Research Article

Integrated Bioinformatics Analysis of Hub Genes and Pathways in Anaplastic Thyroid Carcinomas

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Anaplastic thyroid carcinoma (ATC) is a very rare malignancy; the pathogenesis of which is still not fully understood. The aim of the present study was to identify hub genes and pathways in ATC by microarray expression profiling. Two independent datasets (GSE27155 and GSE53072) were downloaded from GEO database. The differentially expressed genes (DEGs) between ATC tissues and normal thyroid tissues were screened out by the limma package and then enriched by gene ontology (GO) and KEGG pathway analysis. The hub genes were selected by protein-protein interaction (PPI) analysis. A total of 141 common upregulated and 87 common downregulated genes were screened out. These DEGs were significantly enriched in the phagosome and NF-kappa B signaling pathway. Through PPI analysis, TOP2A, TYMS, CCNB1, RACGAP1, FEN1, PRC1, and UBE2C were selected as hub genes, which were highly expressed in ATC tissues. TCGA data suggested that the expression levels of TOP2A, TYMS, FEN1, and PRC1 genes were also upregulated in other histological subtypes of thyroid carcinoma. High expression of TOP2A, TYMS, FEN1, PRC1, or UBE2C gene significantly decreased disease-free survival of patients with other thyroid carcinomas. In conclusion, the present study identified several hub genes and pathways, which will contribute to elucidating the pathogenesis of ATC and providing therapeutic targets for ATC.

1. Introduction

Thyroid carcinoma is a common endocrine cancer accounting for approximately 1.7% of total cancer diagnoses [1]. The main histologic types of thyroid carcinoma include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, medullary thyroid carcinoma, and anaplastic thyroid carcinoma (ATC). Approximately 80% of thyroid cancers are PTCs, which are usually curable with a 5-year survival of over 95% [2]. In contrast, ATC constitutes only a small part (1–2%) of all thyroid carcinomas, but it is the most malignant with a median survival of 3–5 months [3, 4]. To date, there is no standard or effective therapy for ATC. Thus, it is very urgent to understand the pathogenesis of ATC, which will contribute to the discovery of the attractive therapeutic targets.

In the past decades, traditional and molecular biological techniques have been used to reveal ATC-related genes and pathways [5–7]. For instances, Yin et al. found that the downregulated expression of the forkhead box D3 (FOXD3) transcription factor in ATC cells promoted invasiveness and epithelial-to-mesenchymal transition (EMT) and decreased cellular apoptosis. FOXD3 silencing also enhanced p-ERK levels in the ATC cells, suggesting it negatively regulated MAPK/ERK signaling [5]. Zhang et al. observed that S100A4 was highly expressed in ATC tissues. Knockdown of S100A4 significantly decreased proliferation, increased apoptosis, and inhibited the invasive potential of ATC cells [6]. Salerno et al. found that TWIST1 played a pleiotropic role in determining the ATC phenotype. The ectopic expression of TWIST1 induced resistance to apoptosis and increased cellular migration and invasion [7]. Although major efforts to clarify the pathogenesis of ATC are ongoing, the relevant progress is still not obvious. Considering the complexity and heterogeneity of ATC, we adopted microarray technology and bioinformatics methods to systematically explore
2. Materials and Methods

2.1. Microarray Data. The raw data of gene expression profiles of 9 ATC and 7 normal thyroid tissues were downloaded from Gene Expression Omnibus database (GEO accession numbers: GSE27155 and GSE53072). GSE27155 and GSE53072 datasets were submitted by Giordano et al. and Pita et al., respectively [9–11]. Among these analyzed tissues, 4 ATC and 4 normal thyroid tissues from GSE27155 were detected by Affymetrix Human Genome U133A Array (GPL96 platform). Other tissues from GSE53072 were detected by Affymetrix Human Genome 1.0 ST Array (GPL6244 platform).

The raw data of GSE27155 and GSE53072 were preprocessed using the affy and oligo packages with the robust multichip averaging (RMA) algorithm, respectively [12–16]. The probeset IDs were converted into the corresponding gene symbols using the annotation information derived from platforms. If multiple probesets correspond to the same gene, the mean expression values of those probesets were obtained.

