Driver Genes of Locally Advanced Well-differentiated Thyroid Cancer: Genetic Landscape Based on the TCGA Database

Ah Ra Jung
Eulji University Hospital
Dong Hyuk Jang
Eulji University Hospital
Jung Ho Choi
Eulji University Hospital
Yoon Se Lee (manselee@gmail.com)
Asan Medical Center

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Abstract

Background: Despite the usually favorable prognosis of well-differentiated thyroid cancer (WDTC) following appropriate treatment, advanced T-staged WDTCs are associated with poor prognosis. This study focused on identifying the driver genes of locally advanced WDTC by analyzing the TCGA cohort.

Methods: We analyzed data on 501 patients with WDTC from the TCGA cohort. Patients were classified into two subgroups of pathological T4 stage or T1-3 stage (Cluster1 and Cluster2, respectively). The mRNA expression differences between subgroups were compared for several genes in the TCGA cohorts.

Results: Cluster1 included 23 patients with pathological T4 classification (Papillary=21/Follicular=2) and Cluster2 included 478 patients (Papillary=371/Follicular=100/Others=7). The Cluster1 subgroup showed worse overall survival than the Cluster2 subgroup ($p < 0.05$). The two subgroups were analyzed for 34 genes reported in previous studies. Known genetic thyroid cancer alterations, including *BRAF*, *RAS*, *RET*, and *ALK*, were not different in the two subgroups. In Cluster1, *MET*, *SERPINA1*, *TIMP1*, *PROS1*, *FN1*, *CDKN2A*, and *CDKN2B* were significantly elevated, while *TG*, *DNAH9*, *TFF3*, *CRABP1*, *TPO*, *JAK2*, *KIT*, *KDR*, and *NFE2L2* were significantly lower compared with the Cluster2 (all, $p < 0.05$). A *TERT*, *EIF1AX*, and *ATM* showed significantly frequent somatic mutations in Cluster1 compared to Cluster2. We also identified seven pathways related to the 16 genetic markers.

Conclusions: Locally advanced WDTC presented 16 genetic alterations compared to less aggressive thyroid cancers. Somatic mutations associated with local invasion transformation were identified. Genetic profiles associated with locally advanced WDTC have prognostic significance, but these findings must be validated to further understand the pathway.

Background

Resectable well-differentiated thyroid cancers (WDTC), including papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), have a favorable prognosis after surgical treatment with or without adjuvant radioactive iodine therapy. WDTC treatment results are affected by age, gender, aggressive subtypes, and various molecular markers.(1, 2) About 10% of WDTC are composed of locally advanced and resectable WDTC (T4a) invading surrounding structures.(3) It has a two times higher recurrence rate than lower staged WDTC, and about 30% of advanced thyroid cancer died within a decade.(4, 5) Among patients with distant metastasis, T4-staged tumors increased the risk of distant metastasis and decreased recurrence-free survival rate five times compared to early staged WDTC.(6, 7) Compared to confined diseases, locally advanced WDTC requires adjuvant radioactive iodine therapy (RAI), which reduces the risk of recurrence/disease-specific mortality in high-risk patients.(8) External beam radiation therapy (EBRT) is also used to control local recurrence or residual tumors after surgical resection.(9) Despite these treatment modalities, the five-year disease-specific survival rate of T4-staged WDTC ranges from 67.9–87.6%, dependent on the resection margin status.(10) Targeted therapy-based on molecular biomarker investigation may improve treatment outcomes in locally advanced WDTC.

Most WDTC are associated with alterations in a limited number of driver genes, including point mutations in the *BRAF* or *RAS* genes or rearrangements of *RET/PTC* or *PAX8/PPARγ*, which activate the receptor tyrosine kinase (RTK)/mitogen-activated protein kinase (MAPK) pathway.(11) Accumulated genetic and epigenetic alterations initiate and progress thyroid cancer. Currently, these genetic alterations have been used as a molecular signature to discriminate the type of cancer. Next-generation sequencing (NGS) has recently revealed various genes involved in carcinogenesis and metastasis in various solid cancers.(12)

A pan-cancer study using The Cancer Genome Atlas (TCGA) study has defined genetic alterations in papillary thyroid cancer (PTC) with a focus on low-to-intermediate risk tumors.(13) Previous studies have shown the potential contribution of *TERT* and various oncogenes (*AKT1*/ *PIK3CA* and *EIF1AX*) in the early progression of WDTC and, in particular, report that loss of *CDKN2A* may be a strong prognostic factor for patients with advanced WDTC.(14) Although the molecular characteristics of WDTC have been analyzed, the underlying mechanism of its progression to locally advanced and resectable WDTC has not been fully elucidated. To overcome the limitations of current treatments for advanced thyroid cancer, the genetic landscape suggests molecular tools for diagnosis and treatment. In this study, we focused on analyzing the TCGA cohort to identify the driver genes of locally advanced WDTC and we evaluated the associations between genetic markers and the prognosis of patients with advanced WDTC.

