TSHZ3 is A Prognostic Biomarker with Immune Infiltration in Stomach Adenocarcinoma and Bladder Urothelial Carcinoma

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Research

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Abstract

Background: This study aimed to investigate the expressions of Teashirt zinc finger homeobox 3 (TSHZ3) in different cancer types and identify the cancers of which prognosis can be predicted by TSHZ3 expression. Furthermore, we aimed to explore the correlations between tumor-infiltrating immune cells (TIICs) and TSHZ3 expression in these cancers.

Methods: TSHZ3 expression was analyzed by the Oncomine database and Gene Expression Profiling Interactive Analysis (GEPIA). We examined the influence of TSHZ3 on the clinical prognosis with Kaplan-Meier plotter and TIMER (Tumor Immune Estimation Resource). The correlations between tumor immune infiltrates and TSHZ3 expressions were investigated with TIMER and validated by GEPIA.

Results: TSHZ3 expressions were significantly higher in stomach adenocarcinoma (STAD) and bladder urothelial carcinoma (BLCA) when compared to normal tissues. Survival analysis results showed that high TSHZ3 expression was correlated with poor overall survival (OS) in STAD and BLCA. The infiltrations of pro-tumorigenic TIICs (M2 macrophages, monocytes, B cells and pDCs) were positively correlated with TSHZ3 expression in STAD and BLCA. Besides, the infiltration of other pro-tumorigenic TIICs (tumor-associated macrophages (TAMs), Th2 cells and Tregs) and expression of T cell exhaustion markers (PD-1 and TIM-3) were positively correlated with TSHZ3 expression in STAD.

Conclusions: TSHZ3 expression was up-regulated in STAD and BLCA. High TSHZ3 expression predicted poor prognosis of patients with STAD or BLCA. In addition, TSHZ3 expression potentially contributes to the increased infiltrations of pro-tumorigenic TIICs. TSHZ3 can be used as a prognostic biomarker for predicting prognosis and immune infiltration in STAD and BLCA.

Background

Gastric cancer (GC) is the second most common cause of cancer-related deaths in the world. It has a high incidence and poor prognosis [1]. Urinary bladder cancer is the most common malignancy involving the urinary system. The bladder urothelial carcinoma (BLCA) is the most common histological type of bladder cancer, which represents more than 90% of all the bladder carcinomas cases [2, 3].

Solid tumors including GC and BLCA are commonly infiltrated by immune cells. The immune infiltration strongly influences the development of GC and BLCA [4, 5]. Modulating the immune infiltration is a promising therapeutic strategy for GC and BLCA [6, 7]. Indeed, growing evidences suggest that the tumor-infiltrating immune cells (TIICs), such as CD8 + tumor-infiltrating T cells (TITCs) and CD68 + tumor-associated macrophages (TAMs), strongly influence the prognosis and efficacy of immunotherapy and chemotherapy [8–11]. Meanwhile, it has been widely reported that different subsets of some TIICs have diverse biological effects. For example, M2 macrophages, plasmacytoid dendritic cells (pDCs), Treg cells and Th2 cells are pro-tumorigenic while M1 macrophages, one subsets of conventional DCs (cDC1s), Tfh cells and Th1 cells are anti-tumorigenic [12, 13]. Therefore, it is important to elucidate the TIIC profile and identify novel therapeutic targets for GC and BLCA.

Teashirt zinc finger homeobox 3 (TSHZ3) is a gene encoding a zinc finger transcription factor and has been widely studied in the nervous system and the differentiation of smooth muscle cell [14–16]. Moreover, TSHZ3 also functions as tumor suppressor in brain glioma and ovarian cancer but as it is an oncogene in breast cancer [17–19]. A recent study suggests that TSHZ3 predict poor prognosis in tongue squamous cell carcinomas because it modulates the immune infiltration [20]. However, the role of TSHZ3 in other cancer growth and the underlying mechanism of action is not known. In our present study, we employed systemic biology approach, Oncomine and Gene Expression Profiling Interactive Analysis (GEPIA), to analyze the expressions of TSHZ3 in various cancers, and evaluated the correlations between TSHZ3 expression and the prognosis of malignancy patients in databases such as Kaplan-Meier plotter and Tumor Immune Estimation Resource (TIMER) [21–24]. Furthermore, we investigated the correlation of TSHZ3 and TIICs in the diverse tumor microenvironments by using TIMER. Our study not only reveals the critical role of TSHZ3 in BLCA and GC development, but also suggests a potential association and a regulatory role of TSHZ3 in TIICs.

