Estimating Disease-Free Survival of Thyroid Cancer Based on Novel Cuprotosis-Related Gene Model

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Research Article

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Abstract

**Background:** Cuprotosis is a newly discovered form of cell death that differs from other types of cell death. The aim of this study was to investigate the functional role and a possible prognostic model for thyroid cancer.

**Methods:** TCGA and GEO were used to investigate the differential expression of CRGs in THCA. KEGG and GO enrichment analyses were applied to investigate the possible molecular functions. The features of CRGs were selected by LASSO regression. 20 pairs of samples were randomly collected from the hospital to compare expression between tumor and normal.

**Results:** Among the 19 CRGs related to thyroid cancer recurrence, 16 genes were differentially expressed in thyroid cancer. KEGG analysis showed that the 19 CRGs were mainly enriched in cell death, cell cycle and ribosomal pathways. K-M survival analysis and subsequent multiple logistic regression revealed that the expression of BUB1 and GINS2 were potential risk factors for disease-free survival (DFS) of thyroid cancer. In addition, further LASSO-regression selected the following three DFS-related CRGs: FDX1, BUB1 and RPL3. A novel prognostic prediction model was constructed by nomogram, and the prediction probability for 1-, 3- and 5-year survival approached the actual time. As for the possible mechanisms, FDX1, BUB1 and RPL3 were associated with immune infiltration. The cell model experiment illustrated that the ATM signaling pathway might be involved in thyroid cancer cell death.

**Conclusion:** Three CRG models (FDX1, BUB1, RPL3) could better predict the prognosis of thyroid cancer. Immune cell infiltration and the ATM pathway were the possible mechanisms.

1. Introduction

The incidence of thyroid cancer has increased in recent years [1]. The mortality rate of thyroid cancer is low, but the recurrence rate is high, which is a major challenge for patients and surgeons. Moreover, the specific molecular mechanisms of thyroid cancer (THCA) are still unclear. New efficient prognostic model using biomarker panels are required.

Cuprotosis is a newly discovered cell death that depends on the accumulation of copper ions and leads to programmed cell death (Table S2). Copper and other trace metals are essential for life. Excessive accumulation of metal ions is life-threatening. However, abnormal concentrations of copper and zinc ions have been found in the serum of thyroid cancer patients[2, 3], so we hypothesized that the disturbance of copper ion metabolism in thyroid cancer patients may be one of the risk factors for tumor development. Furthermore, there is more than one type of programmed cell death, including necrosis, apoptosis, autophagy and iron death, and all types of cell death often interact directly with each other. The potential relationships and interactions with other types of cell death in thyroid cancer may provide us with some new ideas.
The aim of this study was first to investigate the expression of Cuprotosis-related Genes (CRGs) in thyroid cancer and then to build a prognostic model to predict the prognosis of thyroid cancer. Finally, the possible molecular mechanism in the cell model is also investigated.

2. Materials And Methods

2.1 Data collection

RNA sequencing expression profiles and corresponding clinical information for thyroid cancer were downloaded from TCGA (Table S1). Statistical analyses were performed using R software v4.0.3. \( P < 0.05 \) was considered statistically significant. CRGs were downloaded from Tsvetkov's research. The GSE54958 dataset is from the GEO database.

2.2 Functional enrichment analysis

We used the GO annotation of genes in the R software as a background to assign genes to the background set. The R software package cluster Profiler was used for enrichment analysis to obtain the gene set enrichment results. For gene set enrichment function analysis by using gene annotation of the latest KEGG Pathway. The minimum gene set was set to 5, the maximum gene set was set to 5000, and the \( P < 0.05 \) and FDR < 0.25 were considered statistically significant.

2.3 Analysis of immune infiltration

We used the immune genes module of the TIMER2 web server to investigate the association between the expression of BUB1, FDX1 and RPL3 and immune infiltration in thyroid tumors. Immune cells B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils and dendritic cells were selected. P-values and partial correlation values (cor) were determined using the purity-adjusted Spearman rank correlation test. We used the TIDE platform (http://tide.dfci.harvard.edu) to evaluate the rank of the gene set in another database.

2.4 Protein-protein interaction network analysis

STRING was used to perform the PPI network analysis for different genes. The PPI network was visualized using Cytoscape software (version 3.9.0).