Methods

Data sources

For this study, clinical and genomic data were collected from TCGA thyroid carcinoma. These data can be downloaded from the cBioPortal Browser (https://cbioportal.org/). Clinical information included age, sex, tumor site, TNM staging, recurrence, and survival outcomes. We generated gene expression data for the TCGA cohort by Illumina HiSeq2000 RNA sequencing Version 2 Analysis and transformed and mapped log2-via RSEM.(15) In the cBioPortal thyroid study, there were a total of four cohorts, three of which were WDTC cohorts. The first large-scale integrative genomic analysis of WDTC took place in 2014 (TCGA, Cell 2014). We selected the cohort with the most T4 stage patients (TCGA, Firehose Legacy). Finally, the selected cohort (n = 501) included 392 cases of papillary thyroid cancer, 102 cases of follicular thyroid cancer, and seven others. We divided enrolled patients into Cluster1 (T4) and Cluster2 (T1, T2, and T3) according to pathological T classification (Fig. 1).

Pathway analysis

We used the functional annotation tools from the Database for Annotation, Visualization, and Integrated Discovery (DAVID) bioinformatics resources 6.8.(16) We used the default setting from the tool to map the 16 gene lists to the reference set of direct and indirect relationships, which is a differentially expressed gene list between the two subgroups. P-values were adjusted for multiple testing using the Benjamini-Hochberg procedure within the dataset and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway module. Significant results were constrained at the 0.05 level after adjustment for multiple testing corrections.

Statistical analysis

To test the prognostic significance, only gene expression information with available survival data were used. Overall survival (OS) was defined as the day of surgery to death due to any cause or the last visit. The disease-free survival (DFS) was defined as the day of surgery to the first recurrence at any site or the last visit. Prognostic significance between the two subgroups was estimated by the Kaplan–Meier method.

Fisher's exact test was used to assess the difference in frequency of somatic mutations. P-values of less than 0.05 were considered statistically significant and all statistical tests were two-tailed. Statistical analyses were performed using the IBM® SPSS® Statistics version 24.0 for Windows (IBM Corp., Armonk, NY) and R package version 3.4.4 (http://www.r-project.org).

Results

Cohort characteristics

The 501 patients included 135 (26.9%) males and 366 (73.1%) females with a median age of 46 years (range, 15–89 years). Cohort characteristics are summarized in Table 1. The most common tumor site was the right lobe (*n* = 214, 42.7%), followed by the left lobe (*n* = 173, 34.5%), bilateral (*n* = 86, 17.2%), isthmus (*n* = 22, 4.4%), and others (*n* = 6, 1.2%). Advanced T classification was found in 192 patients (38.3%), nodal positivity in 225 patients (44.9%), and an overall III–IV stage in 165 patients (33.0%). The most common histological finding was the papillary (*n* = 392, 78.2%), followed by the follicular (*n* = 102, 20.4%), and others (*n* = 7, 1.4%).

Table 1
Patient characteristics (*N* = 501)

Variable	N (%)
Gender	
Male	135 (26.9)
Female	366 (73.1)
Age (years), median (IQR)	46 (35–58)
Laterality	
Right lobe	214 (42.7)
Left lobe	173 (34.5)
Bilateral	86 (17.2)
Isthmus	22 (4.4)
Others	6 (1.2)
pT classification	
T1	142 (28.3)
T2	167 (33.4)
T3	169 (33.7)
T4	23 (4.6)
pN classification	
N0	226 (45.1)
N1a	150 (29.9)
N1b	75 (15.0)
NX	50 (10.0)
Overall pTNM stage	
I	285 (56.8)
II	51 (10.2)
III	110 (22.0)
IV	55 (11.0)
Histological	
Papillary	392 (78.2)

Variable	N (%)
Follicular	102 (20.4)
Others	7 (1.4)

Survival outcome of Cluster1 and Cluster2

Cluster1 and Cluster2 consisted of 23 patients (papillary = 21/follicular = 2) and 478 patients (papillary = 371 / follicular = 100 / others = 7), respectively. The Kaplan–Meier plots showed that the overall survival (OS) of Cluster1 was significantly worse than Cluster2 ($p < 0.05$, Fig. 2A). The disease-free survival (DFS) of patients in Cluster1 was worse than that of patients in the Cluster2 subgroup ($p = 0.12$, Fig. 2B).

