Identification of an Autophagy-Related Signature Predicting Overall Survival for Papillary Thyroid Carcinoma

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Abstract

Background: Papillary thyroid carcinoma usually shows an excellent prognosis. However, its recurrence or persistence rate is high. In this study, we used bioinformatics to identify autophagy-related genes (ARGs) and establish a novel scoring system for papillary thyroid carcinoma.

Methods: We collected ARGs sequencing data of patients with papillary thyroid carcinoma from The Cancer Genome Atlas database. Differentially expressed ARGs were identified by the “Limma” package in R language. After univariate and multivariate Cox regression analysis, an ARG signature was developed. The established prognostic signature was evaluated by Kaplan-Meier curve and time-dependent receiver operating characteristic.

Results: A sum of 26 differentially expressed ARGs were identified. Gene set enrichment analysis revealed that several significantly oncological signatures were enriched, such as autophagy, p53 signaling pathway, apoptosis, human cytomegalovirus infection, and platinum drug resistance. After univariate and multivariate analysis, 3 ARGs (ITPR1, CCL2, and CDKN2A) were selected to develop autophagy-related signature. Patients with high risk had significantly shorter overall survival than those with low risk. The areas under the curve indicated that the signature showed good accuracy of prediction.

Conclusions: We established a novel scoring system based on 3 ARGs, which provides a promising tool for the development of personalized therapy.

Keywords
papillary thyroid carcinoma, autophagy-related genes, prognostic signature, RNA-seq

Introduction

Thyroid cancer (TC) is the most common endocrine malignancy. The incidence of TC has been gradually increasing over the past few decades, and its morbidity has also been on a rising tendency worldwide, mainly due to the increasing use of diagnostic equipment.¹,² Papillary thyroid carcinoma is the most common and least aggressive type, taking up approximately 75% of total thyroid malignancies.³ Papillary thyroid carcinoma includes several histological subtypes with distinctive architectural tumor features, such as the follicular variant and the tall cell variant.⁴ Although it is a well-differentiated papillary carcinoma with an excellent prognosis, about half of patients with papillary thyroid carcinoma exhibit lymph node metastases, which are reported to be associated with increased risk of recurrence.⁴,⁵ Additionally, the treatment of papillary thyroid carcinoma has not been significantly improved yet. Therefore, novel molecular biomarkers or prognostic models are urgently needed to provide accurate treatment and to improve prognosis for patients with papillary thyroid carcinoma.

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Autophagy is an important physiological process for cells to degrade cytoplasmic components and maintain the stability of the intracellular environment.\(^\text{6}\) Abnormal levels of autophagy are associated with the pathogenesis of various diseases, including inflammation, neurodegenerative diseases, and tumors.\(^\text{7,8}\) However, the role of autophagy in tumorigenesis remains controversial, and the underlying mechanism is still rudimentary and inconclusive. In recent years, autophagy has been targeted in the search for new therapeutic strategies, such as therapies that inhibit or stimulate autophagy.\(^\text{9,10}\) However, few studies have used autophagy-associated gene expression profiles as a tool to assess the prognosis of patients with papillary thyroid carcinoma. In addition, while most studies have focused on the selection of treatment options for the general patient, few studies have been able to analyze and address the condition of individual patients.

At present, the most important risk factor for predicting patient survival is based on the tumor–node–metastasis (TNM) classification system.\(^\text{11}\) However, this provides only limited information for the clinical prognostication since even patients within the same stage display a strong heterogeneity for prognosis and treatment response. Therefore, finding the ideal biomarker or model to predict the prognosis of patients with papillary thyroid carcinoma has been a long-standing goal. Moreover, few have considered the analysis of genetic characteristics to establish a risk stratification system for papillary thyroid carcinoma. Recently, several studies indicated that gene signature can well predict the prognosis of cancer.\(^\text{12,13}\) However, no prior prognostic models for papillary thyroid carcinoma were established based on autophagy-related genes (ARGs). Therefore, the current study aimed to identify differentially expressed ARGs and build a novel prognostic signature to predict the prognosis of patients with papillary thyroid carcinoma.

**Materials and Methods**

**Data Collection**

We collected messenger RNA (mRNA) expression data and the corresponding clinical data of papillary thyroid carcinoma tissues and normal tissues from The Cancer Genome Atlas (TCGA) database. The autophagy gene list was downloaded from the Human Autophagy Database (HADb; http://autophagy.lu/clustering/index.html). The current study did not require ethics approval as all study data sets were downloaded and analyzed in accordance with the corresponding data policies of previous databases.

