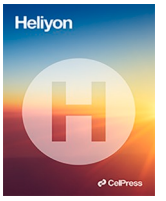
Heliyon 10 (2024) e27651

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

# GDF10 and IDO1 as a thyroid cancer prognostic biomarker associated with immune infiltration

Zhao-bao Lv [a], Jun-jing Zhang [a], Cheng Xiang [b,*]

[a] Breast and Thyroid Surgery, The Second Hospital of Liaocheng, Lingqing, 252600, Shandong, China
[b] Thyroid Surgery, The Second Affiliated Hospital Zhejiang University School of Medicine, Hangzhou, 310009, Zhejiang, China

ARTICLE INFO

*Keywords:*
Immunity gene
Immune checkpoint
Thyroid cancer
Prognosis biomarker

ABSTRACT

*Objection:* The aim of this work is to screen the immune-related genes to predict the prognosis and provide a new direction of treatment for patients with thyroid cancer (THCA).
*Methods:* The mRNA and clinical features of THCA patients were collected from the Cancer Genome Atlas (TCGA) databases. The immune-related genes were obtained from the ImmPort databases. The bio-information methods were performed to screen the differential expression genes (DEGs) and genes related to immunity between the THCA patients and normal individuals. On this basis, the hub prognosis immunity genes were screened by Veen. The related genes were obtained by constructing the protein-protein interaction network. The enrichment analyses were performed based on the protein and protein interaction (PPI) related genes. The hub immune checkpoint was screened by correlation analysis. Finally, the hub gene and the immunity checkpoint-miRNA (or transcription factor, drug) interaction network were constructed. A drug-sensitive analysis also was performed.
*Results:* The GDF10 was screened. The PPI genes were enriched in the TGF-beta signaling pathway, signaling pathways regulating, the pluripotency of stem cells, Cytokine-cytokine receptor interaction, and so on. The hub immunity checkpoint IDO1 was obtained. The joint indicator of two hub genes was positively related to the thyroid differentiation score. Three interaction factors were found to be related to the two hub genes, and 7 kinds of drugs screened act on the two hub genes at the same time.
*Conclusion:* This work indicated that immune-related gene GDF10 and immune checkpoint IDO1 are important for the prognosis prediction of THCA patients, and immunity is involved in the proliferation, and differentiation of tumor cells.

## 1. Introduction

Thyroid cancer (THCA) is a common endocrine tumor with a high prevalence globally in the past few years [1]. Based on the cancer statistics, 40 thousand females and 12 thousand males were diagnosed with THCA in 2020 in the USA. Commonly, THCA includes four subtypes: anaplastic, follicular, medullary, and papillary thyroid carcinoma [2]. The subtype was defined according to the histological characteristics. Although the age-standardized 5-year could reach 85%, the prognosis of THCA was dissatisfied due to a large number

* Corresponding author. Thyroid Surgery, The Second Affiliated Hospital Zhejiang University School of Medicine, No.88 Jiefang Road, Shangcheng District, Hangzhou, 310009, Zhejiang, China.
*E-mail address:* lvzhaobao0708@163.com (C. Xiang).

https://doi.org/10.1016/j.heliyon.2024.e27651
Received 22 May 2023; Received in revised form 4 March 2024; Accepted 5 March 2024
Available online 6 March 2024

of patients [3]. Moreover, the patients commonly had poor quality of life because they need to accept various treatments including surgery, chemotherapy, and drug. In addition to coping with the physical challenges of cancer diagnosis, individuals also face heightened psychological strain. Consequently, the identification of novel biomarkers becomes imperative in enhancing the prognostic capabilities for THCA.

The immune system plays a crucial role in the development of tumors, and immunotherapy holds promise as a potential treatment for individuals afflicted with cancer. An increasing number of researchers have explored immune-related gene biological functions in various cancers. Jia et al. screened 21 immune-related genes that could serve as underlay biomarkers for patients with glioblastoma multiforme [4]. The study of Yang et al. identified 11 immune-related genes and constructed a risk model to predict the prognosis of patients with cervical cancer [5]. There were many studies showed that immune-related genes are potential prognosis signatures for lung tumors [6], ovarian tumors [7], and endometrial carcinoma [8]. Many researchers indicated that there is an association between Hashimoto's thyroiditis (HT) - a common autoimmune disease, and THCA [9–11]. Xu et al. indicated that autoimmune thyroiditis has a protective function in association with thyroid cancer [12]. The find of Li et al. showed that tissue small extracellular vesicle-mediated miR-142-3p transfer can serve as a communication mode between T lymphocytes and thyrocyte cells in HT, inducing and destructing thyrocyte cell in HT [13]. In summary, immunological systems in the THCA were important. [https://www.mdpi.com/1422-0067/20/18/4413]. However, there was a limited study on the immune-related prognosis gene in THCA.

In our work, we aimed to identify the new immune-related prognosis genes for predicting the prognosis of THCA based on the Cancer Genome Atlas (TCGA) databases. We also identified the hub immune checkpoint as a prognosis biomarker for patients. In addition, we joined the hub immune-related prognosis gene and hub immune checkpoint to predict the outcome and provide a new direction for the immunotherapy treatment of THCA patients.

## 2. Materials and methods

### 2.1. Data source

The thyroid cancer patient's information including mRNA expression data and clinical character data was obtained from TCGA public database. The TCGA-THCA data set contained 502 THCA patients' tumor tissues and 48 normal tissues. The mRNA expression data were normalized via the "limma" package of the R software. A total of 456 immune-associated genes in THCA were collected from the public database the Immunology Database and Analysis Portal (ImmPort, https://www.immport.org/).

### 2.2. Screening of prognostic and immune-associated genes

The differential expression genes (DEG) between the normal and THCA tissues were distinguished by the "limma" package of R software based on $P < 0.05$ and $|\log2 (\text{Fold Change})| > 1$. In addition, the DEGs between the dead and the alive were obtained via the same method. The common genes among the two DEG sets and the immune-associated gene were screened via the "Venn" package of R software.

### 2.3. Protein and protein interaction network

The common-gene related proteins were screened by the Protein and protein interaction (PPI) network based on the STRING Version: 11.5 (https://cn.string-db.org/) databases. The basic settings included the minimum required interaction score was 0.4, and max number of interactors was 50. The related proteins were downloaded and constructed the PPI network via the Cytoscape Version 3.8.2 software.

### 2.4. Enrichment analyses

To explore the related biological function, gene enrichment was performed on common-gene related biomarkers via the "clusterProfiler" R package 15 based on Kyoto Encyclopedia of Genes and Genomes (KEGG) database and Gene Ontology (GO) database. Moreover, P-values were calculated by the Bebjamini and Hochberg (BH) method. Cellular component (CC), biological process (BP), and molecular function (MF) were annotated in GO analysis.

### 2.5. Immune infiltration analysis

The Tumor Immune Estimation Resource (TIMER) method based on the TIMER2.0 database (http://timer.cistrome.org/) is commonly used for exploring the relationship between the expression of hub gene and the immune cell infiltration in different tumors. The immune cell included the B cell, CD8+T cell, CD4+T cell, macrophage, neutrophil, and dendritic cell. In addition, the THCA patients were divided into two groups according to the joint indicators to explore the relationship between the hub genes and the immune microenvironment score of 22 kinds of immune cells. The joint indicators based on the expression of various hub genes were calculated via Hosmer-Lemeshow Goodness-of-Fit.

### 2.6. Survival analysis

The Kaplan-Meier method was used to draw the survival curve. In addition, the log-rank test was used to distinguish the difference between different curves. And receiver operating characteristic (ROC) curve was constructed to assess the prediction in THCA patients dead from THCA patients with alive status. P value of $<0.05$ was considered statistically significant. The bilateral p values $< 0.05$ were considered statistically significant.

### 2.7. Gene set enrichment analysis

Gene set enrichment analysis (GSEA) for both the high and low-joint indicators was performed based on the KEGG.v7.4 gene set via the GSEA software. P value of $<0.05$ and an FDR of $<0.25$ were considered statistically significant.

### 2.8. Construction of gene-miRNA, gene-transcription factor, gene-drug network

The Network analyst (https://www.networkanalyst.ca/,11/06/2022) database commonly is used to explore the correction between the gene a nd genes-transcription factors, miRNA, drug, and other molecules. Gene-miRNA interaction database includes the miRtarBase v8.0, TarBase v8.0, and miRecords. Gene-transcription factor (TF)interaction database includes ENCODE, JASPAR, and ChEA. And the Gene-drug interaction includes Drugbank database.