Discover of sixteen driver genes in locally advanced well-differentiated thyroid cancer

This study analyzed the expression of a variety of genes associated with thyroid cancer, including $BRAF^{V600E}$, *RAS, RET*, and *ALK*, between two clusters. Griffith *et al.*(Griffith, *et al.* 2006) identified 12 important diagnostic biomarkers through a meta-review of gene expression in thyroid cancer. Pozdeyev *et al.*(17) proposed an updated model of thyroid cancer genetic evolution. We analyzed these genetic alterations.

In Cluster1, expression of *MET*, *SERPINA1*, *TIMP1*, *PROS1*, *FN1*, *CDKN2A*, and *CDKN2B* were significantly elevated (all, $p < 0.05$, Fig. 3A), while expression of *TG*, *DNAH9*, *TFF3*, *CRABP1*, *TPO*, *JAK2*, *KIT*, *KDR*, and *NFE2L2* were significantly lower compared with Cluster2 (all, $p < 0.05$, Fig. 3B).

Relationship between clusters and somatic mutation

To investigate somatic mutations between the two clusters in WDTC, we analyzed the somatic mutation data of patients in the TCGA cohort (n = 501). We evaluated genes reportedly altered more frequently in advanced differentiated and anaplastic thyroid cancer than in differentiated thyroid cancer.(18, 19) (Table S1) The signaling pathways and gene groups seen more frequently in advanced differentiated thyroid cancers are tumor suppressors, the cell-cycle pathway, and the PI3K/AKT pathway.(17)) Among these genes, A *TERT, EIF1AX*, and *ATM* showed a significantly higher frequency of somatic mutation in Cluster1 than in Cluster2 (Fig. 4 and Table S1). There was no difference in the frequency of somatic mutations between the two subgroups for $BRAF^{V600E}$ and *NRAS*.

Pathway analysis

The genes analyzed with the DAVID functional annotation tools identified 7 significant KEGG pathways based on mutational enrichment (Table 2): proteogloycans in cancer (p = 5.3e-02), the Rap1 signaling pathway (p = 5.8e-02), and autoimmune thyroid disease (p = 9.4e-2). Moreover, several pathways important for locally advanced cancer were identified, including the PI3K-AKT signaling pathway (p =

3.1e-3), the pathway in cancer (p = 5.0e-3), focal adhesions (p = 5.6e-2), and the Ras signaling pathway (p = 6.6e-2).

Table 2
The significant KEGG pathways.

KEGG pathway	Count	Gene	p-value	FDR
PI3K-Akt signaling pathway	5	*JAK2*, *KIT*, *MET*, *FN1*, *KDR*	3.1E-03	1.6E-01
Pathways in cancer	5	*KIT*, *MET*, *CDKN2A*, *CDKN2B*, *FN1*	5.0E-03	1.3E-01
Proteoglycans in cancer	3	*MET*, *FN1*, *KDR*	5.3E-02	6.3E-01
Focal adhesion	3	*MET*, *FN1*, *KDR*	5.6E-02	5.5E-01
Rap1 signaling pathway	3	*KIT*, *MET*, *KDR*	5.8E-02	4.8E-01
Ras signaling pathway	3	*KIT*, *MET*, *KDR*	6.6E-02	4.7E-01
Autoimmune thyroid disease	2	*TG*, *TPO*	9.4E-02	5.4E-01

Discussion

In this study, we demonstrated that the genetic profiles of T4 staged WDTC has genetic alterations, somatic mutations, and a relevant pathway compared to the less aggressive WDTC by analyzing a TCGA cohort. T4 staged WDTC presented 16 genetic alterations compared to less aggressive thyroid cancers, and we identified somatic mutations associated with locally invasion transformations. We also identified seven pathways related to 16 genetic markers. Moreover, we enhanced the current knowledge about the genetic characteristics of locally advanced and resectable WDTC.

