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TGF-β2 is a Prognostic Biomarker Correlated with Immune Cell Infiltration in Colorectal Cancer

A STROBE-compliant article

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Abstract

Transforming growth factor-beta (TGF-β2) is an important cytokine regulating immune cell function. However, whether TGF-β2 controls the invasion of colorectal cancer (CRC) by immune cells is unknown. Therefore, we evaluated the expression of TGF-β2 using multiple databases and determined the relationship between TGF-β2 expression and tumor immune infiltration defined by a set of genetic markers. The analysis demonstrated that the expression of TGF-β2 is closely related to the outcome of many cancers, and this correlation was particularly strong in CRC. In addition, the increased expression of TGF-β2 was significantly associated with the expression of various markers of specific immune cell subpopulations, and overexpression of TGF-β2 was closely related to the prognosis of colon cancer patients. Moreover, TGF-β2 was related to the prognosis and infiltration of the tumor by immune cells in CRC patients. The obtained results indicate that TGF-β2 is a critical factor regulating the recruitment of immune cells and controls their infiltration into colorectal tumors. Thus, high expression of TGF-β2 not only facilitates the prognosis in CRC patients, but also may provide a new target for the treatment of CRC.

Abbreviations: CRC = colorectal cancer, ACC = adrenocortical carcinoma, COAD = colon adenocarcinoma, CTLA4 = cytotoxic T lymphocyte-associated antigen 4, DCs = dendritic cells, GEPIA = gene expression profile interactive analysis, HR = hazard ratio, KIRC = kidney renal clear cell carcinoma, OS = overall survival, PBMCs = peripheral blood mononuclear cells, PD-1 = programmed death 1, PD-L1 = programmed death ligand 1, PPS = post-progression survival, STAD = stomach adenocarcinoma, TAM = tumor-associated macrophages, TIMER = tumor immunoassay resources.

Keywords: colorectal cancer, lymphocytes, prognosis, TGFβ2, tumor infiltration

1. Introduction

Colorectal cancer (CRC) is one of the most deadly cancers worldwide, second only to lung, liver, and stomach cancers.[1] One of the primary causes of its poor prognosis is tumor metastasis.[2] Immunotherapy has become the main treatment for CRC and holds a great promise for the treatment of mismatch-repair-deficient and microsatellite instability-high metastatic CRC.[3] Immunotherapy has provided antitumor effects in

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All data generated or analyzed during this study are included in this published article [and its supplementary information files]

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malignant melanoma and non-small cell lung cancer by targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4), and inhibitors of programmed death 1 (PD-1) receptor, and programmed death ligand 1 (PD-L1).[4,5] However, in metastatic CRC, anti-CTLA4 shows poor clinical efficacy,[6] and anti-PD-1 and anti-PD-L1 demonstrated only partial improvement in patients with advanced gastric cancer and colon cancer.[7–9] Infiltration of the tumor by immune cells is particularly relevant to patient prognosis, and tumor-associated macrophages (TAM) have an impact on the prognosis and efficacy of chemotherapy and immunotherapy.[10] Therefore, it is essential to elucidate the immune phenotype of CRC and understand how immune cells control this type of cancer. These advances will help to identify novel immunotherapy targets in CRC.

Transforming growth factor-beta (TGF-β) is a cytokine that has an important function in immune responses and is particularly relevant to the development of malignant tumors.[11–13] The TGF-β Family comprises three members, TGF-β1, TGF-β2, and TGF-β3, which have essential functions in vitro.[14] TGF-β1 and TGF-β2 affect tumor progression by controlling the activity of stromal cells and tumor cells.[15,16] Most cancer cells are not susceptible to TGF-β-induced inhibition of growth, and resistance to its inhibitory activity is accompanied by an enhancement of tumor growth-promoting activity of TGF-β.[17,18] A low level of TGF-β1 expression is associated with disease-free and overall survival (OS) of cancer patients and constitutes an independent prognostic factor.[19]

TGF-β signaling can induce strong immunosuppression. In addition to other cells in the tumor microenvironment, TGF-β can be secreted by tumor cells and immune cells.[20,21] It drives the epithelial-mesenchymal transformation of tumor cells, enhancing tumor progression.[22] Inhibition of TGF-β signaling prevents metastasis or further development of certain advanced tumors such as CRC and gastric cancer,[23–25] while TGF-β1 can impair immune cell responsiveness[26,27] and promote angiogenesis.[28]

TGF-β is a potent regulator of the tumor microenvironment. It controls the interactions among tumor, immune, and stromal cells, while simultaneously regulating cytokine production. Peripheral blood mononuclear cells (PBMCs) are key immune cells capable of secreting cytokines. The interaction between PBMCs and cancer cells can either induce or suppress the tumor-specific immune response, thereby determining whether tumor cells undergo apoptosis or cancer progresses more rapidly.[21,29,30] Tumor-PBMC interactions are mediated by both direct intercellular contact and cytokine-dependent signaling pathways. Certain tumors can induce the differentiation of naive peripheral CD4+ T cells into CD4+ CD25+ regulatory T cells by secreting TGF-β.[31–33] Additionally, in certain cancer types, including colon cancer, the release of tumor necrosis factor-α, interleukin-1β, and interferon-γ is elevated upon interaction with lymphocytes.[34] However, it is unclear how TGF-β2 regulates tumor progression and immune cell infiltration in CRC.