Materials And Methods

TSHZ3 Gene Expression Level Analysis

The expression level of TSHZ3 in different types of cancers was identified *via* the Oncomine database (<https://www.oncomine.org/resource/login.html>) and the GEPIA database [22, 24]. In the Oncomine database, benign tumor data was excluded and the rest was included for pooled analysis with the Compare module, the threshold was determined by the following values: P-value of all, fold change of all, and gene ranking of all. TSHZ3 gene expression level analysis was conducted by GEPIA while the cancer types were lacking or samples were small in Oncomine. Significant statistical differences in TSHZ3 expression between tumor and normal tissues was considered to be existed while the p-value of < 0.05.

Survival Analysis

We evaluated the effect of TSHZ3 on clinical prognosis by using Kaplan-Meier plotter and TIMER up to Jan 4th 2020. Kaplan-Meier plotter is able to evaluate the effect of special genes on survival using 13,316 cancer samples, which include 6,234 breast, 1,440 gastric, 3,452 lung and 2,190 ovarian cancer samples. The relationship between expression of TSHZ3 and survival in breast, gastric, lung and ovarian cancers was analyzed by Kaplan-

Meier plotter (<http://kmplot.com/analysis/>) [21]. The log-rank P-value and hazard ratio (HR) with 95% confidence intervals and were presented. TIMER (<https://cistrome.shinyapps.io/timer/>) is a web server that includes 10,897 cancers samples from The Cancer Genome Atlas (TCGA). We carried out the survival analysis of different cancer types with the Survival module of TIMER. Results from both cox proportional hazard model method and Kaplan-Meier method were included for further evaluation. Database with larger sample number was selected to present Kaplan-Meier plot to increase the credibility of the results.

Immune Infiltration Analysis

TIMER is a comprehensive web resource for systematic evaluation of immune infiltrations across different cancer types [23], which infers the abundance of TIICs from gene expression profiles by applying a deconvolution published statistical method [25]. Thus, we roughly evaluated the immune infiltrates abundance, including B cell, CD8 + T cell, CD4 + T cell, dendritic cell, neutrophil and macrophage by the Gene module at first. Next, we further confirmed the correlations between TSHZ3 expression and TIICs and its' subsets with gene markers by using the Correlation module of TIMER. These immune gene markers are commonly referenced in recent studies [12, 26, 27]. Gene expression levels were adjusted by tumor purity [28]. Except for the exhausted T cell, we regarded correlations between TSHZ3 expression and TIICs as significant in the Correlation module only when all the correlations of the TIIC gene markers were consistent and significant. Significant correlations revealed by TIMER were further validated for the given sets of TCGA data via GEPIA. The correlation coefficient was determined by Spearman method. The X-axis presented the TSHZ3 expression, and Y-axis presented the gene markers of the immune cells. The datasets of cancer and normal tissues were used for analysis.

Statistical Analysis

All analyses were performed using the independent sample t-test or Spearman correlation analysis, as appropriate. Survival analysis in TIMER were conducted with the cox proportional hazard model method and Kaplan-Meier method. Survival curves were produced via the Kaplan-Meier method and the log rank test. P-values < 0.05 were considered statistically significant.

Results

1. The Expression Levels of TSHZ3 in Different Human Cancer Types

To investigate the differences of TSHZ3 expressions in cancer and normal tissues, TSHZ3 expression levels in cancers and related normal tissues were evaluated with the Oncomine database and GEPIA. The analysis revealed that TSHZ3 expressions were higher in 14 cancer types or subtypes when compared with the normal tissues. These cancers include bladder urothelial carcinoma (BLCA), cervical cancer, gastric cancer, stomach adenocarcinoma (STAD), liver cancer, lung cancer, lung squamous cell carcinoma (LUSC), melanoma, myeloma (multiple myeloma), skin cancer, ovarian serous cystadenocarcinoma (OV), pancreatic cancer, pancreatic adenocarcinoma (PAAD), and pheochromocytoma and paraganglioma (PCPG). However, expressions of TSHZ3 were lower in adrenal cortex carcinoma (ACC), breast invasive carcinoma (BRCA), leukemia, ovarian cancer, prostate cancer, prostate adenocarcinoma (PRAD), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS) compared with normal tissues (Table 1, Fig. 1). The details of TSHZ3 expression in different cancer types in Oncomine and GEPIA were summarized in **Supplementary Table 1** and **Supplementary Figure S1**, respectively.