WDTC, such as follicular and papillary carcinomas, account for 95% of all thyroid cancer cases and are generally have a survival rate of almost 100% when diagnosed early.(20) Approximately 25% of patients with WDTC develop locally advanced or metastatic disease and locally advanced WDTC is managed differently with adjuvant therapy and surgical treatment.(21) Post-surgical management includes RAI therapy to reduce these locoregional and distant metastasis. When RAI therapy becomes ineffective against differentiated thyroid cancer (DTC), the five-year survival is < 50% and 10-year survival is < 10%. (21, 22) Of 80 patients with radioactive iodine-refractory DTC, 38 had somatic mutations (47.5%), including *BRAF*^{V600E}, *RAS, TP53, MET, PIK3CA, GNAS*, and *TPO*.(23) The current study identified the incidence of somatic mutation in *BRAF*^{V600E} at 59.8%, which is similar to the TCGA cohort (58.0%) in 2014, whereas only 8.4%, 3.5%, and 0.7% of tumors had *RAS* mutations (*NRAS*, *HRAS*, and *KRAS*, respectively). The first TCGA cohort discovered two molecular subtypes, *BRAF*^{V600E}-like and *RAS*-like, in the papillary thyroid carcinoma.(13) Dedifferentiation is likely to play a role in mitigating responses to RAI treatment and is consistent with the *BRAF* mutation.(24) However, our results showed no difference in the *BRAF* mRNA expression level and the frequency of somatic mutations between the two clusters.

In our study, 23 patients with pathological T4 classification had significantly elevated expression of *CDKN2A* and significantly lower *JAK2* compared with Cluster2. Expression of *BRAF*, *RAS*, *RET*, and *ALK* was not different between the two subgroups. *CDKN2*A and *CDKN2B* are negative cell-cycle regulators, and their loss due to copy number alterations, inactivating mutations, or epigenetic silencing is one of the most frequently encountered genetic events in human cancer.(25) In thyroid cancer, genetic alterations of *CDKN2A* and *CDKN2B* were seen more frequently in anaplastic thyroid cancer compared with PTC, suggesting a potential role in anaplastic transformation.(17) The prognostic significance of *JAK2* has been well described for a variety of cancers, but not advanced thyroid cancer. *JAK2* plays a critical role in the signaling of prolactin (PRL) hormone, which may be involved in the development of medullary thyroid carcinoma.(26) Peng et al.(27) examined the TCGA database containing 9,315 tumor samples from 31 cancer types to study the relationship between the mRNA expression of *JAK2* and PD-L1 expression. They found that high *JAK2* expression was associated with high mRNA expression of PD-L1, which is likely a good indicator for immunotherapy response, including anti-PD-1/PD-L1 therapy. Our results of significantly lower *JAK2* expression in Cluster1 compared to Cluster2 may contribute to a treatment strategy that can screen patient populations that may benefit from immunotherapy.

A recent study identified 676 genes associated with an increased risk of PTC recurrence in TCGA data.(28) Chien et al. demonstrated that downregulation of the sodium-iodide symporter SLC5A5 (NIS) was the strongest predictor of a decreased recurrence-free survival.(28) Our study and previous studies have shown the associations of *KIT*, *TFF3*, and *TG* low with increased recurrence rates. Most of the recently discovered targeted therapies inhibit the known oncogenic mechanisms in thyroid cancer initiation and progression, such as the MAPK pathway, PI3K/Akt-mTOR pathways, or VEGF. (29) In clinical trials using trametinib with VEGF inhibitors in advanced DTC patients, 33% of subjects showed a partial response (PR) and 50% had stable disease.(30) Bible at el. reported significant activity of single-agent VEGF inhibitor in progressive RAI refractory DTC, with an overall confirmed PR rate of 49%.(31)

The present study revealed that mutations of *TERT, EIF1AX*, and *ATM* were found more frequently in Cluster1 than Cluster2. The *TERT* gene locus was amplified in various cancers, including lung, breast, and cervical cancer,(32) and the prevalence of *TERT* promoter mutations in thyroid cancer was more frequent in poorly-differentiated and anaplastic thyroid cancers.(33) A recent study identified an *EIF1AX* mutation in a nodule ultimately diagnosed as oncocytic thyroid carcinoma.(34) *EIF1AX* mutations are predictive of worse survival in WDTC.(35) This suggests that the treatment strategy can be predicted by markers found in advanced WDTC, unlike the mutations that are the major drivers of WDTC.