The present study utilized the Oncomine and PrognoScan databases and Kaplan-Meier plotters to determine the relationship between the expression of TGF-β2 and patient prognosis. Moreover, the impact of TGF-β2 on tumor-infiltrating immune cells in different tumor microenvironments was assessed using tumor immunoassay resources (TIMER). This investigation provides novel insights into the functional role of TGF-β2 in CRC and proposes a potential mechanism by which TGF-β2 controls the interaction between immune cells and tumors.

2. Materials and methods

2.1. Oncomine database analysis

The Oncomine is a comprehensive database compiled from 86,733 samples and 715 gene expression datasets (https://www.oncomine.org/resource/login.html).[35] This resource was used to assess the association between TGF-β2 expression and prognosis in various tumor types.

2.2. PrognoScan database analysis

We evaluated the relationship between TGF-β2 expression and patient prognosis using the PrognoScan database (http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html).[36] PrognoScan searches the relationship between gene expression and patient prognosis, including OS, post-progression survival(PPS), no-distance survival and disease-free survival, in a large number of publicly available cancer microarray datasets.

2.3. Kaplan-Meier plotter analysis

The Kaplan Meier plotter (http://kmplot.com/analysis/) facilitates the analysis of the effects of multiple genes on the survival of patients in 21 different types of cancer, including breast cancer (n = 6,234), ovary (n = 2,190), lung (n = 3,452), and gastric cancer (n = 1,440).[37] Kaplan-Meier plotter was used to determine the correlation between TGF-β2 expression and survival rates of patients with breast, ovarian, lung, and gastric cancers.

2.4. TIMER database analysis

TIMER is a database designed for the analysis of immune cell infiltration in a variety of cancers (https://cistrome.shinyapps.io/timer/).[38] The database uses statistical methods validated by pathological examination to assess the immune infiltration of tumors by neutrophils, macrophages, dendritic cells, B cells, and CD4/CD8 T cells. In the present analysis, the TIMER database was used first to assess differences in the level of TGF-β2 expression in specific tumor types, and subsequently to explore the association between TGF-β2 expression and the degree of infiltration of specific immune cell subpopulations. Kaplan-Meier curve analysis was performed to investigate changes in the survival of patients with different levels of gene expression or immune cell infiltration. Finally, the correlation of TGF-β2 expression with the expression of specific immune-infiltrating cell subset markers was evaluated.

2.5. Gene expression profile interactive analysis (GEPIA) database analysis

Online Database GEPIA; http://gepia.cancer-pku.cn/index.html) [39] can be used to verify further the significance of genes identified in TIMER. GEPIA is an online database that helps to standardize the analysis of 9,736 tumor samples and 8,587 normal control samples from the TCGA and GTEx RNA-seq data. GEPIA was used to evaluate the association between TGF-β2 expression in multiple tumor types and patient prognosis and between TGF-β2 expression and the level of specific markers of tumor immune cell infiltration.

2.6. Statistical analysis

PrognoScan and Kaplan-Meier plotters were used to generate survival curves, and the results of the PrognoScan, Kaplan-Meier

plotters, TIMER, and GEPIA databases were displayed with the hazard ratio (HR) and *P*-values or Cox *P*-values obtained with the log-rank test. The data from the Oncomine resource provided information on rankings, fold-changes, and P-values. Spearman correlation analysis was used to measure the degree of correlation between specific variables. The following r values were used to determine the strength of the correlation: 0.00-0.19, "very weak"; 0.20-0.39, "weak," 0.40-0.59, "medium," 0.60–0.79, "strong," and 0.80–1.0, "very strong." $P < .05$ was considered statistically significant.

3. Results

The expression of TGF-β2 in various tumors and normal tissues was assessed first using the Oncomine database. This analysis documented that, in comparison with normal tissues, the expression of this gene was increased in CRC, head and neck cancer, liver cancer, myeloma, sarcoma, that is, in 5 of 20 cancers evaluated. Additionally, in the bladder, cervical, lung, melanoma, and ovarian cancers, TGF-β2 was expressed at a lower level than in normal tissue (Fig. 1A). Detailed findings for different tumor types are listed in Supplementary Table 1 (http://links.lww.com/MD/F175). Next, the TCGA and TIMER databases were used to assess the differential expression of TGF-β2 in specific tumor types. In comparison with normal tissues, the expression of TGF-β2 was significantly higher in cholangiocarcinoma, colon adenocarcinoma (COAD), liver hepatocellular carcinoma, stomach adenocarcinoma(STAD), and thyroid carcinoma tissues and significantly lower in bladder urothelial carcinoma, breast invasive carcinoma, kidney chromophobe cell carcinoma, kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, rectum adenocarcinoma, and uterine corpus endometrial carcinoma tissues than in normal tissues. Figure 1B shows the difference between TGF-β2 expression in tumors and normal adjacent tissue samples in the TCGA dataset.

