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Chemokine CCL14 affected the clinical outcome and correlated with immune infiltrates in thyroid carcinoma

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Summary. Background. As an important member of the chemokines, CCL14 plays a vital role in cancer progression. However, the role of CCL14 in THCA has not been investigated. This study aimed to reveal the clinical significance of CCL14 in THCA.

Material and Methods. This study evaluated the expression and prognostic value of CCL14 in THCA. Also, the correlation between CCL14 and immune infiltrates was assessed. Enrichment analysis was finally performed to predict CCL14-associated pathways involved in THCA.

Results. The mRNA and protein expressions of CCL14 in THCA tissues were down-regulated compared with normal tissues. CCL14 high expression predicted favorable DFI and PFI but did not influence the DSS and OS. Further, CCL14 showed a good prediction performance on the PFI of patients. Enrichment analysis found that CCL14 was negatively correlated with migration-related pathways such as Notch signaling, ECM-receptor interaction, and cell adhesion molecules. Further, we found that CCL14 was negatively related to immune infiltrates and their gene markers. A negative relationship was also observed between CCL14 and immune checkpoint genes. These results implied the potential effect of CCL14 on the immune response and immune therapy in THCA.

Conclusions. CCL14 high expression prolonged the DFI and PFI of THCA patients. It was negatively correlated with the migration-related pathways, suggesting that CCL14 might participate in the recurrence of THCA. Further, CCL14 was also shown to be important in immune response and immune therapy in THCA.

Key words: CCL14, Chemokine, Thyroid carcinoma, Expression, Survival, Pathway

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Introduction

Thyroid carcinoma (THCA) is the most prevalent malignancy of the endocrine system, accounting for approximately 1-2% of malignancies and mainly consists of papillary carcinoma, follicular carcinoma, undifferentiated carcinoma, and medullary carcinoma (Liu et al., 2019). With the advancement of medical technology, the clinical prognosis of THCA patients has been improved significantly after therapy. According to the statistics, the incidence rate of THCA in China is approximately 1/100,000-3/100,000, and therefore it is also one of the fastest-growing malignant solid tumors (Zhou et al., 2019). In addition, some THCA patients develop the highly aggressive metastatic disease without the precise clinicopathological diagnosis assay, which decreases the quality of life of THCA patients and increases the mortality rate (Li et al., 2021b). Hence, it is necessary to develop and improve effective diagnostic methods for THCA patients based on the conventional diagnostic method.

Chemokines are defined as a cytokine superfamily with chemotactic and pro-inflammatory characteristics that induce directed chemotaxis and regulate the migration of immune cells during inflammation response (Nakharuthai and Srisapoome, 2020). Generally, chemokines exert their various biological functions by activating 7-transmembrane-domain G protein-coupled receptors on their target cells (Murphy et al., 2000). Currently, chemokines are identified and mainly classified into four categories: CXC, CC, CX3C, and C depending on their cysteine motif (Mizoguchi et al., 2018). Chemokines can regulate immune cell trafficking, the development of stroma and play a key role in inflammation (Mehraj et al., 2022). They also regulate the proliferation, cancer stem-like cell properties, cancer invasiveness and metastasis of cancer cells, which directly and indirectly affect tumor immunity and

Abbreviations. CCL14, C-C motif chemokine ligand 14; THCA, thyroid carcinoma; DFI, disease-free interval; PFI, progression-free interval; DSS, disease-specific survival; OS, overall survival.

influence cancer progression, tumor therapy and patient outcomes (Jiang et al., 2021). It follows that chemokines play a vital role in cancer progression.

The CC chemokine ligand 14 (CCL14) belongs to the CC chemokines family, which is considered as a chemoattractant of monocytes, eosinophils, T lymphoblasts, and neutrophils (Nagarsheth et al., 2017; Korbecki et al., 2020). CCR1, CCR5, and a weak affinity receptor CCR3 are the receptors of CCL14. CCL14 can be detected broadly in various tissues including the spleen, bone marrow, liver, muscle, and gut (Schulz-Knappe et al., 1996). Li et al. suggested that CCL14 might promote angiogenesis and metastasis in breast cancer (Li et al., 2011). CCL14 also inhibits the proliferation and invasion of colon cancer cells through suppressing the formation of M2-like tumor-associated macrophages (Li et al., 2021a). Zhu et al. have demonstrated that CCL14 can inhibit tumor growth by modulating the cell cycle and promoting apoptosis in hepatocellular carcinoma (HCC) (Zhu et al., 2019). Liu et al. found that in HCC patients, CCL14 was significantly associated with overall survival and disease-free survival (Liu et al., 2021). More and more studies have demonstrated the involvement of CCL14 in cancer progression and the clinical prognosis of patients. Up to now, there has been no study reporting the role of CCL14 in THCA.

In this study, we aimed to evaluate the clinical value of CCL4 in THCA. We found that patients with low expression of CCL14 had significantly lower rates of disease-free interval (DFI) and progression-free interval (PFI). And we also investigated the potential mechanism associated with CCL14 in THCA, implying that CCL14 was significantly related to the migration-related pathways. In addition, we observed a significant correlation of CCL14 with immune infiltrates in THCA. Our findings indicated that CCL14 served as a useful biomarker in THCA and deserved further investigation.

Materials and methods

The mRNA expression analysis on CCL14

Initially, the GSCA database (http://bioinfo.life.hust.edu.cn/GSCA/#/) was used to explore the CCL14 mRNA expression level in human cancers. The GSCA database is an integrated database for genomic and immunogenomic gene set cancer analysis and it integrates over 10,000 multi-dimensional genomic data across 33 cancer types from TCGA. Further, the mRNA differential expression of CCL14 in normal and THCA samples was analyzed using the TCGA-THCA dataset which was obtained from the UCSC Xena database (https://xenabrowser.net/). The patients with papillary thyroid carcinoma were enrolled in this study.

Further, the correlation between CCL14 expression and clinical characteristics of patients was evaluated by the UALCAN database (http://ualcan.path.uab.edu/). The clinical characteristics included patients' age, race, gender, cancer stages, tumor histology, and tumor nodal metastasis status. The UALCAN is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data and it provides easy access to publicly available cancer OMICS data such as TCGA and CPTAC.

The protein expression analysis on CCL14

Moreover, we detected the protein expression of CCL14 in THCA and normal thyroid gland tissues (https://www.proteinatlas.org/). The HPA aims to map all the human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology. HPA database provides protein expression data from 44 normal human tissue types and 17 different forms of human cancer which are derived from antibody-based protein profiling using immunohistochemistry, together with millions of in-house generated immunohistochemically stained tissue section images.