2.2. Identification of Differentially Expressed Genes (DEGs). Limma R package was applied to identify the DEGs between ATC and normal thyroid tissues [17]. The Benjamini-Hochberg (BH) method was introduced to adjust the raw p values. The adjusted p value < 0.05 and |log2 fold change (FC)| ≥ 1 were set as the thresholds for identifying DEGs.

2.3. Functional and Pathway Enrichment Analysis of DEGs. In order to systematically explore genes involved in ATC, gene ontology (GO) and pathway enrichment analyses for the common DEGs were performed using the clusterProfiler package in R, which was based on the GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases [18–20]. The criterion for the significant enrichments was set as p value < 0.05.

2.4. Construction of the Protein-Protein Interaction (PPI) Network. The PPI network provides a valuable framework for better understanding of the functional organization of the proteome. In this network, nodes and edges represent proteins and interactions between proteins, respectively. The proteins with high degrees, namely, those highly connected with other proteins, are defined to be located at the network center, which may be regulatory “hubs.” We constructed the PPI network by using STRING database and Cytoscape 3.3 software [21, 22]. The nodes with degree > 1 were reserved in the PPI network. Genes with degree > 25 were considered as hub genes (proteins).

2.5. The Expression Levels of Hub Genes in Other Thyroid Carcinomas. UALCAN (http://ualcan.path.uab.edu) is a user-friendly, interactive web resource for analyzing cancer transcriptome data from The Cancer Genome Atlas (TCGA) [23]. In the current study, UALCAN was used to explore the expression levels of hub genes in other thyroid carcinomas. p value < 0.05 was considered statistically significant.

2.6. The Association of Hub Gene Expression with Disease-Free Survival of Patients with Other Thyroid Carcinomas. Gene expression profiling interactive analysis (GEPIA, http://gepia.cancer-pku.cn/) is a web-based tool to deliver fast and customizable functionalities based on TCGA and GTEx data [24]. These functionalities included differential expression analysis, profiling plotting, correlation analysis, patient survival analysis, similar gene detection, and dimensionality reduction analysis. In the current study, GEPIA was used to explore the association of hub gene expression with disease-free survival of patients with other thyroid carcinomas. Patients were grouped into high expression group and low expression group according to the median value of hub gene expression. p value < 0.05 was considered statistically significant.

3. Results

3.1. Identification of DEGs. As shown in Figure 1 and Table 1, for GSE27155, a total of 404 DEGs, including 263 upregulated and 141 downregulated genes in ATC, were identified. For GSE53072, a total of 2522 DEGs, including 995 upregulated and 1527 downregulated genes in ATC, were screened out. Further analysis showed that the two independent datasets contained 228 common DEGs, including 141 common upregulated and 87 common downregulated genes in ATC.

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Figure 1: Venn diagrams showing the number of differentially expressed genes (DEGs) in ATC tissues compared with normal thyroid tissues ((a) the number of upregulated genes; (b) the number of downregulated genes).

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large-cohort gene expression in ATC tissues, which has been demonstrated to be valuable for molecular mechanism investigation [8].
Table 1: The common differentially expressed genes in GSE27155 and GSE53072 datasets. Note: bold and italic data indicate genes in the phagosome and NF-kappa B signaling pathway, respectively. Bold-italic data indicates the common gene in the phagosome and NF-kappa B signaling pathway.