3.1. Relationship between TGF-β2 expression and prognosis of cancer patients

The relationship between the expression of TGF-β2 and the prognosis of cancer patients was explored using the GEPIA PrognoScan databases (Supplementary Table 2, http://links.lww.com/MD/F176). In multiple types of cancer, including blood, prostate, ovarian, lung, colorectal, breast, and brain cancers, the patient prognosis was significantly correlated with TGF-β2 expression (Fig. 2A-H). Also,Kaplan-Meier plotter databases were used to assess the relationship between the expression of TGF- β2 and the prognosis of various cancer types. This analysis documented that upregulation of TGF- β2 was strongly related to the poor prognosis of patients with gastric cancer (OS HR = 1.88 (95%CI: 1.57–2.24), $P < .0001$; PPS HR = 2.51 (95%CI: 2.01–3.15), $P < .0001$; 209908_s_at), breast cancer (OS HR = 0.77 (95%CI: 0.61–0.99), $P = .039$; no-distance survival HR = 0.78 (95%CI: 0.62–0.99), $P = .043$; 209908_s_at) (2K-L), lung cancer (OS HR = 1.23 (1.08–1.4), $P = .0013$, 220407_s_at; PPS HR = 1.34 (1.04–1.73), $P = .025$, 220406_at), and Ovarian cancer (OS HR = 1.18 (1.04–1.34), $P = .013$, 209909_s_at; progression-free survival, progression-free survival, HR = 1.22 (1.07–1.39), $P = .0036$, 220407_s_at;) (Fig. 2O-P). Conversely, the expression of TGF-β2 appeared as a protective factor in the prostate, breast cancer, colorectal, blood cancer, brain, gastric, lung, and ovarian cancers. The correlation between TGF-β2 expression and patient prognosis in 33 cancer types was further evaluated using the GEPIA database. This analysis demonstrated that the expression of TGF-β2 is related to the OS in adrenocortical carcinoma (ACC), STAD, COAD, KIRC, and lower-grade glioma mesothelioma, and the disease-free survival of ACC, STAD, COAD, lower-grade glioma, uveal melanoma, and pancreatic adenocarcinoma (Supplementary Figure 1-4, http://links.lww.com/MD/F166, http://links.lww.com/MD/F167, http://links.lww.com/MD/F168, http://links.lww.com/MD/F169). Together, these results indicate that the high expression of TGF-β2 is significantly correlated with the poor prognosis of CRC patients.

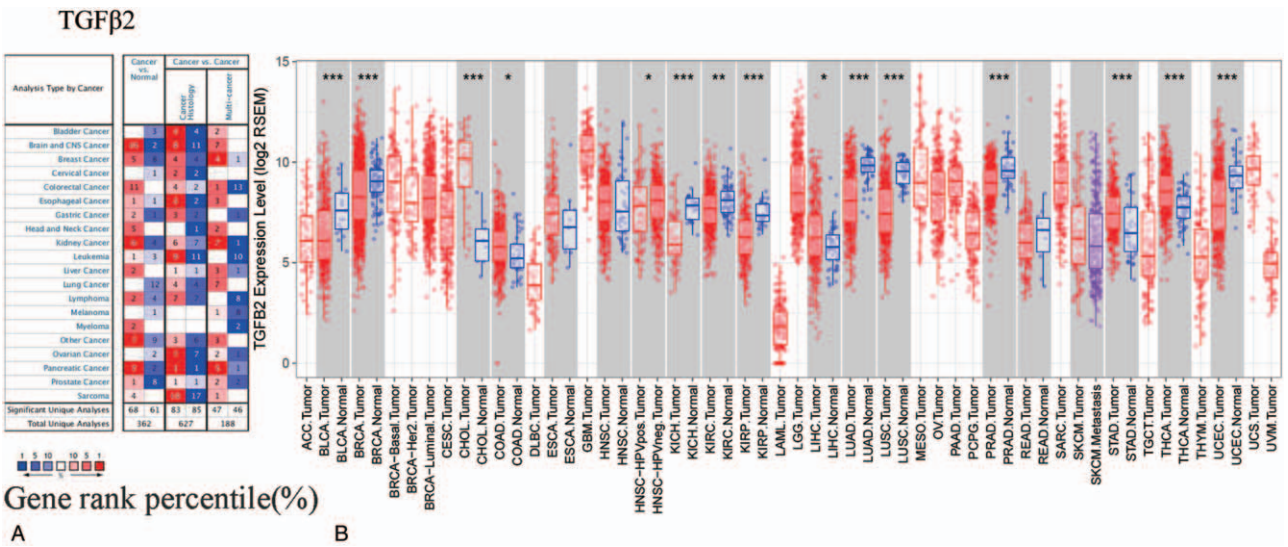

Figure 1. Expression level of TGF-β2 in different types of human cancers. (A) TGF-β2 expression in different cancers is increased or decreased in comparison with normal tissues according to the Oncomine database. (B) The TIMER resource was used to display the expression levels of TGF-β2 in different types of human tumors in the TCGA database. ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$).

