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Original Article

Potential Role of Cuproptosis-Related Lncrna in Prognosis and Immunotherapy of Thyroid Carcinoma

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Abstract

Background: Cuproptosis-related long non-coding RNA (lncRNA) disease is associated with the development and progression of tumors. We aimed to investigate the prediction of cuproptosis-related lncRNA on the prognosis and immunotherapy of patients with thyroid carcinoma (THCA).

Methods: The thyroid cancer-associated expression data and lnc RNAs data were downloaded from The Cancer Genome Atlas (TCGA) and Ensembl database. The prognostic model of cuproptosis-related lncRNAs was successfully constructed through Lasso regression analysis and Cox regression analysis. Then, the prognostic value of prognostic model of cuproptosis-related lncRNAs was tested through the survival analysis, ROC curves and nomographic charts. Finally, the prognostic model of cuproptosis-related lncRNAs associated with immunity and mutational load of tumors was analyzed, and potential targeted drugs for THCA were predicted.

Results: A cuproptosis-related lncRNA model of THCA (AC026100.1, AF235103.3, LNCSRLR) was successfully constructed, which has an independent prognostic value. Moreover, the cuproptosis-related lncRNA model was associated with immune signatures and mutational load in most tumors, showing its high correlation with the sensitivity of targeted drugs such as 5-Fluorouracil, Bleomycin, Rapamycin and Sunitinib.

Conclusion: The cuproptosis-related lncRNA model of THCA has promising applications in the treatment and prognosis of THCA.

Keywords: Cuproptosis; lncRNA; Thyroid carcinoma; Immunotherapy

Introduction

Thyroid carcinoma (THCA) is a tumor which commonly develops in the endocrine system (1). Over the past four decades, the incidence of this disease has been increasing in pets and people due to the increasing proportion of obesity and environmental factors (2). It is expected to be the fourth most common tumor by 2030 (3). According to the histological characteristics, THCA can be divided into well-differentiated THCA (including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), Hŭrthle cell tumor, poorly-differentiated or undifferentiated THCA and medullary thyroid carcinoma (MTC) (4). Among them, the most common one is PTC, accounting for about 80% of all THCA patients, followed by those with FTC, which accounts for about 10%. Undifferentiated THCA and MTC are less common (5). As the best imaging modali-

ty for the diagnosis of high-risk neoplastic lesions, the effect of fine-needle aspiration biopsy (FNAB) depends heavily on the technical level and experience of the operator (6). Although FNAB is simple, safe, and accurate, uncertainty still remains in 15 – 30% of cases (7). The treatments of surgical surgery, thyroid hormone and radioactive iodine have better effects in most patients, which have the best prognosis in those with PTC, with the metastases consistently observed in cervical lymph nodes, but less regularly detected in lungs (8). Also, the most common type of PTC is PTMC. With the development of ultrasound technology, ultrasound-guided thermal ablation of PTMC can also achieve the same therapeutic effect as surgery. Currently, although THCA patients have high overall survival rate, worryingly, nearly 30% of whom have a 10-year recurrence rate, while the survival rate in these high-risk patients can be about 50% (9). It calls for innovative approaches to find critical biomarkers with sufficient sensitivity in the research on THCA.

An abnormal rise of copper ions in human cells might induce cell death with a pathway distinct from known mechanisms of regulated cell death, and copper ions can still induce cell death when blocking known cell death patterns, including apoptosis, ferroptosis, pyroptosis and necroptosis (10). Thus, this copper-dependent modulated mode of cell death is named cuproptosis. Long non-coding RNA (lncRNA) is widely involved in the biological behaviors such as proliferation, apoptosis, invasion and metastasis of THCA (11). Interestingly, lncRNA also plays a crucial role in regulating cuproptosis (12). Increasing evidences suggest the use of cuproptosis-related lncRNAs to predict outcomes in patients with tumors (13,14). On THCA, several models have been published with the aim of assessing the efficiency of treatment procedures and identifying prognostic factors in differentiated carcinomas (15,16). In the face of the complexity of cancer, predictive models are currently considered as a valuable research tool (17). The risk prediction model is a mathematical equation that estimates the risk probability of diseases with patients' data on risk factors (18). It has multiple applications across the medical practice, such as predicting the development of diseases, the response to treatments or the prognosis in patients. A suitable risk prediction model can be developed using different statistical methods according to the research objectives, including logistic regression, artificial neural network, Cox regression and machine learning (19-21).

In this study, first, a cuproptosis-related lncRNA prognosis model was successfully constructed. Then, the prognostic value of the prognostic model of cuproptosis-related lncRNAs was also tested. Finally, it was found that the prognostic model of cuproptosis-related lncRNAs was associated with immunity and mutation load of tumors, and four potential targeted drugs for THCA were predicted.

Materials and Methods

Acquisition of patients' datasets

Rna-seq expression and clinical information of 571 patients with THCA were obtained from The Cancer Genome Atlas (TCGA) data portal (https://cancergenome.nih.gov/) (22). A total of 16,876 lncRNAs and 19,938 protein-coding genes were annotated and classified through the Ensembl Human Genome Browser GRH38.p13 (http://asia.ensembl.org/index.html). Finally, 507 THCA patients were included in this study (Fig. 1).

