TNFRSF12A is associated with immune cell infiltration in thyroid carcinoma

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Abstract

Objective: Comprehensively investigated the immune infiltration of thyroid cancer, to explore key immune-related genes and potential molecular mechanisms for thyroid carcinoma through bioinformatics analysis.

Methods: The thyroid carcinoma (THCA) expression profiles came from the Cancer Genome Atlas (TCGA) databases. The differences in the infiltration levels of immune cells between THCA were analyzed by the CIBERSORT. We analyzed the differentially expressed genes (DEGs) in thyroid, downloaded the immunologically relevant list of genes from IMMPORT, and then selected the differentially expressed immune genes. Gene ontology (GO) terms and pathways associated with these DEGs were identified, and protein–protein interactions (PPIs) were analyzed. The expression of TNFRSF12A in THCA was analyzed in the cBioPortal database. Additionally, we used the Tumor Immune Estimation Resource (TIMER) database evaluated correlation between TNFRSF12A expression and immune cell infiltration. Finally, Kaplan-Meier survival analysis was presented to predict the survival times between high and low TNFRSF12A expression groups for THCA.

Results: Differentially expressed immune genes associated with prognosis included BMP2, SEMA6B, INHBB and TNFRSF12A. There were distinct differences in the infiltration levels of immune cells between THCA and normal tissues. TNFRSF12A was identified to be highly expressed in THCA compared to normal tissues. TNFRSF12A mutation occurred in 5% of THCA samples, and low mRNA expression was the most common type of mutation. TNFRSF12A expression was significantly correlated to the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, neutrophils and dendritic cells. Gene Set Enrichment Analysis (GSEA) results indicated that TNFRSF12A could be involved in key biological processes and pathways of cytokine-cytokine receptor interaction. Patients with low TNFRSF12A expression usually experienced shorten overall survival time than those with its high expression.

Conclusion: Our findings revealed that TNFRSF12A was significantly related to immune cell infiltration, which may provide potential value for immunotherapy.

Introduction

Thyroid carcinoma (THCA) is one of the most common malignant neoplasms in the endocrine system, which arises from the follicular epithelium of the thyroid gland. It is one of the fastest growing malignant neoplasms and can occur at any age. THCA including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), anaplastic thyroid carcinoma (ATC), poorly differentiated thyroid carcinoma (PDTC) and medullary carcinoma (MTC) [1, 2]. Until now, the exact cause of thyroid carcinoma is not entirely clear. Over the past 10 years, the overall survival rate of patients with thyroid cancer has been close to 10%, with no significant improvement [3]. Therefore, searching for new targets and therapies is required [4].

It has been reported that the composition and function of tumor infiltrating immune cells vary with the host immune status, which has potential prognostic value. So far, immunotherapy has achieved
significant results in a variety of tumors, which is expected to be used to improve the prognosis of THCA [5, 6]. Tumor-infiltrating immune cells play an important role in tumor-related immune responses. Several studies have shown that tumor infiltrating immune cells can effectively improve the prognosis of various tumors [7-9]. Tumor immunotherapy is a treatment that eliminates tumor cells by restoring and maintaining normal anti-tumor immune responses, including immune checkpoint inhibitors, therapeutic antibodies, cell therapy, and small molecule inhibitors. In particular, immune checkpoint inhibitors (ICIs) have changed the therapeutic landscape of advanced malignancies. Therefore, it is of great clinical significance to explore the infiltration of immune cells in THCA [10].

Several studies have revealed immune cell infiltration and immune checkpoint expression in thyroid cancer [11, 12]. Previous studies have shown that dendritic cell, which is rare in normal thyroid tissue, increases in thyroid cancer tissue and inhibits the immune response [7, 13]. The purpose of our study was to identify key gene that are associated with survival in patients with thyroid carcinoma, and to investigate their expression in relation to immune cell infiltration, providing evidence for clinical decision making and prognosis. A hub gene, TNFRSF12A gene was up-regulated in THCA tissues compared to normal tissues and significantly correlated with the infiltration levels of dendritic cells and CD8 + T cells. In patients with THCA, low TNFRSF12A gene expression has a poor prognosis [14, 15]. Our research may provide potential value for the immunotherapy of THCA.

Materials and Methods

Data collection

The fragments per kilobase of per million (FPKM) of thyroid carcinoma transcriptome and corresponding clinical data of thyroid carcinoma were derived from TCGA (https://portal.gdc.cancer.gov/) database. We downloaded 2,498 immune-related genes from the IMMPORT database (http://www.immport.org) [16]. The GEPIA2 tool was used to pick out the top 500 genes that most influenced survival in patients with thyroid cancer and download them. All data derived from the TCGA were normalized to gene expression data through the R software package “limma”.

