Research Article

Identification of Three Prognosis-Related Differentially Expressed lncRNAs Driven by Copy Number Variation in Thyroid Cancer

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Thyroid cancer as the malignant tumor with the highest incidence in the endocrine system also shows a fast growth and development. In this work, we developed a new method to identify copy number variation (CNV)–driven differentially expressed lncRNAs in thyroid cancer for predicting cancer prognosis. The data of RNA sequencing, CNV, methylation, mutation, and clinical details of thyroid cancer were obtained from the Cancer Genome Atlas database (TCGA). Molecular subtypes were clustered by iClusterPlus. Weighted gene co-expression network analysis (WGCNA) was employed to show co-expression modules. DEseq2 was conducted to identify protein coding genes (PCGs) and differentially expressed lncRNAs. CNV was detected using GISTIC 2.0. Three molecular subtypes were identified, and 68 differentially expressed lncRNAs (DElncRNAs) related to cancer were found among different molecular subtypes. CNV of FOXD2-AS1, FAM181A-AS1, and RNF157-AS1 was associated with overall survival and was involved in cancer-related pathways. These three DElncRNAs discovered based on CNV could serve as prognostic biomarkers to predict prognosis for thyroid cancer and new targets to explore molecular drugs.

1. Introduction

Thyroid cancer, which is a common thyroid malignancy, can be classified into medullary cancer, papillary cancer, undifferentiated cancer, and follicular cancer by pathological classification. According to the statistics of cancer in 2018, 567,000 new cases of thyroid cancer occurred worldwide, accounting for 3.1% of all new cancer cases of the year. Follicular carcinoma and papillary thyroid carcinoma composed the majority of all thyroid cancer [1, 2]. Although most patients have a positive prognosis and survival after treatment, lymph node metastasis is common as well, and patients are prone to develop into advanced cancer and recurrence. Therefore, it is important to study its specific pathogenesis to guide new treatments [3]. Sufficient evidence indicated that the development and progression of papillary thyroid carcinoma is not only affected by environmental and genetic susceptibility factors, but also by genetic changes [4].

Long noncoding RNAs (lncRNAs), a group of RNAs with a length over than 200 nucleotides, play a critical role in gene expression regulation. In recent years, a number of studies showed that lncRNAs are closely involved in the development and progression of cancers [5–7]. LncRNAs have been considered promising biomarkers for cancer detection and prognosis follow-up [8–10]. For example, the expression level of H19 is promoted in multiple cancers, for example, cervical cancer, breast cancer, and bladder cancer. H19 expression is also higher in thyroid cancer tissue than in normal tissue, which may promote the migration of thyroid cancer cells [11, 12]. Abnormal expression of lncRNA OIP5-AS1 in thyroid cancer tissues suggests its potential of serving as a molecular biomarker for thyroid cancer [13].
As an important genetic variation, genome copy number variation (CNV) has specific changes in progression of certain cancers and has become an important characteristic of tumors. CNV refers to the submicroscopic mutation of DNA fragments from KB to Mb, mainly including replication, deletion, embedding, and complex multisite variation [14]. Redon et al. [15] filtered about 1500 CNVs regions in 270 samples, accounting for about 10% of the human genome and included coding and noncoding regions. In recent years, copy number variation has been found in tumors, and some studies have made breakthrough discoveries. For example, Ye et al. reported that medullary thyroid cancer is relevant to the changes in DNA copy number [16]. One study found that DNA copy number variation is a characteristic of malignant and benign thyroid tumors and that the segmentary expansion of chromosomes (Ch)7 and 12 is less common in follicular variant papillary thyroid cancers or typical papillary thyroid cancers than in follicular adenomas. In addition, the deletion in Ch22 has been detected in a subgroup of follicular adenoma and follicular variant thyroid papillary carcinoma [17]. However, the role of genome-level changes in the number of DNA copies in the pathogenesis of tumors is still controversial and can only explain the pathogenesis of certain patients [18], indicating that the mechanism of CNVs for tumor genesis is not well understood, especially the high-frequency genome variation in the desert region of the tumor, which is less studied. Therefore, the pathogenic mechanism of CNVs for abnormal expression of noncoding RNA, especially Inc RNA, requires further research.