Table 1
TSHZ3 expression in different cancer types.

Cancer types	N (normal)	N(cancer)	Up/Down	Significance	Database
ACC	128	77	Down	#	GEPIA
BLCA	204	339	Up	*	ONCOMINE
Brain and Central Nervous System Cancer	796	1309	Down	#	ONCOMINE
GBM	207	163	Up	#	GEPIA
LGG	207	518	Up	#	GEPIA
Breast Cancer	1141	3628	Down	#	ONCOMINE
BRCA	1134	3318	Down	*	ONCOMINE
Cervical Cancer	132	245	Up	*	ONCOMINE
CESC	110	225	Up	#	ONCOMINE
Colorectal Cancer	728	1102	Down	#	ONCOMINE
COAD	627	542	Down	#	ONCOMINE
READ	631	278	Down	#	ONCOMINE
CHOL	9	36	Up	#	GEPIA
DLBC	337	47	Down	#	GEPIA
ESCA	286	182	Up	#	GEPIA
Gastric Cancer	573	688	Up	*	ONCOMINE
STAD	478	554	Up	*	ONCOMINE
Head and Neck Cancer	934	1068	Up	#	ONCOMINE
THCA	551	607	Down	#	ONCOMINE
HNSC	530	516	Up	#	ONCOMINE
Kidney Cancer	553	598	Down	#	ONCOMINE
KICH	53	66	Down	#	GEPIA
KIRC	553	502	Up	#	ONCOMINE
KIRP	544	62	Down	#	ONCOMINE
Leukemia	304	2073	Down	*	ONCOMINE
LAML	304	757	Down	#	ONCOMINE
Liver Cancer	281	323	Up	*	ONCOMINE
Lung Cancer	1018	1389	Up	*	ONCOMINE
LUAD	1018	811	Down	#	ONCOMINE
LUSC	940	551	Up	*	ONCOMINE
Lymphoma	129	247	Up	#	ONCOMINE
Melanoma	339	95	Up	*	ONCOMINE
Myeloma(multiple myeloma)	84	84	Up	*	ONCOMINE
Endometrial Cancer	363	354	Up	#	ONCOMINE

*P < 0.05, #P ≥ 0.05, ACC(Adrenocortical carcinoma), BLCA(Bladder urothelial carcinoma), BRCA (Breast invasive carcinoma), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma), CHOL (Cholangio carcinoma), COAD (Colon adenocarcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), ESCA (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIRC (Kidney renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LAML (Acute Myeloid Leukemia), LGG (Brain Lower Grade Glioma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), MESO (Mesothelioma), OV (Ovarian serous cystadenocarcinoma), PAAD (Pancreatic adenocarcinoma), PCPG (Pheochromocytoma and Paraganglioma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SARC (Sarcoma), SKCM (Skin Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumors), THCA (Thyroid carcinoma), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma), UCS (Uterine Carcinosarcoma) .

Cancer types	N (normal)	N(cancer)	Up/Down	Significance	Database
Skin Cancer	4	26	Up	*	ONCOMINE
SKCM	558	461	Down	#	GEPIA
TGCT	165	137	Up	#	GEPIA
THYM	339	118	Down	#	GEPIA
Ovarian Cancer	576	695	Down	*	ONCOMINE
OV	561	607	Up	*	ONCOMINE
Pancreatic Cancer	150	175	Up	*	ONCOMINE
PAAD	171	179	Up	*	GEPIA
PCPG	3	182	Up	*	GEPIA
Prostate Cancer	364	515	Down	*	ONCOMINE
PRAD	152	492	Down	*	GEPIA
SARC	2	262	Up	#	GEPIA
UCEC	91	174	Down	*	GEPIA
UCS	78	57	Down	*	GEPIA
<p>*P < 0.05, #P ≥ 0.05, ACC(Adrenocortical carcinoma), BLCA(Bladder urothelial carcinoma), BRCA (Breast invasive carcinoma), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma), CHOL (Cholangio carcinoma), COAD (Colon adenocarcinoma), DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma), ESCA (Esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIRC (Kidney renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LAML (Acute Myeloid Leukemia), LGG (Brain Lower Grade Glioma), LIHC (Liver hepatocellular carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), MESO (Mesothelioma), OV (Ovarian serous cystadenocarcinoma), PAAD (Pancreatic adenocarcinoma), PCPG (Pheochromocytoma and Paraganglioma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SARC (Sarcoma), SKCM (Skin Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumors), THCA (Thyroid carcinoma), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma), UCS (Uterine Carcinosarcoma) .</p>					