Assessment of immune cells

We used the CIBERSORT website (https://cibersort.stanford.edu/) to estimate the relative proportion of 22 infiltrating immune cell types based on gene expression, with the algorithm to 1000 permutations. P < 0.05 was considered to be statistically significant [17].

Screening immune-related genes that influence survival

DEGs were analyzed through the R software package “DESeq”, “limma” and “gplots”. The DEGs, 500 genes that most influenced survival and the immune-related genes were crossed.

Protein-Protein Interaction
The PPI network was used the STRING (version 11.0; http://string-db.org) database. STRING is an online database used to predict interactions between proteins\[^{18}\]. And the PPI network was visualized by the Cytoscape software (version 3.7.2; http://www.cytoscape.org/) \[^{19}\].

**TNFRSF12A and infiltrating immune cells**

The expression of TNFRSF12A in THCA was analyzed in the cBioPortal database (https://www.cbioportal.org/) \[^{20, 21}\]. Additionally, we used the TIMER database evaluated correlation between TNFRSF12A expression and the 6 types of immune cell infiltration (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells). TIMER is an online tool that can analyze the infiltration of immune cells in different tumors (https://cistrome.shinyapps.io/timer/) \[^{22}\].

**GO, KEGG pathway, and GSEA**

The WebGestalt (WEB-based Gene SeT AnaLysis Toolkit) database is a functional enrichment analysis web tool (http://www.webgestalt.org/), which supports three well-established and complementary methods for enrichment analysis, including ORA (Over-Representation Analysis), GSEA (Gene Set Enrichment Analysis), NTA (Network Topology-based Analysis) \[^{23, 24}\]. We performed functional enrichment analysis of immune-related DEGs in THCA, and analyzed the possible pathways involved in TNFRSF12A based on this database, including GO and Kyoto Encyclopedia of Genes and Genomes (KEGG).

**Survival Analysis**

The differences in overall survival and recurrence-free survival were analyzed between the high and low expression of TNFRSF12A groups and Kaplan–Meier curves were depicted via the tool GEPIA2 (Gene Expression Profiling Interactive Analysis, http://gepia2.cancer-pku.cn/) \[^{25}\]. The impact of 6 types of immune cell infiltration on the overall survival of THCA patients was also analyzed using the TIMER.

**Statistical analysis**

All statistical analysis was carried out using R language (version 3.6.2) packages including “limma”, “pheatmap”, “corrplot”, “vioplot”, “DESeq”, “survival” and “gplots”. \( p \) value < 0.05 was considered statistically significant.

**Results**

**Immune infiltration in THCA**

We calculated the proportion in 22 types of immune cell infiltration between THCA and normal tissues using the CIBERSORT algorithm (Fig.1a). We also analyzed the connection between each type of immune cell in THCA via a correlation matrix (Fig. 1b). We further determined the distributions in immune cell infiltration between THCA and normal tissues. We revealed that the proportions of naïve B cells \( p < \)
0.001), memory B cells \((p = 0.004)\), CD8+ T cells \((p < 0.001)\), gamma delta T cells \((\gamma \delta \text{ cells, } p < 0.001)\), follicular helper T (TFH) cells \((p = 0.001)\), and M1 macrophages \((p = 0.006)\) were significantly decreased in THCA compared to normal tissues. Additionally, the proportions of M0 macrophages \((p < 0.001)\), M2 macrophages \((p < 0.001)\), resting dendritic cells (DCs, \(p < 0.001\)), activated DCs \((p = 0.002)\), and resting mast cells \((p < 0.001)\) were significantly increased in THCA (Fig. 1c). Furthermore, we investigated the relationship between each immune cell type and overall survival (OS) in THCA patients. We found that a high proportion of dendritic cells resting \((p = 0.028)\), M0 macrophages \((p = 0.018)\) and monocytes \((p = 0.033)\) were associated with worse OS. And a low proportion of M1 macrophages \((p = 0.024)\), plasma cells \((p = 0.02)\) and CD8+ T cells \((p = 0.028)\) were associated with worse OS (Fig. 1d).

**Immune-related genes that influence survival**

We crossed the 500 DEGs that most influenced survival with immune-related genes. Four immune-related differentially expressed genes affecting survival were identified, finally (Fig. 2). The four genes are TNFRSF12A, SEMA6B, INHBB and BMP2.

**Protein-Protein Interaction**

DEGs were analyzed in the STRING database, A PPI network was constructed by the Cytoscape software, including 170 nodes and 1039 edges (Fig.3 (a)). Then, we took TNFRSF12A as the core position to construct its connected with other genes (Fig. 3 (b)).