This study has been approved by the Ethics Committee of the First People's Hospital of Yunnan Province (Approval No.: KHLL2020-KY034).

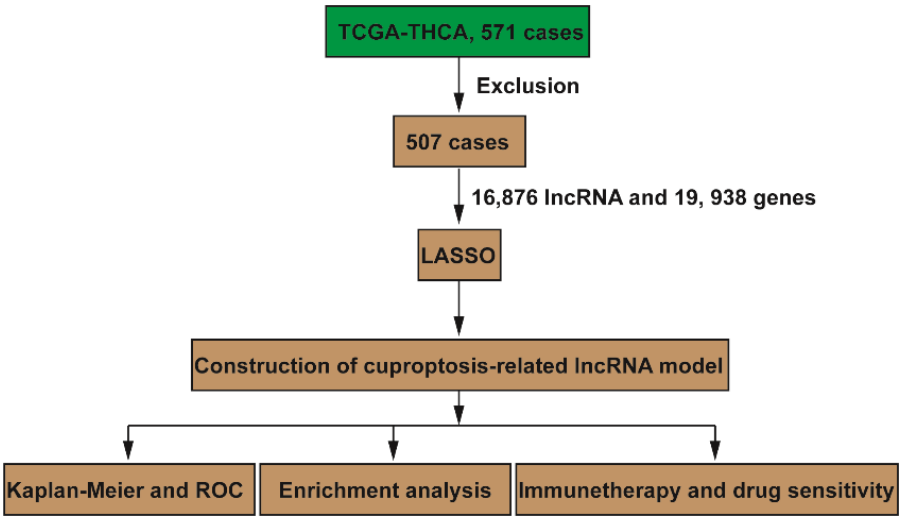

Fig. 1: Comprehensive analysis flow chart

Identification of co-expression networks of cuproptosis-related lncRNA and lncRNA-mRNA in thyroid carcinoma

Nineteen cuproptosis-related genes were obtained from previous related studies (10,23). Pearson correlation coefficient method was used to screen cuproptosis-related lncRNAs, $|R| > 0.6$ and $P<0.001$. Sankey diagrams were used to visualize the lncRNA-mRNA co-expression network.

Construction of a prognostic signature of cuproptosis-related lncRNA in thyroid carcinoma

The association between cuproptosis-related lncRNA and overall survival (OS) in THCA patients was built through a univariate Cox regression model ($P<0.05$). Minimum absolute shrinkage and selection operator (Lasso) Cox regression analysis on the prognostic-related cuproptosis-related lncRNAs was performed with the "glmnet" package in the R software. To assess its independent prognostic impact on survival, a multivariate Cox regression analysis was conducted to analyze the candidates for cuproptosis-related lncRNAs. For the prognostic model of cuproptosis-related lncRNAs, the best lncRNA prognostic marker was selected according to the lowest Akaike information criterion (AIC) value. The risk score for each patient was calculated according to the following formula (24): Risk Score = $\sum n\ k = 0$ coef (k) $*$ x (k), where coef (k) and x (k) represent the regression coefficients, representing values are cuproptosis-related lncRNAs. Subsequently, patients were divided into high- and low-risk groups according to the median of risk score.

Independent prognostic analysis and ROC curves

The Kaplan-Meier survival curves were plotted by the R package "ggsurvplot" to compare OS or PFS between the high- and low-risk groups. ROC curves were calculated with the R package "survivalROC" to present the predictive capability (25).

Nomographic charts

Nomographic charts (26) were plotted to predict possible 1-, 3- and 5-year survival in THCA patients. Potential differences among subgroups by age, gender and tumor stage were investigated. Enhanced regression nomographic charts on risk scores and other clinical covariates of the cuproptosis-related lncRNA model in THCA patients were constructed with the R package "regplot". In calibration curves of risk scores and other clinical covariates of the model for THCA patients, deviation correction estimates of predicted and observed values were determined with

1000 bootstraps and analyzed with the R package "rms".

Principal component analysis (PCA), GO and KEGG

PCA (27) was used to investigate the distribution of THCA patients who had different risk scores. The Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were adopted as a reference, which were then studied through enrichment analysis by the R package "clusterProfiler". The Benjamini-Hochberg method was applied for multiple correction, and the false discovery rate (FDR) < 0.05 was considered significant.

Immune correlation analysis

The ssGSEA analysis was conducted to examine the association between the immune of tumors (Type II IFN response, Cytolytic activity, Inflammation-promoting, Type I IFN response, APC co-inhibition, MHC class I, APC co-stimulation, CCR, Parainflammation, HLA, T cell co-inhibition, Check-point, and T cell co-stimulation) and the cuproptosis-related lncRNA model.