2.5 Immunohistochemistry

The Human Protein Atlas database (www.proteinatlas.org) was used to obtain the immunohistochemistry of FDX1, RPL3, AURKA, CDKN2A, DERL2, NOM1, PDHA1, GINS2, GPI, RPL10, PSMA6, RAD50, RAD9A, QARS1, SLC26A6, ZNHIT2 and TTK in tumor and normal tissues. Only BUB1 and TUT1 are not found in the Atlas database.

2.6 Survival analysis
The KM survival analysis with the log-rank test was also used to compare the survival differences between the two groups mentioned above and to plot the DFS curves. Cox regression analysis was performed to find out the risk factors that may predict DFS.\[^{[8,9]}\] For the Kaplan-Meier curves, p-values and hazard ratio (HR) with 95% confidence interval (CI) were obtained by log-rank tests and univariate Cox proportional hazards regression. All the above analysis methods and R packages were performed using R software version v4.0.3.

### 2.7 Prognosis Model

The Least Absolute Shrinkage and Selection Operator regression algorithm (LASSO) was used for feature selection, 10-fold cross-validation was performed, and the R package GLMNET was used for analysis\[^{[8,10-12]}\]. Further, there are 360 samples were used for the subsequent analysis. The log-rank test was used to compare survival differences between these groups. The time analysis ROC (v 0.4) was used to compare the predictive accuracy of the BUB1, FDX1 and RPL3 genes with the risk score. Multivariate Cox regression analysis was used to build a prediction model, and the R package survival was used for the analysis.

Univariate and multivariate Cox regression analyses were performed to determine the correct terms to construct the nomogram. The R package forest plot was used to plot the P-value, HR and 95% CI for each variable. Based on the results of the multivariate Cox proportional hazards analysis, a nomogram was developed to predict X-year overall recurrence\[^{[12]}\]. The nomogram provided a graphical representation of the factors that can be used to calculate the risk of recurrence for an individual patient based on the scores associated with each risk factor using the R package "RMS".

### 2.8 CCK-8

Cell viability was assessed using the CCK-8 assay. Cells were seeded in 96-well plates and collected 24 hours after I-131 treatment.

### 2.9 Western blot

Expression of the specific proteins was detected by western blot, including P-ATM, ATM, MAPLC3, P62, PI3KC3 and γ-H2AX. GAPDH was used as a control.

### 2.10 Sample collection

20 pairs of samples were collected from the Department of Thyroid Surgery, China-Japan Union Hospital, Jilin University. Patients with PTC who had undergone surgery and had a pathological diagnosis were included in the study. Both tumor samples and para-carcinoma normal tissue samples were collected 30 minutes after surgery, immediately placed in sterilized vials, frozen in liquid nitrogen and stored at -80°C.

The study was conducted in accordance with the Declaration of Helsinki and approved by the China-Japan Union Hospital Institutional Review Board (No.20220804014). Informed consent was obtained from all patients.
2.11 Statistical analysis

Continuous variables were described as mean ± standard deviation. Categorical variables were described as frequencies and proportions. Statistical differences between 2 populations were calculated using t-tests (2-sided) including multiple t-tests, unpaired t-tests or paired t-tests. Categorical data were analyzed using the chi-square test or the chi-square test with continuous correction. P< 0.05 was considered statistically significant.

3. Results

3.1 Differential expression of CRGs in THCA

To investigate the expression of CRGs in thyroid cancer, both the mRNA level and the protein level were analyzed. First, 148 previously reported significant CRGs were compiled from the literature(Elesclomol Cu and Cu-DDC)[5]. We also analyzed the survival probability of thyroid cancer patients’ gene expression (2014) using the Cancer Genome Atlas (TCGA) database. As shown in the Venn diagram (Fig. 1a), 19 CRGs overlapped with the recurrence-relevant genes in thyroid cancer. Therefore, we focused on these 19 CRGs associated with thyroid cancer recurrences in the following study. Based on the TCGA database, we first collected and compared the mRNA expression levels. From the heatmap (Fig. 1b) and boxplot (Fig. 1c), we found that of the 19 CRGs associated with thyroid cancer, 16 genes were differentially expressed in thyroid cancer. Compared with normal thyroid tissue, 7 CRGs were more highly expressed in thyroid cancer, namely BUB1, RPL3, TTK, GINS2, SLC26A6, CDKN2A and ZNHIT2. Meanwhile, 9 CRGs were less expressed, such as FDX1, DERL2, RAD50, RAD9A, NOM1, TUT1, AURKA, PSMA6 and PDHA1. In addition, we further compared and validated the protein level based on the human protein database ATLAS. As shown in Fig. 1d, the majority of proteins were differentially expressed in thyroid cancer, which was consistent with the mRNA expression level. The above data suggest that the 19 CRGs may play an important role in thyroid cancer.