2. Prognostic Potential of TSHZ3 in Tumors

We evaluated the prognostic potential of TSHZ3 in cancers by presenting survival curves via Kaplan-Meier plotter and TIMER. Among the cancers with TSHZ3 differential expressions compared to normal tissues, elevated expression levels of TSHZ3 were correlated with poor prognosis of BLCA (Fig. 2A1), gastric cancer (Fig. 2A2-**A3**), STAD (Fig. 2A4) and ovarian cancer (Fig. 2B4-**C1**) but with good prognosis of lung cancer (Fig. 2C1). However, the TSHZ3 expression level has no significance correlation with lung squamous cell carcinoma (LUSC) or ovarian serous cystadenocarcinoma (OV), the main histological types of lung cancer and ovarian cancer, respectively (Fig. 2B3, **C2**). Notably, high TSHZ3 expression levels were correlated with poor prognosis of OS and PFS in GC (OS HR = 1.96, 95% CI = 1.57 to 2.44, P = 1.2e-9; PFS HR = 2.05, 95% CI = 1.61 to 2.60, P = 2.2e-9) and ovarian cancer (OS HR = 1.42, 95% CI = 1.15 to 1.76, P = 0.0013; PFS HR = 1.49, 95% CI = 1.23 to 1.80, P = 3.5e-5) (Fig. 2A2,**A3**). High TSHZ3 expression also shows poor OS in BLCA (OS HR = 1.96, 95% CI = 1.008 to 1.217, P = 0.006) and STAD (OS HR = 1.178, 95% CI = 1.039 to 1.336, P = 0.009) (Fig. 2A1, **A4**, **Supplementary Table S2**).

The relationships between TSHZ3 expression and prognosis of diverse cancers are presented in Fig. 2 and **Supplementary Table S2**. These results strongly suggest that TSHZ3 is a prognostic factor in many cancer types. The reduced or elevated levels of TSHZ3 may have diverse prognostic value depending on the type of cancer.

3. High TSHZ3 Expression Influences the Prognosis of Gastric Cancer Patients with Lymphatic Metastasis

To better understand the association and underlying mechanisms of TSHZ3 expression in GC, we investigated the correlations between the TSHZ3 expression and the related clinical features in the GC patients with the Kaplan-Meier plotter databases. Our results showed that overexpression of TSHZ3 was correlated with worse OS and PFS in female and male patients as well as patients with two types of Lauren classification (diffuse type and intestinal type) (P < 0.05). Specifically, in GC patients, high TSHZ3 expression was associated with worse OS and PFS in stage 3 to 4 but was not correlated with OS and PFS of stage 1 (OS HR = 2.16, P = 0.20; PFS HR = 1.97, P = 0.26) to 2 (OS HR = 1.74, P = 0.09; PFS HR = 1.81, P = 0.054) and PFS of stage M1 patients (PFS HR = 1.57, P = 0.16) (Table 2). Here the M category refers to distant metastases involvement; M0 indicates no distant metastases and M1 indicate distant metastases [29]. In addition, high TSHZ3 expression level has the highest HR values of OS and PFS in all five N categories. These findings suggest that TSHZ3 expression level is associated with the prognosis of GC patients with lymph node metastases and distant metastases.

Table 2
Correlation between TSHZ3 expression and prognosis in gastric cancer with different clinicopathological characteristics in Kaplan-Meier plotter

