Conjoint analysis of m6A regulators and copy number variations in thyroid carcinoma

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Research

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Abstract

**Background:** Most patients with thyroid carcinoma have a good prognosis, but some thyroid carcinomas are aggressive and prone to recurrence and metastasis. Further, concomitant overdiagnosis and overtreatment are important issues. This study aimed to investigate the relationship between mutations and copy number variations in m6A regulatory genes and the clinicopathological features of thyroid carcinoma.

**Results:** Advanced pathological stage and T stage were significantly correlated with changes in m6A regulatory genes (p<0.05). Patients with abnormal copy numbers of m6A regulatory genes had a significantly shorter progression-free interval than patients with normal copy numbers. Mutations and copy number variations in m6A regulatory genes were significantly correlated with advanced pathological stage and T stage.

**Conclusions:** Copy number variations were a poor prognostic factor for thyroid carcinoma as evidenced by the significantly short progression-free interval in patients with copy number variations in m6A regulatory genes. Further, the ZC3H13 gene may be involved in the occurrence and development of thyroid carcinoma by regulating cancer-associated signaling pathways and biological processes. These findings may help distinguish patients with poor prognoses who may need more aggressive treatment from patients who have better prognoses.

Background

Thyroid carcinoma (THCA) is the most common malignant tumor of the thyroid gland, accounting for approximately 1% of systemic malignancies. THCA is classified into papillary carcinoma, follicular carcinoma, undifferentiated carcinoma, and medullary carcinoma, with papillary carcinoma being the most common type and having the best prognosis. Except for medullary carcinoma, most THCAs originate from follicular epithelial cells. The diverse forms of RNA, such as rRNA, tRNA, mRNA, snRNA, and other chemical modifications of RNA, have received increasing research attention in recent years for their roles in cell biology processes. Among these processes, mRNA modification plays a crucial role in regulating posttranscriptional levels of gene expression.

In eukaryotes, m6A is the most common form of mRNA modification. Studies have shown that m6A is widely present in the transcriptome and modifies more than 7,600 genes and 300 noncoding RNAs [1]. Regulators of m6A RNA methylation can be divided into the following three categories according to their functions in the methylation process [2, 3]: writers (methyltransferases): METTL3, METTL14, WTAP, KIAA1429, RBM15, ZC3H13; readers (binding proteins): YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC; and erasers (demethylases): FTO, ALKBH5. Research has shown that changes and defects in genes of m6A regulators are closely related to the malignant progression of various cancers [4–6]. However, although m6A modification is involved in tumorigenesis [7], proliferation [8], differentiation, invasion, and metastasis in different types of tumors, its function in THCA remains unclear.

Copy number variations (CNVs) are a kind of structural variation phenomenon and are ubiquitous in the human genome. CNVs are mainly caused by copy number amplification, deletions, missing, insertions, recombinations, and complex mutations at multiple sites with resultant abnormal fragments ranging in size from dozens of bases (>50 bp) to several megabases. The American geneticist Calvin Bridges first discovered CNVs in drosophila in 1936, and subsequent studies found that CNVs also exist in other species [9]. Accordingly, CNVs have been determined to directly affect and cause diseases by changing gene dosing, disturbing coding sequences, and interfering with remote regulation.

Therefore, this study aimed to investigate the relationship of mutations and CNVs in m6A regulatory genes with the clinicopathological features, including disease progression-free intervals (PFI), of THCA. Toward this goal, we analyzed the clinical and sequencing data of THCAs from The Cancer Genome Atlas (TCGA) database and further evaluated the mutation profiles of 14 m6A-regulated genes in patients with THCA.
Results

Characterization of mutations and CNVs in m6A regulatory genes

Using the TCGAbiolinks R package, data of 492 patients with THCA with m6A regulatory gene mutations were downloaded (Table 1). Of these patients, only twelve had mutation information on all 6 m6A regulatory genes. The KIAA1429 gene was found to have a frame shift insertion in two patients. The METTL14 gene underwent a silent mutation in the 5'UTR region in one patient. The RBM15 gene had a missense mutation in one patient, resulting in an amino acid replacement (Ser to Arg). The ZC3H13 gene had missense mutations in three patients, with two kinds of mutations in one patient, and seed codon changes in another patient (R646*). The readers YTHDC1 and YTHDC2 had diverse mutations in three patients. Overall, functional changes, including frameshift mutations in KIAA1429 and YTHDC1, a splice donor variant in YTHDC2, and a stop codon mutation in ZC3H13, were the most significant.