The expression of these 19 genes was analyzed between tumor and normal tissues (Fig. 1b). We found that the expression of 16 genes differed significantly (adjusted P< 0.05, Fig. 1c). Our study showed that 7 CRGs (BUB1, RPL3, TTK, GINS2, SLC26A6, CDKN2A, ZNHIT2) were upregulated and 9 CRGs (FDX1, DERL2, RAD50, RAD9A, NOM1, TUT1, AURKA, PSMA6, PDHA1) were downregulated. The results of RT-qPCR showed that the mRNA expression of BUB1, RPL3, TTK, GINS2, SLC26A6, CDKN2A and ZNHIT2 was significantly higher in tumor samples compared to the corresponding normal tissues (Fig. 1c). We found that 19 genes correlated with each other. We validated the expression of the 16 CRGs in THCA and normal tissues using the database ATLAS. Immunohistochemistry results showed the expression of RPL3, FDX1, RPL10, GPI, DERL2, RAD50, RAD9A, GINS2, SLC26A6, QARS1, CDKN2A, NOM1, AURKA, ZNHIT2, PSMA6 and PDHA1. Differential expressions between thyroid cancer tissue and normal thyroid tissue were detected for most CRGs. These results were consistent with the results of RT-qPCR.
3.2 Functional enrichment and KEGG analysis of CRGs in THCA

To investigate the correlation between the 19 genes, we created a PPI network using the STRING database and Cytoscape 3.9.0 software. The PPI network (Fig. 2b) has 39 nodes and 277 edges. The interaction value > 0.15 was considered an interaction relationship with high reliability. The network showed that BUB1, RPL3 and FDX1 are the core genes. In addition, the correlation of the CRGs was examined using the ACLBI database (Fig. 2c). Analysis of the genetic variants revealed that a total of 15 genes were mutated, with the most common type of mutation being a missense mutation (Fig. 2a). To further investigate their potential biological functions, GO and KEGG analyses were performed within the 19 CRGs (Fig. 2d, e, f, g). In the analysis of GO, the biological processes of the 19 CRGs were mainly cell death, regulation of programmed cell death, negative regulation of the mitotic cell cycle and regulation of the intrinsic apoptotic signaling pathway in response to DNA damage. However, according to KEGG analysis, they may also be involved in the cell cycle, cellular senescence, oocyte meiosis and glycolysis/gluconeogenesis. The above analysis suggests that these 19 CRGs may play an important role in the progression and development of THCA.

3.3 Predictive efficacy of CRGs for Survival in THCA

To uncover the relationship between prognosis and expression of CRGs and to find the best predictors for disease-free survival of thyroid cancer, K-M curve analysis and Cox regression were applied. As shown in Fig. 3a, four CRGs correlated positively with disease-free survival of THCA as follows: BUB1 (HR = 4.5867 (1.7368–12.1126), \( P = 0.0021 \)), TTK (HR = 3.0436 (1.2868–7.1989), \( P = 0.0113 \)), CDKN2A (HR = 2.3511 (1.0291–5.3712), \( P = 0.0426 \)), AURKA (HR = 2.9729 (1.2569–7.0315), \( P = 0.0131 \)). Taking BUB1 as an example, patients with higher expression of BUB1 could have poorer survival. However, there were seven other CRGs that correlated negatively with DFS. These were RPL3, FDX1, RPL10, DERL2, RAD9A, TUT1 and ZNHIT2. Further multivariate regression analysis (Fig. 3b) revealed that BUB1 and GINS2 were positively associated with the DFS of THCA. Of note, THCA with high BUB1 expression was 6.623 times more likely to have a worse prognosis than patients with low expression. This suggests that these CRGs are a potential risk factor for DFS in THCA.