| Downregulated genes | Common differentially expressed genes | Upregulated genes |
|---------------------|---------------------------------------|-------------------|
| MAN1C1              | CA4                                   | RAB3I             |
| PBX1                | KCNJ16                                | COL5A2            |
| Clorfil5            | PTPRM                                 | CCNB1             |
| MARC2               | TXNL1                                 | IRAK1             |
| MARC1               | CIRBP                                 | NF-kappa B signaling pathway |
| RAP1GAP             | BCAM                                  | NF-kappa B signaling pathway |
| ECHDC2              | TLE2                                  | NF-kappa B signaling pathway |
| RGS5                | ACADL                                 | NF-kappa B signaling pathway |
| ALDH9A1             | RAB17                                 | NF-kappa B signaling pathway |
| RGS16               | SNTA1                                 | NF-kappa B signaling pathway |
| HHEX                | INPP5J                                | NF-kappa B signaling pathway |
| PRKCQ               | CBX7                                  | NF-kappa B signaling pathway |
| NEBL                | ACOX2                                 | NF-kappa B signaling pathway |
| OGDH1               | ZBED2                                 | NF-kappa B signaling pathway |
| ABLIM1              | HGD                                   | NF-kappa B signaling pathway |
| FGFR2               | LIMCH1                                | NF-kappa B signaling pathway |
| GLB1L2              | PPARG1CA                              | NF-kappa B signaling pathway |
| METTL7A             | HOPX                                  | NF-kappa B signaling pathway |
| PEBP1               | SORBS2                                | NF-kappa B signaling pathway |
| IFT88               | PDE8B                                 | NF-kappa B signaling pathway |
3.2. Integrated Analysis of the Common DEGs. By GO analysis, we identified 146 significant enrichments terms, which were classified in 3 GO categories, including biological processes (BP, 92), molecular functions (MF, 20), and cellular components (CC, 34). The top 10 GO terms of BP, MF, and CC were shown in Figures 2(a)–2(c). For BP, those common DEGs are significantly enriched in neutrophil activation, neutrophil-mediated immunity, neutrophil activation involved in immune response, neutrophil degranulation, hormone metabolic process, mitotic sister chromatid segregation, microtubule cytoskeleton organization involved in mitosis, mitotic spindle organization, thyroid hormone metabolic process, thyroid hormone generation, and so on. For MF, those common DEGs are mainly enriched in cell adhesion molecule binding, actin binding, nucleoside binding, GTPase activity, cofactor binding, coenzyme binding, pattern recognition receptor activity, signaling pattern recognition activity, aldehyde dehydrogenase (NAD) activity, and IgG binding. For CC, those common DEGs are significantly enriched in the side of the membrane, secretory granule membrane, cell-substrate junction, cell-substrate adherens junction, focal adhesion, cell leading edge, spindle,
endocytic vesicle, external side of plasma membrane, extracellular matrix component, and so on. Furthermore, KEGG pathway enrichment analysis showed that these common DEGs were significantly enriched in the phagosome and NF-kappa B signaling pathway (Figure 2(d)). There were 180 nodes and 737 edges in the PPI network (Figure 3(a)). Thereinto, 7 upregulated genes in ATC, including TOP2A, TYMS, CCNB1, RACGAP1, FEN1, PRC1, and UBE2C, were selected as the hub genes (Figure 3(b)). TOP2A gene had the highest degree (degree = 37) in the network. UALCAN and GEPIA online tools were used to explore TCGA data. Results suggested that the expression levels of hub genes, TOP2A, TYMS, FEN1, and PRC1, were also upregulated in at least one histological subtype of thyroid carcinoma (Figure 4). However, the expression levels of CCNB1 and RACGAP1 genes were significantly downregulated in at

| Genes     | Expression | Degree |
|-----------|------------|--------|
| TOP2A     | Up         | 37     |
| TYMS      | Up         | 29     |
| CCNB1     | Up         | 29     |
| RACGAP1   | Up         | 27     |
| FEN1      | Up         | 26     |
| PRC1      | Up         | 26     |
| UBE2C     | Up         | 26     |

Figure 3: Protein-protein interaction (PPI) network of differentially expressed genes (DEGs) ((a) PPI network; (b) hub genes).
least one histological subtype of thyroid carcinoma. UBE2C gene expression did not significantly change in other thyroid carcinomas (figure not shown). Survival analysis indicated that high expression of TOP2A, TYMS, FEN1, PRC1, or UBE2C gene significantly decreased disease-free survival of patients with other thyroid carcinomas (Figure 5).