**Screening of the Differentially Expressed ARGs**

All genes were downloaded in fragments per kilobase million format. The differentially expressed genes (DEGs) were identified by Wilcoxon-rank sum test and fold change (FC) methods, with the thresholds of adjusted \(P\) value < .05 and |log FC| > 1.\(^\text{14}\) The heatmap of DEGs was created using “pheatmap” package of R language (https://cran.r-project.org/web/packages/pheatmap).

**Functional Enrichment Analysis**

Gene ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted by “clusterProfiler” package in R/Bioconductor to predict the possible function of differentially expressed ARGs. Adjusted \(P\) value < .05 was regarded as the cutoff value.

**Construction of ARG Risk Score**

To discover potential protective (hazard ratio (HR) < 1) and hazardous ARGs (HR > 1) that are significantly correlated with the overall survival (OS) of patients with papillary thyroid carcinoma, a univariate Cox proportional hazards model was used to screen out those with a significant \(P\) value < .05. These candidates were then subjected to a multivariate Cox proportional hazards regression with stepwise selection of variables based on the Akaike information criterion. By weighting the estimated Cox regression coefficients, an autophagy-related signature was constructed. The median risk score is considered as classification cutoff value between high- and low-risk papillary thyroid carcinoma subgroups. Between high- and low-risk papillary thyroid carcinoma subgroups, the differences in OS time were compared and analyzed via Kaplan-Meier analysis and log-rank test. To evaluate the predictive value of the ARG signature, we drew the time-dependent receiver operating characteristic (ROC) curve.\(^\text{15}\)

**Statistical Analyses**

All statistical analyses were performed by SPSS version 23.0 (Chicago, Illinois), along with version 3.6.0 of R software (https://www.r-project.org/). Survival curves were created by the Kaplan-Meier method and compared using the log-rank test. The Cox regression coefficients were used to establish a risk score signature. The prediction ability of signature was assessed by area under ROC curve in the package “survivalROC” in R language. Data were considered to be statistically significant with \(P\) value < .05.

**Results**

**Identification of Differentially Expressed ARGs**

The mRNA expression data of 510 papillary thyroid carcinoma tissues and 58 normal tissues were collected from the TCGA. A total of 231 ARGs were obtained from the HADb. Based on the cutoff criteria (adjusted \(P\) value < .05 and |log FC| > 1.0), a total of 26 differentially expressed ARGs (including 15 upregulated and 11 downregulated genes) were identified between papillary thyroid carcinoma and normal thyroid tissues (Figure 1).
Functional Enrichment Analysis

The GO functional analysis of the 26 differentially expressed ARGs can be segmented into biological process (BP), molecular function (MF), and cell component (CC). The top 10 results of the GO functional analysis are shown in Figure 2. The differentially expressed ARGs were primarily enriched in BP including neuronal death, autophagy, and process utilizing autophagic mechanism; in CC including mitochondrial outer membrane, organelle outer membrane, and outer membrane; and in MF including protein heterodimerization activity. Furthermore, we identified certain KEGG pathways positively associated with ARGs such as autophagy, apoptosis, p53 signaling pathway, human cytomegalovirus infection, human immunodeficiency virus 1 infection, and platinum drug resistance (Figure 3).

Figure 1. The volcano plot (A) and heatmap plot (B) of 26 differentially expressed autophagy-related genes (ARGs) between papillary thyroid carcinoma (PTC) and normal tissues.

Figure 2. Enrichment of top 10 Gene ontology (GO) terms of differentially expressed autophagy-related genes (ARGs). The node color changes gradually from red to blue in ascending order according to the adjusted $P$ values. The size of the node represents the number of counts.
Establishment of 3-ARG Signature