Table 3 shows the KEGG pathways of our 16 genes. The growing body of evidence demonstrating the involvement of the PI3K-AKT signaling pathway in thyroid carcinogenesis and drug resistance led to the discovery of several agents targeting key members of this cascade.(29) Multi-kinase inhibitors targeting highly expressed tyrosine kinases in thyroid cancer cells demonstrate a promising anti-tumor activity in vitro and in vivo.(36, 37) The few targeted therapies that have been proven effective for advanced WDTC, sorafenib, selumetinib, pazopanib, and sunitinib, have promising results. Due to its high affinity to the VEGF receptor, lenvatinib showed high anti-angiogenic effects in the thyroid cancer mouse model.(38)

This study has some limitations. High post-ablation stimulated thyroglobulin level (≥ 1ng/mL) and macroscopic ETE were reported as negative prognostic factors in T4a staged WDTC.(39) It is difficult to consider these clinical and pathological factors using TCGA data. Specific clinical factors may help delineate the genomic landscape related to aggressive tumor behavior. Another limitation of this study included not controlling for histologic variants or tumor nodals positive of the well that are differentiated for the thyroid cancer studied. Our study aimed at identifying driver genes and somatic mutations instead of developing a validated prognostic factor in locally advanced WDTC. We hypothesized that using pathway analysis would provide a better understanding of the thyroid carcinogenesis involved in T4 stage tumors.

Conclusions

A refined classification system that more accurately reflects genotypic and phenotypic differences between locally advanced and resectable WDTC (T4a), which invade the surrounding structures, and other WDTCs will lead to more precise medical and surgical treatment. Our study has provided insight into the molecular pathogenesis of T4 beyond its histological classification. Relevant pathways could be candidates for targeted therapy. Further studies are necessary to translate this study into useful clinical applications in the diagnosis and treatment of T4 staged WDTC.

Abbreviations

WDTC: Well-differentiated Thyroid Cancer; PTC: Papillary Thyroid Cancer; FTC: Follicular Thyroid Cancer; RAI: Radioactive Iodine; EBRT: External Beam Radiation Therapy; NGS: Next-generation Sequencing; TCGA: The Cancer Genome Atlas; DAVID: Database for Annotation, Visualization, and Integrated Discovery; KEGG: Kyoto Encyclopedia of Genes and Genomes; OS: Overall Survival; DFS: Disease-free Survival; DTC: Differentiated Thyroid Cancer.

Declarations

Acknowledgments

None to be declared.

Authors' contributions

ARJ and YSL designed of study and drafted the first manuscript. DHJ and JHC were involved in statistical analysis, creating tables and figures, and drafting the manuscript. ARJ and YSL reviewed manuscript drafts and provided critical revisions. All the authors have read the manuscript and have approved this submission.

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Availability of data and materials

The collection of data that supports the findings in this study is available from the cBioPortal Browser (https://cbioportal.org/). Data are available from the authors upon reasonable request and with permission of The Cancer Genome Atlas (TCGA) thyroid carcinoma.

Ethics approval and consent to participate

No applicable.

Consent for publication

No applicable.

Competing interest

The authors have declared no conflicts of interest.

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Table

Table 3 is not available with this version.