3.4 Building and validating a prognostic model based on CRGs in THCA

To build a CRG model to predict the prognosis of thyroid cancer patients, LASSO Cox regression analysis and nomogram were applied. Three CRGs (FDX1, RPL3, BUB1) were selected to build the prognostic model (Fig. 4a) As shown in Fig. 4b, the model had the optimal performance and the least number of independent variables when log \( \lambda \) was set to 0.0287. The risk score was calculated as follows: Risk score = (0.7896) * BUB1 + (0.0151) * RPL3 + (-0.1247) * FDX1. According to the median value of all risk scores, 360 thyroid cancer patients were divided into high and low-risk score groups. (Fig. 4c). A heat map
visualized the expression patterns of the three CRGs between high and low-risk groups (Fig. 4c). As shown by the K-M curves, the risk in the high-risk group was 4.442 times higher than in the low-risk group ($P = 0.00262$; Fig. 4d). To further validate the diagnostic efficacy of the prognostic model, we created a ROC analysis. The result confirmed that the CRG model could predict DFS probability for 1-year ($AUC = 0.789$, $95\% CI (0.654–0.924)$), 3-year ($AUC = 0.733$, $95\% CI (0.633–0.832)$) and 5-year survival ($AUC = 0.757$, $95\% CI (0.653–0.860)$) (Fig. 4e). Overall, this Cuprotosis-related three-gene model could be a robust prognostic model for thyroid cancer.

We further evaluated the performance of the three-gene model for Cuprotosis. First, the results of univariate Cox regression analysis showed that BUB1 (HR: 3.576, 95% CI: $2.14063–5.97377$, $P < 0.001$), FDX1 (HR: 0.4046, 95% CI: $0.22222–0.73668$, $P = 0.00308$), RPL3 (HR: 0.5724, 95% CI: $0.34249–0.95663$, $P = 0.03324$), T stage (HR: 2.2366, 95% CI: $1.31427–3.80621$, $P = 0.003$) and N stage (HR: 2.65774, 95% CI: $1.13725–6.21111$, $P = 0.02401$) were significantly associated with thyroid cancer prognosis (Fig. 5a). According to multivariate Cox regression analysis, BUB1 was an independent risk factor for thyroid cancer (HR: 4.21965, 95% CI: $2.10531–8.45738$, $P = 0.00005$; Fig. 5b). This means that BUB1 could be an independent prognostic factor for thyroid cancer.

In addition, a nomogram model for THCA was constructed based on the clinical features and the three selected CRGs. Figure 5c shows the ability to predict the 1-year, 3-year and 5-year DFS probability of THCA patients. Most importantly, by combining the T stage and BUB1, the calibration curves confirmed that the 1-, 3- and 5-year survival probability predicted by the nomogram (red, yellow, grey) is close to the actual survival. To validate the expression in practice, twenty paired tissue samples were collected from clinical patients at our center. As shown in Fig. 5e, BUB1 was differentially expressed in tumor tissue compared to normal thyroid tissue. This was consistent with the TCGA database. In addition, we compared the expression of BUB1 in the N0 group (without lymph node metastasis) and the N1 group (with lymph node metastasis), unfortunately, there is no significant difference between the two groups. This may be due to the small number of samples. The above analyses suggest that BUB1, FDX1 and RPL3 may be associated with THCA survival.

### 3.5 The three CRGs were involved in immune infiltration

To further investigate the possible mechanisms of CRGs in THCA, we first divided patients into a low-risk group and a high-risk group based on the risk score. As shown in the volcano curve (Fig. 6a), 624 DEGs in the high-risk groups were significantly upregulated, while 267 DEGs were downregulated. Interestingly, further analysis at GO indicated that the biological process of these DEGs was mainly related to the immune system process, immune response and adaptive immune response (Fig. 6b).