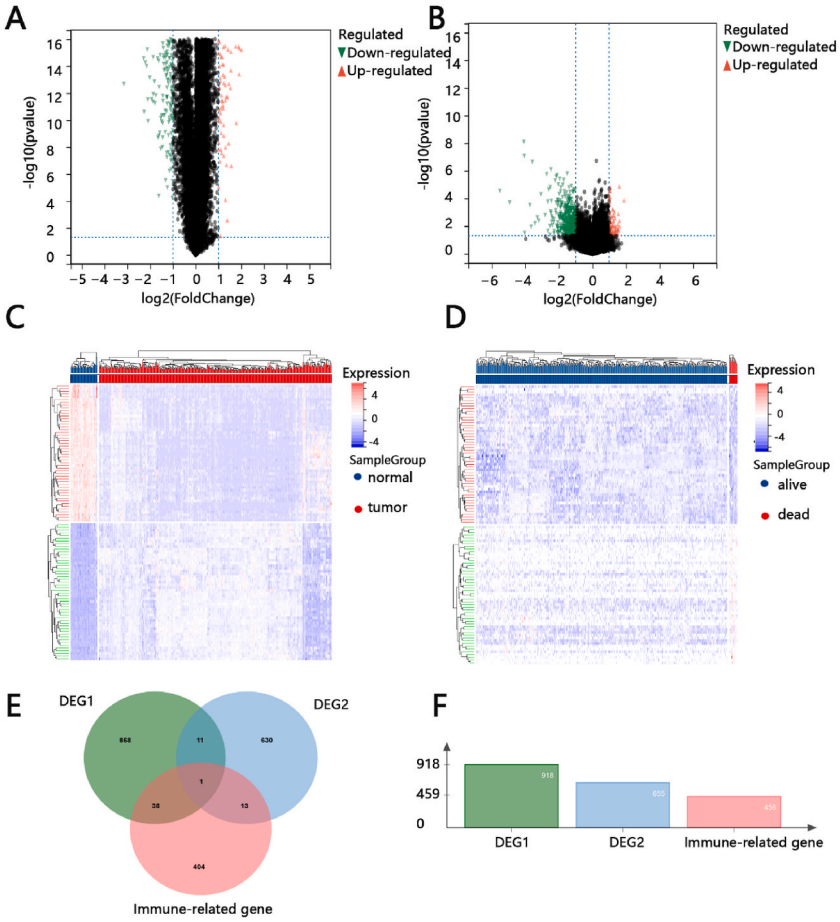



**Fig. 1.** Volcano map of differentially expressed genes (DEG) between the normal tissue and THCA tissue (A). Volcano map of DEG between the dead THCA patient and alive THCA patient (B). Heatmap of top 100 DEGs between the normal tissue and THCA tissue (C). Heatmap of top 100 DEGs between dead THCA patient and alive THCA patient (D). The Venn plot of three gene sets (E). The histogram of three gene sets (F). DEG1 is representative of the DEG between the normal tissue and THCA tissue. DEG2 is representative of the DEG between the dead THCA patient and the alive THCA patient. Immune-related gene were from the website of ImmPort (https://www.immport.org/).

### 2.9. Drug sensitivity

The sensitivity between the common anticancer drugs and the hub gene expression was analyzed via the website of the Gene Set Cancer Analysis (GSCA) (http://bioinfo.life.hust.edu.cn/GSCA/#/). The website is a public online platform for anticancer drug sensitivity and pharmacogenomic analysis.

### 2.10. Statistical analysis

Data analysis was performed using R software and SPSS version 21.0 software (IBM SPSS, Armonk, NY, USA). The categorical data between groups was counted and compared with rank sum test as the data did not conform to a normal distribution. T-test was used in the comparison of continuous variables in the two groups. The relationship analysis between the immune checkpoints and the expression of the hub gene via the Pearson analysis method. P values $< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Identification of immune-related prognosis gene in THCA patients

To explore the immune-related prognosis gene in THCA patients, we screened the 918 different expression genes between the normal tissue and the tumor tissue including 453 regulated-up and 465 regulated-down genes. A total of 655 DEGs including 77 regulated-up genes and 578 regulated-down genes between the dead THCA patients and alive THCA patients were screened according to the standard of $P < 0.05$ and $|$ Flod Change $|>2$. Two DEG sets were visualized via a volcano map (Fig. 1 A, B). Moreover, the top 100 DEGs were plotted into the heatmap, respectively (Fig. 1 C, D). We obtained 404 immune-related genes from the Immunology Database and Analysis Portal (ImmPort, https://www.immport.org/). The hub gene GDF10 was extracted via the Venn graph (Fig. 1 E, F).

### 3.2. Patient characteristics in the high and low GDF10 expression level

Clinical data and expression data of 450 cases of THCA which included 327 female patients (72.7%), and 123 male patients (27.3%), were obtained from TCGA data (Table 1). A total of 13 patients' outcomes were dead in THCA patients and all of them were from the high expression level group. In the high-expression group, most patients were stage I (171 cases) and stage III (73 cases), followed by stage IV (36 cases), and the smallest number was in stage II (26 cases). In the low-expression group, most patients were stage I (78 cases) and stage III (32 cases), followed by stage IV (17 cases) and stage I (17 cases). In the low-expression group, most patients were T2 (55 cases) and T3 (55 cases), followed by T1 (28 cases), and the smallest number was in T4 (6 cases).In the high-expression group, most patients were T1 (103 cases) and T3 (102 cases), followed by T2 (85 cases), and the smallest number was in T4 (16 cases).In summary, there were significant differences between the high-expression group and low-expression group in survival status and T of THCA patients ($p < 0.05$).

**Table 1**
Clinicopathological characteristics of THCA patients in different GDF10 levels.

| character | | group | | total | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| | | low | high | | | |
| sex | female | 103 | 224 | 327 | 0.138 | 0.71 |
| | male | 41 | 82 | 123 | | |
| age | ≥50 | 58 | 144 | 202 | 1.82 | 0.177 |
| | <50 | 86 | 162 | 248 | | |
| event | alive | 144 | 293 | 437 | 6.3 | 0.012 |
| | dead | 0 | 13 | 13 | | |
| type | Thyroid Papillary Carcinoma - Classical | 103 | 226 | 329 | 0.513 | 0.916 |
| | Thyroid Papillary Carcinoma - Follicular | 25 | 52 | 77 | | |
| | Thyroid Papillary Carcinoma - Tall Cell | 13 | 22 | 35 | | |
| | Other, specify | 3 | 6 | 9 | | |
| M stage | M0 | 78 | 194 | 272 | 4.839 | 0.089 |
| | MX | 62 | 109 | 171 | | |
| | M1 | 4 | 3 | 7 | | |
| T stage | T1 | 28 | 103 | 131 | 11.102 | 0.011 |
| | T2 | 55 | 85 | 140 | | |
| | T3 | 55 | 102 | 157 | | |
| | T4 | 6 | 16 | 22 | | |
| N stage | N0 | 68 | 160 | 228 | 1.005 | 0.316 |
| | N1 | 76 | 146 | 222 | | |
| clinical stage | I | 78 | 171 | 249 | 1.286 | 0.732 |
| | II | 17 | 26 | 43 | | |
| | III | 32 | 73 | 105 | | |
| | IV | 17 | 36 | 53 | | |

### 3.3. The expression of the hub gene

In order to investigate the clinical significance of the immune-related prognostic gene GDF10 in THCA, an analysis was conducted to assess its expression levels in both the pan-cancer and THCA contexts. The results showed that the GDF10 expression was lower in various cancer groups than in normal groups (Fig. 2A). Commonly, its lower expression in THCA group than in the normal group was further confirmed via independent sample T-test and paired sample T-test (Fig. 2B and C). The survival analysis showed that the THCA patients with low expression levels had higher survival probability (Fig. 2D). Moreover, the ROC analysis showed that it had the ability