Expression verification of CCL14 in vitro by qPCR

Furthermore, we verified the expression of CCL14 in normal Nthy-ori3-1 cells and THCA cells (BCPAP and TPC-1) by qPCR. All the cells were obtained from the laboratory of the pathology of Xuzhou Medical University. The cells were incubated in DMEM media, supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 1x glutamine at 37°C in 5% CO_2. We selected the cells at the logarithmic phase for further experiment. Quantitative real-time PCR was performed using SYBR Premix Ex Taq (Takara) following the manufacturer's protocol. The indicated gene expression was normalized to GAPDH by $2^{-\Delta\Delta Ct}$. Primers were designed as follows: GAPDH, Forward (5′-3′): CTGGGCTACACTGAGCACC, Reverse (5′-3′): AAGTGGTCGTTGAGGGCAATG. CCL14, Forward (5′-3′), CCAAGCCCGGAATTGTCTTCA, Reverse (5′-3′): GGGTTGGTACAGACGGAATGG.

Survival analysis on CCL14

The clinical data of THCA patients was obtained from the TCGA-THCA dataset which was from the UCSC Xena database. The influence of CCL14 expression on the survival time of THCA patients was estimated by Kaplan-Meier and log-rank test. Before survival analysis, THCA patients were divided into low and high expression groups by setting the best-cutoff value of CCL14 expression level as the grouping threshold. The best-cutoff value indicates that all possible cutoff values between the lower and upper quartiles are computed, and the best performing threshold is used as a cutoff. Then we compared the survival difference of THCA patients between 2 groups in terms of disease-specific survival (DSS), overall

survival (OS), disease-free interval (DFI), and progression-free interval (PFI).

The prognostic performance of CCL14 on the survival probability of THCA patients was predicted by receiver operating characteristic (ROC) curve analysis with measurement of area under the curve (AUC) and P value. The AUC indicated the degree of prediction performance. Further, we performed the univariable Cox regression analysis to evaluate the correlation of several covariates with DFI and PFI. The covariates included CCL14 expression level, age, gender, clinical stage, T stage, N stage, and M stage. We also performed a multivariate Cox regression analysis regarding these covariates in THCA patients to identify independent risk and protective factors for DFI and PFI with the method of ENTER.

Enrichment analysis

LinkedOmics database (http://www.linkedomics.org/login.php) was used to identify co-expressed genes of CCL14 by setting HiSeq RNA of CCL14 from TCGA-THCA as the screening condition for analysis with the Pearson correlation test, and the co-expressed genes were presented in volcano plot and heatmap, which were determined by P-value<0.05. Then WebGestalt database (http://www.webgestalt.org/) was used to interpret the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses for the top 200 co-expressed genes. GO terms were annotated by cellular component (CC), biological process (BP), and molecular function (MF). The filter criteria were as follows: method of analysis: Over-Representation Analysis (ORS); gene ID type: gene symbol; reference set: genome protein-coding; the number of genes for a category:5-2000; multiple test adjustment: BH. Subsequently, the gene set enrichment analysis (GSEA) was conducted to explore the correlation between CCL14 and significant pathways.

Immune infiltration analysis

Considering the important role of chemokines in regulating inflammatory cell infiltration, we investigated the relationship between CCL14 and the infiltration level of immune cells, including B cells, CD4+ T cells, and CD8+T cells, macrophages, neutrophils, and dendritic cells in TIMER database (https://cistrome.shinyapps.io/timer/). We further explored the correlation between CCL14 and gene markers of significant immune cells by Spearman analysis in TIMER. This study also performed the ESTIMATE algorithm to assess the proportion of immune cells and stromal cells in THCA and assessed the influence of CCL14 on the immune score and stromal score. In addition, the effect of CCL14 on the expression of immune checkpoint genes was evaluated as well. Finally, we explored the correlation of CCL14 with immune subtype and drug sensitivity in THCA in the TISIDB (http://cis.hku.hk/TISIDB/) and GSCA (http://bioinfo.life.hust.edu.cn/GSCA/#/) databases.

Statistical analysis

Statistical analyses were performed using SPSS 23.0. The quantitative data between 2 groups were compared by t-test or Mann-Whitney U test. And the one-way ANOVA or Kruskal-Wallis one-way ANOVA was used for comparing the quantitative data among multiple groups, followed by a post-hoc test. Kaplan-Meier method was used to estimate the survival rate and the log-rank test was used to compare the survival difference between groups. The ROC analysis was performed to predict the prognostic performance on survival probability. Cox regression analyses were conducted to estimate the simultaneous effects of prognostic factors on survival. The correlation between 2 variables was analyzed by Spearman or Pearson method. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The expression analysis on CCL14

In this study, we first investigated the CCL14 mRNA expression level in different types of human cancers through the GSCA database. As shown in Fig. 1A, the mRNA expression of CCL14 was down-regulated in almost all cancers. In THCA, the downregulation of CCL14 was observed compared with in normal samples (Fig. 1B). Expression analysis on paired samples also showed the downregulation of CCL14 in THCA (Fig. 1C). We also verified the expression of CCL14 in THCA and normal samples by qPCR. The results (Fig. 1D) showed that the mRNA expression of CCL14 in normal Nthy-ori3-1 cells was statistically higher than in TPC-1 (P=0.0005) and BCPAP cells (P=0.0090). The immunohistochemistry Images from the HPA database presented that the protein staining of CCL14 in normal glandular cells was medium, while in tumor cells it was low (Fig. 1E). These results suggested the downregulation of CCL14 in thyroid carcinoma.

Then we evaluated the association between CCL14 mRNA expression and clinicopathological parameters of THCA patients (Fig. 2). We found that the expression of CCL14 was not related to the patient's age, gender, race, and nodal metastasis status. However, cancer stages and disease subtypes were associated with CCL14 expression. A higher expression of CCL14 was observed in patients with stage 2 and follicular subtypes.

Prognosis analysis on CCL14

The above results showed the abnormal expression of CCL14 in THCA. We then estimated the effects of the expression level of CCL14 on the survival of THCA patients by the Kaplan-Meier analysis (Fig. 3). The

survival analysis showed that the expression of CCL14 did not influence the disease-specific survival (P=0.06) and overall survival (P=0.37). However, higher expression of CCL14 prolonged the disease-free interval (P=0.036) and progression-free interval (P<0.001) time of THCA patients. It followed that the expression of CCL14 was significantly related to the recurrence of THCA. Differentiated thyroid cancer has an excellent prognosis and lower mortality than other cancers, with disease-specific survival of up to 90% at 10-year follow-up. However, the recurrence rate in the neck ranges from 20% to 59%, varying according to the risk of recurrence.