Univariate and multivariate Cox proportional hazards regression analyses were conducted to assess relationship between expression levels of ARGs and OS in patients with papillary thyroid carcinoma (Figure 4A and B). A predictive signature model was established according to 3 ARGs (ITPR1, CCL2, and CDKN2A) selected from further multivariate Cox regression analysis. The risk score for OS was calculated as follows: risk score = (0.12337693147 × expression value of ITPR1) + (0.028524 × expression value of CCL2) + (0.192202 × expression value of CDKN2A). We then used this model to calculate the risk score for each patient. Using the median of the risk score as the cutoff value, patients were stratified into high-risk (n = 233) and low-risk group (n = 234), respectively. The Kaplan-Meier curves, risk score, and survival status of these 3 prognostic ARGs are shown in Figure 5. The prognostic power of the autophagy-related signature was evaluated via Kaplan-Meier curve and areas under the curve (AUC) value of the ROC curve. Kaplan-Meier curves showed that patients in the high-risk group had a significantly lower OS than those in
the low-risk group \((P < .001)\). Areas under the curve value of the signature predicting the 5- and 10-year OS rates were 0.839 and 0.886, respectively (Figure 6). Furthermore, comparison showed that the discrimination of the signature was higher than that of the American Joint Committee on Cancer (AJCC) TNM classification (Figure 6).

**Discussion**

Although papillary thyroid carcinoma is a well-differentiated papillary carcinoma with an excellent prognosis, its recurrence rate after treatment is relatively high. Moreover, the TNM staging system does not adequately predict the risk of papillary thyroid carcinoma. Therefore, there is urgent need for better prognostic methods to predict the risk of patients with papillary thyroid carcinoma. In this study, we explored the ARG expression profiles of 467 patients with papillary thyroid carcinoma from TCGA and established a prognostic signature for stratifying the risk of papillary thyroid carcinoma. We identified 26 differentially expressed ARGs between papillary thyroid carcinoma tissues and normal thyroid tissues. Considering that these genes may be involved in the initiation of papillary thyroid carcinoma, we further analyzed the functions of the differentially expressed ARGs through GO and KEGG pathway analysis. The result indicated several significantly enriched oncological signatures, such as autophagy, apoptosis, p53 signaling pathway, human cytomegalovirus infection, human immunodeficiency virus 1 infection, and platinum drug resistance. After univariate and multivariate analysis, ARGs with independent prognostic value were selected to define a
risk-score model using their expression levels weighted by corresponding correlation coefficient in the multivariate Cox analysis. According to median risk scores, the patients with papillary thyroid carcinoma were assigned to either low- or high-risk group. Patients with high-risk score have significantly worse OS than those with low-risk score. The prognostic power of the autophagy-related signature was evaluated via AUC value of the ROC curve. The results showed that the risk-score model can effectively stratify the risk of patients with papillary thyroid carcinoma.

The proposed autophagy-related signature included 3 ARGs (ITPR1, CCL2, and CDKN2A). All genes in the signature were associated with OS of patients with papillary thyroid carcinoma. Inositol 1,4,5-trisphosphate receptor, type 1 (ITPR1), a member of IP3 receptor family, encodes an intracellular receptor mediating calcium release from the endoplasmic reticulum and plays a role in inducing autophagy.\(^\text{16-18}\) Messai et al\(^\text{17}\) reported that ITPR1 is a new direct target of hypoxia-inducible factors 2α (HIF2α), and that targeting ITPR1 significantly increased the sensitivity of renal cancer cells to natural killer (NK)-mediated lysis. Mechanistically, ITPR1 overexpression regulated NK-mediated killing via autophagy activation in target cells by NK-derived signal. Silencing ITPR1 in renal cancer cells inhibited NK-induced autophagy. In addition, in vivo ITPR1 targeting significantly enhanced NK-mediated tumor regression. ITPR1-induced autophagy was also reported in other tumors, including papillary thyroid carcinoma.\(^\text{19,20}\)

Yu et al analyzed the transcriptomics data from 3 microarray data sets and identified ITPR1 as a key candidate gene in papillary thyroid carcinoma, consistent with our results. CCL2 (chemokine C-C motif ligand 2), also named monocyte chemotactic protein-1, is a well-known cytokine, which binds to CCR2 G-protein-coupled receptors to regulate macrophage recruitment during acute inflammation.\(^\text{21}\) Xu et al\(^\text{22}\) found that drug-resistant gastric cancer cells can secrete more CCL2 than drug-sensitive gastric cancer cells. CCL2 attenuated drug-induced cytotoxicity by activating PI3K–Akt–mTOR signaling to inhibit proapoptotic autophagy. Knockdown of CCL2 or autophagy induction successfully reversed the drug resistance of gastric cancer cells. Fang et al\(^\text{23}\) reported that CCL2 is overexpressed in luminal B breast cancer cells and increases cell growth and survival by inhibiting necrosis and autophagy. In anaplastic thyroid carcinoma,\(^\text{24}\) CCL2 can be secreted after cytokines stimulation, leading to a different modulation. However, it was not assessed whether CCL2 could be used as a marker in the follow-up of patients with anaplastic thyroid carcinoma. Cyclin-dependent kinase inhibitor 2A (CDKN2A) has been reported to induce autophagy in multiple malignancies.\(^\text{25,26}\) However, there are no reports of CDKN2A-induced autophagy in papillary thyroid carcinoma.