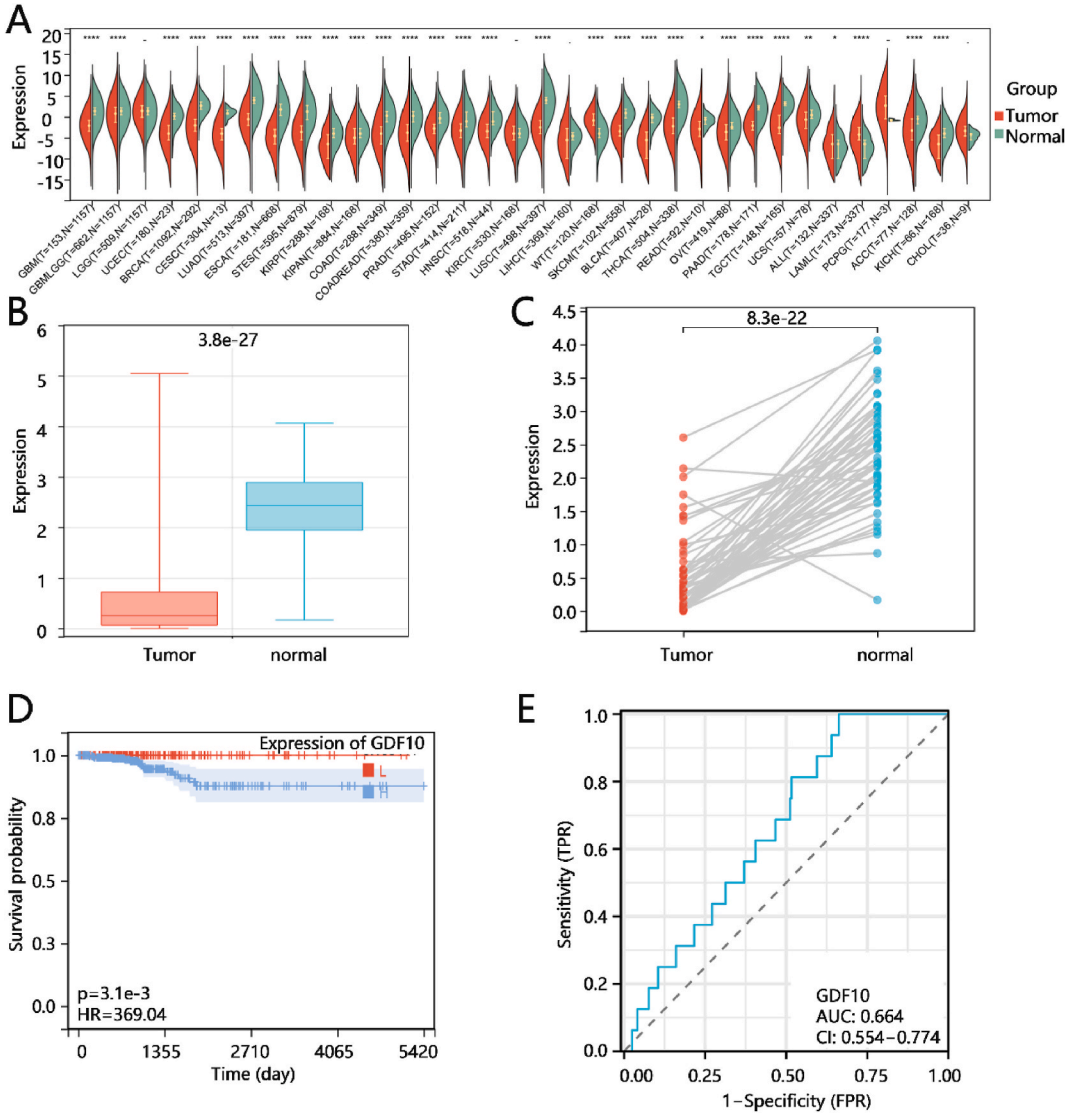



**Fig. 2.** The expression of GDF10 in pan-cancer (A). The comparison of the expression between the normal and the tumor (B). The comparison of matched pairs of the expression the normal and the tumor (C). The survival analysis of the GDF10 in patients with THCA (D). The ROC analysis of the GDF10 (E). GBM: Esophageal carcinoma; GBMLGG: Glioblastoma multiforme; LGG: Acute Myeloid Leukemia; UCEC: Thymoma; BRCA: Bladder Urothelial Carcinoma; CESC: Breast invasive carcinoma; LUAD: Liver hepatocellular carcinoma; ESCA: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; STES: Stomach adenocarcinoma; KIRP: Kidney renal clear cell carcinoma; KIPAN: Kidney Chromophobe; COAD: Cholangio carcinoma; COADREAD: Colon adenocarcinoma; PRAD: Pheochromocytoma and Paraganglioma; STAD: Skin Cutaneous Melanoma; HNSC: lioblastoma multiforme; KIRC: Kidney Chromophobe; LUSC: Lung adenocarcinoma; LIHC: Brain Lower Grade Glioma; WT: Uveal Melanoma; SKCM: Sarcoma; BLCA: Adrenocortical carcinoma; THCA: Testicular Germ Cell Tumors; READ: Prostate adenocarcinoma; OV: Mesothelioma; PAAD: Ovarian serous cystadenocarcinoma; TGCT: Stomach adenocarcinoma; UCS: Uterine Carcinosarcoma
ALL: Adrenocortical carcinoma; LAML: Kidney renal papillary cell carcinoma; PCPG: Pancreatic adenocarcinoma; ACC: Adrenocortical carcinoma; KICH: Head and Neck squamous cell carcinoma; CHOL: Cervical squamous cell carcinoma and endocervical adenocarcinoma. The '*' represents the significant difference between the tumor group and the normal group. L represents low expression group. H represents high expression group. AUC: Areas Under Curve.

to distinguish the dead patients from the THCA patients with alive status based on the expression of the GDF10. The area under the curve (AUC) for ROC was 0.664(95% confidence interval: 0.554–0.774) (Fig. 2E).

### 3.4. Construction of protein-protein network

A total of 34 genes were screened based on the STRING database. The protein-protein network (PPI)network information includes 64 nodes, 66 edges, and 2 seeds. The network plot was constructed via Cytoscape software (Fig. 3).

### 3.5. Gene enrichment analysis

To analysis the biological function and related pathways of GDF10-associated genes, we performed the gene enrichment analysis based on the KEGG and GO database. The results showed that those genes were enriched in the TGF-beta signaling pathway, signaling pathways regulating, pluripotency of stem cells, Cytokine-cytokine receptor interaction, Fluid shear stress and atherosclerosis, Hippo signaling pathway, Hepatocellular carcinoma, Axon guidance, Human T-cell leukemia virus 1infection, Pancreatic cancer, Colorectal cancer (Fig. 4A). Regarding the GO analysis, those genes related to transmembrane receptor protein serine/threonine kinase activity, transmembrane receptor protein kinase activity, protein serine/threonine kinase activity, SMAD binding, activin-activated receptor activity, activin binding, BMP receptor activity, transforming growth factor beta receptor, cytoplasmic mediator activity in MF (Fig. 4B); enzyme linked receptor protein signaling pathway, transmembrane receptor protein serine/threonine kinase signaling pathway, BMP signaling pathway, response to BMP, cellular response to BMP stimulus, regulation of transmembrane receptor protein serine/threonine kinase signaling pathway, positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway, pathway-restricted SMAD protein phosphorylation in BP (Fig. 4C); neuron projection, receptor complex, plasma membrane protein complex, plasma membrane receptor complex, SMAD protein complex, activin responsive factor complex, inhibin-beta glycan-

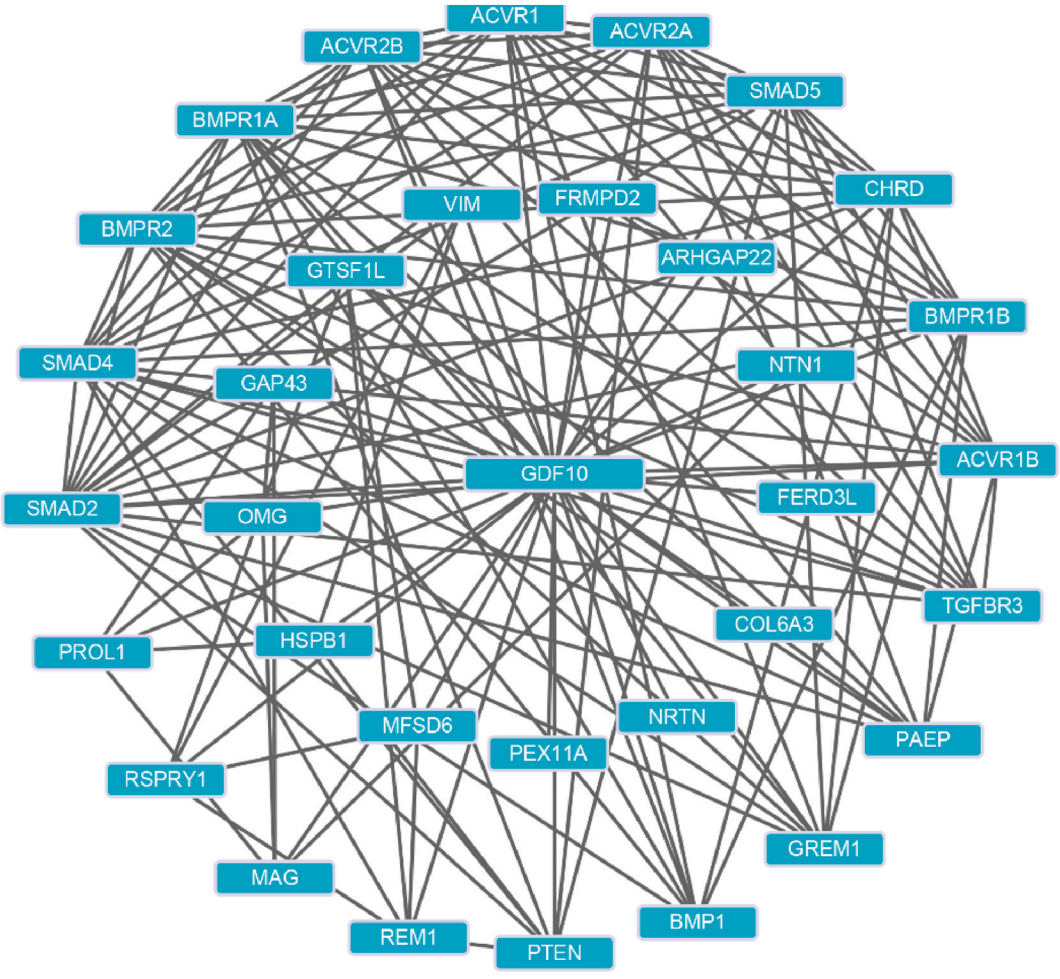



**Fig. 3.** The PPI of the GDF10. The PPI network information includes 64 nodes, 66 edges, and 2 seeds and is obtained from the STRING website. PPI: protein-protein network.