Combining correlation analysis of CNV and IncRNA expression, we found that IncRNAs showing a transcriptional imbalance played a critical role in the biological process of thyroid cancer. We identified three CNV-related IncRNAs as prognostic biomarkers for predicting prognosis of thyroid cancer. Moreover, these prognostic IncRNAs could provide inspiration to discover novel molecular drugs for thyroid cancer treatment.

2. Materials and Methods

2.1. Large-Scale Data Selection. A total of 500 thyroid cancer samples were included in this study. 354 (70.8%) had thyroid papillary-classical/usual carcinoma, 101 cases (20.2%) had thyroid papillary carcinoma-follicular patterned (≥99.0%), 36 cases (7.2%) had thyroid papillary carcinoma-tall cell (≥50.0% tall cell features) type, and 9 cases (1.8%) were diagnosed with other unknown tissue type. From the Cancer Genome Atlas (TCGA) Genomic Data Commons (GDC) database (https://gdc.cancer.gov/) and the Cancer Genome Atlas (TCGA) Genomic Data Commons (GDC) database (https://gdc.cancer.gov/swagger), CNV data, CpG data, RNA-seq data, clinical follow-up information, and mutation data of thyroid cancer were collected. Firstly, to process RNA-Seq data, counts data and all fragments per kilobase million (FPKM) data were obtained. FPKM was converted to transcripts per kilobase millions (TPM). Messenger RNA of IncRNA, 3prime_overlapping_ncRNA, sense_overlapping, antisense, processed_transcript, and sense_intronic were regarded as IncRNAs according to the gene types in genome.v22.gtf file. In this way, the FPKM expression profile of IncRNAs was extracted. Also, the FPKM expression profiling of protein-coding genes (PCGs) was acquired. After obtaining the expression profile of all 450 k samples, CpG probes with NA in the expression of samples, CpG probes with cross-reactive site samples, and CpG probes on sex chromosomes and single nucleotide sites were removed from the CpG data. From TCGA GDC, the CNVs of all samples without type differences were downloaded. MuTect [19] software processed-single nucleotide mutation data were obtained from TCGA GDC (see Figure 1 for the flow chart of this study).

2.2. Univariate Survival Analysis. The influence of CNV, methylation, and coding genes on the cancer prognosis was analyzed independently to better classify the samples. We selected samples with a follow-up time longer than 30 days. Based on univariate Cox proportional hazard regression (threshold: \( P = 0.05 \)), a model was established. 3074 CNV regions, 40,150 CpG sites, and 1447 coding genes were finally screened.

2.3. Identification of Molecular Subtypes and Differential Analysis of IncRNAs and PCGs. The CNV, prognostic coding genes and methylation sites were obtained by univariate survival analysis, and from the three common omics samples, a total of 500 samples were obtained. Multi-cluster analysis in R software package iClusterPlus [20] was conducted, and the number of classification was selected to be 3. Next, differentially expressed IncRNAs and PCGs in different subtypes were analyzed using R software package DEseq2 [21]. In the expression profile, the genes showing an average Count<1 were removed. In each subtype, the threshold of false discovery rate (FDR)<0.05 and fold change more than twice was applied to identify PCGs and the differentially expressed IncRNAs (DEIncRNAs).

2.4. Weighted Gene Co-Expression Network Construction. To mine the co-expression module based on IncRNA expression profiles and differentially expressed PCGs, WGCNA co-expression algorithm in the R package WGCNA (http://www.r-project.org/) was employed [22]. By approximate scale-free topology criteria, we determined the soft threshold. Adjacency matrix was converted to topological matrix (TOM). Next, average-linkage hierarchical clustering was employed to cluster genes. Finally, we used dynamic tree cut method to determine module eigengenes with at least 30 co-expressed genes.