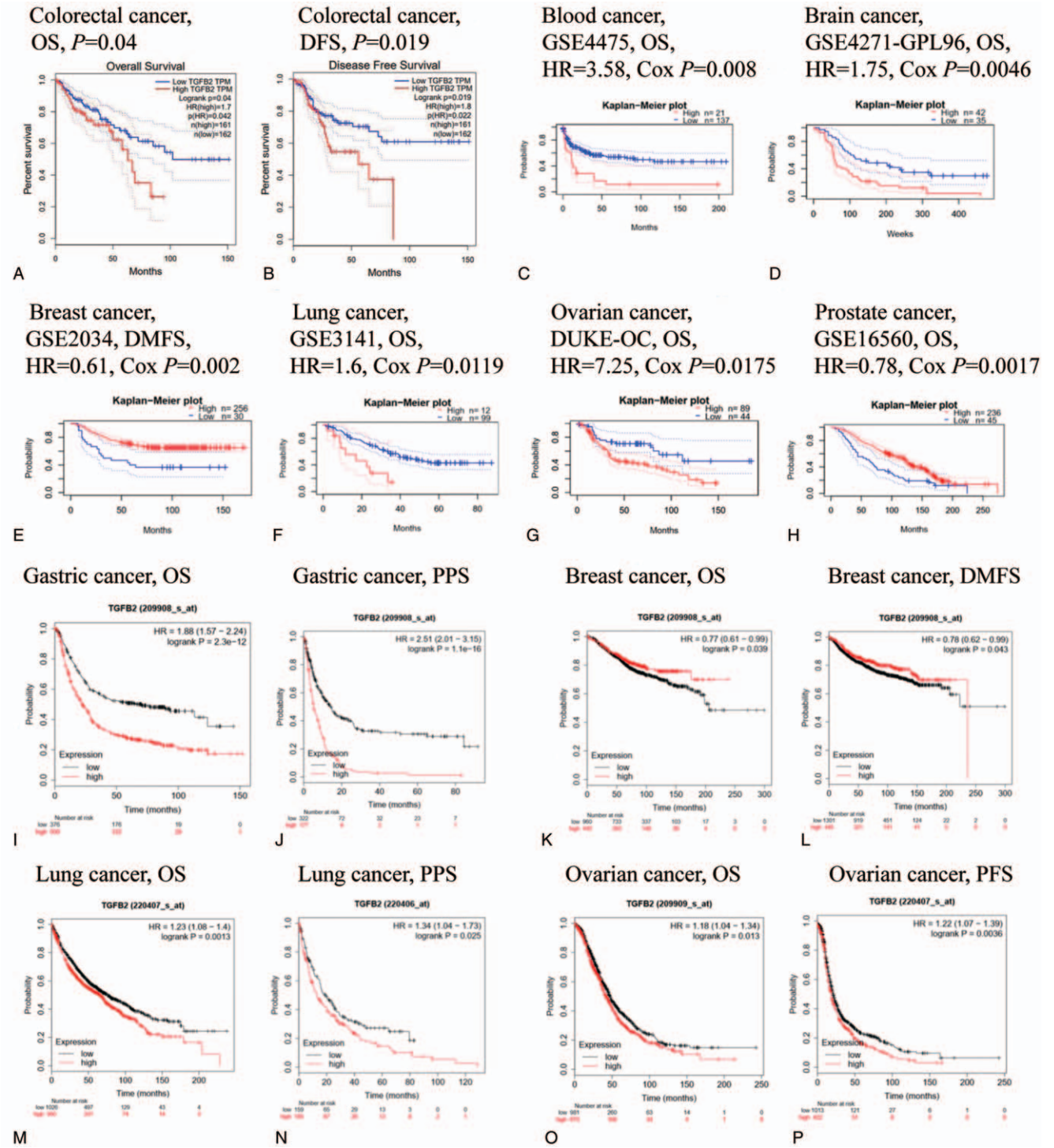

Figure 2. Correlation between TGF-β2 expression and prognosis of patients with various types of cancer. (A-H) GEPIA and PrognoScan databases. (I-P) Kaplan-Meier plotter database. DFS, disease-free survival; OS; PPS, post-progression survival; DMFS, no-distance survival; PFS, progression-free survival.

3.2. Relationship between TGF-β2 expression and infiltration of CRC by immune cells

In cancer patients, tumor infiltration and lymph node metastasis are independent prognostic factors.[40–42] Therefore, the TIMER database was used to establish whether the expression of TGF-β2 correlates with the degree of immune cell infiltration in 39 tumor types (Supplementary Figures 5-9, http://links.lww.com/MD/F170, http://links.lww.com/MD/F171, http://links.lww.com/MD/F172, http://links.lww.com/MD/F173, http://links.lww.com/MD/F174). A statistically significant correlation has been identified between the expression of TGF-β2 and tumor purity in 26 cancer types and between the expression of TGF-β2 and B-cell infiltration in 14 cancer types. Acorrelation was also present between TGF-β2 expression and the magnitude of invasion by

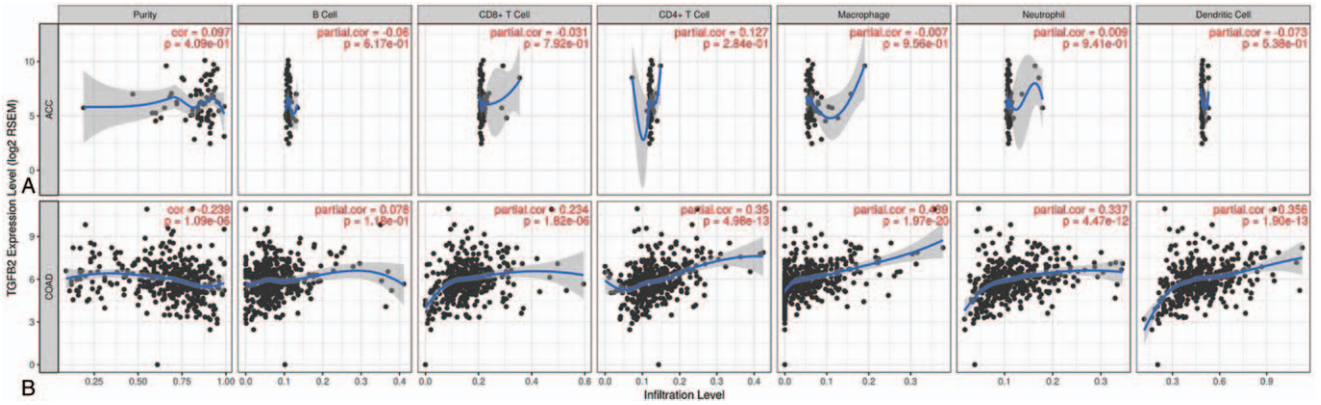

Figure 3. Correlation between TGF-β2 expression and infiltration by immune cells of COAD (colon adenocarcinoma) and ACC (adrenocortical carcinoma) (A) The expression of TGF-β2 in COAD (n = 457) was significantly negatively correlated with tumor purity, and infiltration by with CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and CDs in COAD. Significantly positive correlation was present, except for B cells. (B) In ACC (n = 79), TGF-β2 expression was not significantly correlated with tumor purity and Bcell, CD8+ T cell, macrophage, neutrophil, and DC infiltration.