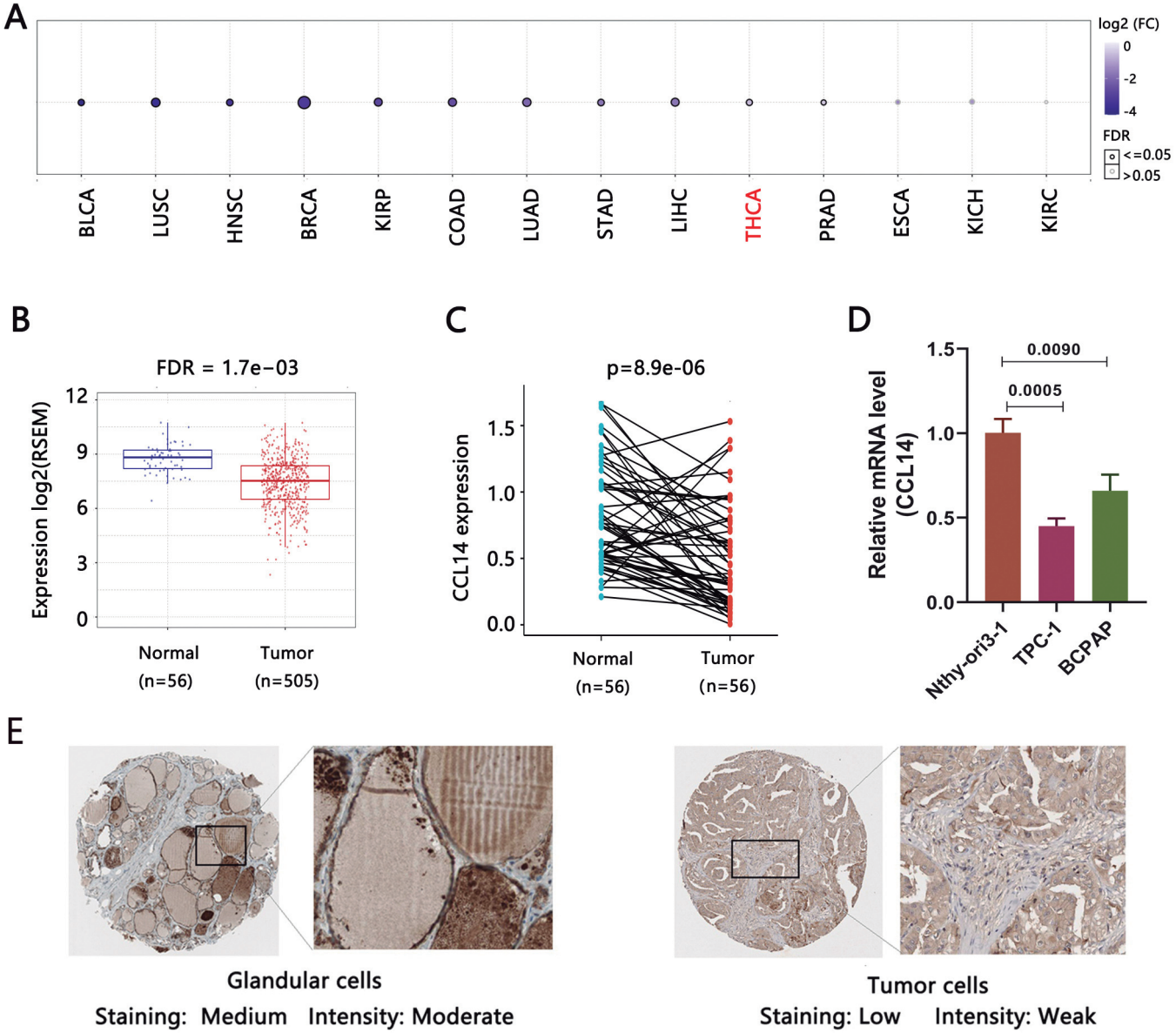

Fig. 1. The expression analysis of CCL14. **A.** The mRNA expression of CCL14 in pan-cancer in GSCA database. **B.** The mRNA expression of CCL14 in normal and THCA tissues based on the TCGA data. The RSEM indicated the calculation method of relative gene expression level. **C.** The mRNA expression of CCL14 in tumor and pericarcinomatous tissues in THCA based on the TCGA data. **D.** The mRNA expression verification of CCL14 in normal and THCA cells by qPCR. **E.** The protein expression of CCL14 in normal and THCA tissues based on the HPA database. BLCA, Bladder Urothelial Carcinoma; LUSC, Lung squamous cell carcinoma; HNSC, Head and Neck squamous cell carcinoma; BRCA, Breast invasive carcinoma; KIRP, Kidney renal papillary cell carcinoma; COAD, Colon adenocarcinoma; LUAD, Lung adenocarcinoma; STAD, Stomach adenocarcinoma; LIHC, Liver hepatocellular carcinoma; THCA, Thyroid carcinoma; PRAD, Prostate adenocarcinoma; ESCA, Esophageal carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma. FC, fold change; FDR, false discovery rate. The P value or FDR <0.05 was considered to indicate a statistically significant difference.

Therefore, this study was more concerned with the recurrence and deterioration of the disease.

The prediction performance of CCL14 on the survival probability of THCA patients was further estimated by ROC analysis (Fig. 4). The results showed that CCL14 possessed favorable prediction performance regarding progression-free interval (AUC=0.652, P<0.001) of THCA patients, which further showed the significant prediction role of CCL14 on the development of THCA. But the prediction performance of CCL14 in terms of disease-free interval was not found (AUC=0.591, P=0.114).

Subsequently, we used the univariate Cox regression to explore the prognosis-related factors (Table 1). The univariate analysis showed that CCL14 expression, age, clinical stage, T stage, N stage, and M stage were related to the DFI of THCA patients. However, only the independent prognostic value of the M stage was observed in multivariate analysis. In terms of PFI, CCL14 expression, age, clinical stage, T stage, and M stage were associated with the clinical outcome of patients, but we found no independent prognostic value for any variables in multivariate analysis.

Association between CCL14 and immune cell infiltration in THCA

Tumor-infiltrating lymphocytes (TIL) were defined as an important factor influencing tumor development. Thus, we analyzed the correlation between the expression of CCL14 and common immune cell infiltration in THCA by the TIMER database (Fig. 5). The results showed that CCL14 expression was negatively correlated with CD4+ T cells (P<0.05), neutrophils (P<0.01), and dendritic cells (P<0.001).

We further explored the relationship between CCL14 expression and marker genes of immune cells including neutrophils, dendritic cells, and exhausted T cells in THCA (Table 2). The analysis results showed that CCL14 expression was negatively correlated with the expression of neutrophil marker (CD11b), and all the dendritic cell makers. CCL14 also had a negative association with the exhausted T cell makers (PDL-1, CTLA4). These results suggested that CCL14 was closely related to the immune response and tumor immune escape in THCA.