2.5. Identification of CNV-Driven IncRNAs. Visualization of regions in the genome by the Genomic Identification of Significant Targets in Cancer (GISTIC) [23] shows the deletion or amplification in thousands of samples. Thus, the copy number data of 505 TCGA thyroid cancer cases were analyzed by GISTIC 2.0 software. Firstly, the copy number spectrum of IncRNAs was developed. The copy number was amplified when >1, and a copy number deletion was defined when and the copy number ≤-1. The ratio of amplification to deletion in the IncRNAs was determined, and the distribution in genome was observed.
2.6. Functional Pathway Enrichment Analysis in Module. The functions of hub genes in the module were examined. By loading "anRichment" package in R [20], the gene ontology (GO) enrichment analysis was conducted. $P < 0.05$ was the threshold.

2.7. Gene Set Enrichment Analysis. Gene set enrichment analysis (GSEA) [24] was performed and mapped into pathway enrichment database of Kyoto Encyclopedia of Genes and Genomes (KEGG) to study mechanisms of DElncRNAs in TCGA and GSE33630. Here, the cutoff was FDR < 0.05.

2.8. Statistical Analysis. Kaplan-Meier was used to plot survival curves using log-rank test. A significance was defined as $P < 0.05$. ROC analysis was conducted by using pROC R package [25]. Unless otherwise stated, all the analyses were carried out in R 3.4.3 under default parameters.

### 3. Results

3.1. Identification of Three Molecular Subtypes Based on Prognostic Methylation Sites, CNV, and PCGs. We identified differentially expressed CNV, methylation sites, and PCGs related to prognosis by comparing cancer samples to normal samples, and they were used as a basis to construct molecular subtypes using iClusterPlus. Three molecular subtypes were obtained (Table 1). From the Kaplan-Meier survival curve, obvious prognostic differences among the three molecular subtypes could be observed (Figure 2(a)). Furthermore, gene mutations in different subtypes were determined. In each subtype, 20 genes showing the highest mutation were selected, and a total of 42 genes were acquired. The intersection between the 20 genes with the highest mutation frequency was determined from the three subtype samples (Figure 2(b)). Also, the mutations of these 42 genes in various subtypes were visualized (Figure 2(c)). In addition, we used the R software package estimate to evaluate the immune microenvironment infiltration score of each patient. By analyzing the difference of the immune microenvironment infiltration scores of the three subtypes, we can observe that cluster2 has the significantly highest immune infiltration (Figure 2(d)). Using the R software package cibersort to evaluate the score of immune cell infiltration in 22, it can be observed that T_cells_regulatory_(Tregs) and Macrophages_M0 in cluster2 have the significantly highest infiltration score and cluster2 has the significantly lowest immune infiltration score in T_cells_CD4_memory_resting and Macrophages_M2. These results show that these three molecular subtypes have different immune microenvironment characteristics.

3.2. DElncRNAs and PCGs Were Identified from the Three Molecular Subtypes. We obtained 3254 PCGs and 2396...
Survival time in day

Survival probabilities

Cluster1 (N = 194)
Cluster2 (N = 175)
Cluster3 (N = 131)

(a)

RYR1 ZNF292 CSMD2 DNAH9 MACF1 GOLGA4 KMT2A ADGRV1 JMJD1C MALAT1 LRRK2 BRAF ZFHX3 TTN TBC1D12 DNAH2 PKHD1 MUC16 SPTA1 SUOX PIK3CG C2CD3 CSMD3 GAPVD1 WHSC1L1 AIM1 ADAMTS16 BDP1 HRAS RAI14 DOCK7 C5orf42 CNTLN SMC3 DST CSF2RA XIRP2 TG INTS2 NRAS HMCN1 EIF1AX

(b)

\( \rho = 0.05 \)