In our study, functional enrichment analysis of 26 ARGs was performed to further elucidate molecular and biological mechanisms in papillary thyroid carcinoma. The results demonstrated that these ARGs were mainly enriched in autophagy, apoptosis, p53 signaling pathway, human cytomegalovirus infection, human immunodeficiency virus 1 infection, and platinum drug resistance. Several signaling pathways identified in this study have been investigated in papillary thyroid carcinoma in recent years. Autophagy is a key cellular process that not only protects cells and organisms from stress but also ensures the maintenance and development of various cancers. Several autophagy-related pathways have been reported in TC.\(^\text{27-29}\) Wang et al\(^\text{27}\) showed that baicalein-induced autophagy of undifferentiated TC cells via the ERK/P38/Akt pathway. Xu et al\(^\text{29}\) indicated that transmembrane protein 21 (TMP21) modulates cell growth of papillary thyroid carcinoma cells by inducing autophagy through activation of the adenosine monophosphate-activated protein kinase/(AMPK) mTOR pathway. P53 is a classic tumor suppressor that often mutates between multiple cancer types. As a key guardian in tumorigenesis, p53 is activated in response to carcinogenesis and regulates a large number of genes involved in cell cycle arrest, DNA repair, and apoptosis.\(^\text{30}\) Mutations of p53 are highly frequent in colorectal cancer, and p53 mutation status is associated with disease outcome.\(^\text{31}\) Taken together, these differentially expressed ARGs are involved in many important papillary thyroid carcinoma–associated biological functions and pathways.

Our study is the first to establish an autophagy-related signature and to show favorable predictive ability. Our results are of great relevance to clinicians and patients because they allow to predict individual patient outcomes. When patients who were previously diagnosed with a tumor ask for a clinician’s help, they are likely more concerned about their individual survival risk at that specific moment, than the risk since diagnosis, as traditionally considered.

Nonetheless, there are still some limitations of this study. First, the proposed signature was established based on data from the TCGA database. Additional validation with independent external mRNA expression data are needed to investigate the performance of the signature. In addition, the signature was established based on bioinformatics data. Further experimental studies are needed to validate the functions and predictive value of the 3 ARGs.

**Conclusions**

This study successfully identified 3 prognostic ARGs and developed a novel scoring system to predict OS in patients with papillary thyroid carcinoma. It may help in clinical decision-making for individualized therapeutic regimen design.

**Authors’ Note**

G.H. and H.-F.F. contributed equally to this work. G.H. and H.Z. conceived and designed the original study. G.H. and H.-F.F. collected and analyzed the data. G.H., H.-F.F., and H.Z. contributed to the interpretation of data. G.H. and H.-F.F. drafted the manuscript. H.Z. revised the manuscript. All authors read and approved the final manuscript.