Clinicopathological characteristics	Overall survival (n = 882)			Progression-free survival (n = 646)		
	N	Hazard ratio	P	N	Hazard ratio	P
GENDER						
Male	349	2.15(1.60 – 2.89)	2.1e-07	341	2.15(1.61 – 2.88)	1.2e – 07
Female	187	2.52(1.57 – 4.04)	7.7e – 05	179	2.27(1.50 – 3.46)	7.9e – 05
STAGE						
1	62	2.16 (0.65 – 7.22)	0.20	60	1.97(0.59 – 6.59)	0.26
2	135	1.74 (0.91 – 3.31)	0.09	131	1.81(0.98 – 3.34)	0.054
3	197	1.99 (1.36 – 2.91)	0.00031	186	2.24(1.52 – 3.3)	2.8e – 05
4	140	1.98 (1.31 – 3.01)	0.0011	141	1.51(1.03 – 2.23)	0.034
STAGE T						
2	241	2.08 (1.36 – 3.2)	0.00062	239	1.98(1.31–3.00)	0.001
3	204	1.70(1.17 – 2.47)	0.0047	204	1.49(1.04 – 2.12)	0.027
4	38	3.06 (1.25 – 7.46)	0.0099	39	4.39(1.75 – 11.02)	0.00074
STAGE N						
0	74	2.66(1.1 – 6.4)	0.025	72	2.62(1.09 – 6.29)	0.025
1	225	2.53(1.68 – 3.82)	4.6e – 06	222	2.38(1.61 – 3.52)	7.8e – 06
2	121	2.14(1.36 – 3.37)	0.00077	125	2.06(1.32 – 3.23)	0.0012
3	76	2.34(1.35 – 4.06)	0.0019	76	2(1.16 – 3.45)	0.012
1 + 2 + 3	442	2.29(1.75 – 2.98)	3.6e – 10	423	2.12(1.65 – 2.74)	2.9e – 09
STAGE M						
0	444	2.2(1.67 – 2.92)	1.5e – 08	443	2.07(1.59 – 2.71)	5.3e – 08
1	56	2.46(1.33 – 4.56)	0.0033	56	1.57(0.84 – 2.93)	0.16
LAUREN CLASSIFICATION						
Diffuse	240	1.95(1.36 – 2.8)	0.00022	231	2.4(1.64 – 3.52)	3.4e – 06
Intestinal	169	2.18(1.51 – 3.13)	1.8e – 05	263	2.17(1.52 – 3.08)	1.1e – 05
Mixed	29	3.84(1.24 – 11.9)	0.013	28	1.98(0.56 – 7)	0.28
DIFFERENTIATION						
Poor	121	1.57(0.96 – 2.59)	0.071	121	1.52(0.95 – 2.43)	0.08
Moderate	67	2.17(0.94 – 5.02)	0.064	67	1.83(0.98 – 3.41)	0.055
Bold values indicate P < 0.05.						

4. TSHZ3 Expression is Associated with Immune Infiltrates Level in STAD and BLCA

We evaluated the correlations between TSHZ3 expression and immune infiltration profiles in 39 cancer types with TIMER. The rough evaluation results revealed significant correlations between TSHZ3 expression and the tumor purity in 25 cancer types, B cell infiltration levels in 10 cancer types, CD8 + T cells infiltrating levels in 18 cancer types, CD4 + T cells infiltrating levels in 24 cancer types, macrophages infiltrating levels in 25 cancer types, neutrophils infiltrating levels in 26 cancer types, and dendritic cells infiltrating levels in 27 cancer types (Fig. 3 and **Supplementary Fig. 2**).

Based on the correlation between TSHZ3 expression and immune infiltration profiles in different cancer types, we next focused on the distinct cancer types in which TSHZ3 was correlated with prognosis and immune infiltrates. Tumor purity can affect the results of immune infiltration analysis in clinical cancer samples with genomic approaches [30]. Thus, we selected the cancer types with significant negative correlation between TSHZ3 expression and tumor purity in TIMER to further exploration. Interestingly, we found there were significant positive correlations between TSHZ3

expression levels and infiltrating levels of CD8 + T cells ($r = 0.235$, $P = 4.89 \times 10^{-6}$), CD4 + T cells ($r = 0.455$, $P = 3.92 \times 10^{-20}$), macrophages ($r = 0.696$, $P = 5.68 \times 10^{-55}$), neutrophils ($r = 0.332$, $P = 5.54 \times 10^{-11}$) and DCs ($r = 0.476$, $P = 2.28 \times 10^{-22}$) in STAD (Fig. 3-C). Similarly, significant positive correlations existed between TSHZ3 expression and infiltrating levels of CD4 + T cells ($r = 0.186$, $P = 3.62 \times 10^{-4}$), macrophages ($r = 0.360$, $P = 1.25 \times 10^{-12}$), neutrophils ($r = 0.163$, $P = 1.79 \times 10^{-3}$), and DCs ($r = 0.130$, $P = 1.26 \times 10^{-2}$) in BLCA (Fig. 3-A). Furthermore, there was no significant associations between TSHZ3 expression and infiltrating levels of CD8 + T cells, B cells, CD4 + T cells, macrophages and DCs in kidney chromophobe (KICH) (Fig. 3-B).