Figure 2: Continued.
DElncRNAs in the molecular subtypes using DEseq2. (Table 2) and constructed a volcano map based on DElncRNAs screened from the three subtypes (Figures 3(a)–3(d)). In each subtype, the number of lncRNA and PCGs differences was counted, and we found that lncRNAs differences were generally smaller than PCGs differences (Figure 3(e)). Also, lncRNAs associated with the cancer were downloaded from Lnc2Cancer and LncRNADisease. 611 lncRNAs were acquired and compared with 2396 lncRNAs in different subtypes. There are 68 shared lncRNAs (Figure 3(f)). Further, we performed GSEA analysis according to the absolute value of the difference multiple of lncRNA in each subtype as the rank. The data indicated that the differentially expressed lncRNA was clustered in the gene concentration with large difference multiple (Figures 3(g)–3(j)). Finally, between the tumorous and healthy samples, the intersection of the three subtypes and DElncRNAs was analyzed; here, the two showed a large intersection (Figure 3(k)).

3.3. Identification of Co-Expression Modules Based on Differentially Expressed PCGs and lncRNAs. Firstly, hierarchical clustering on the samples was performed. Samples with a distance of more than 100,000 were screened as outliers, and 560 samples were obtained (Figure 4(a)). The co-expression network conformed to the scale-free network, as shown by the current data. To further ensure a scale-free nature of the network, \( \beta = 4 \) was selected (Figures 4(b) and 4(c)). R package WGCNA showed 24 modules (Figure 4(d), Table 3). Note that the grey module cannot be aggregated into other modules. In 23 modules, the histogram of relative multiples of lncRNA ratio to PCG showed no significant difference between lncRNA and genes in each module (Figure 4(e)). Further, the relationship of clinical status with modules was analyzed, and the modules are related to at least one phenotype, except dark red, cyan, tan, black, royal blue, and light yellow (Figure 4(f)). Modules related to at least three phenotypes were selected, yellow and purple, for subsequent analysis. Next, the unional enrichment analysis was conducted on yellow and purple modules. In the yellow module, the top 20 GO terms enriched were

![Figure 2: Molecular subtypes were identified by multi-omics analysis. (a) KM curves for disease-free survival of 3 molecular subtypes. (b) A Venn diagram for the top 20 genes showing the highest mutations in each molecular subtype. (c) Heat maps of the top 20 genes with the highest mutations in each molecular subtype. (d) The distribution of immune infiltration microenvironment scores of the three subtypes was different. (e) Distribution difference of three subtypes in 22 immune cell infiltration scores.](image-url)

![Table 2: Differential expression of PCG and lncRNA.](table-url)
Figure 3: Continued.
mainly associated with cell proliferation and vascular system (Figure S1A). The top 20 pathways were mainly related to ligand-receptor interaction, PI3K–Akt, MAPK, and other pathways (Figure S1B). In the purple module, the top 20 GO terms enriched were mainly correlated with extracellular matrix and cell differentiation (Figure S1C).

Among the top 20 pathways enriched, the most significant pathways were ECM-receptor interaction, PI3K-Akt, and Wnt signaling pathways (Figure S1D).

### 3.4. Identification of lncRNAs Driven by CNV

A sum of 505 TCGA thyroid cancer copy number were analyzed by GIS-TIC. The distribution of copy numbers across the genome were observed and found that the proportion of copy number amplification on chromosomes 1, 7, 12, 12, 16, and 17 was larger and the copy number deletion was the highest on chromosomes 9, 13, and 22 (Figure 5(a)). Furthermore, the correlation distribution of copy number and lncRNA expression profile was calculated. An overall positive correlation trend of the copy number and lncRNA expression and a significantly higher distribution than random were observed (Figure 5(b)). We identified frequently changing regions in the genome of thyroid cancer. Many lncRNAs showed significant multiple copies or significant deletions, while compared with copy amplification regions, lncRNA deletions were more frequent (Figures 5(c) and 5(d)). To further reveal the correlation of copy number with lncRNA expression, in each sample, 119 lncRNAs showing a copy number ratio of more than 7% were selected. Differences in expression of lncRNAs in the copy-amplified or copy-deficient samples as well as in the samples with normal copies were explored. LncRNAs whose expression was greater than 0 in at least 50% of the samples were selected, and finally 50 lncRNAs that met the conditions were retained. Among them, 20 lncRNAs were copy-amplified (Figure 6(a)), most of which were high-expressed in the copy-amplified samples. 30 lncRNAs were copy-deleted (Figure 6(b)), a great majority of which were significantly low-expressed in the copy-deleted samples. These results suggested that lncRNA copy changes were positively correlated with lncRNA expression.