multiple immune cells, such as CD8+ T cells (in 19 cancer types), CD4+ T cells (in 21 cancer types), macrophages (in 24 cancer types), neutrophils (in 23 cancer types), and dendritic cells (in 25 cancer types). In ACC, no significant correlation was found between TGF-β2 levels and infiltration by B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, or dendritic cells (Fig. 3A).Similarly, there was no relationship between TGF-β2 levels in CRC and infiltration by B cells; however, in this tumor type, the expression of TGF-β2 was significantly associated with the levels of CD8+ T cells ($r = 0.234259$, $P < .0001$), CD4+ T cells ($r = 0.350021$, $P < .0001$), macrophages ($r = 0.438681$, $P < .0001$), neutrophils ($r = 0.336562$, $P < .0001$), and dendritic cells ($r = 0.355884$, $P < .0001$) (Fig. 3B). These findings indicate that TGF-β2 plays an essential function in regulating the infiltration of CRC by immune cells, and has a particularly potent effect on macrophage infiltration.

3.3. Correlation between TGF-β2 and the expression of immune markers

The TIMER and GEPIA databases were used to explore further the relationship between TGF-β2 expression and immune cell infiltration, represented by the expression of sets of markers of various immune cells in COAD, including CD8+ T cell subsets, total T cells, B cells, monocytes, TAM, M1 and M2 macrophages, neutrophils, natural killer cells, dendritic cells (DCs), Th1 cells, Th2 cells, Tfh cells, Th17 cells, and T cell subsets. ACC was used as a control group. The results were adjusted according to tumor purity in order to identify the correlation between TGF-β2 expression in COAD and markers of monocytes (CD86, CSF1R), TAMs (CCL2, CD68, IL10), M1 macrophages (NOS2, PTGS2), M2 macrophages (CD163, VSIG4, MS4A4A), neutrophils (CEACAM8, ITGAM, CCR7), dendritic cells (HLA-DPB1, HLA-DRA, HLA-DPA1, CD1C, NRP1, ITGAX), Th1 cells (TBX21, STAT4, STAT1), Th2 cells (GATA3, STAT6, IL13) Tfhcells (BCL6, IL21), Th17 cells (STAT3, IL17A) and Tregs (FOXP3, CCR8, STAT5B, TGF-β1). For all markers, the correlation was statistically significant ($P < 0.05$; Table 1). In contrast, only 10 immune cell markers were associated with differential expression of TGF-β2 in ACC (Table 1). In COAD, the expression of TGF-β2 was significantly correlated with markers of monocytes (CD86, CSF1R), TAMs (CCL2), M1 macrophages (NOS2, PTGS2), and M2 macrophages (CD163, VSIG4, MS4A4A). In all cases, the association was statistically significant ($P < 0.05$; Fig. 4). Therefore, the relationship between the expression of TGF-β2 and markers of immune cells in COAD was evaluated using the GEPIA database. The correlation between TGF-β2 and markers of monocytes, TAMs, and M1 and M2 macrophages was similar to that obtained using TIMER, except for the absence of a statistically significant correlation with NOS2 (Table 2). These results suggest that TGF-β2 may regulate the polarization of macrophages in COAD. Data on the expression of markers of DCs (CD1C, NRP1, and ITGAX) indicate that high TGF-β2 expression increases DC infiltration in COAD. Importantly, DCs can increase tumor metastasis by activating the response of Tregs and inhibiting tumor-specific cytolytic CD8+ T cells,[43] highlighting the key role of TGF-β2 in the regulation of tumor metastasis.Also, a significant correlation was observed between the expression of TGF-β2 and markers of Tregs and exhausted T cells, including FOXP3, CCR8, STAT5B, and TGF-β (Table 1), suggesting that TGF-β2 may play a role in the immune escape of CRC.

4. Discussion

4.1. TGF-β2 is one of the three members of the TGF-β

Family of cytokines that have a wide regulatory function in cancer. TGF-β cytokines signal through type I and type II receptors (TGF-βR1 and TGF-βR2), and the signal is transmitted through downstream regulatory SMAD proteins, which control critical cellular activities, such as migration, proliferation, and differentiation. TGF-β cytokines are capable of suppressing IL-2-stimulated growth of T cells. The present work demonstrated that TGF-β2 expression correlates with the prognosis of patients with multiple types of cancer, and particularly strong correlation is present between high TGF-β2 expression and the prognosis of CRC patients. The upregulation of TGF-β2 is also a reliable predictor of lymph node metastasis in CRC patients. Additionally, the performed analyses documented that the expression of TGF-β2 is related to the expression of several markers of immune cell subspecies in tumors. Together the current investigation

Table 1

Analysis of the correlation between TGFβ2 and immune cell related genes and markers in TIMER.