| THCA Sample ID | Writers | Readers |
|----------------|---------|---------|
| TCGA-BJ-A18Y   | KIAA1429| R756T, E757Q|
| TCGA-DJ-A13R   | METTL14  | R384G |
| TCGA-E8-A432   | RBM15    | R646*  |
| TCGA-E8-A436   | ZC3H13   | X281_splice|
| TCGA-EL-A3MZ   | YTHDC1   | YTHDC2 |
| TCGA-EL-A3TB   | YTHDC2   |         |
| TCGA-EL-A3ZG   | F455delins | intron_variant |
| TCGA-EL-A3ZP   | S212R    |         |
| TCGA-EL-A3ZQ   | F455Sfs*11 | YKLQIRLFFQIL |
| TCGA-EL-A4JZ   | Q555Ffs*23 |         |
| TCGA-ET-A40S   | A486T    |         |
| TCGA-FY-A40L   | NA (5'UTR)|         |
| TCGA-FY-A40M   | Y298Sfs*4 |         |

Table 1: Mutations in the m6A Regulatory Genes in the 492 patients with THCA

CNV data of the 399 patients were downloaded from the cBioportal platform (https://www.cbioportal.org/) (Table 2). The 14 m6A regulators all had slight CNVs, ranging from 1–3%, that is, of the 399 patients, 5–10 had CNVs. Notably, the copy number abnormality of the writer gene ZC3H13 reached 5.01%. This affected 20 patients, 15 of whom had missing copy numbers and 5 of whom had increased copy numbers. The second most frequent gene with CNVs was the eraser ALKBH5. It was mutated in 19 patients, 4 of whom had missing copy numbers and 15 of whom had increased copy numbers. The abnormal frequency distribution of copy numbers for all m6A methylation regulators is shown in Fig. 1. We found that m6A methylation regulators mainly mutated through copy number gain (Fig. 2). Figure 3 shows the most common patterns of CNVs in m6A regulatory genes among the 399 patients with THCA.
Table 2
Different CNV Patterns in the 399 patients with THCA

| Type       | m6A | Diploid | Deep Deletion | Shallow Deletion | Copy Number Gain | Amplification | CNV Sum | Percentage |
|------------|-----|---------|---------------|------------------|------------------|---------------|---------|------------|
| Writers    |     |         |               |                  |                  |               |         |            |
| WTAP       | 393 | 0       | 4             | 2                | 0                | 6             | 1.50%   |            |
| METTL3     | 389 | 0       | 0             | 10               | 0                | 10            | 2.51%   |            |
| METTL14    | 393 | 0       | 1             | 4                | 1                | 6             | 1.50%   |            |
| RBM15      | 393 | 0       | 3             | 3                | 0                | 6             | 1.50%   |            |
| ZC3H13     | 379 | 2       | 13            | 5                | 0                | 20            | 5.01%   |            |
| KIAA1429   | 390 | 0       | 2             | 7                | 0                | 9             | 2.26%   |            |
| Erasers    |     |         |               |                  |                  |               |         |            |
| FTO        | 385 | 0       | 2             | 12               | 0                | 14            | 3.51%   |            |
| ALKBH5     | 380 | 0       | 4             | 14               | 1                | 19            | 4.76%   |            |
| Readers    |     |         |               |                  |                  |               |         |            |
| YTHDF1     | 388 | 0       | 0             | 11               | 0                | 11            | 2.76%   |            |
| YTHDF2     | 394 | 0       | 3             | 2                | 0                | 5             | 1.25%   |            |
| YTHDF3     | 390 | 0       | 2             | 7                | 0                | 9             | 2.26%   |            |
| YTHDC1     | 394 | 0       | 1             | 4                | 0                | 5             | 1.25%   |            |
| YTHDC2     | 386 | 0       | 0             | 13               | 0                | 13            | 3.26%   |            |
| HNRNPC     | 389 | 0       | 0             | 10               | 0                | 10            | 2.51%   |            |

CNV, copy number variation; THCA, thyroid carcinoma

Analysis of the relationship of changes in m6A regulatory genes with clinical pathology and molecular characteristics

We evaluated whether there were significant differences in clinical factors such as age distribution, sex, and pathological stage between patients with mutations or CNVs and those without. In total, 56 patients with mutations in m6A regulatory genes or CNVs were included in the analysis (Table 3). The chi-square test showed that advanced pathological stage and T stage significantly correlated with changes in m6A regulatory genes (p < 0.05). Analysis of BRAF and RAS genes, which are known to be related to THCA, showed that changes in these two genes did not significantly correlate with changes in m6A regulators (Table 4).
Table 3
Clinicopathologic Characteristics of Patients with THCA according to the Presence of Mutation/CNV in m6A Regulatory Genes