5. Refined Analysis on the Correlation between TSHZ3 Expression and Immune Infiltrates

To further investigate the correlations between TSHZ3 expression and immune infiltrates, we focused on the correlations between TSHZ3 and immune marker genes of diverse TIICs and its' subtypes of BLCA, STAD in TIMER using KICH as the control. These TIICs included TAMs, B cells, monocytes, neutrophils, NK cells, CD8 + T cells and T cells (general). As different subsets of some TIICs have diverse effects in the progression of cancers, we also analyzed subsets of different TIICs, including M1 and M2 macrophages, pDCs and cDC1s, Th1 cells, Th2 cells, Th17 cells, Tregs, Tfh cells as well as T cells exhaustion. After adjusted with purity, our results revealed significant correlations between TSHZ3 expression levels and all listed immune marker sets in some pro-tumorigenic TIICs in STAD and BLCA, such as M2 macrophages, monocytes, B cells and pDCs. Besides, the expressions of all the immune marker sets of other TIICs (TAMs, Th2 cells and Tregs) were positively correlated with the TSHZ3 expression level in STAD. However, TSHZ3 expression level had no significant correlation with TIICs in KICH (Table 3). The validation results via GEPIA were similar to the refined analysis results in TIMER (Table 4).

Table 3
Correlation analysis between TSHZ3 and gene markers of immune cells in TIMER.

Immune Cells	Gene Markers	STAD		BLCA				KICH					
		None		Purity		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P
TAM	CCL2	0.539	***	0.511	***	0.500	***	0.327	***	-0.078	0.531	-0.076	0.546
	CD68	0.231	***	0.203	***	0.284	***	0.097	0.063	0.001	0.995	0.017	0.893
	IL6	0.297	***	0.271	***	0.426	***	0.242	***	0.128	0.307	0.144	0.252
	IL10	0.457	***	0.440	***	0.562	***	0.413	***	0.044	0.726	0.068	0.593
B Cell	CD19	0.332	***	0.307	***	0.332	***	0.141	**	0.067	0.594	0.076	0.549
	CD79A	0.345	***	0.302	***	0.398	***	0.203	***	0.165	0.186	0.179	0.154
	PNOC	0.383	***	0.349	***	0.380	***	0.217	***	0.092	0.462	0.102	0.419
pDC	CD303(CLEC4C)	0.336	***	0.355	***	0.275	***	0.153	**	0.119	0.341	0.126	0.319
	BDCA-4(NRP1)	0.674	***	0.660	***	0.445	***	0.334	***	0.162	0.192	0.201	0.108
	CD304(IL3RA)	0.632	***	0.609	***	0.483	***	0.318	***	0.036	0.776	0.040	0.755
	CCR2	0.475	***	0.451	***	0.463	***	0.225	***	0.030	0.809	0.053	0.676
	CXCR3	0.255	***	0.230	***	0.362	***	0.128	*	0.011	0.933	0.025	0.842
cDC1	HLA-DRA	0.205	***	0.168	**	0.333	***	0.098	0.059	-0.147	0.238	-0.164	0.192
	CD141(THBD)	0.613	***	0.599	***	0.121	*	0.029	0.578	0.061	0.625	0.075	0.554
	XCR1	0.342	***	0.317	***	0.218	***	0.164	**	0.311	*	0.311	*
	CLEC9A	0.363	***	0.347	***	0.336	***	0.182	***	-0.330	**	-0.346	**
	DEC-205(LY75)	-0.132	**	-0.138	**	-0.045	0.368	-0.032	0.544	-0.083	0.508	-0.081	0.522
Monocyte	CD155(CSF1R)	0.537	***	0.520	***	0.553	***	0.385	***	-0.171	0.170	-0.199	0.112
	CD86	0.409	***	0.382	***	0.478	***	0.275	***	-0.080	0.532	-0.081	0.519
	CCR1	0.415	***	0.388	***	0.454	***	0.258	***	-0.095	0.446	-0.097	0.442
M1 Macrophage	IRF5	0.219	***	0.196	***	-0.030	0.545	-0.025	0.628	-0.221	0.075	-0.238	0.056
	INOS(NOS2)	-0.012	0.813	-0.025	0.628	0.123	*	0.049	0.350	-0.186	0.134	-0.192	0.125
	COX2(PTGS2)	0.270	***	0.254	***	0.178	***	0.115	*	0.068	0.584	0.068	0.592
M2 Macrophage	IRF4	0.327	***	0.285	***	0.411	***	0.183	***	-0.021	0.866	-0.011	0.931
	CCL17	0.271	***	0.231	***	0.204	***	0.134	*	0.039	0.754	0.048	0.703
	CD163	0.469	***	0.455	***	0.535	***	0.352	***	0.003	0.982	0.018	0.888
	VSIG4	0.495	***	0.497	***	0.530	***	0.351	***	-0.019	0.877	-0.010	0.936
	MS4A4A	0.515	***	0.504	***	0.539	***	0.360	***	-0.072	0.565	-0.074	0.560
Natural Killer Cell	KIR2DL1	0.140	**	0.130	*	0.125	*	-0.022	0.680	-0.085	0.499	-0.082	0.519
	KIR2DL3	0.061	0.213	0.023	0.658	0.174	***	-0.001	0.991	-0.211	0.089	-0.210	0.926
	KIR2DL4	-0.074	0.131	-0.114	*	0.118	*	-0.049	0.351	-0.185	0.137	-0.184	0.143
	KIR3DL1	0.100	*	0.080	0.119	0.168	***	0.077	0.141	-0.120	0.338	-0.118	0.350
	KIR3DL2	0.139	**	0.110	*	0.095	0.056	-0.057	0.273	-0.051	0.682	-0.047	0.710
	KIR3DL3	-0.078	0.112	-0.069	0.177	-0.038	0.445	-0.112	*	-0.250	*	-0.250	*
	KIR2DS4	0.034	0.487	0.014	0.791	0.103	*	-0.053	0.309	-0.131	0.295	-0.130	0.302