Figures

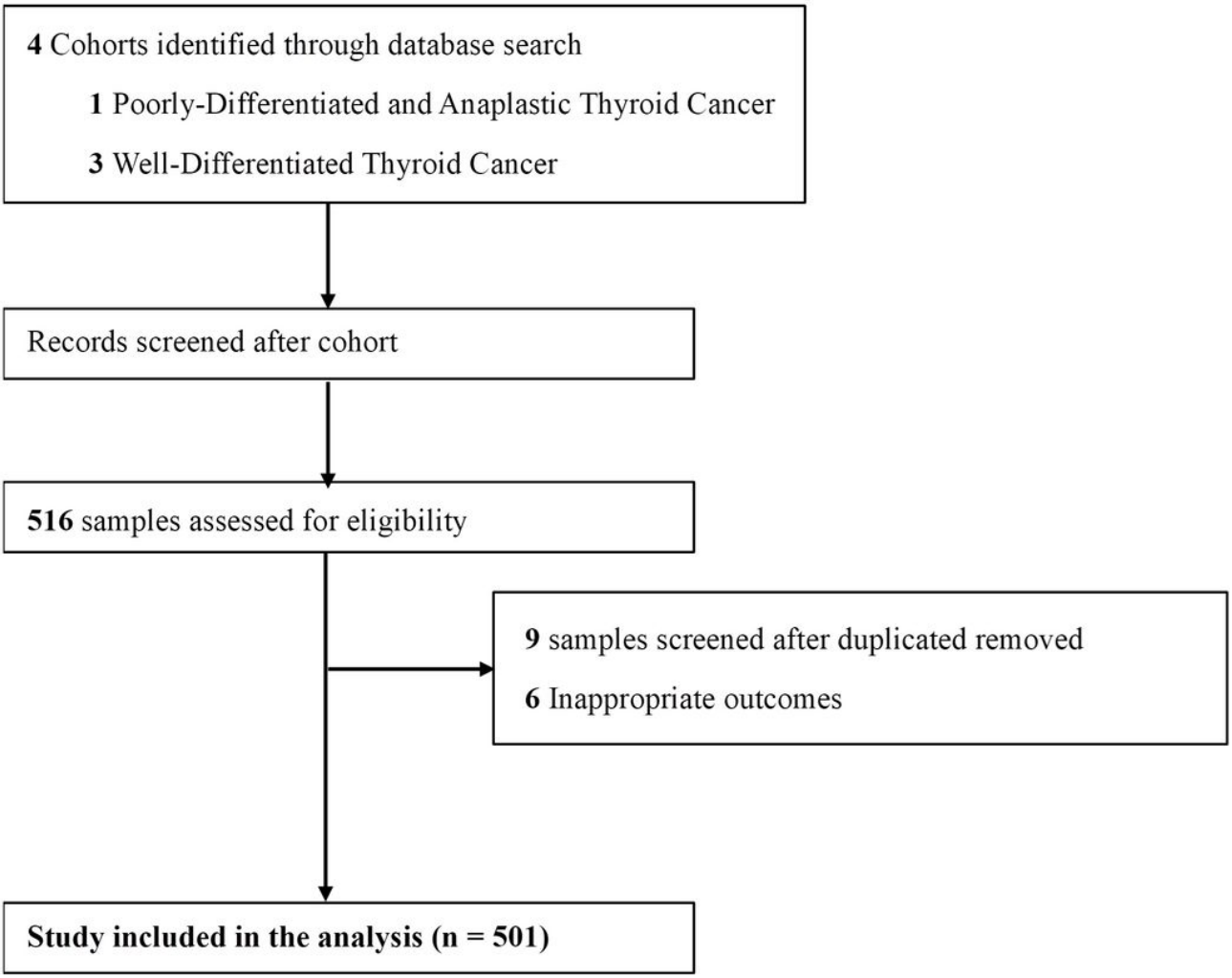

Figure 1

Flow diagram of the study selection process.