Therefore, immune infiltration analysis was applied to investigate their possible roles and relationships (Fig. 6c). The expression level of FDX1 was positively associated with the immune infiltration level of macrophages ($P = 1.77 \times 10^{-2}$), but negatively correlated with CD8 + T cells ($P = 1.6 \times 10^{-11}$), neutrophils ($P = 2.74 \times 10^{-3}$) and dendritic cells ($P = 1.11 \times 10^{-6}$). However, RPL3 was positively correlated with B cells ($P = 6.04 \times 10^{-6}$), CD4 + cells ($P = 9.36 \times 10^{-7}$) and negatively correlated with CD8 + T cells ($P = 1.71 \times 10^{-8}$).
addition, BUB1 was positively associated with the frequency of B cells \( (P = 1.78 \times 10^{-35}) \), CD8+ T cells \( (P = 3.27 \times 10^{-8}) \), CD4+ cells \( (P = 1.6 \times 10^{-13}) \), macrophages \( (P = 3.31 \times 10^{-27}) \), neutrophils \( (P = 9.95 \times 10^{-45}) \) and dendritic cells \( (P = 5.35 \times 10^{-59}) \).

Finally, the database OASIS [13] was annotated to investigate the potential therapeutic targets in synergy with immune checkpoint blockade (ICB) (Fig. 6d). Interestingly, BUB1 was among the top targets of this module, rendering the tumor microenvironment resistant to ICB. High RPL3 expression was associated with T cell dysfunction phenotypes in TCGA endometrial datasets (Fig. 6d left panel). Low expression of BUB1 was also associated with poorer ICB outcomes in renal and bladder cancers and in treatment-naïve melanomas treated with ICB (Fig. 6d, second panel from left). Among the cell types promoting T cell exclusion, myeloid suppressor cells had very high levels of BUB1 expression (Fig. 6d, right). This module prioritizes BUB1 with the best potential for developing combination immunotherapies. These data suggest that the three CRGs may be involved in regulating immune infiltration.

3.6 ATM pathway involved in the regulation of cell death in THCA.

Our previous work had confirmed that the ATM pathway mainly regulates autophagy induced by irradiation. However, I-131 treatment is the usual therapy, especially for complex and aggressive thyroid cancer. Excitingly, ATM was enriched based on a serious enrichment analysis and was present in the relative functions and pathways of CRGs (Fig. 7a). This aroused our desire to further investigate the possible role of ATM in regulating cell death in THCA. First, we validated the differential expression of ATM in the paired tissue samples. The expression of ATM in tumor tissue is lower than in normal tissue \( (P = 0.062) \). In addition, we compared the expression of ATM between the N0 group and N1 group, the median of ATM expression in N1 group is lower than N0 group, but there is no significant difference (Fig. 7b). Then, we investigated its functional role in the thyroid cancer cell line TPC-1. As shown in Fig. 7c, cell viability was inhibited by increasing I-131 dose in a dose-dependent manner. Considering the minimum effective dose, we chose 14.8 MBq/ml as the treatment dose in the following experiment. According to the Western blot in Fig. 7d, phosphorylation of ATM was significantly increased after I-131 treatment, implying that the ATM signaling pathway could be activated by I-131 treatment in thyroid cancer. However, as a classic marker of autophagy, an increase in MAPLC3-II / MAPLC3-I was also detected \( (1.62 \text{ vs. } 1.00) \). The common autophagy inhibitor (3- MA) was selected to further investigate the specific role of autophagy in I-131-induced cell death. As shown in Fig. 7e, only treatment with I-131 could significantly inhibit cell viability, but this could be reversed by combined pre-treatment with 3- MA. Further Western blot showed that ATM is indeed involved in the regulation of autophagy. In addition, gamma-H2AX, the DNA damage response marker, was also found to be affected. This suggests that autophagy may be involved in I-131-induced cell death. As there were no standard methods and markers to directly indicate Cuprotosis levels. Whether the ATM pathway specifically regulates Cuprotosis needs further validation in the future.
4. Discussion

Cuprotosis is a novel cell death mechanism in recent years, a type of copper-dependent cell death that differs from apoptosis, necroptosis and ferroptosis. Our research could provide a new diagnostic model and new insights into the mechanisms of thyroid cancer development and immunotherapy.