Mutation analysis and tumor mutation load

The TMB score was defined as the total number of somatic mutations/exon length (28). The TMB score (mutation frequency) was calculated with Perl scripts based on the Java8 platform. THCA patients were defined as the high and low TMB groups based on the median TMB score. After combining the TMB score with clinical characteristics data of THCA patients, the Kaplan-Meier survival analysis was performed to analyze the correlation between TMB and clinical characteristics. The waterfall plots were used to visualize the frequency of gene mutations in patients in both high- and low-risk groups.

Identification of potential drugs

The potential THCA-targeted drugs were screened with the R package "prophetic" based on the cuproptosis-related lncRNA model (29). The Spearman correlation analysis was performed to examine the correlation coefficients of THCA-targeted drugs and the cuproptosis-related lncRNA model.

Statistical analysis

A descriptive statistical analysis was conducted on THCA patients in the TCGA. Continuous variables were described as the mean ± standard deviation, and categorical variables were described as frequency and proportions. The Benjamini-Hochberg method was adopted for multiple correction, and FDR <0.05 was taken as the criterion for statistical dominance. All statistical analyses were performed with 4.1.1 in R version. The p-value was bidirectional, and $P<0.05$ was statistically significant.

Results

Determination of prognostic signature of cuproptosis-related lncRNA in thyroid carcinoma

Overall, 580 cuproptosis-related lncRNAs were identified from 16,876 lncRNAs and 19 cuproptosis-related genes by the criteria of $|R|> 0.6$ and $P<0.001$ (Fig. 2A). Univariate Cox proportional hazards analysis showed that 18 cuproptosis-related lncRNAs were significantly correlated with the survival rate of THCA patients (Fig. 2B). LASSO Cox regression was conducted to screen for prognostic cuproptosis-related lncRNAs based on a ten-fold cross-validation at 1000 × (Fig. 2C, D). Multivariate Cox analysis further identified three lncRNAs with prognostic significance (AC026100.1, AF235103.3 and LNCSRLR), which were then used to construct cuproptosis prognostic model.

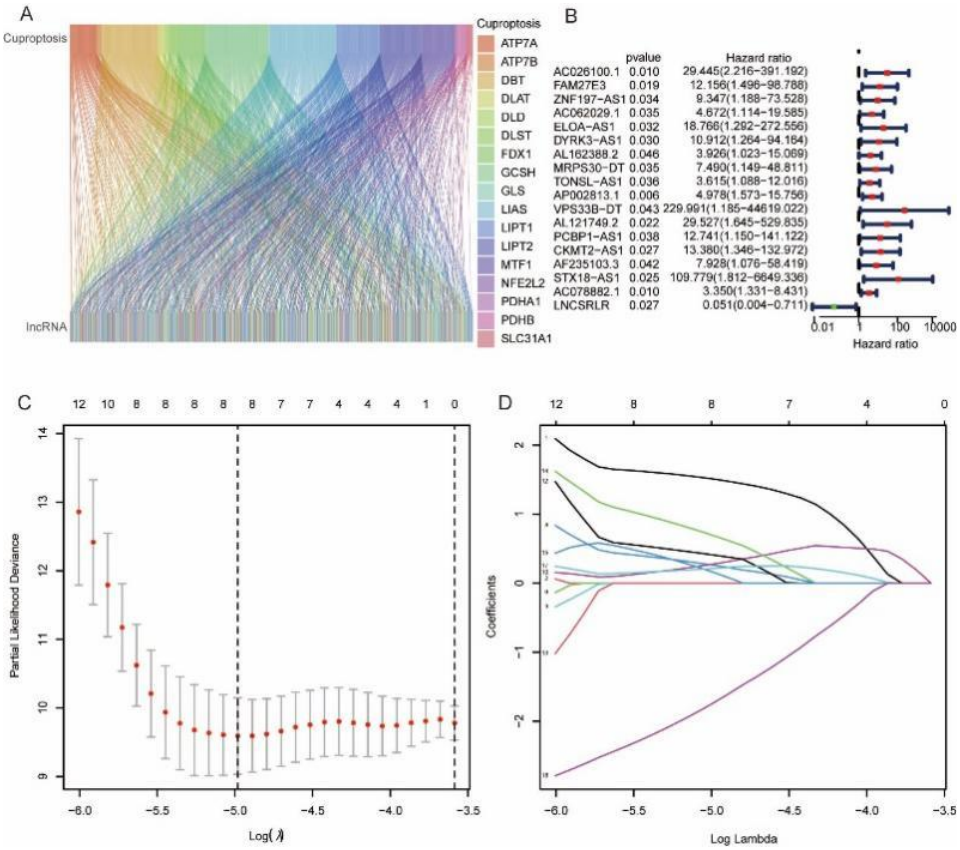

Fig. 2: Cuproptosis-related lncRNA selection utilizing Lasso model.