### 3.5. Three lncRNAs Were Associated with Prognostic in Thyroid Cancer Were Determined

In order to systematically identify the prognostic markers of lncRNA, the criteria for inclusion were that lncRNAs showing a copy number
Sample clustering to detect outlier

(a)

(b)

(c)

(d)

(e)

Figure 4: Continued.
Figure 4: Identification of co-expression modules based on differentially expressed PCGs and lncRNAs. (a) Cluster analysis on the samples. (b–c) Network topology analysis for different soft-thresholding powers. (d) Module colors and gene dendrogram. (e) Histogram of relative multiples of lncRNA ratio to PCG ratio in 23 modules. The horizontal axis is the multiple of the lncRNA ratio to the PCG ratio in the module, vertical axis is module, and the values on the right are significant P values. (f) Correlation between 24 modules and clinical phenotype.

Table 3: PCGs and lncRNA in modules.

| Module       | All | Lnc | PCG | P value | Fc  |
|--------------|-----|-----|-----|---------|-----|
| Blue         | 579 | 229 | 350 | 0.935   | 0.889 |
| Light cyan   | 61  | 23  | 38  | 0.809   | 0.822 |
| Black        | 162 | 74  | 88  | 0.219   | 1.142 |
| Royal blue   | 48  | 18  | 26  | 0.636   | 0.940 |
| Green yellow | 90  | 30  | 60  | 0.970   | 0.679 |
| Grey60       | 58  | 23  | 35  | 0.710   | 0.892 |
| Grey         | 744 | 316 | 428 | 0.500   | 1.003 |
| Turquoise    | 1832| 790| 1042| 0.234   | 1.030 |
| Green        | 239 | 105 | 134 | 0.336   | 1.064 |
| Brown        | 562 | 256| 306 | 0.061   | 1.136 |
| Cyan         | 70  | 35  | 35  | 0.121   | 1.358 |
| Red          | 188 | 83  | 105 | 0.338   | 1.074 |
| Purple       | 105 | 47  | 58  | 0.346   | 1.101 |
| Yellow       | 276 | 115 | 161 | 0.624   | 0.970 |
| Salmon       | 77  | 36  | 41  | 0.253   | 1.192 |
| Midnight blue| 67  | 29  | 38  | 0.489   | 1.036 |
| Magenta      | 106 | 43  | 63  | 0.685   | 0.927 |
| Dark red     | 36  | 15  | 21  | 0.599   | 0.970 |
| Dark green   | 35  | 12  | 23  | 0.875   | 0.709 |
| Tan          | 78  | 33  | 45  | 0.551   | 0.996 |
| Light yellow | 50  | 21  | 29  | 0.577   | 0.983 |
| Light green  | 51  | 13  | 38  | 0.996   | 0.465 |
| Dark turquoise| 33 | 13  | 20  | 0.699   | 0.883 |
| Pink         | 107 | 37  | 70  | 0.961   | 0.718 |
3.6. Validation of Three lncRNAs in GSE33630. For further verifying the role of the three CNV-related lncRNAs in thyroid cancer, a dataset GSE33630 including 60 thyroid samples and 45 normal samples was downloaded from GEO. The reannotation data revealed a significantly high expression of FOXD2-AS1 in thyroid cancer samples (Figure 8(a)). FAM181A-AS1 and NF157-AS1 showed a low expression in tumor samples (Figures 8(b)–8(c)). Meanwhile, functional enrichment analysis demonstrated that the correlation coefficients of the top 20 KEGG pathway were above 0.36, 0.24 and 0.71, respectively (Figures 8(d)–8(f)). Among them, FOXD2-AS1 was positively correlated with 11 pathways, including ECM receptor interaction, MAPK signaling pathway, and some other cancer-related pathways, and negatively correlated with 9 pathways, mainly including REGULATION_OF_AUTOPHAGY and metabolism-related pathways. In FAM181A-AS1 and RNF157-AS1, P53 signaling pathway and ECM receptor interaction were negatively correlated with cancer-related pathways, which further indicated that the expression levels of the three lncRNAs were closely associated with cancer initiation and progression.