|                      | With Mutations and/or CNVs* | Without Mutations and/or CNVs** | $\chi^2$ | p-Value |
|----------------------|-----------------------------|---------------------------------|---------|---------|
| Age, years           |                             |                                 |         |         |
| ≤ 60                 | 38                          | 356                             |         |         |
| > 60                 | 18                          | 96                              | 2.8061  | 0.09391 |
| Sex                  |                             |                                 |         |         |
| Female               | 38                          | 333                             |         |         |
| Male                 | 18                          | 119                             | 0.58578 | 0.4441  |
| Pathological stage   |                             |                                 |         |         |
| Stage I              | 19                          | 266                             |         |         |
| Stage II             | 15                          | 38                              |         |         |
| Stage III            | 13                          | 100                             |         |         |
| Stage IV             | 9                           | 46                              |         |         |
| NA                   | 0                           | 2                               | 23.192  | 3.68E-05|
| T stage              |                             |                                 |         |         |
| T1                   | 9                           | 135                             |         |         |
| T2                   | 23                          | 145                             |         |         |
| T3                   | 17                          | 154                             |         |         |
| T4                   | 7                           | 16                              |         |         |
| TX                   | 0                           | 2                               | 13.555  | 0.003577|
| N stage              |                             |                                 |         |         |
| N0                   | 31                          | 201                             |         |         |
| N1                   | 21                          | 205                             |         |         |
| Nx                   | 4                           | 46                              | 1.5015  | 0.2204  |
| M stage              |                             |                                 |         |         |
| M0                   | 23                          | 260                             |         |         |
| M1                   | 0                           | 9                               |         |         |
| Mx                   | 33                          | 182                             | 0.06895 | 0.7929  |

*Cases of mutations or CNVs or both as confirmed through the TCGA database.

**Cases of neither mutations nor CNVs as confirmed through the TCGA database.

Ambiguous variables (Nx, Mx, N/A, discrepancy, and Gx) were excluded from the chi-square test and nonparametric test. CNV, copy number variation, THCA, thyroid carcinoma.
Table 4
Relationship between Molecular Characteristics and Alteration in m6A Regulatory Genes in Patients with Thyroid Carcinoma

|                      | With Mutations and/or CNVs* | Without Mutations and/or CNVs** | $\chi^2$  | p-Value |
|----------------------|----------------------------|---------------------------------|-----------|---------|
| BRAF Wt              | 54                         | 427                             | 0.090502  | 0.7635  |
| Alteration           | 2                          | 25                              |           |         |
| RAS Wt               | 55                         | 442                             | 3.99E-31  | 1       |
| Alteration           | 1                          | 10                              |           |         |

*Cases of mutations or CNVs or both as confirmed through the TCGA database.

**Cases of neither mutations nor CNVs as confirmed through the TCGA database.

Correlation between different CNV patterns and mRNA expression of m6A-regulated genes

As only a few samples had CNVs, we combined loss type (i.e., deep deletion and shallow deletion) with copy number loss and combined gain type (i.e., copy numbers gain and amplification type) with copy number increase. Then, t-tests were performed to compare the differences in expression between samples with different copy number type (loss, diploid, gain) for each m6A methylation regulator (Fig. 4). Results with no significant or increased copy numbers and low expression are shown in Additional File 1.

Next, we further estimated the effect of changes in m6A regulatory genes on their expression profiles. As shown in Fig. 4, the expression of six m6A regulatory genes was significantly correlated with their different copy number states. Increased copy number was associated with increased mRNA expression, while copy deletion resulted in decreased mRNA expression. The m6A regulatory genes whose expression significantly differed between samples with different copy numbers (three or two, some samples have only missing or increased copy numbers) included FTO, METTL3, YTHDF1, YTHDF2, ZC3H13, and WTAP. In addition, we also found that for ZC3H13 and WTAP, the median expression in samples with increased copy numbers was lower than that in samples with normal copy numbers. This may be caused by differences in the number of samples, that is, the number of samples with increased copy numbers being lower than that of samples with normal copy numbers.

Analysis of the association between CNVs of m6A regulatory genes and patient survival

The comparison of PFI between patients with normal copy numbers and those with abnormal copy numbers is shown in Fig. 5. We found that patients with abnormal copy numbers had significantly lower PFI than patients with normal copy numbers (p < 0.05), indicating that an abnormal copy number was associated with a worse prognosis in patients with THCA. However, we found no significant difference in PFI between patients with copy number gain and those with copy number loss (Additional File 2). We also analyzed different status of PFI in different states of each gene (Additional File 3) and found no significant difference, possibly because the number of patients with CNV changes in a single gene was too small to yield a measurable effect.

Multivariate cox regression analysis of m6A regulatory genes
Univariate and multivariate Cox regression analyses of patients with THCA showed that pathological stage and M stage were correlated with PFI (Table 5). Because only pathological stage and M stage significantly correlated with PFI in the univariate analysis (p < 0.05), they were the only factors included in the multivariate Cox analysis. These results, in turn, showed that pathological stage remained associated with PFI; thus, it was deemed an independent prognostic factor in THCA.