**Expression and mutation of TNFRSF12A in THCA**

In the cBioPortal database, we analyzed the expression of THFRSF12A in 56 cancers. THFRSF12A expression in THCA was higher than other tumors (Fig. 4 (a)). We analyzed the genetic alteration of THFRSF12A in THCA, we founded that the TNFRSF12A mutation occurred in 5% of THCA samples. Among them, low mRNA expression was the most common type of mutation (Figure 4(b)).

**THFRSF12A expression is associated with immune cell infiltration in THCA**

Using the TIMER, We analyzed the correlation between different somatic copy number alterations and immune cell infiltration in THCA samples. As shown in Fig. 5(a), our data indicated that somatic copy number alterations were significantly associated with the infiltration of B cells \((p < 0.01)\), CD8+ T cells \((p < 0.01)\), CD4+ T cells \((p < 0.01)\), macrophages \((p < 0.05)\), neutrophil \((p < 0.01)\) and dendritic cells \((p < 0.001)\). The correlation between THFRSF12A expression and immune cell infiltration was analyzed across patients with THCA (Fig. 5(b)). THFRSF12A expression was distinctly correlated to B cells \((\text{cor} = 0.282; \ p = 3.06e - 10)\), CD8+ T cells \((\text{cor} = -0.128; \ p = 4.62e - 03)\), CD4+ T cells \((\text{cor} = 0.268; \ p = 1.86e - 09)\), neutrophils \((\text{cor} = 0.283; \ p = 1.99e - 10)\) and dendritic cells \((\text{cor} = 0.234; \ p = 1.83e - 07)\). Using the WebGestalt database for enrichment analysis, we founded that the DEGs were involved in pathways like cytokine-cytokine receptor interaction \((\text{FDR} < 2.2e-16, \ p < 2.2e-16)\), T cell receptor signaling pathway \((\text{FDR} = 2.5441e-8, \ p = 1.5608e-10)\) and Th17 cell differentiation \((\text{FDR} = 3.9366e-8, \ p = 3.6226e-10)\). GSEA including GO and KEGG was performed for TNFRSF12A. Our results showed that TNFRSF12A was
immunocytes in the TME have been proven to play a crucial role in the development of various
cancers \cite{26-28}. Different types of tumors have different immune cell subpopulations. In this study, we
performed a comprehensive and detailed assessment of immune infiltration in THCA. We demonstrated
that THCA infiltrates locally with various immune cell subsets \cite{5, 29}. We found that the proportions of
naïve B cells, memory B cells, CD8$^+$ T cells, gamma delta T cells, follicular helper T (TFH) cells and M1
macrophages were significantly decreased in THCA compared to normal tissues. The proportions of M0
macrophages, M2 macrophages, resting dendritic cells, activated DCs, and resting mast cells were
significantly increased in THCA \cite{30}. Additionally, we found that a high proportion of dendritic cells resting,
M0 macrophages and monocytes were associated with worse OS. And a low proportion of M1
macrophages, plasma cells and CD8$^+$ T cells were associated with worse OS \cite{7}.

**THFRSF12A expression is associated with THCA patients’ prognosis**

Cox proportional hazards model was constructed to evaluate the prognostic value of THFRSF12A
expression on the survival of THCA patients using TIMER (Table 1). Using the online tool GEPIA 2, we
explore the association between THFRSF12A expression and patients’ survival. The data showed that
patients with low THFRSF12A expression indicated poorer overall survival time compared to those with
its high expression (p = 0.007; Fig 6(a)). And there was no significant difference in disease-free survival
between the high- and low-expression groups of THFRSF12A for THCA patients (Fig 6(b)).

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Abbreviation: coef: coefficient; CI: confidence interval; HR: hazard ratio

**Discussion**

Immune cells in the TME have been proven to play a crucial role in the development of various
cancers \cite{26-28}. Different types of tumors have different immune cell subpopulations. In this study, we
performed a comprehensive and detailed assessment of immune infiltration in THCA. We demonstrated
that THCA infiltrates locally with various immune cell subsets \cite{5, 29}. We found that the proportions of
naïve B cells, memory B cells, CD8$^+$ T cells, gamma delta T cells, follicular helper T (TFH) cells and M1
macrophages were significantly decreased in THCA compared to normal tissues. The proportions of M0
macrophages, M2 macrophages, resting dendritic cells, activated DCs, and resting mast cells were
significantly increased in THCA \cite{30}. Additionally, we found that a high proportion of dendritic cells resting,
M0 macrophages and monocytes were associated with worse OS. And a low proportion of M1
macrophages, plasma cells and CD8$^+$ T cells were associated with worse OS \cite{7}.
To further understand the THCA tumor immune microenvironment, we identified 170 THCA immune-related genes in combination with the TCGA dataset and the IMMPORT dataset. Then we crossed the 500 differentially expressed genes that most influenced survival with immune-related genes, four immune-related DEGs affecting survival for THCA were identified\[^{26}\]. There are TNFRSF12A, SEMA6B, INHBB and BMP2. We chose TNFRSF12A as the research object of this study.