We then used the ESTIMATE algorithm to assess the

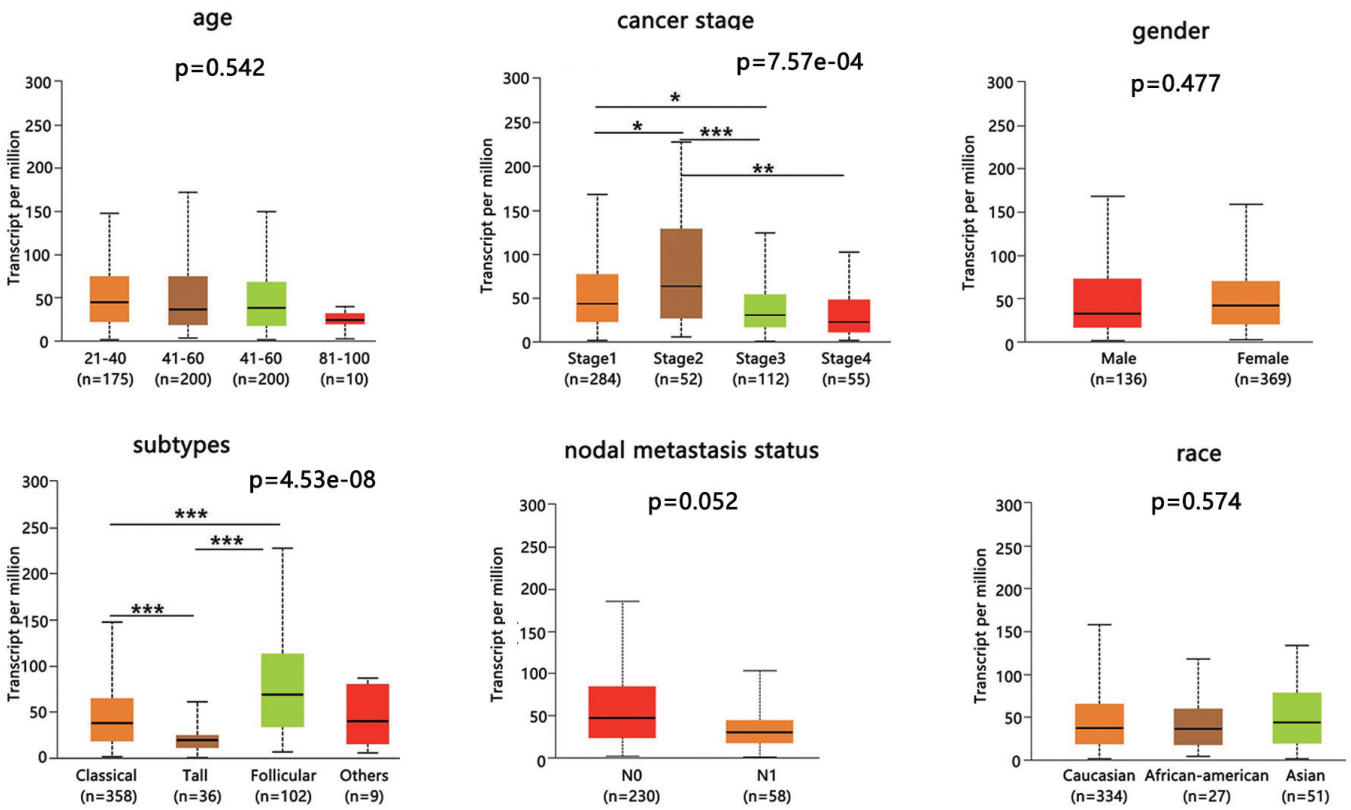

Fig. 2. The correlation between CCL14 mRNA expression and clinicopathological parameters of THCA patients in the UALCAN database based on TCGA data. *P<0.05, **P<0.01, ***P<0.001. P<0.05 was considered to indicate a statistically significant difference.

proportion of immune cells and stromal cells in THCA. The results in Fig. 6A demonstrate that the expression of CCL14 had a significant positive correlation with stromal scores and the stromal score in the CCL14 high expression group was higher than in the CCL14 low expression. The results further confirmed the importance of CCL14 on immune infiltrates in THCA. The above results in Table 2 have indicated the correlation of CCL14 with gene markers of exhausted T cells (PDL1 and CTLA4), and we also explored the relationship of CCL14 with immune checkpoint genes. Fig. 6B showed that CCL14 was negatively correlated with immune checkpoints including CTLA4 and PDL-1, and the CCL14 high expression group had lower expression of CTLA4 and PDL-1. These results suggested the significance of CCL14 in the immune therapy in THCA through immune checkpoint genes. A positive correlation between CCL14 and the sensitivity of chemotherapeutic drugs was also observed (Fig. 6D). However, no correlation was found between CCL14 expression and immune subtypes in THCA (Fig. 6C).

Functional annotation of CCL14

To explore the underlying functional regulation of CCL14 in THCA, we detected the co-expressed genes with CCL14 in THCA through the Linkedomics database. All the co-expressed genes were presented in Fig. 7A with a volcano plot. And the heatmap presented the top 50 positive and negative co-expressed genes associated with CCL14 (Fig. 7B).

Then, the top 200 co-expressed genes were selected for exploring the enriched GO terms and KEGG pathways (Fig. 8). For the CC category, the co-expressed genes were mainly located at the receptor complex, apical plasma membrane, cell-cell junction, and apical part of the cell. In terms of MF, they were primarily involved in protein binding of heterotypic cell-cell adhesion, regulating the activity of transmembrane receptor protein tyrosine kinase and transmembrane receptor protein tyrosine kinase activity. The significant BP mainly referred to vasculogenesis. Additionally, we explored the potential KEGG