		COAD				STAD				ACC			
		None		Purity		None		Purity		None		Purity	
Description	Gene markers	cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*	Cor	*P*
CD8+ T cell	CD8A	0.13	***	0.04	0.26	0.12	**	0.12	*	0.01	.91	0.12	.31
	CD8B	0.09	*	0.02	0.49	0.11	*	0.12	*	0.10	.37	0.03	.78
T cell (general)	CD3D	0.06	.18	0.05	0.36	0.05	.28	0.04	.43	0.05	.68	0.15	.20
	CD3E	0.14	*	0.05	0.31	0.06	.19	0.05	.35	0.06	.61	0.05	.65
	CD2	0.18	**	0.10	0.05	0.11	.02	0.11	.03	0.04	.74	0.10	.40
B cell	CD19	0.04	.35	0.05	0.33	0.13	*	0.10	.06	0.04	.70	0.06	.63
	CD79A	0.15	*	0.04	0.42	0.14	*	0.12	.02	0.04	.70	0.11	.37
Monocyte	CD86	0.39	***	0.33	***	0.18	**	0.18	**	0.06	.58	0.19	.11
	CSF1R	0.38	***	0.30	***	0.31	***	0.29	***	0.13	.25	0.07	.58
TAM	CCL2	0.50	***	0.45	***	0.32	***	0.33	***	0.20	.07	0.13	.26
	CD68	0.18	***	0.12	0.01	0.05	.28	0.06	.25	0.16	.15	0.10	.40
	IL10	0.26	***	0.20	***	0.27	***	0.29	***	0.20	.08	0.15	.21
M1 Macrophage	NOS2	0.25	***	0.27	***	0.18	**	0.18	**	0.46	***	0.48	***
	IRF5	0.07	.11	0.07	0.13	0.17	**	0.17	**	0.00	.97	0.07	.58
	PTGS2	0.27	***	0.23	***	0.33	***	0.33	***	0.10	.39	0.13	.28
M2 Macrophage	CD163	0.40	***	0.34	***	0.24	***	0.23	***	0.13	.27	0.06	.60
	VSIG4	0.35	***	0.27	***	0.27	***	0.30	***	0.07	.51	0.02	.86
	MS4A4A	0.35	***	0.28	***	0.26	***	0.27	***	0.21	.06	0.15	.20
Neutrophils	CEACAM8	0.21	***	0.20	***	0.02	.74	0.02	.74	0.01	.90	0.02	.89
	ITGAM	0.38	***	0.33	***	0.26	***	0.26	***	0.22	.05	0.20	.09
	CCR7	0.20	***	0.12	0.01	0.22	***	0.20	***	0.01	.96	0.05	.65
Natural killer cell	KIR2DL1	0.05	.28	0.01	0.85	0.09	.08	0.07	.15	0.18	.11	0.16	.19
	KIR2DL3	0.03	.55	0.00	0.99	0.07	.14	0.05	.34	0.14	.22	0.07	.54
	KIR2DL4	0.03	.56	0.09	0.07	0.12	.01	0.14	*	0.08	.48	0.19	.10
	KIR3DL1	0.04	.35	0.01	0.91	0.08	.11	0.07	.16	0.15	.20	0.11	.36
	KIR3DL2	0.09	.07	0.02	0.72	0.01	.87	0.00	.94	0.07	.51	0.07	.55
	KIR3DL3	0.03	.58	0.04	0.46	0.10	.03	0.12	.02	0.04	.75	0.06	.63
	KIR2DS4	0.08	.08	0.06	0.24	0.02	.72	0.00	.99	0.21	.07	0.15	.19
Dendritic cell	HLA-DPB1	0.21	***	0.12	0.01	0.05	.30	0.04	.47	0.13	.24	0.04	.72
	HLA-DQB1	0.10	.03	0.00	0.98	0.09	.07	0.11	.03	0.05	.68	0.03	.83
	HLA-DRA	0.23	***	0.14	*	0.04	.44	0.05	.35	0.14	.21	0.05	.69
	HLA-DPA1	0.26	***	0.18	**	0.01	.84	0.00	.99	0.14	.20	0.05	.66
	CD1C	0.37	***	0.31	***	0.31	***	0.32	***	−0.18	.11	-0.11	.35
	NRP1	0.67	***	0.64	***	0.57	***	0.57	***	0.38	**	0.32	*
	ITGAX	0.42	***	0.37	***	0.28	***	0.28	***	0.03	.80	0.08	.50
Th1	TBX21	0.12	.02	0.08	0.10	0.07	.15	0.03	.78	0.06	.62	0.12	.02
	STAT4	0.21	***	0.22	***	0.20	**	0.01	.90	0.11	.33	0.21	***
	STAT1	0.25	***	0.03	0.52	0.05	.38	0.20	.08	0.27	.02	0.25	***
	IFNG	0.02	.74	0.08	0.10	0.09	.09	0.21	.07	0.31	*	0.02	.74
	TNF	0.07	.17	0.09	0.06	0.09	.09	0.03	.77	0.09	.45	0.07	.17
Th2	GATA3	0.21	***	0.25	***	0.26	***	0.35	*	0.33	*	0.21	***
	STAT6	0.16	**	0.05	0.29	0.05	.32	0.37	**	0.38	**	0.16	**
	STAT5A	0.01	.81	0.17	**	0.17	**	0.28	.01	0.24	.04	0.01	.81
	IL13	0.11	.03	0.08	0.11	0.07	.15	0.22	.05	0.19	.10	0.11	.03
Tfh	BCL6	0.24	***	0.39	***	0.37	***	0.01	.92	0.03	.80	0.24	***
	IL21	0.11	.02	0.02	0.75	0.01	.81	NA	NA	NA	NA	0.11	.02
Th17	STAT3	0.14	*	0.29	***	0.27	***	0.12	.31	0.11	.37	0.14	*
	IL17A	0.12	.01	0.08	0.09	0.09	.07	0.18	.10	0.18	.13	0.12	.01
Treg	FOXP3	0.26	***	0.05	0.29	0.03	.52	0.31	*	0.32	*	0.26	***
	CCR8	0.35	***	0.21	***	0.21	***	0.16	.16	0.21	.08	0.35	***
	STAT5B	0.25	***	0.37	***	0.36	***	0.29	*	0.26	.03	0.25	***
	TGFB1	0.22	***	0.38	***	0.37	***	0.26	.02	0.31	*	0.22	***
T cell exhaustion	PDCD1	0.03	.56	0.07	0.18	0.07	.21	0.12	.29	0.26	.03	0.03	.56
	CTLA4	0.22	***	0.10	0.05	0.10	.06	0.07	.54	0.17	.14	0.22	***
	LAG3	0.04	.39	0.01	0.86	0.00	.94	0.29	.01	0.34	*	0.04	.39
	HAVCR2	0.30	***	0.13	*	0.13	.01	0.08	.46	0.02	.88	0.30	***
	GZMB	0.05	.34	0.08	0.09	0.10	.04	0.03	.79	0.02	.84	0.05	.34