Past research has shown that tumor necrosis factor receptor superfamily member 12A (THFRSF12A), receptor for TNFSF12/TWEAK, weak inducer of apoptosis in some cell types. It is mainly in the positive regulation of exogenous apoptosis signaling pathway. Promotes angiogenesis and the proliferation of endothelial cells, May modulate cellular adhesion to matrix proteins\[^{15,31,32}\]. Through the cBioPortal database, TNFRSF12A had a 5% genetic alteration across THCA samples, and low mRNA expression was the most common type of mutation. TNFRSF12A expression was markedly associated with THCA patients’ clinical outcomes, which could become a potential prognostic marker\[^{33}\]. In our study, the results showed that the mutation of TNFRSF12A had a distinct correlation with the infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophil and dendritic cells. Meanwhile, TNFRSF12A expression exhibited a significant association with B cells, CD8+ T cells, CD4+ T cells, neutrophils and dendritic cells. Thus, TNFRSF12A expression might be related to THCA tumor immune microenvironment.

In GSEA, we found that TNFRSF12A was significantly related with biological regulation. TNFRSF12A located in plasma membrane and enables protein binding. For KEGG pathway enrichment analysis results, cytokine-cytokine receptor interaction were related to high TNFRSF12A expression, indicating that TNFRSF12A might affect the local infiltration of immune cells by interacting with other cytokine receptors. But the interesting thing is, Cox proportional hazard model analysis results demonstrated that TNFRSF12A expression was in relationship with THCA patients’ prognosis. While, low TNFRSF12A expression predicted a poorer prognosis for THCA patients, which was consistent with the results of previous research\[^{14,15}\]. It may be related to the fact that low mRNA expression is the most common type of mutation. Thus, TNFRSF12A expression could be a promising immune-related prognostic marker for THCA\[^{34}\].

Our study has several limitations. Firstly, we only analyzed the infiltration of immune cell infiltration between normal and THCA tissues, without paying attention to the differences between subtypes. Secondly, the specific interactions of TNFRSF12A with other cytokine receptors need further exploration. In addition, more relevant clinical samples should be collected to confirm the biological functions of TNFRSF12A in THCA.

**Conclusion**

Through bioinformatics analysis, we found that TNFRSF12A and normal tissue showed significant differences in immune cell infiltration. Differences in immune cell infiltration were significantly associated with the prognosis of THCA patients. The expression of TNFRSF12A gene in THCA tissues was higher
than that in normal tissues. The expression of TNFRSF12A is related to the immune infiltration cells in THCA samples. Patients with low TNFRSF12A expression usually have a worse prognosis than those with high TNFRSF12A expression. Therefore, TNFRSF12A may be a potential immune-related prognostic marker for THCA.

Declarations

Data Availability Statement

Data openly available in a public repository.

Conflicts of Interest

The authors declare no conflicts of interest.

References


**Figures**

![Figure 1](image-url)
Landscape of immune cell infiltration in THCA and normal tissues by CIBERSORT. (a) The composition of 22 types of immune cells in THCA and normal tissues. (b) corHeatmap visualizing the correlation between 22 types of immune cells across THCA and normal tissues. (c) Violin plots visualizing the distributions of immune cells between MESO and normal tissues. Blue represents normal samples and red indicates tumor samples. (d) The correlation of immune cells and overall survival (OS).

**Figure 2**

Venn diagram depicting DEGs that most influenced survival and the immune-related genes.
Figure 3

Identification of DEGs associated with immunity for THCA. (a) A PPI network based on DEGs using Cytoscape. (b) TNFRSF12A as the core position to construct its connected with other genes.
Figure 4

(a) the expression of THFRSF12A across 56 kinds of cancers. (b) THFRSF12A genetic alteration in THCA.
Figure 5

(a) the correlation between somatic copy number alterations and immune cell infiltration. (b) The correlation between THFRSF12A expression and immune cell infiltration in THCA samples. Functional enrichment analysis of immune-related differentially expressed genes including THFRSF12A. (c) Biological Process, Cellular Component and Molecular Function category. (d) KEGG, 10 categories are identified as enriched categories.
Figure 6

(a) Disease-free survival. (b) Overall survival analyses of THFRSF12A expression for THCA patients using the GEPIA 2 online platform.