A. Co-expression network of cuproptosis-related genes and lncRNAs. B. The univariate Cox regression analysis results show that cuproptosis-related lncRNAs. C. Plots on the ten-fold cross-validation error rates. D. LASSO coefficient profiles on cuproptosis-related lncRNAs

Evaluation on the value of a prognostic model of cuproptosis-related lncRNAs

THCA patients were classified into high- and low-risk groups by the median risk score based on the cuproptosis prognostic model. Risk curves and scatter plots were drawn to illustrate the risk score and corresponding survival status of THCA patients. The higher the risk score, the higher the patient's mortality was (Fig. 3A, B). The heat map also showed that AC026100.1 and AF235103.3 were upregulated in patients with high-risk THCA, while LNCSRLR was up-regulated in low-risk THCA (Fig. 3C). The heat map on the correlation with cuproptosis-related genes showed that AC026100.1, AF235103.3 and LNCSRLR were highly associated with cuproptosis-related genes, respectively (Fig. 3D). The Kaplan-Meier survival analysis showed that both OS and PFS in the high-risk group were lower than those in the low-risk group (Fig. 3E, F; OS: $P = 0.009$, PFS: $P = 0.010$).

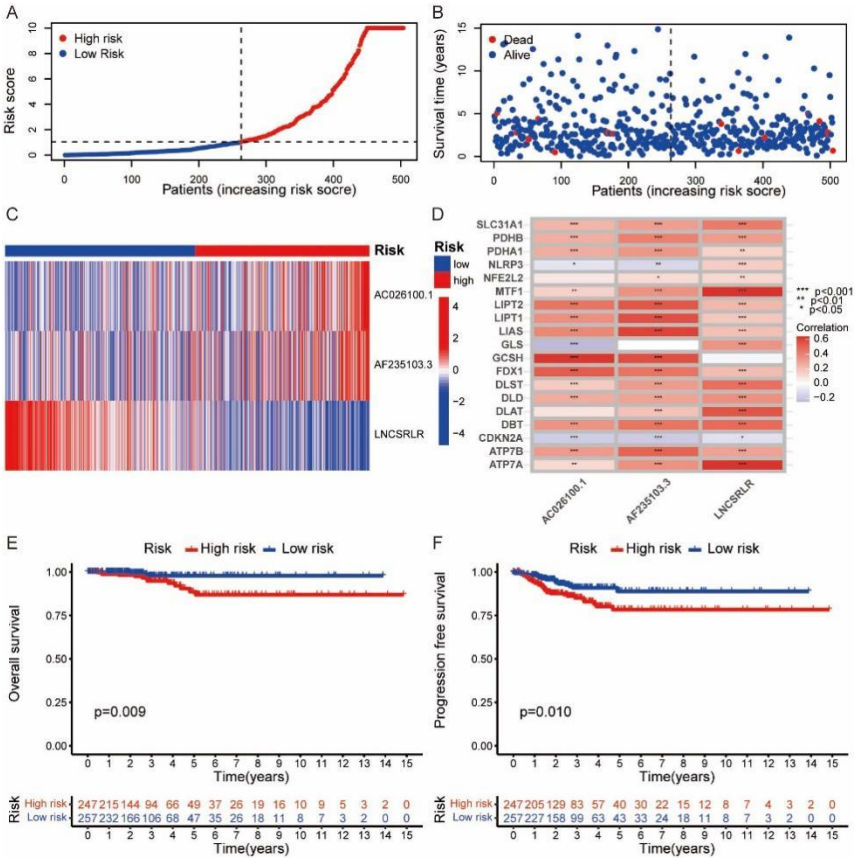

Fig. 3: the Construction and validation of the The Overall Survival and Progression free survival of Cuproptosis-related lncRNA model in THCA patients.

A-C. Distribution and survival status of THCA patients with different risk scores, and Heatmap of the lncRNA signature. D. Heatmap of the correlation between AC026100.1, AF235103.3, LNCSRLR and cuproptosis- related genes. E-F. The Overall Survival and Progression free survival of cuproptosis-related lncRNA model in THCA patients. THCA, Thyroid carcinoma

The prognostic model of cuproptosis-related lncRNAs is an independent prognostic factor for patients with thyroid carcinoma

Both univariate and multivariate Cox regression analyses were conducted to determine whether a prognostic model of cuproptosis-related lncRNAs could be used as an independent risk factor for THCA patients. Multivariate Cox regression analysis showed that age (HR=1.162, 1.101-1.226, P = 0.001) and risk scores of prognostic model of cuproptosis-related lncRNAs (HR= 1.051, 1.006-1.097, P = 0.026) were independently associated with OS (Fig. 4A, B). ROC curve analysis showed that AUC value based on risk scores of prognostic model of cuproptosis-related lncRNAs was greater than 0.7 (1 year, AUC=0.947; 3 years, AUC=0.722; 5 years, AUC=0.778), which was greater than most other clinical prognostic indexes, such as stage, age, gender, T stage, etc. (Fig. 4C-D). In addition, C-index scores (Fig. 5A) were also performed to verify the reliability of prognostic model of cuproptosis-related lncRNAs (C-index>0.8) and stage, T stage, M stage, gender, age and risk scores were applied to predict 1-, 3-, and 5-year survival in THCA patients (Fig. 5B, C). Kaplan-Meier survival analysis showed that OS was lower in high-risk patients either in stages I-II or in stages III-IV (Fig. 5D, E; I-II: P = 0.015, III-IV: P = 0.004). These data suggest that the prognostic model of cuproptosis-related lncRNAs is an independent prognostic factor in THCA patients.