*P < 0.05, **P < 0.01, ***P < 0.001; NA or NaN means no association (the expression level of marker was 0); STAD, stomach adenocarcinoma; BLCA, bladder urothelial carcinoma; KICH, kidney chromophobe; TAM, tumor-associated macrophage; pDC, plasmacytoid dendritic cell; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; None, correlation without adjustment. Purity, correlation adjusted by purity; Cor, R value of Spearman's correlation.

Neutrophils	CCR7	0.428	***	0.389	***	0.139	**	0.078	0.133	0.100	0.424	0.125	0.320
	CD66b(CEACAM8)	0.007	0.890	0.018	0.727	0.017	0.739	0.023	0.654	-0.011	0.928	-0.011	0.929
	CD11b(ITGAM)	0.513	***	0.502	***	0.514	***	0.328	***	-0.135	0.278	-0.158	0.208
CD8 + T	CD8A	0.260	***	0.228	***	0.260	***	0.028	0.592	0.058	0.631	0.092	0.467
Cell	CD8B	0.108	*	0.081	0.114	0.148	**	-0.025	0.638	0.233	0.060	0.290	*
T Cell (general)	CD2	0.270	***	0.231	***	0.349	***	0.105	*	0.069	0.578	0.100	0.427
	CD3D	0.225	***	0.174	***	0.314	***	0.098	0.061	0.040	0.747	0.063	0.617
	CD3E	0.253	***	0.206	***	0.371	***	0.125	*	0.028	0.821	0.048	0.707
Th1	STAT1	-0.061	0.214	-0.067	0.192	0.158	**	-0.046	0.378	-0.119	0.341	-0.117	0.354
	STAT4	0.329	***	0.300	***	0.352	***	0.118	*	-0.070	0.575	-0.069	0.584
	T-bet(TBX21)	0.268	***	0.235	***	0.315	***	0.087	0.097	-0.205	0.099	-0.221	0.077
	IFN- γ (IFNG)	-0.067	0.17	-0.096	0.061	0.135	**	-0.069	0.187	0.159	0.203	0.195	0.120
	TNF- α (TNF)	0.128	**	0.085	0.099	0.154	**	0.006	0.907	-0.206	0.097	-0.205	0.085
Th2	IL13	0.142	**	0.146	**	0.169	***	0.037	0.485	-0.039	0.755	-0.040	0.750
	STAT5A	0.409	***	0.403	***	0.173	***	-0.001	0.990	-0.201	0.106	-0.216	0.084
	STAT6	0.122	*	0.120	*	-0.089	0.074	-0.003	0.957	-0.194	0.118	-0.201	0.108
	GATA3	0.361	***	0.346	***	-0.215	***	-0.08	0.126	-0.374	**	-0.374	**
Th17	IL17A	-0.197	***	-0.227	***	-0.065	0.190	-0.127	*	NA	NA	NaN	NaN
	STAT3	0.454	***	0.437	***	0.231	***	0.072	0.166	-0.135	0.277	-0.140	0.267
Tfh	IL21	0.076	0.124	0.057	0.273	0.114	*	0.035	0.505	NA	NA	NaN	NaN
	BCL6	0.534	***	0.512	***	-0.046	0.353	-0.009	0.856	0.137	0.272	0.135	0.283
Treg	CCR8	0.358	***	0.343	***	0.426	***	0.282	***	0.167	0.179	0.179	0.154
	FOXP3	0.242	***	0.199	***	0.393	***	0.220	***	0.212	0.087	0.230	0.065
	STAT5B	0.608	***	0.591	***	0.098	*	0.077	0.142	-0.193	0.121	-0.192	0.125
	TGF β (TGFB1)	0.641	***	0.633	***	0.308	***	0.226	***	0.528	***	0.555	***
T Cell Exhaustion	CTLA4	0.104	*	0.061	0.235	0.300	***	0.071	0.177	0.053	0.672	0.073	0.565
	GZMB	0.011	0.817	0.044	0.388	0.286	***	0.035	0.504	-0.060	0.631	-0.056	0.659
	LAG3	0.112	*	0.076	0.137	0.259	***	0.037	0.438	0.075	0.548	0.086	0.498
	PD-1(PDCD1)	0.174	***	0.139	**	0.311	***	0.081	0.123	0.057	0.651	0.075	0.551
	TIM-3(HAVCR2)	0.392	***	0.379	***	0.458	***	0.239	***	-0.127	0.309	-0.131	0.299
*P < 0.05,**P < 0.01,***P < 0.001; NA or NaN means no association (the expression level of marker was 0); STAD, stomach adenocarcinoma; BLCA, bladder urothelial carcinoma; KICH, kidney chromophobe; TAM, tumor-associated macrophage; pDC, plasmacytoid dendritic cell; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; None, correlation without adjustment. Purity, correlation adjusted by purity; Cor, R value of Spearman's correlation.													