### Table 5
Univariate and Multivariate Cox Regression Analyses of the Effect of m6A Regulatory Genes on Progression-Free Interval

| Variable                     | Univariate | Multivariate |
|------------------------------|------------|--------------|
|                              | HR (95% CI)| P            | HR (95% CI)| P          |
| Age, years (> 60 vs. ≤60)    | 1.990 (0.917–4.319) | 0.082        |            |            |
| Sex (male vs. female)        | 0.564 (0.298–1.065)  | 0.078        |            |            |
| Pathological stage (III-IV vs. I-II) | 2.802 (1.511–5.196) | 0.001        | 2.557 (1.177–5.558) | 0.018 |
| M (M1 vs. M0)                | 3.396 (1.014–11.381) | 0.048        | 2.925 (0.865–9.887) | 0.084 |
| N (N1 vs. N0)                | 1.537 (0.798–2.958)  | 0.198        |            |            |
| T (T3-T4 vs. T1-T2)          | 1.613 (0.865–3.007)  | 0.133        |            |            |
| m6A regulator genes (altered vs. diploid) | 1.523 (0.673–3.449) | 0.313        |            |            |

Note: Ambiguous variables (Nx, Mx, N/A, discrepancy, and Gx) were excluded. HR, hazard ratio; CI, confidence interval

### Enrichment analysis of the loss of m6A regulatory gene function

Considering that *ZC3H13* plays an important role in the methylation process and the frequency of mutations and that this gene showed the highest CNVs in patients with THCA, we explored the biological function of this gene in the pathogenesis of THCA. We found that low *ZC3H13* expression was significantly correlated with oxidative phosphorylation, Parkinson's disease, Alzheimer's disease, and glutathione metabolism (Table 6, Fig. 6). High *ZC3H13* expression was associated with cancer-related pathways such as the TGF-β signaling pathway and the Wnt signaling pathway, as shown in Table 7.

### Table 6
Gene Set Enrichment Analysis of Low *ZC3H13* mRNA Expression in the Thyroid Carcinoma Cohort

| Name                           | Size | ES        | NOM p-Value | FDR q-Value |
|--------------------------------|------|-----------|-------------|-------------|
| KEGG_OXIDATIVE_PHOSPHORYLATION | 101  | 0.745129  | 0.005882353 | 0.10500215  |
| KEGG_PARKINSONS_DISEASE        | 100  | 0.70253545| 0.007858546 | 0.088924155 |
| KEGG_ALZHEIMERS_DISEASE       | 144  | 0.53214025| 0.007797271 | 0.080741264 |
| KEGG_GLUTATHIONE_METABOLISM   | 49   | 0.5197475 | 0.00952381  | 0.1901218   |

ES, enrichment score; NOM, nominal; FDR, false discovery rate
Table 7  
Gene Set Enrichment Analysis of High ZC3H13 mRNA Expression in the Thyroid Carcinoma Cohort

| Name                                                      | Size | ES             | NOM p-Value | FDR q-Value |
|------------------------------------------------------------|------|----------------|-------------|-------------|
| KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS                        | 130  | -0.52496415    | 0           | 0.15935099  |
| KEGG_ADHERENS_JUNCTION                                     | 68   | -0.5882908     | 0.001919386 | 0.09284918  |
| KEGG_PROSTATE_CANCER                                       | 89   | -0.5100872     | 0           | 0.063088976 |
| KEGG_TGF_BETA_SIGNALING_PATHWAY                            | 85   | -0.51799953    | 0.001930502 | 0.056011233 |
| KEGG_WNT_SIGNALING_PATHWAY                                 | 149  | -0.4610484     | 0           | 0.06603799  |
| KEGG_INOSITOL_PHOSPHATE_METABOLISM                         | 54   | -0.5282841     | 0           | 0.07000477  |
| KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM                 | 76   | -0.5091506     | 0           | 0.065082096 |
| KEGG_PATHWAYS_IN_CANCER                                    | 321  | -0.44432777    | 0           | 0.06613078  |
| KEGG_NEUROTROPHIN_SIGNALING_PATHWAY                        | 125  | -0.46585009    | 0           | 0.06720902  |
| KEGG_RENAL_CELL_CARCINOMA                                  | 66   | -0.5130015     | 0           | 0.062297847 |
| KEGG_ENDOMETRIAL_CANCER                                    | 52   | -0.53427994    | 0.001996008 | 0.06384516  |
| KEGG_SMALL_CELL_LUNG_CANCER                                | 84   | -0.505915      | 0.003984064 | 0.07345369  |
| KEGG_O_GLYCAN_BIOSYNTHESIS                                 | 26   | -0.58248794    | 0.005847953 | 0.06954466  |
| KEGG_COLORECTAL_CANCER                                     | 62   | -0.497784      | 0.005988024 | 0.06539087  |
| KEGG_ERBB_SIGNALING_PATHWAY                                | 86   | -0.47092432    | 0           | 0.06411188  |
| KEGG_MTOR_SIGNALING_PATHWAY                                | 51   | -0.4858755     | 0.002       | 0.06421729  |
| KEGG_ENDOCYTOSIS                                           | 176  | -0.4077002     | 0.007751938 | 0.08138933  |
| KEGG_PANCREATIC_CANCER                                     | 69   | -0.48465937    | 0.008016032 | 0.08156461  |
| KEGG_DORSO_VENTRAL_AXISFORMATION                           | 24   | -0.55215466    | 0.00984252  | 0.08260301  |
| KEGG_LONG_TERM_POTENTIATION                                | 69   | -0.43423492    | 0.001934236 | 0.09778976  |
| KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION               | 85   | -0.4270458     | 0.001960784 | 0.10496421  |
| KEGG_MAPK_SIGNALING_PATHWAY                                | 265  | -0.38500428    | 0           | 0.105129935 |
| KEGG_HEDGEHEGOG_SIGNALING_PATHWAY                          | 56   | -0.4695593     | 0.009689922 | 0.10340274  |
| KEGG_GAP_JUNCTION                                          | 87   | -0.4244276     | 0.009708738 | 0.10714945  |
| KEGG_INSULIN_SIGNALING_PATHWAY                             | 137  | -0.38877195    | 0.00407332  | 0.1035778   |
| KEGG_OOCYTE_MEIOSIS                                        | 110  | -0.41317105    | 0.007782101 | 0.1045717   |
| KEGG_GNRH_SIGNALING_PATHWAY                                | 101  | -0.39601892    | 0.004081633 | 0.110016145 |