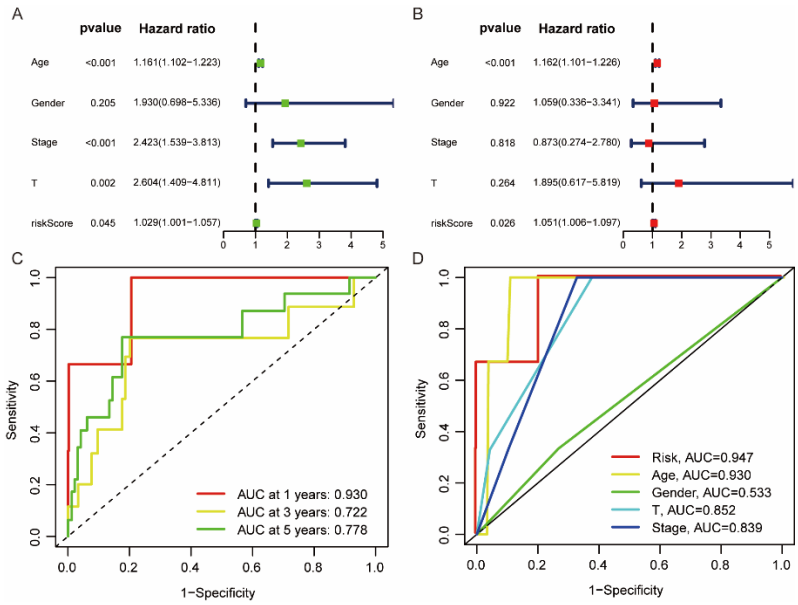

Fig. 4: Cuproptosis-related lncRNA model was an independent prognostic factor.

A-B. The univariate and multivariate Cox regression analysis of risk model score and clinical feature prognostic value. C. ROC curve used to predict prognosis in patients with THCA at 1, 3, and 5 years. D. ROC curve used to predict prognosis in patients with THCA at 1 year based on risk score, age, AJCC stage, and T stage. THCA, Thyroid carcinoma

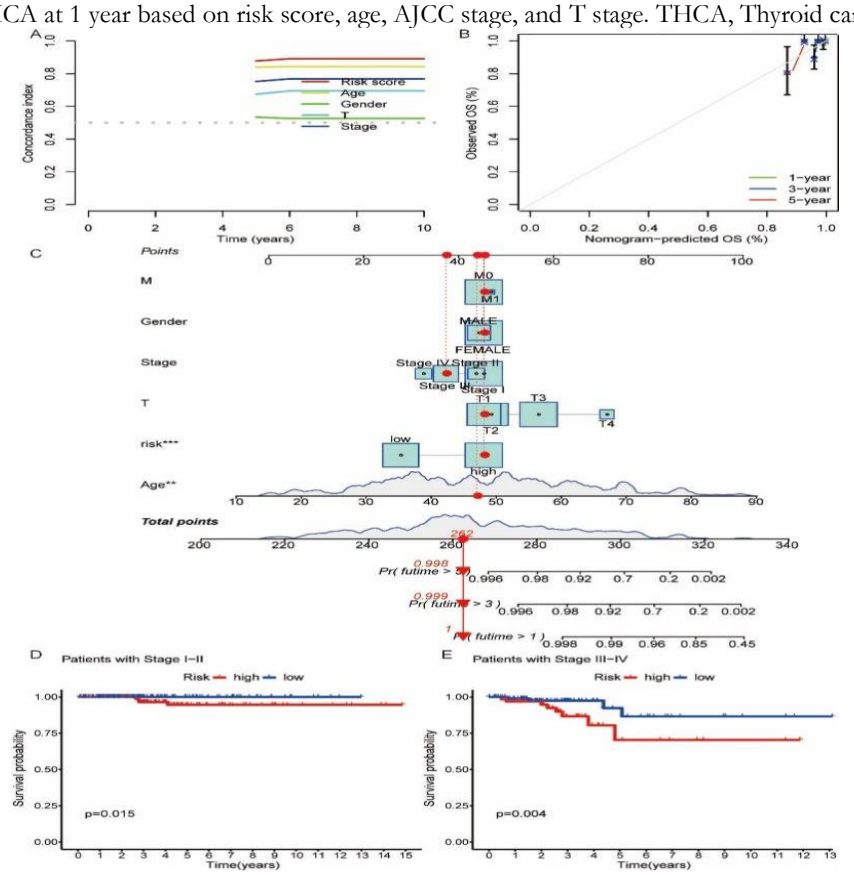

Fig. 5: Cuproptosis-related lncRNA model used to predict prognosis in THCA patients