Current advances in the field of Cuprotosis are an exciting and growing area of research field\textsuperscript{14–19}. (Fig.S2 a) Much evidence suggests that CRGs are expressed in several tumors and correlate with the stage of tumor progression and poor prognosis, including renal cell carcinoma, pancreatic cancer, lung adenocarcinoma, bladder cancer, hepatocellular carcinoma and gastric cancer (Table S2). Several other studies have found that FDX1 and DLAT are associated with tumor progression. In this study, we compiled the original data and analysis of the dataset published in Tsvetkov's research. The dataset included not only 10 genes selected by Tsvetkov, but also 148 genes that log FC > 1 or <-1. We investigated signature genes for prognosis (OS, DFS, PFS, DSS) in THCA patients based on the TCGA database. Considering this information, we investigated 19 CRGs related to prognosis in THCA. Finally, 16 CRGs were found to be differentially expressed in tumor and normal tissues, which is consistent with the immunohistochemical results. In addition, the 19 genes showed close interaction in the STRING analysis. BRAF, TERT and RAS gene mutations were frequently observed in thyroid cancer. In this study, we found that GPI, QARS, TUT1 and BUB1 were predominant in CRGs. The biological process (BP) of CRGs mainly focuses on cell death. This is considered a logical next step in our ongoing research.

The mortality rate of THCA is lower and largely different from that of other tumors. Metastasis and recurrence in THCA significantly affect prognosis. Life expectancy is an important parameter reflecting the progression of THCA. In this study, we found 11 genes whose expression is indicative of prognosis in DFS. In multivariate analysis, we confirmed that two genes (BUB1 and GINS2) were of prognostic significance. Studies by Ge MQ confirmed that AKR1C1 candidates have differential expression in THCA compared to normal tissue\textsuperscript{20}.

Overexpression of BUB1 contributes to the morphological progression of clear cell renal carcinoma\textsuperscript{21}. Shen YL's research showed that GINS2 can accelerate the growth of glioma cells\textsuperscript{22}. According to the above results, we analyzed 19 genes with LASSO regression. The three-gene model (FDX1, BUB1, RPL3) was constructed to predict DFS of 1, 3 and 5 years in thyroid cancer (AUC: 0.789, 0.733, 0.757). Some predictive models for thyroid cancer from other studies showed an AUC of OS (0.621, 0.859, 0.842)\textsuperscript{20,23,24}. By combining the T-stage, the calibration curves confirmed that the 1-, 3- and 5-year survival probability predicted by the nomogram (red, yellow, grey) is close to the actual survival. The nomogram could potentially be a clinically predictive tool for THCA prognosis. Further investigation into the molecular mechanisms of the 3-gene model revealed that immune infiltration plays a role.

There are a large number of bio-informational articles on THCA, most of which focus on overall survival and ferroptosis. In our manuscript, we chose to study DFS, which is more meaningful to patients, based on our clinical experience. Cuprotosis was discovered in March 2022, there are still many doubts about
the mechanism of THCA. We tried to investigate some studies on the treatment of Cuprotosis in THCA. In addition, we built a prediction model based on CRGs.

Although we have verified the expression of 19 genes by immunohistochemistry using bio information, we still need to investigate the expression of patients in our area by RT-qPCR and immunohistochemistry. Further studies include the investigation of FDX1, BUB1 and RPL3 in THCA cell lines and the investigation of Cuprotosis signs in THCA. We found that the molecular mechanisms were related to autophagy by analyzing the signaling pathways of 300 genes of the 3-gene model. ATM might be the central gene of the mechanism. So we conducted an independent experiment. We found that I-131 activates the autophagy pathway via the ATM pathway. Benkafadar's research revealed that ATM triggers apoptosis via the P53 pathway\[25\]. Therefore, ATM can be used to inhibit cancer cells.

However, there are some limits. First, the data of our study relied mainly on the public database. Therefore, more basic experimental validation might be needed. Furthermore, we need to collect more samples to reduce statistical errors. At the same time, the mechanism of action between Cuproptosis and immune cells needs to be further explored.

In summary, as shown in Fig. 8, we found different expressions of CRGs in THCA. In addition, we constructed a novel prognostic model to predict the disease-free survival of THCA. Furthermore, we collected 20 pairs of samples of THCA patients, to verify the expression of CRGs. Additionally, we investigated that CRGs might regulate cell death in THCA through the ATM pathway.

5. Conclusion

In conclusion, as shown in Fig. 8, there are different expressions of CRGs in THCA. We found A novel Prognostic Model to Predict the disease-free survival of THCA. Additionally, we investigated that CRGs might regulate cell death in THCA through the ATM pathway.

Declarations

Data availability statement

Without undue reservation, the authors will make available the raw data that supports the conclusions of this article.