ES, enrichment score; NOM, nominal; FDR, false discovery rate

Discussion
Although THCA generally has a good prognosis, some high-risk patients develop metastasis and recurrence and can thus die from the disease. Therefore, relevant diagnostic and prognostic indicators of THCA need to be identified. Our analysis of the relationship between mutations and CNVs in m6A regulatory genes and clinical pathology showed that advanced pathological stage and T stage were significantly associated with changes in m6A regulatory genes. We then evaluated the effect of changes in m6A regulatory genes on their expression profiles and found that the expression of six m6A regulatory genes (i.e., FTO, METTL3, YTHDF1, YTHDF2, ZC3H13, and WTAP) significantly correlated with their different copy number states. An increased copy number was associated with mRNA expression, while copy deletion was associated with reduced mRNA expression. We also found a significantly lower PFI in patients with abnormal copy numbers than in those with normal copy numbers, indicating that abnormal copy number is a factor indicative of a poorer prognosis in patients with THCA. We also analyzed the PFI in different states of each gene. Because the number of patients with a single-gene CNV change was too small, the results were not significant. Furthermore, pathologic stage was an independent prognostic factor for patients with THCA. Therefore, we speculated that CNVs are involved in the occurrence of thyroid cancer by affecting cancer-related signaling pathways in which m6A regulatory genes are involved by changing their mRNA expression.

The global incidence of THCA has continued to increase in recent decades. Data from the National Cancer Registry of China show that the annual incidence markedly increased by 4.9% during 2000–2003 to 20.1% during 2003–2011 [10]. A series of cancer-related pathways are dysregulated in THCA development. Several valuable THCA molecular markers such as BRAF, RAS point mutations, RET/PTC, TERT, and PAX8/PPAR-γ are increasingly being used in clinical practice [11, 12]. However, there are no concise prospective data to support the use of molecular markers alone to determine the degree of treatment or to predict the prognosis of patients with THCA.

New evidence indicates that m6A is involved in various aspects of RNA metabolism, including pre-splicing of mRNA, 3′-end processing, nuclear export, translation regulation, mRNA attenuation, and noncoding RNA processing [7, 13–15]. m6A RNA modification affects tumor proliferation [16], differentiation, tumorigenesis, invasion [17], and metastasis [18] by regulating proto-oncogenes and tumor suppressor genes. Similarly, CNVs play a crucial role in the occurrence, development, and outcome of several cancers, such as lung, endometrial, prostate, and gastric cancers [19–22]. However, the role of m6A methylation modification and CNVs in THCA is unknown. In this study, we analyzed mutation data and CNV data of m6A regulatory genes in 492 patients with THCA from the TCGA database and found that KIAA1429 and YTHDC1 with frameshift mutations, YTHDC2 with splice donor variant changes, and ZC3H13 with stop codon mutations had the greatest impact on gene function, indicating that “writers” and “readers” may play an important role in the occurrence of thyroid cancer. The “writers” METTL3 and METTL14 are more likely to be mutated or undergoing CNVs in other genes in clear cell renal cell carcinoma [23], while the changes of “erasers” FTO and ALKBH5 have been proven to be more important in breast cancer, glioblastoma, and hematological malignancies [24–26]. The differences in genes related to different tumor types suggest that the regulation of m6A at the cellular level is complicated, and further studies are needed to investigate the regulatory mechanism of m6A in thyroid cancer.