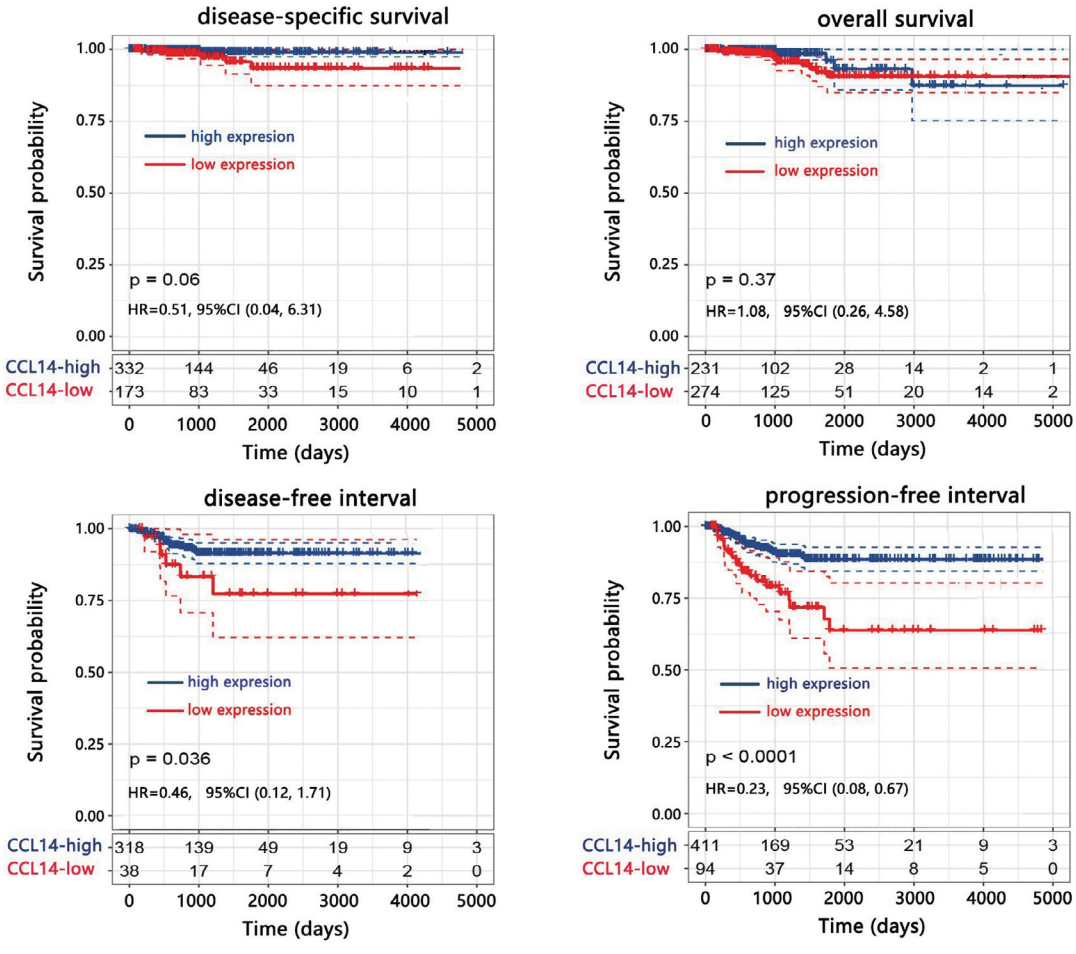

Fig. 3. The effects of CCL14 mRNA expression on the prognosis of THCA patients based on TCGA data. HR, hazard ratios; CI, confidence intervals. Disease-specific survival (DSS): The percent of people who died from a specific disease in a defined period. Patients who died from causes other than the disease being studied are not counted. Overall survival (OS): Time to death. Disease-free interval (DFI): The measure of time after treatment during which no sign of cancer is found. Progression-free interval (PFI): The length of time during and after the treatment of a disease, such as cancer, that a patient lives with the disease but it does not get worse. HR: hazard ratio. CI: confidence interval. P<0.05 was considered to indicate a statistically significant difference.

pathways, finding that these genes were enriched in the Notch signaling pathway, ECM-receptor interaction, cell adhesion molecules (CAM), and focal adhesion pathway.

Further, the correlation between CCL14 expression and related significant pathways was explored through GSEA analysis (Fig. 9). The results showed that CCL14 expression was negatively correlated with the Notch signaling pathway (NES=-1.5636, P<0.001) and cell adhesion molecules (CAM) pathway (NES=-1.9532, P<0.001).

Discussion

Thyroid carcinoma (THCA) is one of the most common malignant endocrine tumors with an increasing incidence rate annually. Although THCA patients have a relatively better prognosis compared with other solid cancers, about 10% of cases develop a more clinically aggressive and invasive phenotype of THCA, which promotes local recurrence and metastatic disease with poor prognosis (He et al., 2018). Thus, novel potential diagnostic and therapeutic biomarkers are critically needed for developing effective and accurate treatment strategies for THCA patients.

Currently, several studies reported the abnormal expression of CCL14 and its influence on cancer progression in human cancer. CCL14 expression was significantly lower in hepatocellular carcinoma and low CCL14 expression was associated with poorer disease-

Table 1. Association between clinicopathologic characteristics and patient clinical outcomes through univariate and multivariate Cox regression analysis.

Characteristics	disease-free interval (DFI)			progression-free interval (PFI)	
	HR	95% CI	P value	HR	95% CI
Univariate analysis					
CCL14 expression	0.500	0.27-0.91	0.022	0.234	0.082-0.670
Age	2.110	1.19-3.76	0.011	1.019	1.002-1.036
Gender	1.630	0.90-2.94	0.108	0.578	0.331-1.007
Clinical stage	2.600	1.48-4.59	0.001	1.529	1.21601.922
T stage	2.410	1.35-4.30	0.003	1.848	1.349-2.531
N stage	1.870	1.05-3.33	0.035	1.590	0.895-2.826
M stage	6.140	2.20-17.14	0.001	5.895	2.036-17.012
Multivariate analysis					
CCL14 expression	0.707	0.355-1.401	0.323	0.302	0.075-1.212
Age	1.454	0.668-3.167	0.236	1.024	0.911-1.057
Gender	1.144	0.571-2.193	0.704	0.840	0.382-1.845
Clinical stage	1.484	0.636-3.487	0.346	1.266	0.720-20225
T stage	1.613	0.750-3.472	0.221	1.105	0.652-1.871
N stage	1.503	0.766-2.950	0.236	0.950	0.427-2.116
M stage	3.960	1.186-13.222	0.025	2.731	0.711-10.481

CI, confidence interval; HR, hazard ratio; M, distant metastasis; N, lymph node metastasis; T, topography distribution. P<0.05 indicates statistical significance.

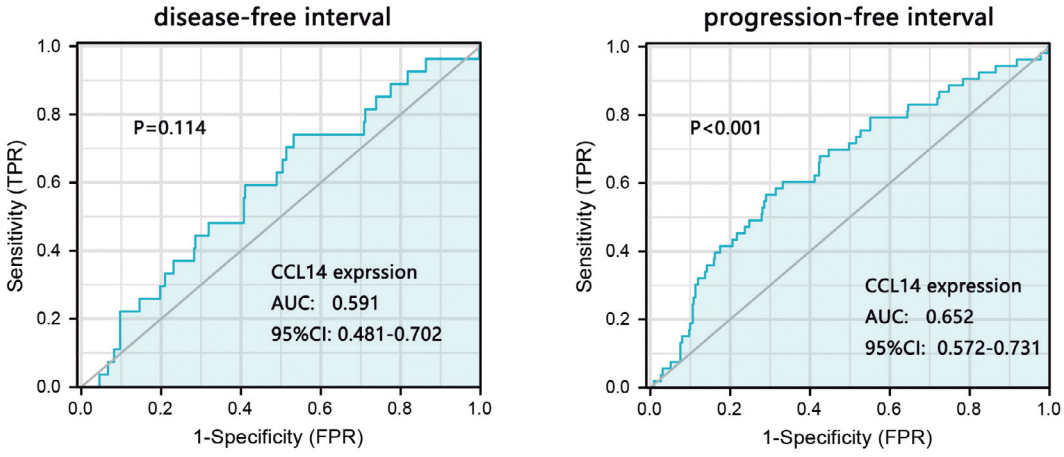

Fig. 4. The ROC curve for predicting the prognostic performance of CCL14 in THCA. ROC, receiver operating characteristic; AUC, area under the curve. CI: confidence interval. P<0.05 was considered to indicate a statistically significant difference.

specific survival, overall survival, progression-free survival, and relapse-free survival of patients (Gu et al., 2020). CCL14 was also poorly expressed in colorectal cancer, and upregulation of CCL14 suppressed the colony formation and invasiveness of tumor cells (Yan et al., 2021). In this study, we found that CCL14 mRNA expression was down-regulated in THCA samples, and low expression of CCL14 was associated with poor disease-free interval (DFI) and progression-free interval (PFI). It followed that CCL14 might be significantly related to the recurrence of THCA. The unfavorable prognostic role of CCL14 in THCA was the same as that in other cancer types (Cai et al., 2020; Li et al., 2021a). In addition, CCL14 showed better prediction performance on DFI and PFI. Unfortunately, we found no independent prognostic value of CCL14 in THCA.