CODA, colon adenocarcinoma; CTLA4 = cytotoxic T lymphocyte-associated antigen, IFN-γ = interferon-γ, 4 LUSC, lung squamous cell carcinoma; STAD, stomach adenocarcinoma; Th, T helper cell; TAM, tumor-associated macrophage; Tfh, Follicular helper T cell; TNF-α = tumor necrosis factor-α, Treg, regulatory T cell; Cor, R value of Spearman's correlation; Purity, correlation adjusted by purity. None, correlation without adjustment. Values are retained to two decimal places. $^{*}P<.01$; $^{**}P<.001$; $^{***}P<.0001$.

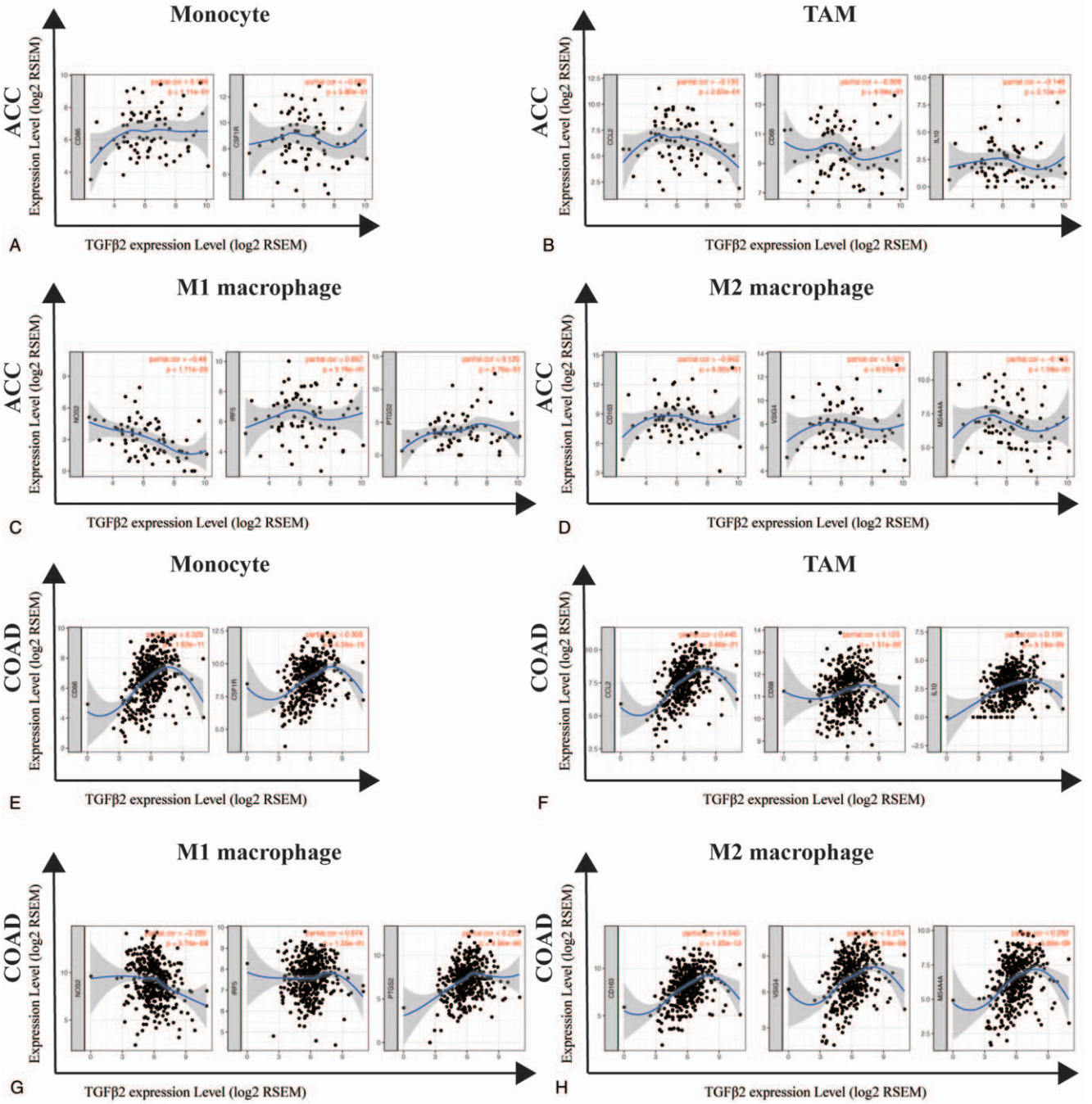

Figure 4. Analysis of the correlation between TGF-β2 expression and immunological markers in COAD (colon adenocarcinoma) and adrenocortical carcinoma (ACC). (A–D) Scatterplots of correlations between TGF-β2 expression and markers of monocytes (A), TAMs (B), and M1 (C) and M2 (D) macrophages in ACC. (E–H) Scatterplots of correlations between TGF-β2 expression and markers of monocytes (E), TAMs (F), and M1 (G) and M2 (H) macrophages in COAD.