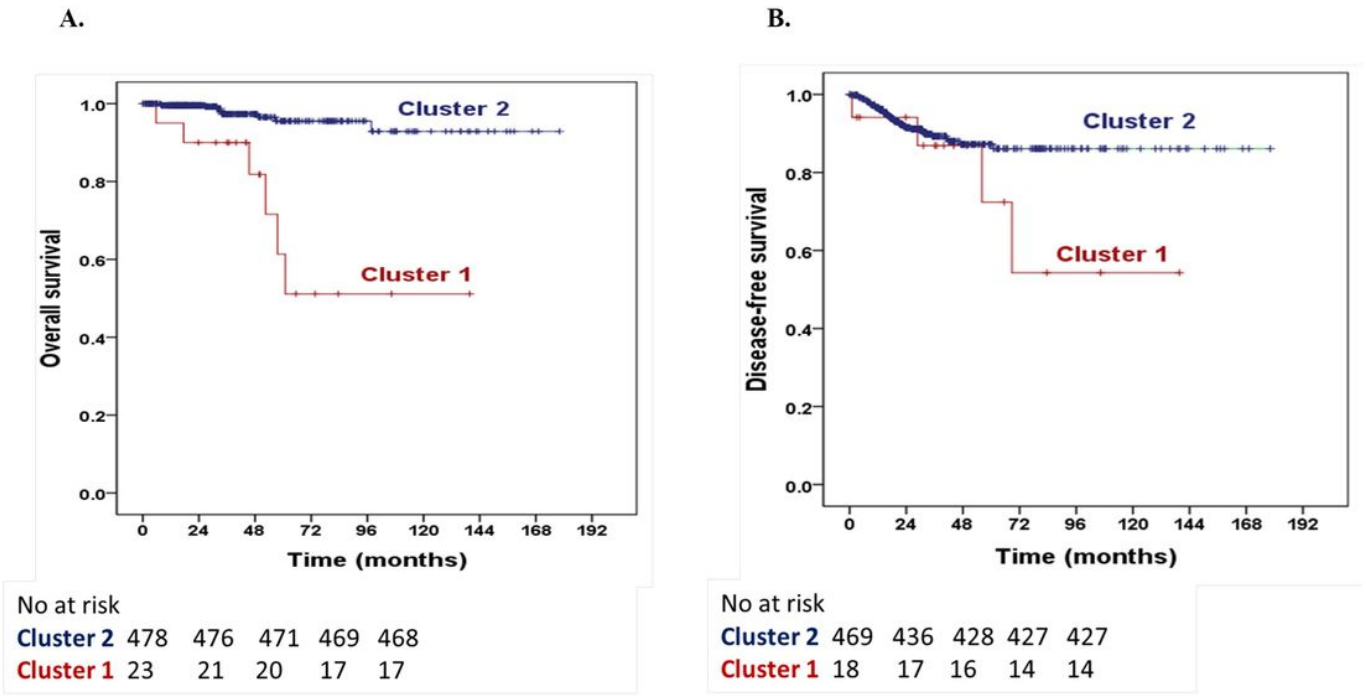

Figure 2

Kaplan-Meier curves estimating survival according to pathological T4 classification and T1-3 classification. A–B, Overall survival and disease-free survival between two subgroups. Log-rank test, P<.05.