Additionally, it should be noted that chemokines are capable of regulating various immune cells in the tumor

Table 2. Correlation analysis between gene markers of immune cells and CCL14 expressions in THCA.

	Marker	Correlation coefficient (no adjustment)	P value	Correlation coefficient (adjustment)	P value
Neutrophils	CD11b	-0.187	***	-0.185	***
	CCR7	-0.059	0.181	-0.056	0.213
Dendritic cell	HLA-DPB1	-0.216	***	-0.220	***
	HLA-DQB1	-0.262	***	-0.269	***
	HLA-DRA	-0.262	***	-0.258	***
	HLA-DPA1	-0.260	***	-0.262	***
	BDCA-1 (CD1C)	-0.175	***	-0.175	***
	BDCA-4 (NRP1)	-0.303	***	-0.310	***
	CD11c (ITGAX)	-0.154	***	-0.155	***
Exhausted T cell	PD-1 (PDCD1)	-0.002	0.957	-0.010	0.827
	PDL-1 (CD274)	-0.234	***	-0.223	***
	CTLA4	-0.218	***	-0.221	***

The correlation between expressions of 2 genes was analyzed by Spearman analysis with or without tumor purity adjustment. Note: ***P<0.001. P<0.05 was considered to indicate a statistically significant difference.

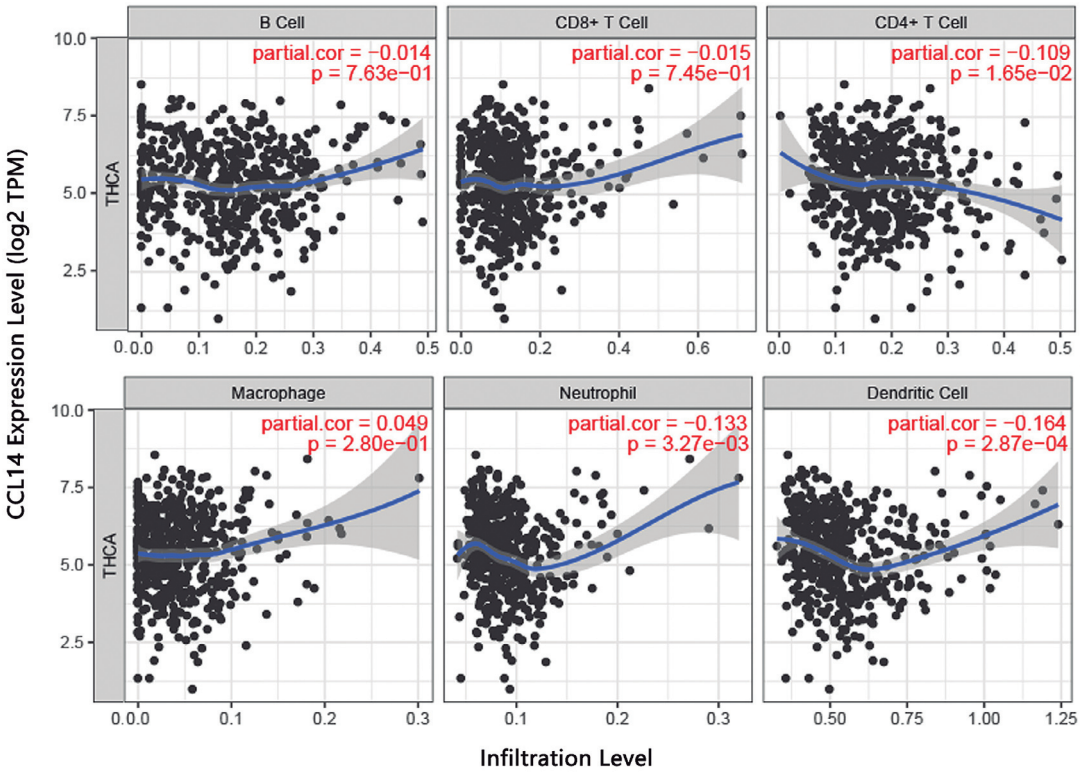

Fig. 5. The correlation between CCL14 expression and infiltration level of common immune cells in THCA by Spearman analysis in TIMER database. P<0.05 was considered to indicate a statistically significant difference.

microenvironment (Scharping and Delgoffe, 2016). In this study, we demonstrated the significant relationship between CCL14 expression and infiltration level of immune cells in THCA. We also found that there was a significant correlation between CCL14 and immune marker genes in THCA. Gu et al. found that CCL14 showed a strong correlation with tumor-infiltrating immune cells and CCL14 expression correlated with the