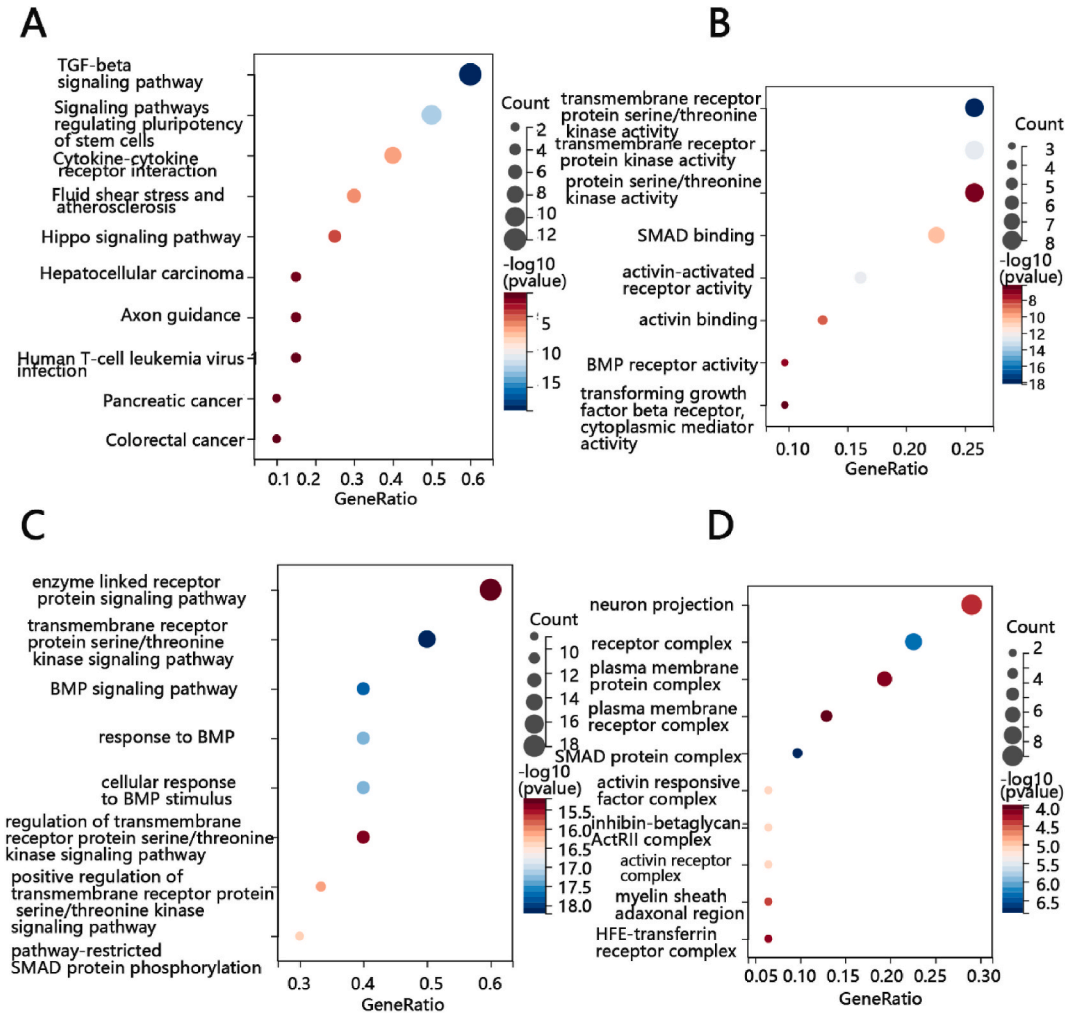



**Fig. 4.** Significant KEGG pathway of the PPI genes (A). The GO analysis of PPI genes in terms of (B) molecular function (MF); (C) biological process (BP); and (D) cellular component (CC).

ActRII complex, activin receptor complex, myelin sheath adaxonal region, HFE-transferrin receptor complex in cellular component CC (Fig. 4D).

### 3.6. Immune infiltration analysis

Previous studies have posited the significance of tumor infiltrating immune cells in tumor development. Gene enrichment analysis findings indicate that pathways related to immune processes exhibit enrichment of GDF10-related genes. Consequently, we investigated the association between GDF10 expression and immune characteristics utilizing the TIMER2.0 database. The results showed that GDF10 expression was positively related to B cell ($r = 0.313$, $p < 0.05$), $CD8^+$ T cell ($r = 0.118$, $p < 0.05$), $CD4^+$ T cell ($r = 0.113$, $p < 0.05$), macrophages ($r = 0.161$, $p < 0.05$), neutrophils ($r = 0.119$, $p < 0.05$), dendritic cell ($r = 0.181$, $p < 0.05$) (Fig. 5A). Moreover, the immune cells score between the high expression of GDF10 and the low expression of the GDF10 group were compared. The results indicated that plasma cells, CD8 T cells, T cells follicular helper, dendritic cells resting, and M1were higher in the high expression of GDF10 than in the low expression of the GDF10 group with a significant difference. DC activated, M2, M0, monocytes, and T cell regulatory (Tregs) were higher in the low expression of GDF10 than in the high expression of GDF10 group with obvious difference (Fig. 5B).

### 3.7. The relationship between the hub gene expression and immune checkpoint

The pivotal role of immune checkpoints in the immune response process is of utmost importance. In light of the putative oncogenic function of GDF10 in THCA, an investigation was conducted to examine the correlation between the expression levels of GDF10 and

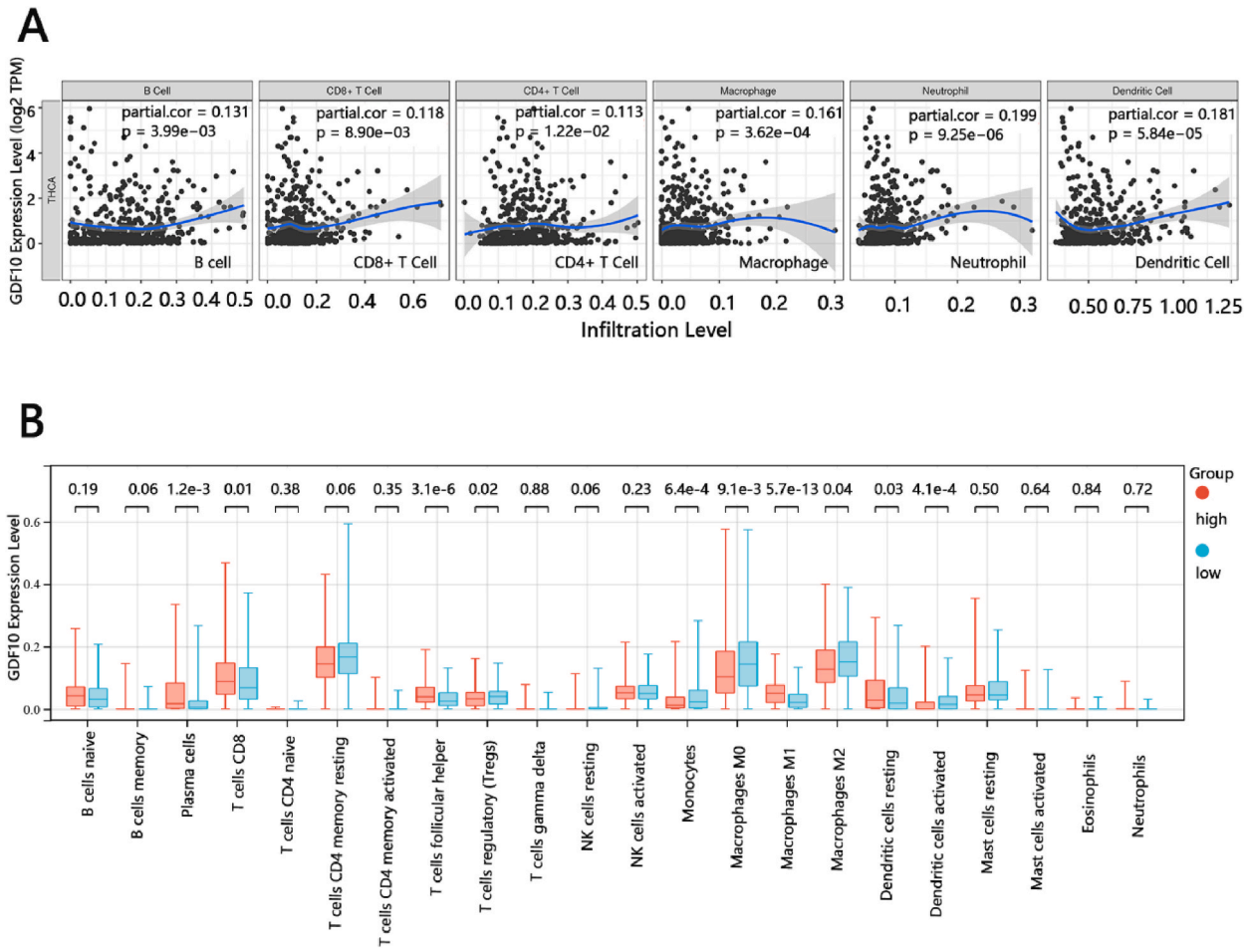



**Fig. 5.** Correlation of GDF10 expression with six infiltrating immune cells (B cells, CD4 + T cells, CD8 + T cells, macrophages, neutrophils, and dendritic cells) based on the TIMER2.0 database (A). The comparison of the 22 kinds of immune cell scores between the high expression of GDF10 and the low expression of GDF10 (B).

immune checkpoint genes using Pearson analysis (Fig. 6A–G). The results showed that GDF10 expression was positively related to the expression of IDO1($p = 2.3e\text{-}16$, $r = 0.33$), and was negatively related to the expression of SIGLEC15 ($p = 4.2e\text{-}9$, $r = -0.24$), CD274 ($p = 5.2e\text{-}4$, $r = -0.15$), CD276 ($p = 1.3e\text{-}7$, $r = -0.22$), and HMGB1 ($p = 0.76$, $r = -0.01$) with obvious differences. The expression of CD274 was substantially higher in the GDF10 low expression group than in the GDF10 high expression group. The expressions of IDO1 and HMGB1 were substantially higher in the GDF10 high-expression group than in the GDF10 low-expression group (Fig. 6H).