A.C-index of cuproptosis-related lncRNA model in THCA patients. B-C. Calibration and nomogram used to predict prognosis in THCA patients at 1, 3, and 5 years based on risk score, age, AJCC stage and T stage. D-E. The overall survival of cuproptosis-related lncRNA model in THCA patients with stage I-II and stage III-IV. THCA, Thyroid carcinoma

Principal component analysis and enrichment analysis

PCA maps were performed based on the three lncRNAs from the prognostic model of cuproptosis-related lncRNAs including whole genome, cuproptosis-related genome and cuproptosis-related lncRNAs to visualize the distribution of patients (Fig. 6A-D). GO analysis (Fig. 7A,B) showed that the prognostic model of cuproptosis-related lncRNAs participated in biological behaviors such as signaling receptor activator activity, receptor ligand activity, collagen containing extracellular matrix, endoplasmic reticulum lumen, external encapsulating structure organization and response to copper ion. KEGG analysis (Fig. 7C,D) showed that the prognostic model of cuproptosis-related lncRNAs was involved in signaling pathways such as extracellular matrix organization, response to metal ion, sensory perception of mechanical stimulus and response to copper ion.

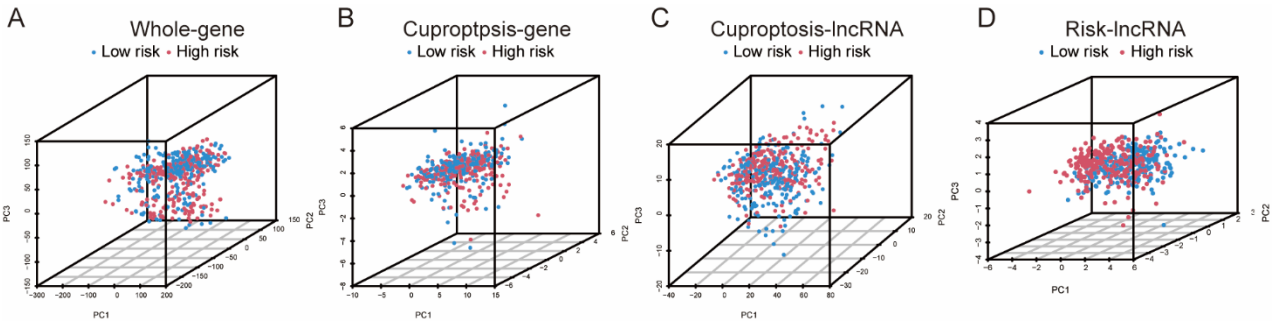

Fig. 6: Patients with high and lowrisk scores have different cuproptosis statuses.
A-D: PCA maps show the distribution of patients based on the whole genome; cuproptosis-related gene sets; cuproptosis-related lncRNAs; and cuproptosis-related lncRNA model

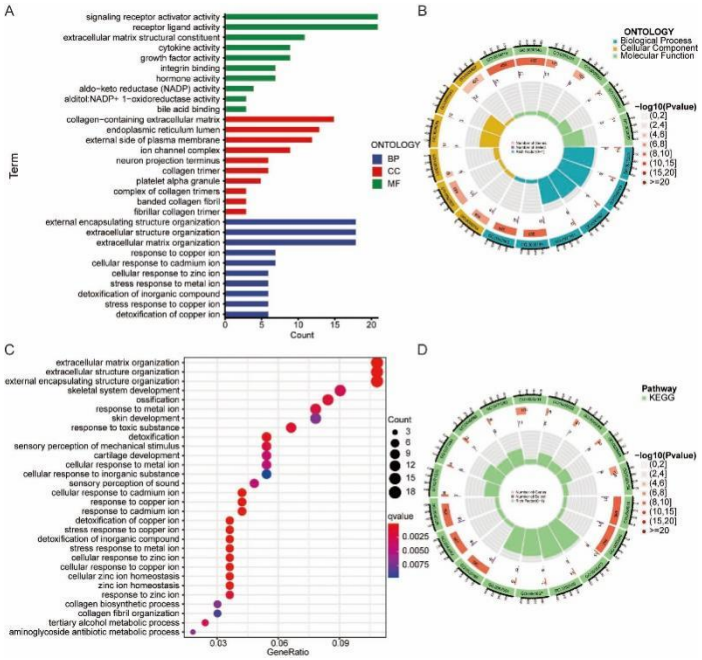

Fig. 7: GO and KEGG analysis of cuproptosis-related lncRNA model in THCA patients.
A-B. GO analysis of cuproptosis-related lncRNA model in THCA patients.C-D. KEGG analysis of cuproptosis-related lncRNA model in THCA patients. THCA, Thyroid carcinoma

Analysis of tumor immunity and mutational load

To further explore the role of prognostic model of cuproptosis-related lncRNAs in THCA, its relationship with tumor immunity was validated based on a prognostic model. The results showed that the risk score of prognostic model of cuproptosis-related lncRNAs was correlated with inflammation-promoting, Type I IFN response, APC co-inhibition, MHC class I, CCR, Parainflammation, HLA, T cell co-inhibition, Checkpoint and T cell co−stimulation (Fig. 8A). In addition, a tumor mutation load analysis was performed based on a prognostic model. The results showed that the BRAF gene mutation rate of patients in the low-risk group (71%) was higher than that of patients in the high-risk group (49%) (Fig. 8B, C), while patients in the high-risk group had higher tumor mutation load and lower OS than that in the low-risk group (Fig. 8D-F).