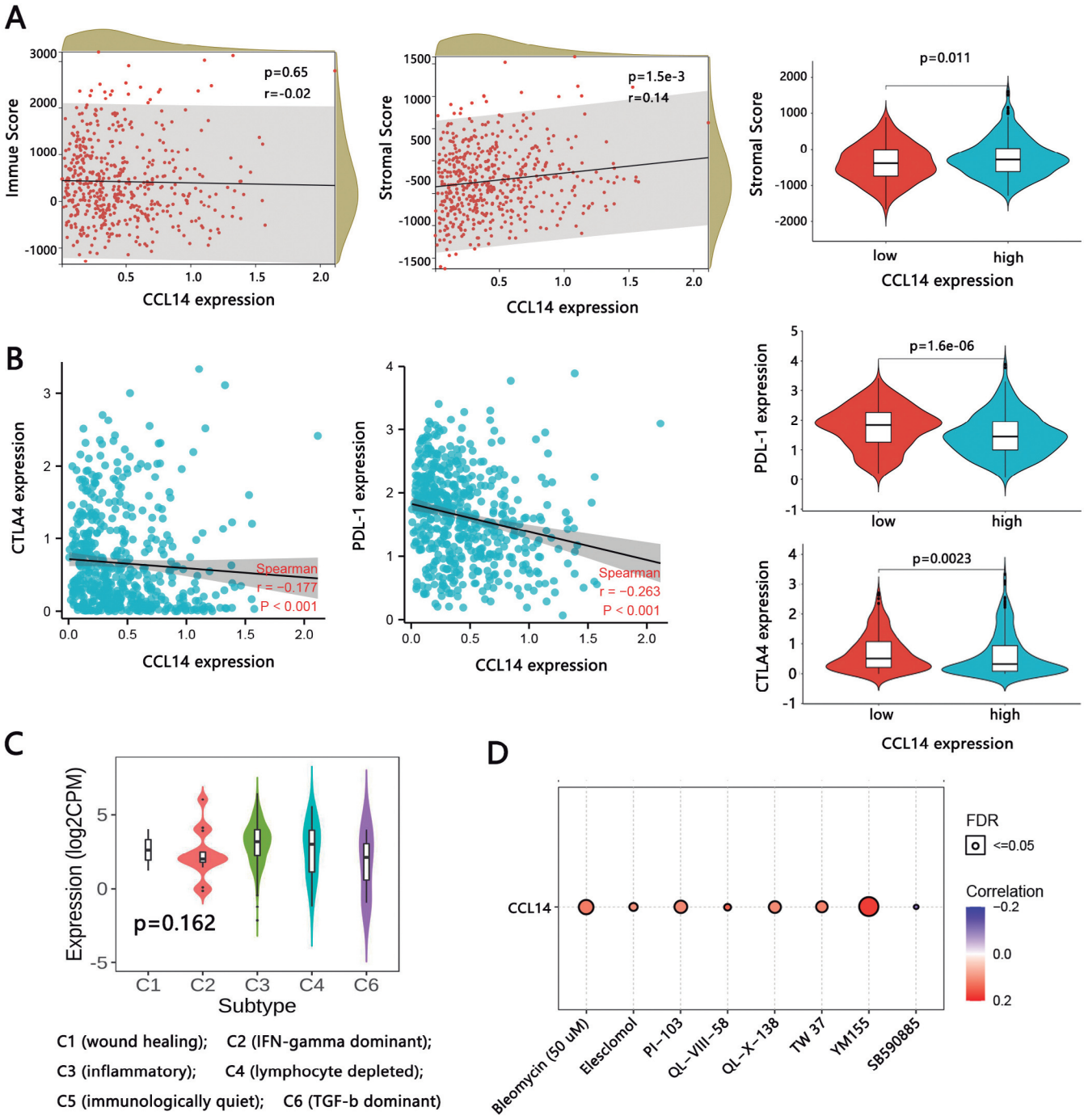

Fig. 6. Correlation analysis on CCL14 with immune infiltrates in THCA. **A.** The correlation between CCL14 expression and Immune score or stromal score. **B.** The correlation between CCL14 expression and immune checkpoint genes (PDL-1/CTLA4). **C.** Correlation between CCL14 expression and immune subtypes in THCA in TISIDB database. **D.** Correlation between GDSC drug sensitivity and CCL14 mRNA expression in GSCA database. The correlation was analyzed by the Spearman method.

expression of several immune cell markers, suggesting its role in regulating tumor immunity (Gu et al., 2020). In addition, we also observed a significant relationship between CCL14 and immune checkpoint (PDL-1, CTLA4). Immune checkpoints referred to a group of molecules present in immune cells that can participate in preventing the activation of the immune system (Li et al., 2021c). Tumor cells can inhibit the function of T cells by activating immune checkpoints, thereby achieving tumor immune escape. Typically, anti- PD-1,

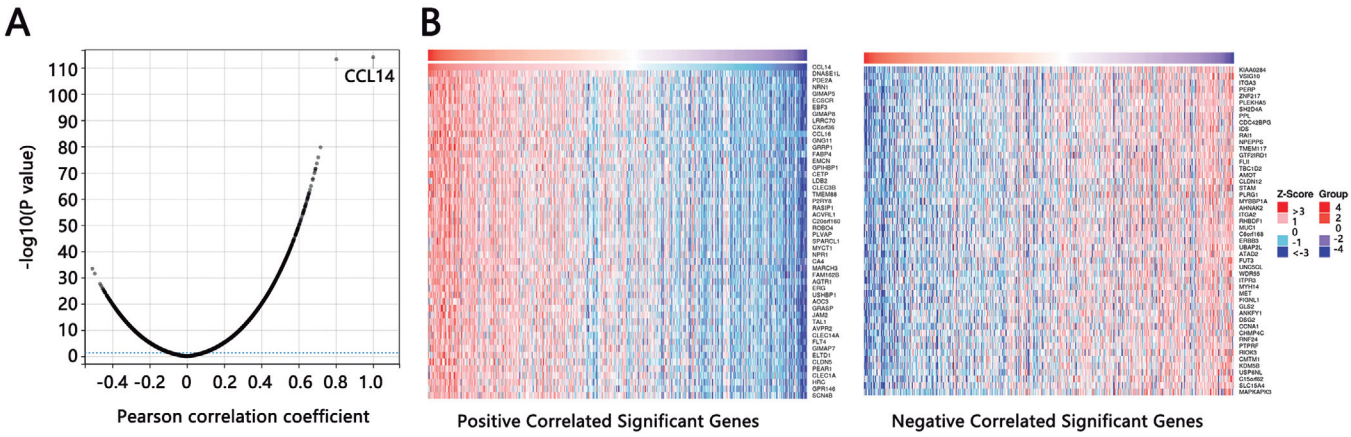

Fig. 7. The co-expressed genes of CCL14 in THCA. (A) Co-expressed genes of CCL14 were identified by Pearson correlation analysis. (B) The top 50 positively and negatively correlated genes with CCL14 in THCA in the LinkedOmics database.