3.7. Verification of the Relationship between the Three lncRNAs and Prognosis. The overall survival rate of thyroid cancer was noticeably high, and follow-up to the end of the event was more than 10 years. Therefore, the cohort data with survival information are rare at present. We also evaluated the relationship between the lncRNAs and prognosis from the clinical characteristics of the samples. We obtained...
Figure 6: Different expression of lncRNA in copy amplified or deleted and normal copied samples. (a) 20 lncRNAs were copy-amplified, most of which were highly expressed in the copy-amplified samples. (b) 30 lncRNAs were copy-deleted, and the vast majority of which were significantly lowly expressed in the copy-deleted samples.
In this study, we provided a comprehensive set of dysregulated lncRNAs driven by CNV in thyroid cancer. Three molecular subtypes with differential prognosis of thyroid cancer patients were categorized using multi-omic data. These three subtypes have significantly different molecular characteristics and prognosis. Cluster2 patients have the highest immune microenvironment score, and cluster2 has the lowest immune microenvironment score. These differences may lead to different benefits in immunotherapy and may also be an important reason for the different prognosis of these patients. 2396 DElncRNAs from the molecular subtypes were obtained. There were many regions in the thyroid cancer genome with multiple copies or significant deletions of lncRNAs. Furthermore, three CNV-driven lncRNAs showed a significant correlation to overall survival, and the dysregulated lncRNAs driven by CNV were involved in some critical biological functions in thyroid cancer, such as cell cycle, metabolism pathways, and P53 signaling pathway, indicating that they may be potential clinical biomarkers of prognosis.

As a common structural variation in human genome, CNV has been regarded as an important contributing factor to cancer development [26, 27]. Panebianco et al. found a higher TERT promoter CNV in advanced thyroid cancer [28]. Five CNV-related genes (VEZT, NDUFA12, GDF3, FG6, and NR2C1) have been identified as the most effective genes to distinguish FAs from PTCs/FVPTCs, as they could correctly classify 90% of the cases [17]. CNV loss on the FMN2 gene promotes lymph node metastasis in medullary thyroid carcinoma family [29]. However, no studies have been performed on lncRNA CVA in thyroid cancer. In ovarian cancer prognosis-correlated, lncRNAs as novel biomolecular markers have been discovered for based CNV analysis [30]. In this study, for the first time, the prognostic indicators of thyroid cancer were identified based on lncRNA CNV. CNV-driven lncRNAs may contribute to thyroid cancer development and have the potential of serving as biomarker for thyroid cancer diagnosis.

Three copy number-related lncRNAs (FOXD2-AS1, FAM181A-AS1, and RNF157-AS1) significantly correlated with thyroid cancer survival were identified. FOXD2-AS1 was high-expressed in thyroid cancer tissues and cell lines, and this was related to tumor progression and metastasis [31–33]. This was consistent with our findings that high-expressed FOXD2-AS1 could predict a poor survival and that tumor related pathways such as P53 signaling pathway and cell cycle were mainly the KEGG enrichment pathways of target genes regulated by FOXD2-AS1, indicating that the analysis of whole genome IncRNA CNV to identify prognosis of thyroid cancer was an effective and reliable approach. However, no previous study investigated FAM181A-AS1 and RNF157-AS1. Based on the current literature review and the current findings, we hypothesized that CNV caused changes in the three lncRNA expressions and affected the progression of thyroid cancer through the regulatory mechanism of ceRNA. We inversely searched the miRNAs targeting these three lncRNAs from the Starbase database and observed that there were 16 miRNAs targeting ENSG000000237424 (FOXD2-AS1). Further, we matched the protein coding genes targeted by these 16 miRNAs. According to the Cerna