### *3.8. The expression of the hub immune checkpoint*

We obtained the hub immune checkpoint gene IDO1 according to the forward results. The expression analysis of IDO1 was performed. The results showed that it was higher expression in some kinds of cancer tissue such as lung adenocarcinoma and stomach cancer than in corresponding normal tissue, while it was lower expression in some kinds of cancer tissue than in corresponding normal tissue, for instance, THCA and acute lymphoblastic leukemia (Fig. 7A). It was a lower expression in the THCA group than in the normal group via independent sample T-test and paired sample T-test (Fig. 7 B C). The results of the survival analysis showed that IDO1 was an obstruction factor for THCA. The ROC analysis showed that it has a poor ability to distinguish dead patients from THCA patients. Hence, we used the joint indicators to perform the survival analysis and ROC analysis. The results showed that joint indicators were associated with overall survival time, in addition, the THCA patients with high joint indicators had shorter OS than those with low joint indicators (Fig. 7F). The ROC indicated that it has a good ability to distinguish the dead patients from the THCA patients with alive status. The area under the curve (AUC) for ROC was 0.664 (95% confidence interval:0.554–0.774) (Fig. 7G).

### *3.9. The relationship analysis between the hub gene expression and differentiation score*

Thyroid differentiation score (TDS) is a commonly used algorithm to evaluate the differentiation status [14]. The corrections

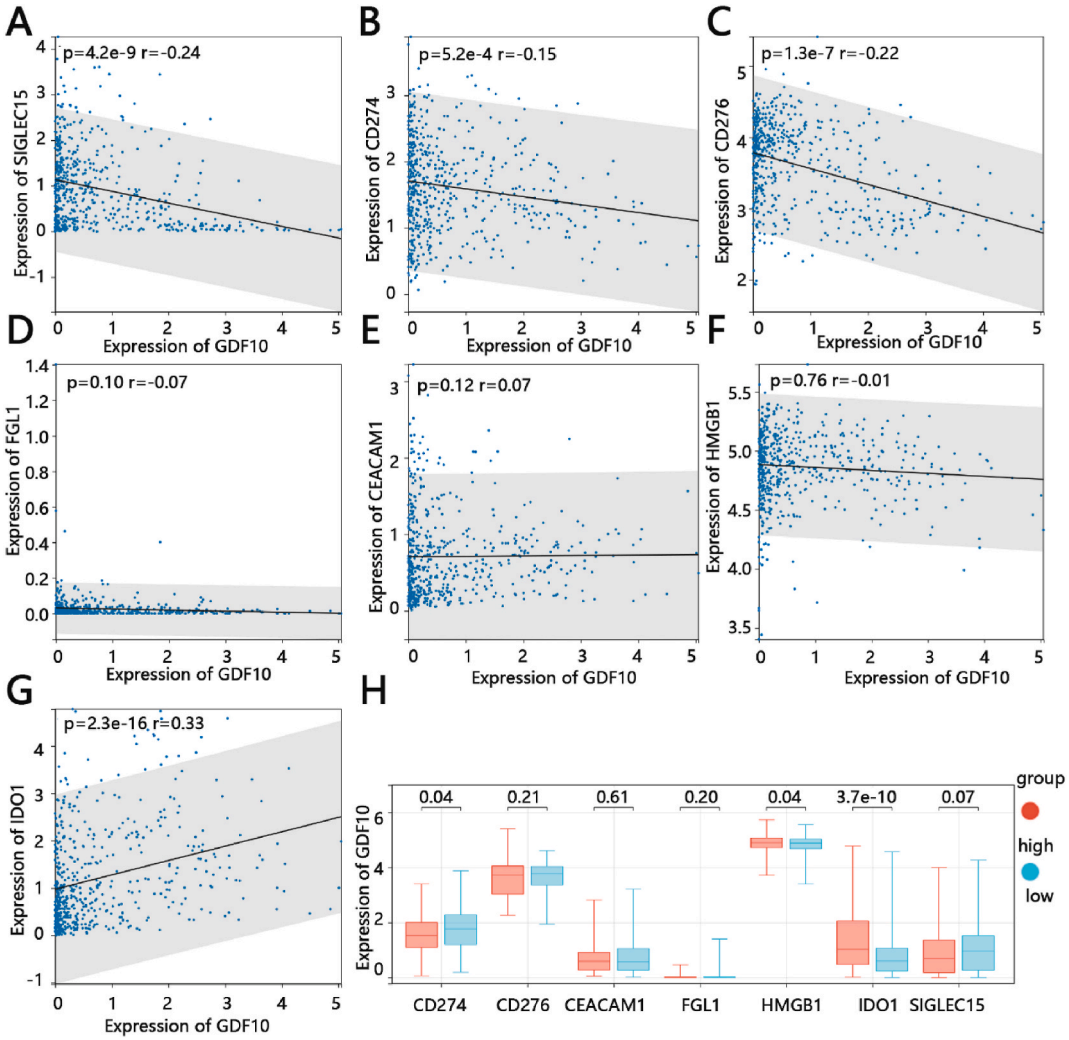



**Fig. 6.** The correction between the expression of GDF10 and immune checkpoint (A: SIGLEC15; B:CD274; C:CD276; D: FGL1; E: CEACAM1; F: HMGB1; G: IDO1). The comparison of the expression of immune checkpoint between the high expression of GDF10 and the low expression of GDF10 (H). r: correlation coefficient.

between the expression of the hub gene and TDS were analyzed. The results showed that IDO1 expression was negatively related to the TDS ($r = -0.09$, $p > 0.05$) and GDF10 expression was positively related to the TDS with obvious differences in statistics ($r = 0.42$, $p < 0.05$) (Fig. 8 A B). In addition, there was a positive relationship between the joint indicators and the TDS, and the TDS was higher in the high joint indicators group than in the low group ($r = 0.51$, $p < 0.05$) (Fig. 8C D) with a significant difference in statistics.

### 3.10. Gene set enrichment analysis

To explore the related pathway in different joint indicators groups, the GSEA was performed. The results showed that the low joint indicator was enriched in DNA replication (NES = 1.9037, FDR = 0.0694), autoimmune thyroid disease (NES = 1.8475, FDR = 0.0716), cell cycle (NES = 1.7644, FDR = 0.0750) P53 signal pathway (NES = 1.7799, FDR = 0.0785) (Fig. 8E).

### 3.11. Results of gene-miRNA and gene-TF

The interaction network of GDF10/IDO1 and miRNA or TF was constructed to further explore the relationship between two hub genes. The result showed that there were 64 nodes and 66 edges. BACH1, STAT3, and RNF2 were common TF of the two genes (Fig. 9), and no common miRNA was observed.