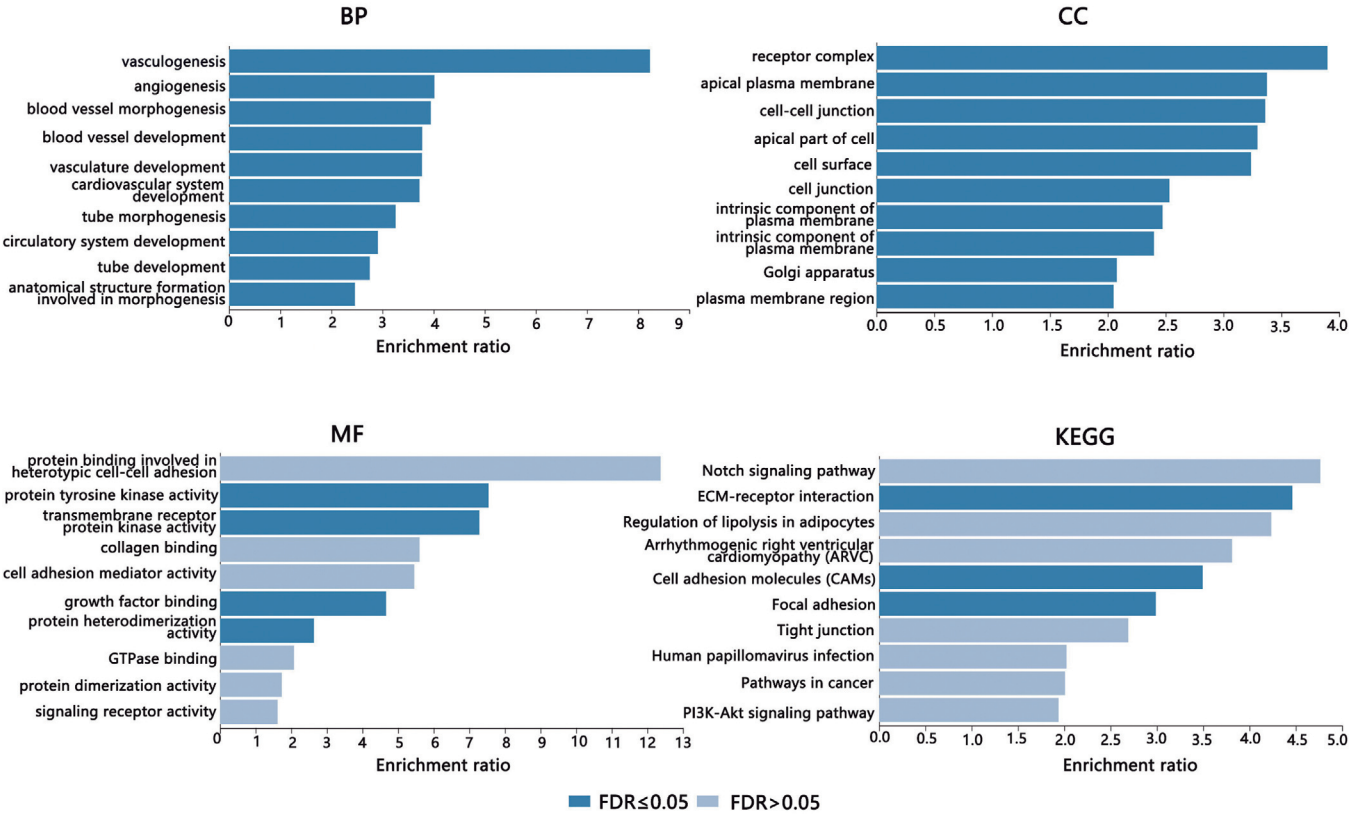

Fig. 8. The Gene Ontology (GO) and KEGG pathway analyses on co-expressed genes. CC, cellular component; BP, biological process; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

anti- PD-L1, and anti-CTLA-4 therapy were administered in the treatment of tumors (Knudsen et al., 2021). Our results indicated that CCL14 affected the expression of immune checkpoint genes, indicating that CCL14 may affect immune therapy in THCA by influencing immune checkpoint genes.

Due to the significant role of CCL14 in THCA, we also explored the potential biological process associated with CCL14. It is known that chemokines can direct the migration of leukocytes throughout the body both under physiological and inflammatory conditions (Yoshie et al., 2001). They are also involved in processes like angiogenesis, tumor growth, and metastasis (Yoshie et al., 2001). In this study, GO-BP analysis confirmed that the co-expressed genes of CCL14 in THCA were mainly involved in the blood vessel formation process. The KEGG pathway analysis showed that co-expressed genes were significantly enriched in ECM-receptor interaction and cell adhesion molecules (CAM). Previous studies have reported the involvement of ECM-receptor interaction pathways in the migration and invasion of human cancer (Zhang et al., 2016; Machackova et al., 2020). In terms of cell adhesion molecules (CAM), many cell adhesion molecules (CAM) conventionally act as tumor suppressors as they are involved in suppressing cancer cell growth. However, Moh et al. indicated the oncogenic role of CAM as they can be able to induce tumor migration (Moh and Shen, 2009). It followed that ECM-receptor interaction and cell adhesion molecules (CAM) pathways were closely related to the migration of cancers. The KEGG analysis in this study implied that CCL14 may be closely related to the migration of THCA. In addition, the majority of co-expressed genes were also enriched in the Notch signaling pathway. The abnormal activation of Notch signaling has been observed in breast cancer, non-small cell lung cancer, and blood cancers (Katoh and Katoh, 2020). Accumulating evidence has indicated that tumor progress was closely linked to aberrant Notch activation, which may induce cell proliferation, metastasis, and epithelial-mesenchymal transformation (Li et al., 2017). The pathway analysis indicated the potential of CCL14 in the migration of THCA. In our study, high expression of CCL14 correlated with a good prognosis of THCA patients, and we speculated that CCL14 may inhibit the abnormal activation of Notch signaling and CAM pathway, thus suppressing the migration of THCA. The GSEA analysis then revealed that CCL14 was negatively related to the Notch signaling pathway and migration-related pathway, which further supported our speculation. However, the potential regulation of CCL14 on the migration of THCA should be verified by function analysis *in vivo* and *in vitro* in future work.

Conclusion

This study revealed that high expression of CCL14 was favorable to the disease-free interval and progression-free interval of THCA patients, and cancer stage and disease subtypes affected the CCL14 expression. Pathway analysis found that CCL14 was negatively associated with migration-related pathways such as Notch and CAM pathways, implying that CCL14 may regulate the migration of THCA. Further, we found that CCL14 was significantly correlated with the immune infiltrates and gene markers of immune cells, and CCL14 high expression had a higher stromal score. These results suggested that CCL14 was closely related to the immune response in THCA. In addition, CCL14 was found to significantly correlate with the immune checkpoint, and CCL14 high expression had

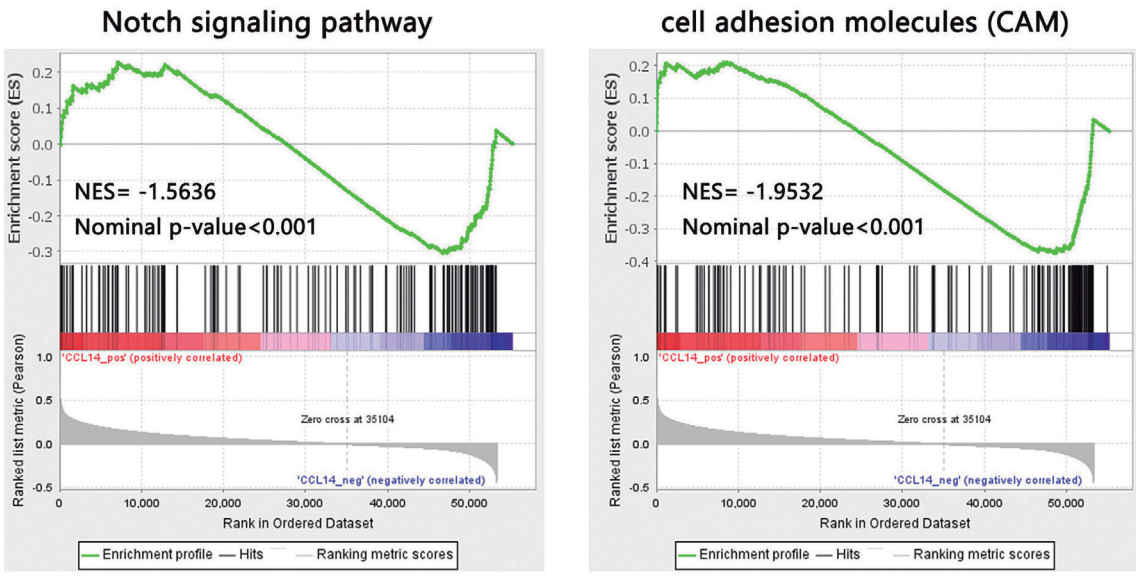

Fig. 9. Enrichment plots from GSEA analysis associated with Notch signaling pathway and cell adhesion molecules. GSEA results showed differential enrichment of CCL14 related to 2 pathways. GSEA, gene set enrichment analysis; NES, Normalized Enrichment Score.

lower expression of immune checkpoint genes, suggesting that CCL14 contributed to the immune therapy in THCA through mediating immune checkpoint genes.

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