highlights multiple functions of TGF-β2 in the progression of CRC, making it a valuable prognostic biomarker in colorectal tumors.

The use of independent Oncomine and GEPIA databases to assess the correlation between TGF-β2 expression and the prognosis of 33 different types of cancer revealed significant differences in TGF-β2 expression between normal and cancer tissues. Oncomine data have shown elevated levels of TGF-β2 in normal tissues, including lymphomas and brain, breast, colorectal, esophageal, gastric, head and neck, kidney, liver, pancreatic, cancers. Conversely, the levels of TGF-β2 levels are decreased in breast, kidney, lung, and prostate cancer (Fig. 1A). According to the TCGA database, expression of TGF-β2 is increased in cholangiocarcinoma, COAD, liver hepatocellular carcinoma, STAD, Thyroid thyroid carcinoma, and relatively lower in bladder urothelial carcinoma, breast invasive carcinoma, kidney chromophobe cell carcinoma, kidney renal papillary cell carcinoma, KIRC, lung adenocarcinoma, lung squamous cell carcinoma, prostate adenocarcinoma, and uterine corpus endometrial carcinoma (Fig. 1B). Variations in TGF-β2 expres-

Table 2

Correlation analysis between TGFβ2 and related genes and markers of monocyte, TAM and macrophages in GEPIA.

		COAD		STAD		ACC	
Description	**Gene markers**	**cor**	***p***	**Cor**	***P***	**Cor**	***P***
Monocyte	CD86	0.21	.00039	0.079	.11	0.16	.18
	CD115 (CSF1R)	0.24	5.1e–05	0.12	.012	0.14	.23
TAM	CCL2	0.22	.00024	0.09	.069	-0.13	.26
	CD68	0.18	.0036	0.029	.56	0.12	.29
	IL10	0.14	.02	−0.022	.66	0.18	.13
M1 Macrophage	INOS (NOS2)	−0.092	.13	−0.088	.076	-0.13	.26
	IRF5	0.019	.76	0.11	.021	0.059	.16
	COX2 (PTGS2)	0.45	6.7e–15	0.11	.022	0.14	.23
M2 Macrophage	CD163	0.19	.0015	0.13	.0084	0.23	.048
	VSIG4	0.23	.00015	0.15	.0025	0.24	.033
	MS4A4A	0.2	8e–04	0.11	.033	0.18	.12

CODA, colon adenocarcinoma; GEPIA = gene expression profile interactive analysis, LUSC, lung squamous cell carcinoma; STAD, stomach adenocarcinoma. TAM, Tumor-associated macrophages. Normal, correlation analysis in normal tissue of TCGA. Tumor, correlation analysis in tumor tissue of TCGA. NA, No correlation value in database. $^{*}P < .01$; $^{**}P < .001$; $^{***}P < .0001$.

sion in a range of different cancers may be related to discrepancies in data collection methods between individual studies or differences in underlying biological mechanisms. In the database used in the current work, a correlation between high TGF-β2 expression and poor prognosis of CRC was observed.

Another important finding of this study is the demonstration that the expression of TGF-β2 is related to the degree of immune infiltration in many cancer types, particularly in CRC. TGF-β2 expression was positively correlated with the degree of macrophage infiltration, but weakly positively correlated with the degree of CD8+ T cell, CD4+ T cell, DC, and neutrophil infiltration in COAD (Fig. 3B). In addition, the correlation between TGF-β2 and the expression of certain immunological marker genes strongly suggests that TGF-β2 can control immune cell infiltration and interactions in the colorectal tumor microenvironment. Markers of M2 macrophages, such as VSIG4 and MS4A4A, correlated weakly with TGF-β2 expression, while the expression of the marker of M1 macrophages, PTGS2, was moderately and strongly correlated (Table 2). These results reveal a potential regulatory role of TGF-β2 in TAM polarization. Also, TGF-β2 was found to have the potential to activate Tregs and induce T cell exhaustion. The increase in TGF-β2 expression was positively correlated with the expression of Treg and T cell exhaustion markers (FOXP3, CCR8, STAT5B, TGFB1, CTLA4, and HAVCR2) (Table 1). Thus, TGF-β2 expression may participate in the regulation of tumor-associated T cell exhaustion and Tregs. In addition, the level of TGF-β2 in COAD is correlated with the expression of multiple markers of T cells (Th1, Th2, Tfh, and Th17). This finding may reflect the ability of TGF-β2 to control T cell responses in CRC. Collectively, these results highlight the potential of TGF-β2 to regulate the recruitment and activation of immune cells in colorectal tumors.

In summary, the expression of TGF-β2 is related to the magnitude of the infiltration of CRC by immune cells, and is significantly correlated with the prognosis of CRC. TGF-β2 may be an important regulator of immune cell infiltration in CRC cancer patients and a valuable prognostic biomarker.

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