**Declaration of Conflicting Interests**

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References
1. La Vecchia C, Negri E. Thyroid cancer: the thyroid cancer epidemic—overdiagnosis or a real increase? Nat Rev Endocrinol. 2017;13(6):318-319.
2. Morris LG, Tuttle RM, Davies L. Changing trends in the incidence of thyroid cancer in the United States. JAMA. 2016;142(7):709-711.
3. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in thyroid cancer incidence and mortality in the United States, 1974-2013. JAMA. 2017;317(13):1338-1348.
4. Sipos JA, Mazzaferrri EL. Thyroid cancer epidemiology and prognostic variables. Clin Oncol. 2010;22(6):395-404.
5. Ito Y, Kudo T, Kobayashi K, Miya A, Ichihara K, Miyauchi A. Prognostic factors for recurrence of papillary thyroid carcinoma in the lymph nodes, lung, and bone: analysis of 5,768 patients with average 10-year follow-up. World J Surg. 2012;36(6):1274-1278.
6. Weidberg H, Shvets E, Elazar Z. Biogenesis and cargo selectivity of autophagosomes. Annu Rev Biochem. 2011;80:125-156.
7. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008;132(1):27-42.
8. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069-1075.
9. Kocaturk NM, Akkoc Y, Kig C, Bayraktar O, Gozuacik D, Kutlu O. Autophagy as a molecular target for cancer treatment. Eur J Pharm Sci. 2019;134:116-137.
10. Janku F, McConkey DJ, Hong DS, Kurzrock R. Autophagy as a molecular target for cancer treatment. Annu Rev Mol Cell Biol. 2011;25:173-194.
11. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: continuing to build a bridge from a population-based to a more “personalized” approach to cancer staging. CA Cancer J Clin. 2017;67(2):93-99.
12. Wu J, Zhao Y, Zhang J, Wu Q, Wang W. Development and validation of an immune-related gene pairs signature in colorectal cancer. Oncotarget. 2019;8(7):1596715.
13. Zuo S, Wei M, Zhang H, et al. A robust six-gene prognostic signature for prediction of both disease-free and overall survival in non-small cell lung cancer. J Transl Med. 2019;17(1):152.
14. Noble WS. How does multiple testing correction work? Nat Biotechnol. 2009;27(12):1135-1137.
15. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics. 2000;56(2):337-344.
16. Santulli G, Marks AR. Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging. Curr Mol Pharmacol. 2015;8(2):206-222.
17. Messia Y, Noman MZ, Hasmim M, et al. ITPR1 protects renal cancer cells against natural killer cells by inducing autophagy. Cancer Res. 2014;74(23):6820-6832.
18. Messia Y, Noman MZ, Janji B, Hasmim M, Escudier B, Chouaib S. The autophagy sensor ITPR1 protects renal carcinoma cells from NK-mediated killing. Autophagy. 2015;14(10):1831-1844.
19. Xu S, Wang P, Zhang J, et al. Al-ncRNA EGOT enhancing autophagy sensitizes paclitaxel cytotoxicity via upregulation of ITPR1 expression by RNA-RNA and RNA-protein interactions in human cancer. Mol Cancer. 2019;18(1):89.
20. Yu X, Zhong P, Han Y, et al. Key candidate genes associated with BRAF in papillary thyroid carcinoma on microarray analysis. J Cell Physiol. 2019;234(12):23369-23378.
21. Roca H, Varsos ZS, Mizutani K, Pienta KJ. CCL2, survivin and autophagy: new links with implications in human cancer. Autophagy. 2008;4(7):969-971.
22. Xu W, Wei Q, Han M, et al. CCL2-SQSTM1 positive feedback loop suppresses autophagy to promote cheemosresistance in gastric cancer. Int J Bio Sci. 2018;14(9):1054-1066.
23. Fang WB, Yao M, Jokar I, et al. The CCL2 chemokine is a negative regulator of autophagy and necrosis in luminal B breast cancer cells. Breast Cancer Res Treat. 2015;150(2):309-320.
24. Ferrari SM, Elia G, Piaggi S, et al. CCL2 is modulated by cytokines and PPAR-γ in anaplastic thyroid cancer. Anticancer Agents Med Chem. 2018;18(3):458-466.
25. Jeong EH, Lee TG, Ko YJ, et al. Anti-tumor effect of CDK inhibitors on CDKN2A-defective squamous cell lung cancer cells. Cell Oncol. 2018;41(6):663-675.
26. Kang R, Tang D, Lotze MT, Zeh HJ. AGER/RAGE-mediated autophagy promotes pancreatic tumorigenesis and bioenergetics through the IL6-pSTAT3 pathway. Autophagy. 2012;8(6):989-991.
27. Wang M, Qiu S, Qin J. Baicalein induced apoptosis and autophagy of undifferentiated thyroid cancer cells by the ERK/PJ3K/Akt pathway. Am J Transl Res. 2019;11(6):3341-3352.
28. Feng H, Cheng X, Kuang J, et al. Apatinib-induced protective autophagy and apoptosis through the AKT-mTOR pathway in anaplastic thyroid cancer. Cell Death Dis. 2018;9(10):1030.
29. Xu X, Gao H, Qin J, He L, Liu W. TMP21 modulates cell growth in papillary thyroid cancer cells by inducing autophagy through activation of the AMPK/mTOR pathway. Int J Clin Exp Pathol. 2015;8(9):10824-10831.
30. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2013;13(2):83-96.
31. Russo A, Bazan V, Iacobetta B, Kerr D, Soussi T, Gebska N. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. J Clin Oncol. 2005;23(30):7518-7528.