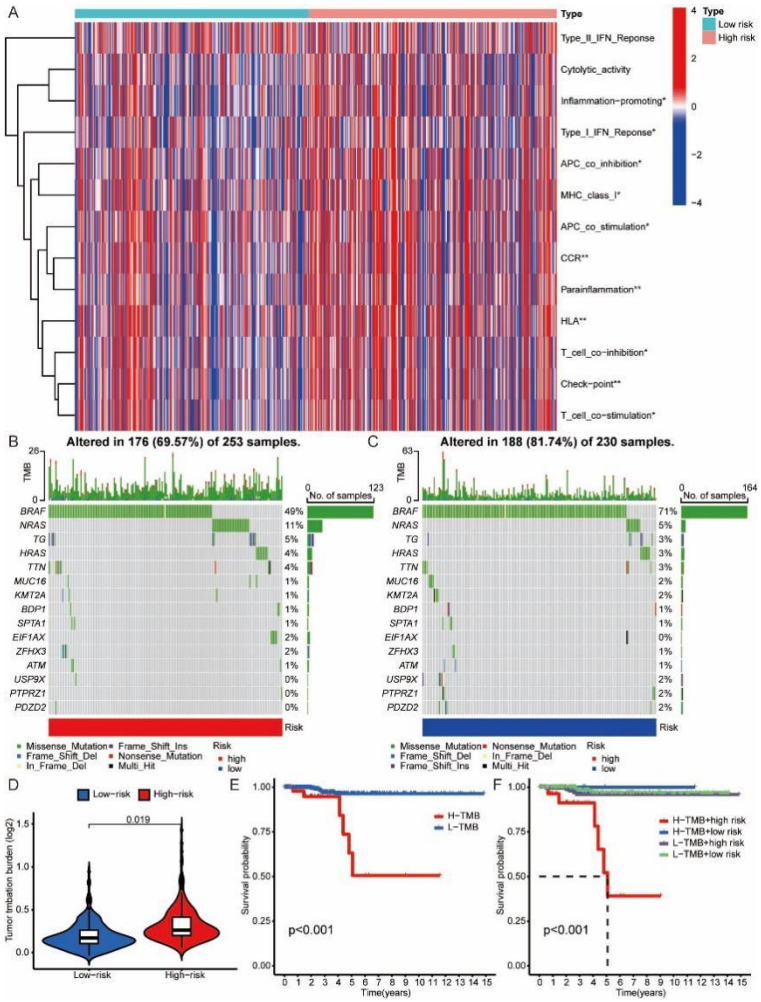

Fig. 8: the Relationship between the risk score and tumor immune

A. Heatmap of the correlation between cuproptosis-related lncRNA model and tumor immune. B-C. Waterfall plot of mutation profiles of each gene in each sample. The legend at the bottom described the mutation types. The plot above the legends showed the mutation burden of each sample. D. Patients in the high-risk group had a higher tumor mutational burden. E-F. Higher TMB level was correlated with worse survival outcome in THCA patients. THCA, Thyroid carcinoma

Potential therapeutic drugs for thyroid carcinoma

Finally, potential targeted drugs for THCA were screened based on a prognostic model of cuproptosis-related lncRNAs. The results showed that THCA patients might be sensitive to drugs such as 5-Fluorouracil, Bleomycin, Rapamycin and Sunitinib (Fig. 9A-H, 5-Fluorouracil: R=0.2, Bleomycin: R=0.23, Rapamycin: R=0.19, Sunitinib: R=0.16).