The “writer” ZC3H13 gene, which is a canonical CCCH zinc finger protein that harbors a somatic frameshift mutation in colorectal cancer, suppresses colorectal cancer proliferation and invasion by inactivating Ras-ERK signaling [27], indicating that ZC3H13 may serve as a tumor suppressor gene. However, Gewurz et al. found that ZC3H13 may be a key upstream factor of NF-κB responsible for its activation [28]. Activation of NF-κB signaling accelerates tumor proliferation and invasion [29], suggesting that ZC3H13 may be an oncogenic protein. ZC3H13 may bind with K-ras, which is frequently mutated in various cancers such as non-small cell lung cancer and colon carcinoma [30, 31] and is strongly associated with cancer progression [32]. Thus far, the expression and biological function of ZC3H13 in malignant tumors are still unclear. It is speculated that the ZC3H13 gene may also play a major part in the occurrence and development of THCA by regulating cancer-associated signaling pathways and biological processes. Further studies are needed to identify the specific impact of ZC3H13 on the regulation of the downstream genes. With improvements in CNV detection
technology, its pathogenic mechanism and relationship with gene mutations are expected to be widely recognized. CNVs in m6A regulatory genes are expected to provide new directions for the diagnosis and prognosis of thyroid cancer.

This study has some limitations that need to be considered when interpreting the findings. First, the number of samples with increased copy numbers and samples with normal copy numbers (26 vs. 240) was not evenly distributed. Second, a relationship between the expression levels of ZC3H13 and the risk of THCA was not observed in this study; thus, further research is necessary to clarify this ambiguity. Despite these limitations, the study remains valuable because to the best of our knowledge, this is the first study to investigate the genetic changes of m6A regulatory genes in THCA and to analyze the relationship of mutations and CNVs in m6A regulatory genes with the clinical pathology of THCA. To further clarify the specific target mRNAs of m6A modification in the occurrence and development of THCA, we plan to conduct m6A-Seq and m6A MERIP studies in clinical samples to support our findings.

**Conclusion**

This study revealed that mutations and CNVs in m6A regulatory genes were significantly correlated with advanced pathological stage and T stage, indicating that CNVs are associated with a poor THCA prognosis. Patients with CNVs in m6A regulatory genes had significantly lower PFI than those without CNVs. *ZC3H13*, the m6A regulatory gene that most frequently had CNVs, may play a major role in the occurrence and development of THCA by regulating cancer-associated signaling pathways and biological processes. These findings provide evidence that elucidates the importance of epigenetic modification of RNA in THCA.

**Methods**

**Data collection and preprocessing**

All the clinical data, mutation profiles, and mRNA expression data are publicly available and from an open source. We systematically searched the TCGA official website ([https://portal.gdc.cancer.gov](https://portal.gdc.cancer.gov)) to collect clinical data, mutation profiles, and mRNA expression data of patients with THCA. Data of 492 patients with THCA with m6A regulatory gene mutations were downloaded through the TCGAbiolinks R package, whereas the CNV data of 399 patients were acquired from the cBioportal platform ([https://www.cbioportal.org/](https://www.cbioportal.org/)). Clinical data for 301 patients with THCA who had both PFI and copy number information were downloaded using TCGAbiolinks R. For patients who developed recurrent progression, we used the PFI from the first occurrence of disease. Thereafter, we excluded patients with PFI less than 30 days. Thus, 266 patients were included in the analysis; of them, 240 patients had normal copy numbers and 26 had abnormal copy numbers (i.e., increased or missing copy numbers).

**Gene set enrichment analysis**

Gene set enrichment analysis (GSEA) is a tool for analyzing data on whole-genome expression profile microarrays and was used to explore the potential molecular mechanisms underlying the prognostic gene signature constructed in this study. GSEA was applied to compare enriched terms between the high- and low-risk groups of patients with THCA as well as to investigate the correlation with the Kyoto Encyclopedia of Genes and Genomes pathway. The patients were divided into two groups, the high expression group and the low expression group, based on the mean *ZC3H13* expression. Then, a single-gene GSEA was performed on *ZC3H13*.

**Statistical analysis**

All statistical analyses were performed using SPSS 20.0 (IBM, Chicago, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). We explored the relationship between CNVs of m6A regulatory genes and clinicopathological characteristics such as age, sex, and TNM stage using the chi-square test or Mann-Whitney U test.
Kaplan-Meier curves and the log-rank test were used to evaluate the prognostic value of alterations in m6A regulatory genes. Cox proportional hazard regression was performed using SPSS. A p-value < 0.01 and a false discovery rate of q < 0.05 were considered to indicate statistical significance.