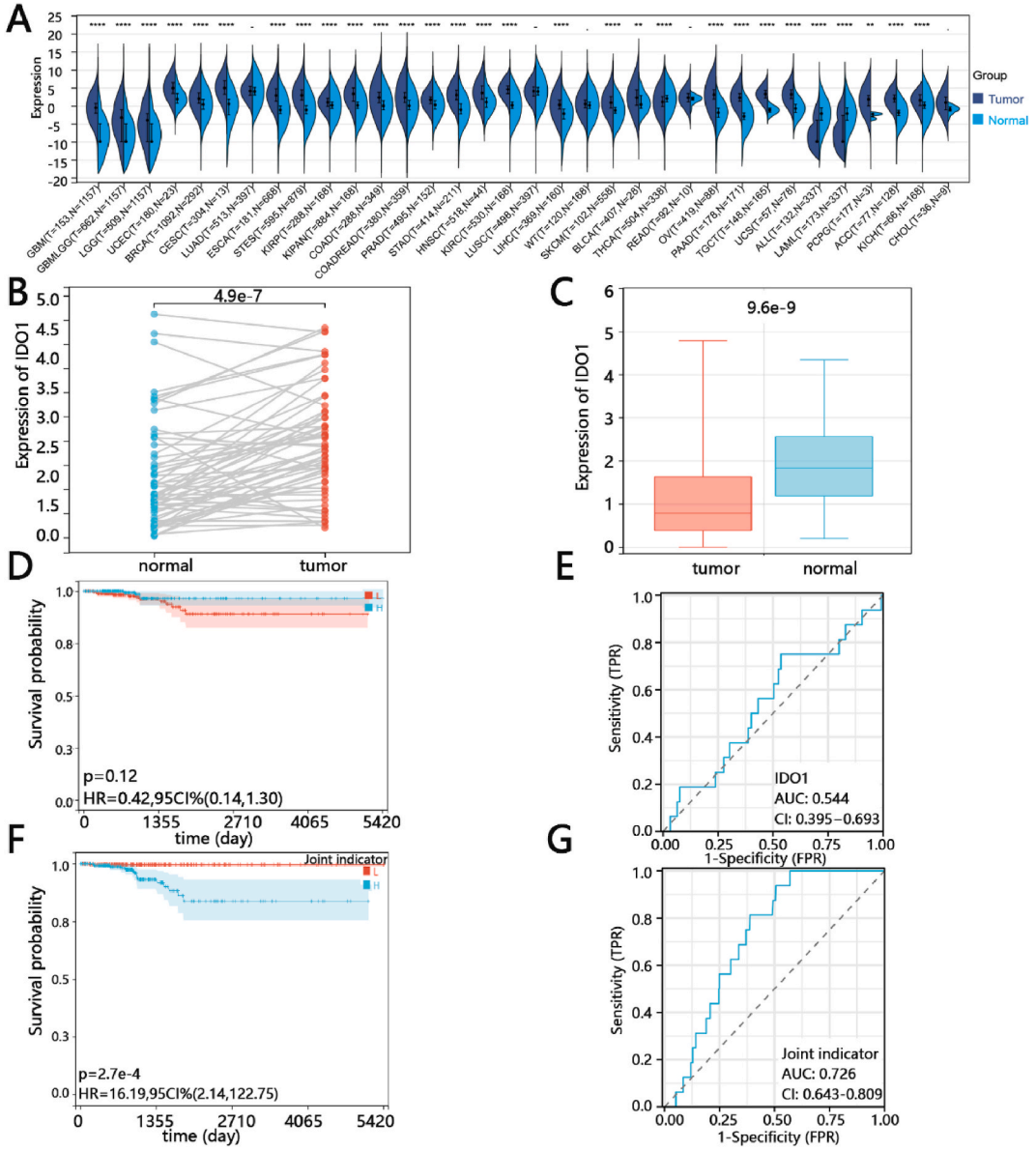



**Fig. 7.** The expression of IDO1 in pan-cancer (A). The comparison of matched pairs of the expression the normal and the tumor (B). The comparison of the expression between the normal and the tumor (C). The survival analysis of the IDO1 in patients with THCA (D). The ROC analysis of the IDO1 (E). The survival analysis of the IDO1 in patients with THCA (F). The ROC analysis of the joint indicators (G). GBM: Esophageal carcinoma; GBMLGG: Glioblastoma multiforme; LGG: Acute Myeloid Leukemia; UCEC: Thymoma; BRCA: Bladder Urothelial Carcinoma; CESC: Breast invasive carcinoma; LUAD: Liver hepatocellular carcinoma; ESCA: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; STES: Stomach adenocarcinoma; KIRP: Kidney renal clear cell carcinoma; KIPAN: Kidney Chromophobe; COAD: Cholangio carcinoma; COADREAD: Colon adenocarcinoma; PRAD: Pheochromocytoma and Paraganglioma; STAD: Skin Cutaneous Melanoma; HNSC: lioblastoma multiforme; KIRC: Kidney Chromophobe; LUSC: Lung adenocarcinoma; LIHC: Brain Lower Grade Glioma; WT: Uveal Melanoma; SKCM: Sarcoma; BLCA: Adrenocortical carcinoma; THCA: Testicular Germ Cell Tumors; READ: Prostate adenocarcinoma; OV: Mesothelioma; PAAD: Ovarian serous cystadenocarcinoma; TGCT: Stomach adenocarcinoma; UCS: Uterine Carcinosarcoma; ALL: Adrenocortical carcinoma; LAML: Kidney renal papillary cell carcinoma; PCPG: Pancreatic adenocarcinoma; ACC: Adrenocortical carcinoma; KICH: Head and Neck squamous cell carcinoma; CHOL: Cervical squamous cell carcinoma and endocervical adenocarcinoma. The '*' represents the significant difference between the tumor group and the normal group. L represents low joint indicator (expression) group. H represents high joint indicator (expression) group. AUC: Areas Under Curve; CI: Confidence Interval. HR: Hazard Ratio.

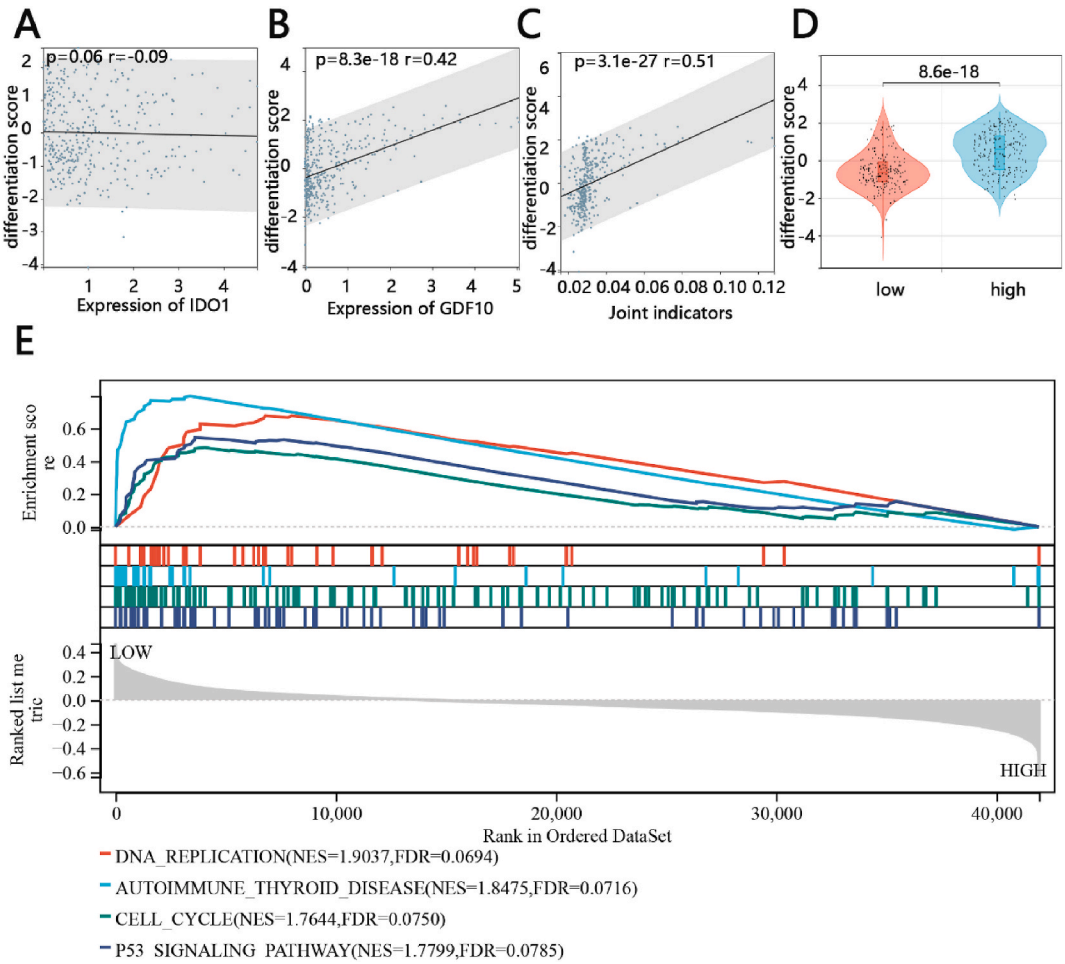



**Fig. 8.** The correction between the expression of IDO1 and THCA differentiation score (A). The correction between the expression of GDF10 and THCA differentiation score (B). The correction between the joint indicator and THCA differentiation score (C). The comparison of the differentiation scores between the high joint indicators and the low joint indicators (D). The GAEA results (E). r: correlation coefficient.

### *3.12. Results of the gene-drug and drug sensitivity*

The interaction network of the gene-drug was constructed. The results showed that there were 53 nodes and 58 edges. Valproic Acid, (6-(4-(2-piperidin-1-ylethoxy)-3-pyridin-4-ylpyrazolo (1,5-a) pyrimidine, trichostatin A, Nickel, decitabine, 4-(5-benzo (1,3) dioxol-5-yl-4-pyridin-2-yl-1H-imidazole-2-yl) benzamide, entinostat were the common drug of the two genes (Fig. 10A). Genes associated with the sensitivity and resistance of cancer drugs have been widely studied. The sensitivity between hub gene and cancer drugs was explored based on the CSCA online databases. The results showed that there was a positive correlation between the expression of GDF10 and PD−0325901, RDEA119, Trametinib,17−AAG, and Docetaxel ($p < 0.01$). However, the expression of the GDF10 was negatively related with AR−42, Docetaxel, Methotrexate, NPK76−II−72−1, THZ−2−102−1, WZ3105, BIX02189, BMS345541, BX−912, Belinostat, CAY10603, CP466722, CX−5461, GSK1070916, Genentech Cpd 10, JW−7−24−1, KIN001−102, KIN001−244, MPS−1−IN−1, PIK−93, QL−X−138, SB52334, SNX−2112, TPCA−1, YM201636, ZSTK474. The expression of IDO1 was positively related with AR−42, Methotrexate, NPK76−II−72−1, THZ−2−102−1, and WZ3105, while was negatively related with 17−AAG and Docetaxel (Fig. 10 B).