Author contributions

Conceptualization, Rui Du, Hui Sun and Nan Liang; Data curation, Rui Du, Jingting Li, Fang Li and Lusi Mi; Formal analysis, Rui Du; Funding acquisition, Hui Sun and Nan Liang; Methodology, Rui Du, Jingting Li and Fang Li; Resources, Rui Du; Supervision, Gianlorenzo Dionigi, Hui Sun and Nan Liang; Writing – original draft, Rui Du; Writing – review & editing, Gianlorenzo Dionigi and Nan Liang.

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Conflict of interest

There were no commercial or financial conflicts of interest.

Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the China-Japan Union Hospital Institutional Review Board (No.20220804014). Informed consent was obtained from all patients.

References


19. <2022-AMJ CANCER research-The potential value of cuprotosis (copper-induced cell death) in the therapy of clear cell renal cell carcinoma.pdf> [J].


Figures
Figure 1

Differential Expression of CRGs in THCA. (a) Venn plot of the prognosis-related genes of THCA and CRGs; (b) Heatmap of the CRGs associated with DFS; (c) The expression of 19 genes in thyroid cancer and normal tissues; (d) The expression of RPL3, FDX1, RPL10, GPI, DERL2, RAD50, RAD9A, GINS2, SLC26A6, QARS1, CDKN2A, NOM1, AURKA, ZNHIT2, PSMA6, and PDHA1 showed by immunohistochemistry in thyroid cancer and normal tissues. Scale bar: 200µm.
Figure 2

Functional enrichment and KEGG analysis of CRGs in THCA. (a) The mutation frequency and classification of 19 CRGs in THCA; (b) The PPI network provided interactive information among the 19 CRGs; (c) The interaction relationship among 19 CRGs; (d) KEGG pathway enrichment analysis; (e, f, g) MF, CC, BP- Gene ontology (GO) pathway enrichment analysis.
Figure 3

Predictive efficacy of CRGs for Survival in THCA. (a) Kaplan-Meier curves for high and low expressed groups in the two subgroups of the CRGs; (b) Multivariate Cox regression analyses established that CRGs expression exerted a critical influence on the Disease-free survival (DFS).
Figure 4

Establishment of a CRG model for predicting the prognosis of thyroid cancer patients. (a,b) Fitting processes of LASSO Cox regression model of CRGs; (c) The ranking of the risk scores among all thyroid cancer samples; (d) Kaplan-Meier disease-free survival analysis for high (red) and low (blue) risk groups; (e) ROC for 1- (red), 2- (blue) and 3-year (yellow) survival time for high and low-risk patients.
Figure 5

Establishment and validation of the Prognostic Model based on CRGs in THCA. (a) The univariate Cox regression of CRGs and clinical characteristics; (b) The multivariate Cox regression of CRGs and clinical characteristics; (c) the Nomogram of 1-year, 2-year, and 3-year DFS prediction of Thyroid cancer patients. C-index: 0.81 (0.721-1) \( P < 0.001 \); (d) Calibration curve for the disease-free survival nomogram model in the discovery group; (e) The validation of relative expression of BUB1 in tumor and normal tissue, N0 group and N1 group by RT-qPCR.
Figure 6

The three CRGs were involved in Immune Infiltration. (a) Volcano plots showing DEGs (Fold change <1.5); (b) GO enrichment analysis of DEGs; (c) Correlation between FDX1, RPL3, BUB1 expression and immune infiltration in THCA in the TIMER database; (d) Rank(ascendingly/descendingly) of FDX1, RPL3, BUB1 based on the average score of a group with multiple cohorts.
Figure 7

ATM pathway involved in the regulation of cell death in THCA. (a) KEGG enrichment analysis of BUB1, FDX1, RPL3 related genes set; (b) The expression of ATM in thyroid cancer tissue and normal tissue, N0 group and N1 group; (c) Cell viability was estimated in the different doses of I-131; (d) The effect of I-131 in ATM, P-ATM and MAPLC3-I/MAPLC3-II; (e) Cell viability of control, I-131, 3-MA, I-131+3-MA group; (f) The expression of P-ATM, ATM, MAPLC3-I/MAPLC3-II, P62, PI3KC3, γ-H2AX, GAPDH of control, I-131, 3-MA, I-131+3-MA group.
Figure 8
The schematic workflow of the study.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
• figS1.tiff
• STable.docx