**Ethical considerations**

All the clinical data, mutation profiles, and mRNA expression data are publicly available from TCGA official website and cBioportal platform, which are open to the public under some guidelines. Therefore, the requirement for ethical approval was waived. The need for informed consent was also waived owing to the retrospective study design.

**Abbreviations**

CNVs: copy number variations

PFI: progression-free intervals

TCGA: The Cancer Genome Atlas

THCA: thyroid carcinoma

**Declarations**

**Ethics approval and consent to participate:** All the clinical data, mutation profiles, and mRNA expression data are publicly available from TCGA official website and cBioportal platform, which are open to the public under some guidelines. Therefore, the requirement for ethical approval was waived. The need for informed consent was waived owing to the retrospective study design

**Consent to participate and for publication:** Not applicable.

**Data availability:** The datasets generated and/or analyzed during the current study are available in the TCGA repository, https://portal.gdc.cancer.gov.

**Competing interest:** The authors declare that they have no competing interests.

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**Authors’ contributions:** HH conceptualized and designed the study, carried out investigations and formal analysis, and prepared the original draft. JYY carried out study validation and formal analysis, and reviewed and edited the manuscript. XDF carried out visualization and investigation. YQN was responsible for data curation and software. DCL was responsible for resources and project administration. ZFZ supervised the study and acquired funding for the study.

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**References**

1. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell. 2012;149:1635–46. https://doi.org/10.1016/j.cell.2012.05.003.
2. Li A, Chen YS, Ping XL, Yang X, Xiao W, Yang Y, et al. Cytoplasmic m6A reader YTHDF3 promotes mRNA translation. Cell Res. 2017;27:444–7. https://doi.org/10.1038/cr.2017.10.

3. Chai RC, Wu F, Wang QX, Zhang S, Zhang KN, Liu YQ, et al. Kang, m6A RNA methylation regulators contribute to malignant progression and have clinical prognostic impact in gliomas. Aging (Albany NY) 2019;11:1204–25. https://doi.org/10.18632/aging.101829.

4. Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, et al. Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res 2017;27:626–41. https://doi.org/10.1038/cr.2017.31.

5. Pan Y, Ma P, Liu Y, Li W, Shu Y. Multiple functions of m6A RNA methylation in cancer. J Hematol Oncol 2018;11:48. https://doi.org/10.1186/s13045-018-0590-8.

6. Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics 2013;11:8–17. https://doi.org/10.1016/j.gpb.2012.12.002.

7. Gilbert WV, Bell TA, Schaening C. Messenger RNA modifications: form, distribution, and function. Science 2016;352:1408–12. https://doi.org/10.1126/science.aad8711.

8. Alarcón CR, Goodarzi H, Lee H, Liu X, Tavazoie S, Tavazoie SF. HNRNPA2B1 Is a mediator of m(6)A-dependent nuclear RNA processing events. Cell 2015;162:1299–308. https://doi.org/10.1016/j.cell.2015.08.011.

9. Dolatabadian A, Patel DA, Edwards D, Batley J. Copy number variation and disease resistance in plants. Theor Appl Genet 2017;130:2479–90. https://doi.org/10.1007/s00122-017-2993-2.

10. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015, CA. Cancer J Clin 2016;66:115–32. https://doi.org/10.3322/caac.21338.

11. Decaussin-Petrucci M, Descotes F, Depaere L, Lapras V, Denier ML, Borson-Chazot F. Molecular testing of BRAF, RAS and TERT on thyroid FNAs with indeterminate cytology improves diagnostic accuracy. Cytopathology 2017;28:482–7. https://doi.org/10.1111/cyt.12493.

12. Insilla AC, Proietti A, Borrelli N, Macerola E, Niccoli C, Vitti P, et al. TERT promoter mutations and their correlation with BRAF and RAS mutations in a consecutive cohort of 145 thyroid cancer cases. Oncol Lett 2018;15:2763–70. https://doi.org/10.3892/ol.2017.7675.

13. Roignant JY, Soller M. m6A in mRNA: an ancient mechanism for fine-tuning gene expression. Trends Genet 2017;33:380–90. https://doi.org/10.1016/j.tig.2017.04.003.

14. Shi H, Wang X, Lu Z, Zhao BS, Ma H, Hsu PJ, et al. YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. Cell Res 2017;27:315–28. https://doi.org/10.1038/cr.2017.15.

15. Lipshitz HD, Claycomb JM, Smibert CA. Post-transcriptional regulation of gene expression. Methods 2017;126:1–2. https://doi.org/10.1016/j.ymeth.2017.08.007.