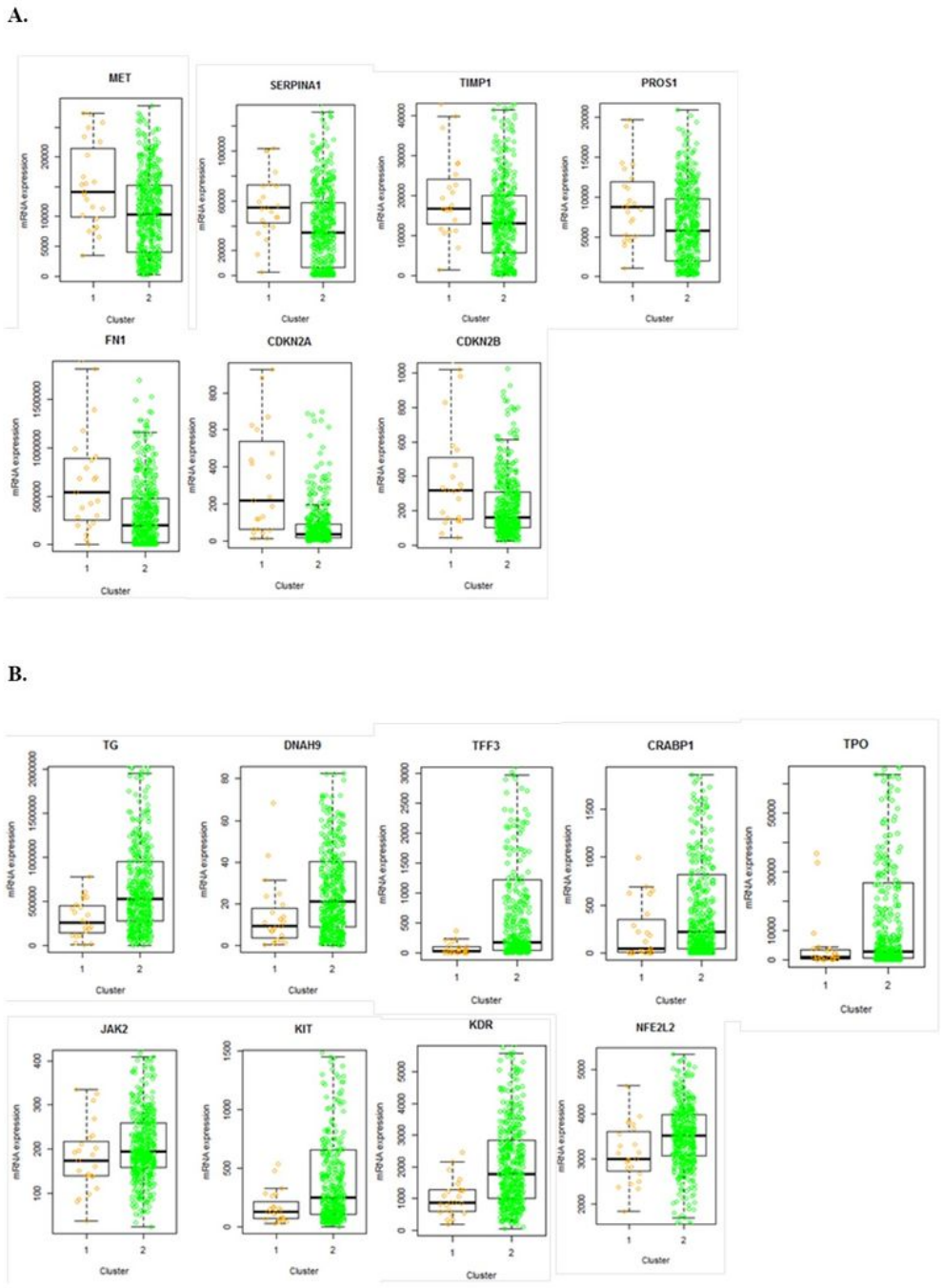

Figure 3

Identified sixteen driver genes of pathological T4 classification. A–B, The significantly gene expression elevated and lowered in Cluster1.

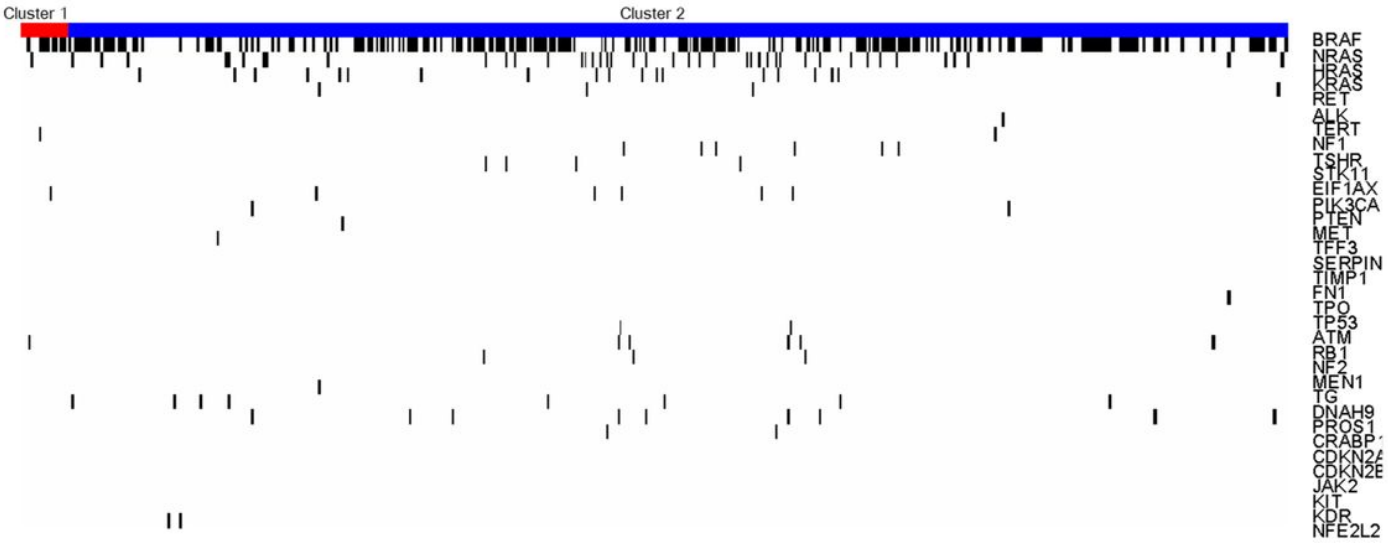

Figure 4

Somatic mutation between two clusters of patients in the TCGA cohorts. Samples are shown in columns and are cluster according to T classification.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx