases. According to the genetic alterations, the patients were categorized into two groups. The KM analysis suggested that the 33 patients with genetic alterations in these 3 genes were likely to have markedly lower survival times than those without the genetic alterations (Fig. 17B, P=0.007). In terms of disease-free survival, despite the fact that there was no significant difference between these two groups, the patients with the genetic alterations were prone to undesirable prognoses (Fig. 17C). Of note, Alternate Terminator was the most significant splicing events of ATG9B (exon 18). Alternate Promoter in exon 2 and exon 3 were the most significant splicing events of BID and DNAJB1 respectively (Fig. 18). However, there was no significant difference between the three alternative splicing events among the different tumor samples.
Discussion

In cellular processes, autophagy is the foundation for homeostatic regulation. An autophagy-perturbed status is common in TC, and it can contribute to tumor progression. The present study highlighted the comprehensive analysis of ARGs in patients with TC. First, we identified the differentially expressed ARGs in TC cases and normal samples. Subsequently, a functional enrichment analysis found that autophagy influenced several tumor-related pathways. We then analyzed the clinical significances of the differentially expressed genes, and found...
Figure 16. The expression relationships of 3 prognosis-relevant autophagy-related genes with the methylation and copy number variations. (A) Relationship between ATG9B mRNA expression level and copy number alterations. (B) Correlation between ATG9B mRNA expression level and methylation level. (C) Relationship between BID mRNA expression level and copy number alterations. (D) Correlation between BID mRNA expression level and methylation level. (E) Relationship between DNAJB1 mRNA expression level and copy number alterations. (F) Correlation between DNAJB1 mRNA expression level and methylation level.
that they were closely related to lymph node metastasis and the tumor stage. It is also interesting that a risk model we proposed exhibited an excellent ability to predict the prognosis of TC. We expected to identify several clinically applicable diagnostic or prognostic biomarkers, and we provided a novel perspective of autophagy in TC.

Due to the indispensable function of autophagy, some researchers have evaluated several effects to reveal the clinical significance and corresponding molecular mechanisms in TC. However, its role in the tumorigenesis of TC remains controversial and unclear. Several scientific researchers have studied autophagy-related agents combined with other clinical treatment techniques in TC (31,32). These strategies regarding autophagy inhibition have achieved certain effects; however, there are still issues that have not been resolved. Hence, it is imperative to analyze the available data to indicate that the role of autophagy in the steps of TC onset, progression and prognosis. In the present study, a differential expression analysis was conducted among a series of ARGs to select the key mediators in the initiation of TC. In addition, we performed a
and developed a signature for predicting survival. The signa-
then submitted these genes to a multivariable Cox regression
levels and survival using a univariate Cox regression model,
the treatment response and progression surveillance.
stage. These findings evidently show that these genes actively
displayed a significant correlation with the advanced tumor
process is under strict control by a series of ARGs (46).
(ATG9B, BID and DNAJB1) included in the prognostic signa-
ture made it possible to separate the patients with TC into
two groups with significantly different survivals. Three genes
were the majority of these research studies focused only on a signal
in vitro or in vivo, is required in the future. These genes also
displayed a significant correlation with the advanced tumor
stage. These findings evidently show that these genes actively
participate in the proliferation and invasion of TC. Therefore,
these genes may be particularly useful for the assessment of
the treatment response and progression surveillance.
We examined the associations between the ARG expression
levels and survival using a univariate Cox regression model,
and identified 6 genes as candidate prognostic biomarkers. We
then submitted these genes to a multivariable prognostic model
and developed a signature for predicting survival. The signa-
ture made it possible to separate the patients with TC into
two groups with significantly different survivals. Three genes
( ATG9B, BID and DNAJB1) included in the prognostic signa-
ture could act as clinically applicable indicators. Autophagy
process is under strict control by a series of ARGs (46).
Previously, it was demonstrated that the dysregulated
autophagy level was closely related to tumor growth, survival
and proliferation (47). Moreover, the deletion of several essen-
tial ARGs compromises the survival of tumor cells in vitro
and in vivo (48,49). Therefore, the stable expression of ARGs is
indispensable for inhibiting the onset of tumors. In this study,
we found that several ARGs were dysregulated in the onset
and progression of PTC. Therefore, these genes may play an
important role in leading uncontrolled autophagy. Previously,
Wang et al reported that the lack of ATG9B may facilitate the
docking of both LC3 and p62 to initiate autophagy-associated
degradation (50). Furthermore, this process may promote the
apoptosis of hepatocytes in the initiation of hepatocellular
carcinoma. Behrends et al first reported that DNAJB1 was
involved in autophagy, which served as the bridge between
WIP2 and ATG2A. WIP2 and ATG2A act with each other
through DNAJB1. And depletion of WIP2 led to reducing
numbers of autophagosomes (51). In MCF-7 breast cancer cells,
BID knockdown has been proved effective in the suppression
of apoptosis and a shift of cell death towards autophagy (52).
These findings displayed different roles of core ARGs in
autophagy. Considering their expression profiles and clinical
significance in PTC, these genes may also contribute to the
progression of PTC via autophagy. ATG9B, BID and DNAJB1
all play multiple functions in autophagy; however, they have
not been reported for their important clinical significance
and molecular function in TC. These genes of less concern
could provide novel insight into the clinical management and
molecular mechanisms responsible for the development and
progression of TC. Therefore, in this study, we used several
bioinformatics methods to dig up the potential regulatory
mechanism. These three genes are all significantly related to
the methylation level of the CpG locus. Of note, the patients
with alterations in these three genes had worse survivals when
compared with the patients without alterations. Thus, we
proposed the hypothesis that epigenetics and genomic altera-
tions change the expression profiles of ARGs, thus influencing
the prognosis of TC. In the present study, we also explored the
associations between alternative splicing events and core ARGs.
We also found the difference of alternative splicing events in
each tumor samples were not huge. These findings hinted that
alternative splicing events may not be the cause of significant
clinical values of the three genes.
In this study, some limitations should be acknowledged.
First, the underlying molecular mechanisms of the key ARGs in
the TC pathogenesis are lacking, and additional experimental
investigations should be conducted to uncover these. Second,
although the prognostic signature we proposed was based
on the expression profiles and clinical information obtained
from the generally recognized TCGA database, it should be
validated in other independent databases. Despite these limita-
tions, this study highlighted the great clinical significance and
potential molecular mechanisms of autophagy. These findings
suggest that autophagy-targeted treatment may have a unique
effect on TC, particularly in patients with lymph node meta-
tases, and they provide insight into the complex biological
functions of autophagy in TC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

The study was designed by GC and PL. PL, HY and YH, XJL, JJJ, WJM, DYW, QL, JBP, YQW, HYL, QYM, YPW and DHP participated in statistical analysis. PL and HY wrote the draft and GC, HY AND YH corrected the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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