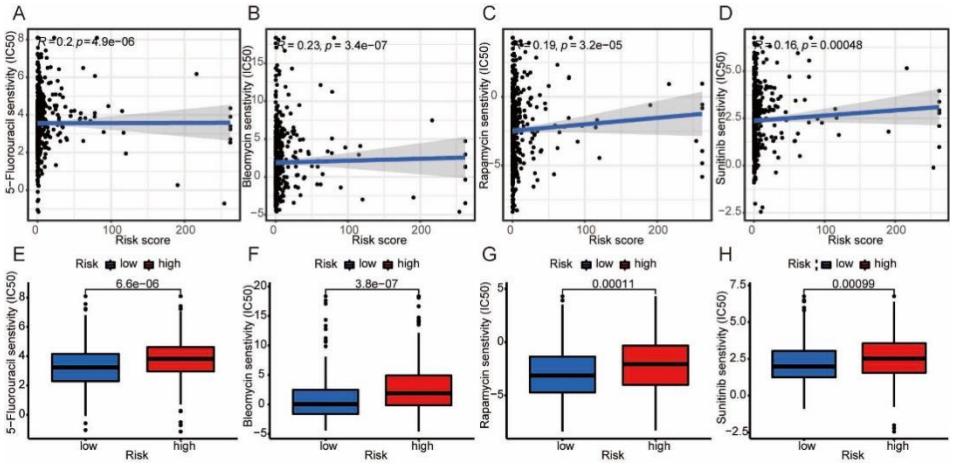

Fig. 9: the Potentially targeted drugs for THCA patients

A-D. 5-Fluorouracil, Bleomycin, Rapamycin, and Sunitinib were correlated with cuproptosis-related lncRNA model in THCA patients. E-H. Cuproptosis-related lncRNA model in THCA patients with high risk were sensitive with 5-Fluorouracil, Bleomycin, Rapamycin and Sunitinib, respectively. THCA, Thyroid carcinoma

Discussion

In this study, we constructed a prognostic model of lncRNA in THCA, successfully validating its independent prognostic value in THCA. The prognostic model of cuproptosis-related lncRNAs could effectively predict the survival of THCA patients. Furthermore, the prognostic model of cuproptosis-related lncRNAs was significantly associated with tumor immunity and mutational load and could predict sensitive potentially targeted drugs for THCA. The prognostic model constructed in this study consisted of three cuproptosis-related lncRNAs (AC026100.1, AF235103.3 and LNCSRLR).

To our knowledge, no previous study has examined the correlation between the prognostic model of cuproptosis-related lncRNAs and the development of THCA. Surprisingly, cuproptosis-related lncRNAs were differentially expressed between tumors and normal tissues. All of these lncRNAs were significantly associated with OS and PFS, suggesting a potential role of cuproptosis in the prognosis of THCA and the predictive value of this model in predicting the survival of THCA. Similar to iron, copper is an essential element in maintaining life activities ranging from microorganisms to plants, nonhuman animals, and human beings, and plays an essential role as a cofactor for essential enzymes (30). Notably, cancer cells have a higher demand for copper compared to normal cells; some cancers express large amounts of thioacylated mitochondrial proteins and show high levels of respiration (31).

Meanwhile, elevated copper concentrations were found in tumor tissues or serum from animal models and multiple cancer patients (32). Therefore, copper chelators are expected to be developed as adjuvant agents in the future. Through a genome-wide CRISPR-Cas9 loss-of-function screen, the researchers have mainly identified 10 regulatory genes specifically related to the metabolic pathway of cuproptosis, including 7 positively regulated genes [ferredoxin 1 (FDX1), lipo-

ic acid synthase (LIAS), lipoyltran-sferase1 (LIPT1), dihydrothiocinamide dehydrogenase (DLD), dihydrolipoamide S-acetyltransferase (DLAT), pyruvate dehydrogenase E1alpha (PDHA1) and pyruvate dehydrogenase β subunit gene (PDHB)], and 3 negatively regulated genes [metal transcription factor1 (MTF), glutaminase (GLS) and cyclin dependent kinase inhibitor 2A (CDKN2A)] (10). The specific role of these genes in the cuproptosis process and the effectiveness of copper toxicity in treatments for cancers will be future research hotspots.

In this study, cuproptosis-related lncRNAs which constitute the prognostic model, showed different degrees of correlation with regulated genes specifically associated with the cuproptosis-related metabolic pathway (including FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1, MTF1, GLS and CDKN2A).

Mutations causing copper overload may trigger serious consequences. Nonetheless, it is feasible to control intracellular copper levels within a range to kill tumor cells selectively(33). We found that the cuproptosis-related lncRNA model was closely related to tumor immunity. The BRAF gene mutation rate was higher in the low-risk group, and the patients with high-risk and high tumor mutation load had the worst prognosis. Some THCA patients have a poor prognosis due to drug resistance (34). Therefore, potential targeted drugs for THCA were screened, including 5-Fluorouracil, Bleomycin, Rapamycin and Sunitinib, according to the cuproptosis-related lncRNA model.

The strengths of this study are the first screening of cuproptosis-related lncRNAs and the successful development of a prognostic model of cuproptosis-related lncRNAs. Furthermore, we predicted potential targeted drugs for THCA with the model. Definitely, this study also has some shortcomings. First, we did not use external datasets to verify the prognostic value of the model. Second, the wet experimental validation of lncRNAs in the model was also not performed. In future experiments, we will design experiments to validate the expression and functional aspects of lncRNAs in this study.

Conclusion

We have successfully constructed a cuproptosis-related lncRNA model for THCA (AC026100.1, AF235103.3 and LNCSRLR) with a prognostic value. It has a high tumor mutational burden and a poor prognosis. The potential targeted drugs for THCA were predicted using this model (5-Fluorouracil, Bleomycin, Rapamycin, and Sunitinib). High-risk thyroid cancer patients are more likely to benefit from the drug. Based on these results, we can speculate that cuproptosis-related lncRNAs have certain value in the diagnosis, treatment and prognosis of thyroid cancer.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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