## 4. Discussion

THCA has emerged as the predominant neoplastic condition affecting the endocrine system, with a notable rise in diagnostic rates in recent years. Despite advancements in precision medicine, the prognosis for THCA patients has remained unsatisfactory thus far. Consequently, there is an imperative need to identify novel biomarkers that can enhance the prognostic accuracy and outcomes for individuals afflicted with THCA. The immune system plays a significant role in both the development and treatment of tumors. However, there has been a scarcity of research focused on immune-related prognosis genes. Therefore, conducting a screening of

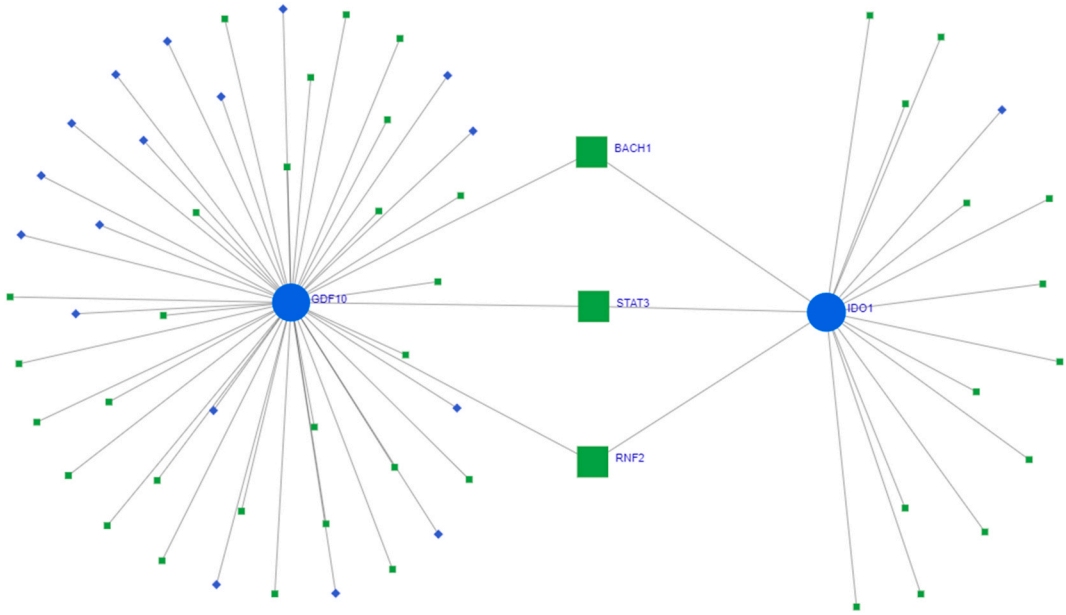



**Fig. 9.** Interactions among hub genes and miRNAs or TF. The blue circle represents the key gene. The blue square represents miRNA. The green square represents TF. TF: transcription factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

immune-related prognosis genes and analyzing their correlation with immune cell infiltration could provide a novel perspective for enhancing the prognosis of patients diagnosed with THCA. One hub gene, GDF10, was identified based on the analysis of three gene sets. Subsequently, an immune infiltration analysis was conducted, revealing a positive correlation between GDF10 expression and the presence of six immune cell types, namely B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. Furthermore, this study also investigated the association between the expression of GDF10 and immune checkpoint genes. The findings revealed a significant correlation between IDO1 and GDF10, indicating that IDO1 plays a crucial role as an immune checkpoint. Additionally, both genes were found to be associated with the prognosis of patients with THCA.

GDF10, growth differentiation factors 10, is a member of the transforming growth factor superfamily [15]. Many researches indicated that there was a correlation between the expression of GDF10 and the human disease. For example, it involved in the process of anti-inflammatory of N-methyl-D-aspartate receptor and relief never injury in neuropathic pain [16,17]. GDF10 have the function of maintain homeostasis and diminishing cardiovascular risk [18]. It also plays a significant role in the development of tumor, and has different function in different tumor. In some cancers including lung cancer [19,20], pleural mesothelioma [21], and breast cancer [22], the expression of GDF10 were lower than in normal tissue. And it plays a critical role in the proliferation and epithelial to mesenchymal transition of tumor cells in oral squamous cell cancer and triple-negative breast cancer [23,24]. Those researches indicate that GDF10 acts as a tumor suppressor in mammary epithelial cells that limits proliferation and inhibit EMT. In this study, we found that it also was lower in THCA tumor than in normal tissue, and the THCA patients with low expression had high survival rate. The findings additionally indicated a positive correlation between the expression of GDF10 and the thyroid differentiation score, which serves as an index for evaluating tumor differentiation. Previous studies have demonstrated that patients with higher thyroid differentiation scores exhibit more favorable prognoses [25,26]. The low expression of GDF10 is not conducive to cellular differentiation, but it is advantageous in inhibiting cell proliferation and the epithelial-mesenchymal transition (EMT) process. This hypothesis requires additional experimental validation in subsequent studies.

The dynamic nature and substantial functional role of immune infiltration, immune cells, immune checkpoints, and tumor microenvironment contribute significantly to tumor development [27]. The immune-related analyses were showed that the expression of GDF10 was positively correlated with B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, dendritic cells. Activated peripheral blood $CD8^+$ T cells can enter the tumor microenvironment, kill tumor cells and inhibit the proliferation of tumor cells. The activating process is performed via circulating $CD4^+$ T cells target tumor cell surface antigens [28,29]. The key immune checkpoint IDO1 was screened.

IDO1, Indoleamine 2,3-dioxygenase 1, IDO1 is helpful for immunosuppressive tumor and tumor immune escape depending on it's the function of catalysis and signal. It catalyzed the commitment and rate-limiting step of the kynurenines (KYN) metabolic pathway that produces the endogenous aryl hydrocarbon receptor (AhR) agonist KYN which apply to IDO + DCs and T cells, converting them into regulatory cells that mediate T cell inhibition function in tumor proliferation, tumor apoptosis and tumor differentiation [30–32]. Its expression is related to significantly worse clinical prognosis and reduced overall survival in many tumors [33]. Moretti et al. indicated that IDO1 play an important role in the suppression of the immune system at the level of thyroid carcinoma microenvironment via inhibiting the proliferation of activated T lymphocytes [34].

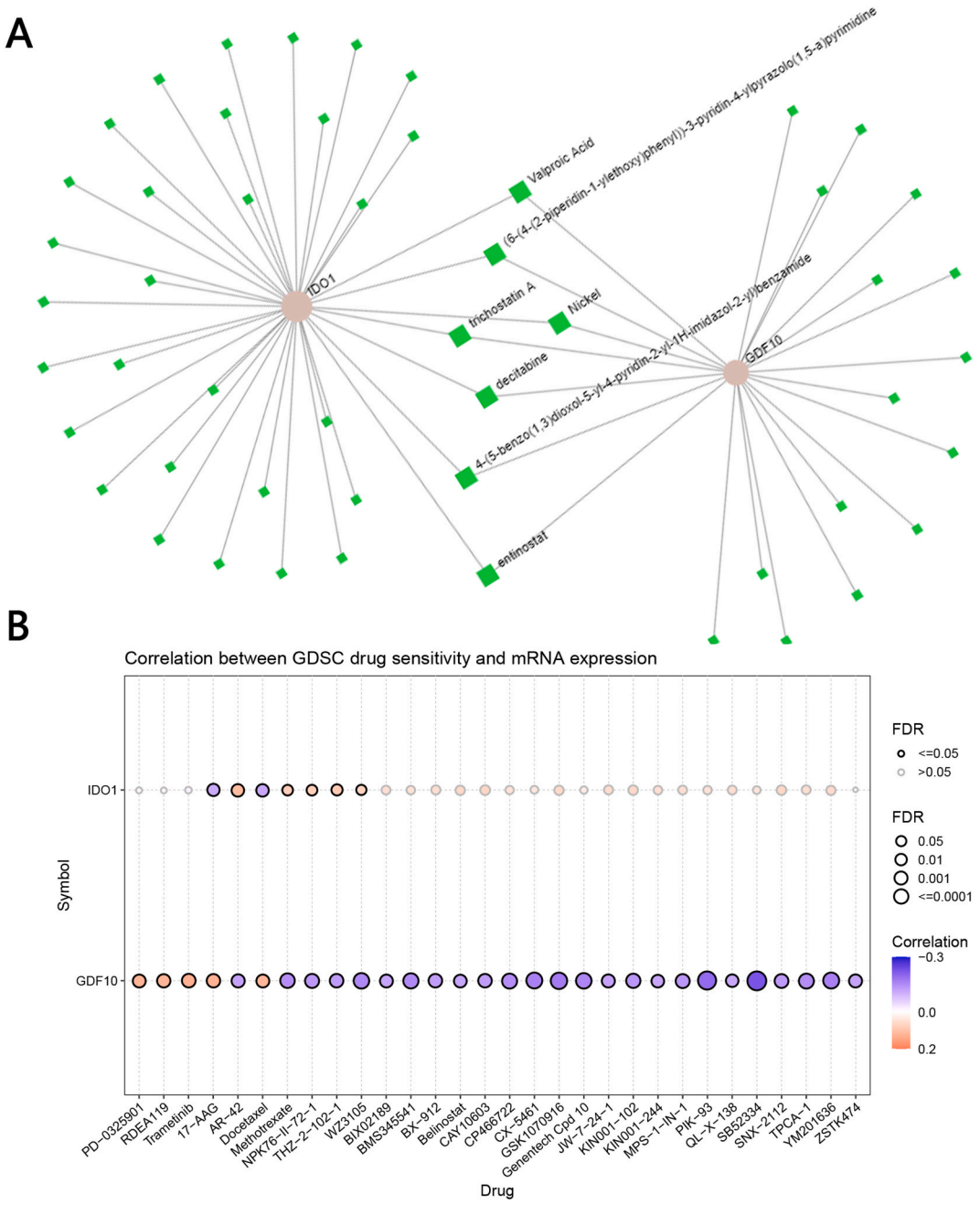



**Fig. 10.** Interactions between hub genes and drug (A). The drug sensitivity of the hub gene (B). FDR: False discovery rate.