4. Discussion

In the present study, we identified two significant pathways (phagosome and NF-kappa B signaling pathway) and seven hub genes (TOP2A, TYMS, CCNB1, RACGAP1, FEN1, PRC1, and UBE2C) related with ATC. In 2017, Huang et al. used GSE33630 data to conduct a similar analysis and considered the Toll-like receptor signaling pathway, extracellular matrix-receptor interaction, and cytokine-cytokine receptor interaction pathway as important pathways implicating ATC. FOS, CXCL10, COL5A1, COL11A1, and CCL28 genes might be used as therapeutic targets for ATC [25]. These findings were different from our findings, which might be attributed to tumor heterogeneity or differences in analytical methods between two studies. Besides the identification of potential hub genes and pathways associated with ACT, we still analyzed the expression levels of these hub genes in other histological subtypes of thyroid carcinoma and found that UBE2C gene expression did not significantly change in other thyroid carcinomas, suggesting that UBE2C might act as a specific diagnostic biomarker for ACT. Further survival analysis showed that high expression of TOP2A, TYMS, FEN1, PRC1, or UBE2C gene significantly decreased disease-free survival of patients with other thyroid carcinomas.

Although there was no study directly linking phagosome to ATC, the phagosome participated in the innate and adaptive immune responses [26, 27]. The present study found ten common DEGs involving in the phagosome. Therefore, the role of the phagosome and phagosome-related genes in ATC was worthy of being further explored. NF-kappa B signaling pathway played an important role in cancer initiation and progression [28, 29]. The present analysis of DEGs showed that most genes of the NF-kappa B signaling pathway were upregulated, suggesting that the pathway was also activated in ATC. Furthermore, the NF-kappa B signaling pathway also participated in an anticancer agent (R-roscovitine) activity, inducing apoptosis of ATC cells [30]. These results indicated that novel agents involving the NF-kappa B signaling pathway could be developed to improve ATC treatment.

Previous studies have indicated that seven hub genes identified by the present study play an important role in the occurrence and development of tumors [31–46]. TOP2A gene encodes a DNA topoisomerase that controls and alters the topologic states of DNA during transcription. Immunohistochemical analysis showed that TOP2A correlated with thyroid tumor histology and it was more frequently expressed in tumors with aggressive clinical behavior [31]. TYMS gene encodes thymidylate synthase, which catalyzes the methylation of deoxyuridylate to deoxythymidylate. Although the role of TYMS in ATC was not reported previously, the enzyme has been of interest as a target for cancer chemotherapeutic agents [32–35]. The protein encoded by CCNB1 gene is involved in mitosis and necessary for proper control of the G2/M transition phase of the cell cycle. Lin et al. found that dinaciclib, a cyclin-dependent kinase (CDK) inhibitor, could inhibit ATC cell proliferation by
decreasing CDK1, CCNB1, and Aurora A expression, inducing cell cycle arrest in the G2/M phase and inducing the accumulation of prophase mitotic cells [36]. RACGAP1 gene encodes a GTPase-activating protein (GAP) that is a component of the centralspindlin complex. This protein played a regulatory role in cytokinesis, cell growth, and differentiation. To date, there is no report linking RACGAP1 to ATC, but its importance in the development of other cancers has been revealed [37, 38].

The protein encoded by FEN1 gene removes 5' overhanging flaps in DNA repair and processes the 5' ends of Okazaki fragments in lagging strand DNA synthesis. Previous studies confirmed that FEN1 not only promoted cancer cell proliferation and progression but also conferred cancer drug resistance [39, 40]. According to these results, we inferred that FEN1 might also play important roles in development or drug resistance of ATC.

PRC1 gene encodes a protein involved in cytokinesis [41, 42]. The protein is present at high levels during the S and G2/M phases of mitosis and enters the G1 phase. Recent studies showed that PRC1 was upregulated in many types of cancer and might serve as a prognostic biomarker of cancer [43–45]. UBE2C gene encodes a member of the E2 ubiquitin-conjugating enzyme family. The encoded protein is required for the destruction of mitotic cyclins and for cell cycle progression. Pallante et al. found that UBE2C overexpression was involved in thyroid cell proliferation and might act as a diagnostic biomarker for ATC [46].

Taken together, the integrated bioinformatics study presented several hub genes and pathways related to ATC, which would provide new insights into the exploration of pathogenesis and therapeutic targets for ATC.

**Data Availability**

The microarray data used to support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) repository (accession numbers: GSE27155 and GSE53072).
**Conflicts of Interest**

The authors declare that they have no competing interests.

**Authors’ Contributions**

Xuereen Gao and Jianguo Wang contributed equally to this work.

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