16. Liu J, Eckert MA, Harada BT, Liu SM, Lu Z, Yu K, et al. m6A mRNA methylation regulates AKT activity to promote the proliferation and tumorigenicity of endometrial cancer. Nat Cell Biol 2018;20:1074–83. https://doi.org/10.1038/s41556-018-0174-4.

17. Lin S, Choe J, Du P, Triboulet R, Gregory RI. The m(6)A methyltransferase METTL3 promotes translation in human cancer cells. Mol Cell 2016;62:335–45. https://doi.org/10.1016/j.molcel.2016.03.021.

18. Ma JZ, Yang F, Zhou CC, Liu F, Yuan JH, Wang F, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinoma by modulating N6-methyladenosine-dependent primary MicroRNA processing. Hepatology 2017;65:529–43. https://doi.org/10.1002/hep.28885.

19. Schumacher SE, Shim BY, Corso G, Ryu MH, Kang YK, Roviello F, et al. Somatic copy number alterations in gastric adenocarcinomas among Asian and Western patients. PLoS One 2017;12:e0176045. https://doi.org/10.1371/journal.pone.0176045.
20. Kaveh F, Baumbusch LO, Nebdal D, Børresen-Dale A.L, Lingjærde OC, Edvardsen H, et al. A systematic comparison of copy number alterations in four types of female cancer. BMC Cancer 2016;16:913. https://doi.org/10.1186/s12885-016-2899-4.

21. VanderWeele DJ, Finney R, Katayama K, Gillard M, Paner G, Imoto S, et al. Genomic heterogeneity within individual prostate cancer foci impacts predictive biomarkers of targeted therapy. Eur Urol Focus 2019;5:416–24. https://doi.org/10.1016/j.euf.2018.01.006.

22. Woo HG, Choi JH, Yoon S, Jee BA, Cho EJ, Lee JH, et al. Integrative analysis of genomic and epigenomic regulation of the transcriptome in liver cancer. Nat Commun 2017;8:839. https://doi.org/10.1038/s41467-017-00991-w.

23. Zhou J, Wang J, Hong B, Ma K, Xie H, Li L, et al. Gene signatures and prognostic values of m6A regulators in clear cell renal cell carcinoma - a retrospective study using TCGA database. Aging (Albany NY) 2019;11:1633–47. https://doi.org/10.18632/aging.101856.

24. Zhang C, Zhi WI, Lu H, Samanta D, Chen I, Gabrielson E, et al. Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. Oncotarget 2016;7:64527–42, https://doi.org/10.18632/oncotarget.11743.

25. Zhang S, Zhao BS, Zhou A, Lin K, Zheng S, Lu Z, et al. m6A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. Cancer Cell 2017;31:591–606.e6. https://doi.org/10.1016/j.ccell.2017.02.013.

26. Li Z, Weng H, Su R, Weng X, Zuo Z, Li C, et al. FTO plays an oncogenic role in acute myeloid leukemia as a N6-methyladenosine RNA demethylase. Cancer Cell 2017;31:127–41. https://doi.org/10.1016/j.ccell.2016.11.017.

27. Zhu D, Zhou J, Zhao J, Jiang G, Zhang X, Zhang Y, et al. ZC3H13 suppresses colorectal cancer proliferation and invasion via inactivating Ras-ERK signaling. J Cell Physiol 2019;234:899–907. https://doi.org/10.1002/jcp.27551.

28. Gewurz BE, Towfic F, Mar JC, Shinners NP, Takasaki K, Zhao B, et al. Genome-wide siRNA screen for mediators of NF-kB activation, Proc. Natl. Acad. Sci. U S A. 2012;109:2467–72. https://doi.org/10.1073/pnas.1120542109.

29. Park MH, Hong JT. Roles of NF-kB in cancer and inflammatory diseases and their therapeutic approaches. Cells 2016;5:15. https://doi.org/10.3390/cells5020015.

30. Chapman AM, Sun KY, Ruestow P, Cowan DM, Madl AK. Lung cancer mutation profile of EGFR, ALK, and KRAS: meta-analysis and comparison of never and ever smokers. Lung Cancer. 2016;102:122–34. https://doi.org/10.1016/j.lungcan.2016.10.010.

31. Cicenas J, Tamosaitis L, Kvaderaviciute K, Tarvydas R, Staniute G, Kalyan K, et al. KRAS, NRAS and BRAF mutations in colorectal cancer and melanoma. Med Oncol 2017;34:26. https://doi.org/10.1007/s12032-016-0879-9.

32. Neuzillet C, Hammel P, Tijeras-Raballand A, Couvelard A, Raymond E. Targeting the Ras-ERK pathway in pancreatic adenocarcinoma. Cancer Metastasis Rev 2013;32:147–62. https://doi.org/10.1007/s10555-012-9396-2.