### Table 4: LncRNAs with significant prognosis.

| Symbol   | P value | HR   | Low 95% CI | High 95% CI | CNV Rate | DECount | Pcc  | GPL570 Probe |
|----------|---------|------|-------------|-------------|----------|---------|------|--------------|
| FOXD2-AS1| 0.007   | 0.454| 0.261       | 0.790       | 0.008    | 4       | 0.01 | 224457_at    |
| PGM5P3-AS1| 0.033   | 2.043| 1.081       | 3.859       | 0.020    | 3       | 0.01 | 239287_at    |
| LOC102724714| 0.013  | 2.141| 1.201       | 3.817       | 0.022    | 4       | 0.03 | NA           |
| HAND2-AS1| 0.015   | 2.158| 1.195       | 3.896       | 0.016    | 4       | 0.02 | 239708_at    |
| LOC100507144| 0.032  | 1.861| 1.069       | 3.240       | 0.026    | 3       | 0.12 | NA           |
| FAM181A-AS1| 0.037  | 1.895| 1.056       | 3.400       | 0.026    | 3       | 0.08 | 1557211_a_at |
| FENDRR   | 0.017   | 2.033| 1.168       | 3.540       | 0.044    | 3       | 0.06 | 243039_at    |
| RNF157-AS1| 0.046   | 1.784| 1.025       | 3.106       | 0.051    | 3       | 0.10 | 230776_at    |
Figure 7: Continued.
Figure 7: Continued.
hypothesis, we constructed a statistical model to evaluate the significance of the competition between lncRNA-mRNAs and selected FDR < 0.01 to obtain 50 genes. Further, we also screened lncRNA-mRNA with significant positive correlation and finally obtained 18 ceRNAs (Figure S2D). It can be observed that FOXD2-AS1 and TEX22 share the most miRNAs and high positive correlation. Those data suggested that the three lncRNAs may be important marker for thyroid cancer. Based on the above analysis, FAM181A-AS1 and RNF157-AS1 showed potential to help further reveal the mechanisms of thyroid cancer.

However, our study still has the following limitations. Clinical data and large datasets on thyroid cancer were insufficient. A lack of basic research was another limitation. Though the present analysis may not be the optimal, it should be sufficient to conclude that CNV leading to lncRNA dysregulation contributed to a poor prognosis of thyroid cancer. Basic experimental research and more in-depth functional research will be our future research direction.

5. Conclusions

At present, no studies have reported the relation of thyroid cancer with changes of lncRNA CNV. This study opened a new methodology to discover prognostic biomarkers for
Figure 8: Continued.
thyroid cancer through combining IncRNA expression profiles with CNV data. FOXD2-AS1, FAM181A-AS1, and RNF157-AS1 with abnormal CNV showed a difference on thyroid cancer survival and may promote the cancer development. Overall, this study identified three IncRNAs that could serve as prognostic biomarkers and provided a new insight into the possible mechanism of thyroid cancer development.

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that they have no competing interest.

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Supplementary Materials
Supplementary 1. Figure S1: functional enrichment analysis. (a) Top 20 GO term in the yellow module. (b) Top 20 KEGG pathways in the yellow module. (c) Top 20 GO term in the purple module. (d) Top 20 KEGG pathways in the purple module.

Supplementary 2. Figure S2: verification of the relationship between three IncRNAs and prognosis. (a) The expression differences of the three IncRNAs in the early and late samples in the GSE60542 dataset. (b) Correlation between FAM181A-AS1 expression and lymph node metastasis in GSE60542 dataset. (c) The KM curve of FAM181A-AS1 and RNF157-AS1 expression and prognosis in the TCGA exon dataset. (d) ceRNA network of 18 mRNAs associated with FOXD2-AS1.

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