In order to assess the joint function of both GDF10 and IDO1, the joint indicators were used to computed. The GSEA results showed that the P53 signal pathway and cell cycle were enriched in the low joint indicator group that had lower TDS than in high joint indicator group. Those results indicated that two genes have relationship with the tumor proliferation and differentiation in THCA tumor, further effect the prognosis of the patients with THCA.

Although our study has identified the hub immune-related prognosis gene GDF10 and immune checkpoint IDO1 and improved understanding of them in THCA, there are still some limitations as follows due to practical conditions. First, we could not clearly evaluate their function in subtypes of THCA. Second, the study is without further validation in clinical samples. Third, the study lacks the experimental validation to verify the function of hub genes in vivo and in vitro.

## 5. Conclusion

The results indicated that immune-related gene GDF10 and immune checkpoint IDO1 are important for prognosis prediction of THCA patients, and immunity involved in the proliferation, differentiation of tumor cells.

## Data availability

Data will be made available on request.

## Ethics approval

The Ethics Committee of The Second Affiliated Hospital Zhejiang University School of Medicine deemed that this research is based on open-source data, so the need for ethics approval was waived.

## Funding

Not applicable.

## Consent to participate

Not applicable.

## Consent to publish

Not applicable.

## CRediT authorship contribution statement

**Zhao-bao Lv:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Jun-jing Zhang:** Writing – original draft, Methodology. **Cheng Xiang:** Writing – review & editing, Writing – original draft, Formal analysis.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

[1] H. Sung, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca - Cancer J. Clin. 71 (3) (2021) 209–249.
[2] M.E. Cabanillas, D.G. McFadden, C. Durante, Thyroid cancer, Lancet 388 (10061) (2016) 2783–2795.
[3] C. Allemani, et al., Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2), Lancet 385 (9972) (2015) 977–1010.
[4] D. Jia, et al., Mining TCGA database for genes of prognostic value in glioblastoma microenvironment, Aging (Albany NY) 10 (4) (2018) 592–605.
[5] S. Yang, et al., Identification of a prognostic immune signature for cervical cancer to predict survival and response to immune checkpoint inhibitors, OncoImmunology 8 (12) (2019) e1659094.
[6] F. Mehraban, et al., Personalizing the safe, appropriate and effective concentration(s) of ozone for a non-diabetic individual and four type II diabetic patients in autohemotherapy through blood hemoglobin analysis, J. Transl. Med. 17 (1) (2019) 227.
[7] S. Shen, et al., Development and validation of an immune gene-set based Prognostic signature in ovarian cancer, EBioMedicine 40 (2019) 318–326.
[8] J. Liu, et al., Exploration of a novel prognostic risk signatures and immune checkpoint molecules in endometrial carcinoma microenvironment, Genomics 112 (5) (2020) 3117–3134.
[9] D. Ahn, et al., Clinical relationship between Hashimoto's thyroiditis and papillary thyroid cancer, Acta Oncol. 50 (8) (2011) 1228–1234.
[10] D. Repplinger, et al., Is Hashimoto's thyroiditis a risk factor for papillary thyroid cancer? J. Surg. Res. 150 (1) (2008) 49–52.
[11] Y.K. Chen, et al., Cancer risk in patients with Hashimoto's thyroiditis: a nationwide cohort study, Br. J. Cancer 109 (9) (2013) 2496–2501.
[12] S. Xu, et al., Prevalence of Hashimoto thyroiditis in adults with papillary thyroid cancer and its association with cancer recurrence and outcomes, JAMA Netw. Open 4 (7) (2021) e2118526.
[13] G. Li, et al., miR-142-3p encapsulated in T lymphocyte-derived tissue small extracellular vesicles induces Treg function defect and thyrocyte destruction in Hashimoto's thyroiditis, BMC Med. 21 (1) (2023) 206.
[14] N. Cancer Genome Atlas Research, et al., Integrated genomic characterization of endometrial carcinoma, Nature 497 (7447) (2013) 67–73.
[15] N. Mecklenburg, et al., Growth and differentiation factor 10 (Gdf10) is involved in Bergmann glial cell development under Shh regulation, Glia 62 (10) (2014) 1713–1723.
[16] S. Echeverry, et al., Transforming growth factor-beta1 impairs neuropathic pain through pleiotropic effects, Mol. Pain 5 (2009) 16.
[17] L. Jiang, et al., Decrease of growth and differentiation factor 10 contributes to neuropathic pain through N-methyl-D-aspartate receptor activation, Neuroreport 28 (8) (2017) 444–450.
[18] K. Addley, Change in Sunday licensing laws and the effect on Monday absenteeism. A short report on a poultry processing plant, Ulster Med. J. 58 (2) (1989) 137–139.
[19] Z. Dai, et al., Bone morphogenetic protein 3B silencing in non-small-cell lung cancer, Oncogene 23 (20) (2004) 3521–3529.
[20] M. Tandon, et al., Runx2 mediates epigenetic silencing of the bone morphogenetic protein-3B (BMP-3B/GDF10) in lung cancer cells, Mol. Cancer 11 (2012) 27.

[21] K. Kimura, et al., The aberrant promoter methylation of BMP3b and BMP6 in malignant pleural mesotheliomas, Oncol. Rep. 20 (5) (2008) 1265–1268.
[22] G. Upadhyay, et al., Stem cell antigen-1 enhances tumorigenicity by disruption of growth differentiation factor-10 (GDF10)-dependent TGF-beta signaling, Proc. Natl. Acad. Sci. U.S.A. 108 (19) (2011) 7820–7825.
[23] C.W. Cheng, et al., Loss of GDF10/BMP3b as a prognostic marker collaborates with TGFBR3 to enhance chemotherapy resistance and epithelial-mesenchymal transition in oral squamous cell carcinoma, Mol. Carcinog. 55 (5) (2016) 499–513.
[24] T. Zhou, et al., GDF10 inhibits proliferation and epithelial-mesenchymal transition in triple-negative breast cancer via upregulation of Smad7, Aging (Albany NY) 11 (10) (2019) 3298–3314.
[25] Y.R. Su, et al., Factors influencing postoperative anxiety and depression following Iodine-131 treatment in patients with differentiated thyroid cancer: a cross-sectional study, World J. Psychiatr. 13 (7) (2023) 486–494.
[26] H. Li, et al., Nodal metastases associated with fusion oncogenes are age dependent in young adult patients with thyroid cancer, J. Clin. Endocrinol. Metab. 109 (1) (2023) 143–150.
[27] D. Zeng, et al., Macrophage correlates with immunophenotype and predicts anti-PD-L1 response of urothelial cancer, Theranostics 10 (15) (2020) 7002–7014.
[28] L. Zhou, M.M. Chong, D.R. Littman, Plasticity of CD4+ T cell lineage differentiation, Immunity 30 (5) (2009) 646–655.
[29] R. Zander, et al., CD4(+) T cell help is required for the formation of a cytolytic CD8(+) T cell subset that protects against chronic infection and cancer, Immunity 51 (6) (2019) 1028–1042 e4.
[30] R. Schwarcz, The kynurenine pathway of tryptophan degradation as a drug target, Curr. Opin. Pharmacol. 4 (1) (2004) 12–17.
[31] U. Grohmann, F. Fallarino, P. Puccetti, Tolerance, DCs and tryptophan: much ado about Ido, Trends Immunol. 24 (5) (2003) 242–248.
[32] M.T. Pallotta, et al., Indoleamine 2,3-dioxygenase 1 (Ido1): an up-to-date overview of an eclectic immunoregulatory enzyme, FEBS J. 289 (20) (2022) 6099–6118.
[33] E. Panfili, et al., The catalytic inhibitor epacadostat can affect the non-enzymatic function of Ido1, Front. Immunol. 14 (2023) 1134551.
[34] S. Moretti, et al., Signal transducer and activator of transcription 1 plays a pivotal role in RET/PTC3 oncogene-induced expression of indoleamine 2,3-dioxygenase 1, J. Biol. Chem. 292 (5) (2017) 1785–1797.