Table 4
Correlation analysis between TSHZ3 and relate gene markers of immune cell in GEPIA.

Immune Cells	Gene Markers	STAD				BLCA				KICH			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
		R	P	R	P	R	P	R	P	R	P	R	P
TAM	CCL2	0.550	***	0.560	***					-0.024	0.980	0.440	*
	CD68	0.300	***	-0.480	**					0.100	0.420	0.510	*
	IL6	0.330	***	0.750	***					0.140	0.260	0.240	0.250
	IL10	0.510	***	-0.021	0.90					0.120	0.340	0.440	*
B Cell	CD19	0.330	***	-0.480	**	0.360	***	-0.092	0.710	0.150	0.240	0.570	**
	CD79A	0.310	***	-0.640	***	0.410	***	-0.360	0.130	0.280	0.024	0.470	*
	PNOC	0.380	***	-0.220	0.210	0.390	***	0.340	***	0.190	0.140	0.130	0.540
pDC	CD303(CLEC4C)	0.370	***	0.098	0.570	0.340	***	-0.150	0.540	0.200	0.110	0.530	***
	BDCA-4(NRP1)	0.710	***	0.800	***	0.470	***	0.730	***	0.300	*	0.710	***
	CD304(IL3RA)	0.680	***	0.720	***	0.500	***	0.350	0.150	0.170	0.170	0.160	0.430
	CCR2	0.520	***	-0.350	*	0.490	***	-0.22	0.360	0.150	0.240	0.640	***
	CXCR3	0.280	***	-0.570	***	0.400	***	-0.700	***	0.120	0.330	0.540	***
Monocyte	CD155(CSF1R)	0.600	***	0.100	0.550	0.590	***	0.580	**	-0.038	0.760	0.740	***
	CD86	0.440	***	-0.290	0.091	0.520	***	-0.320	0.180	0.023	0.860	0.510	**
	CCR1	0.480	***	0.160	0.360	0.500	***	0.370	***	0.025	0.840	0.370	0.068
M2 Macrophage	IRF4	0.350	***	-0.610	***	0.430	***	-0.380	0.100	0.063	0.620	0.730	***
	CCL17	0.290	***	-0.210	0.210	0.210	***	0.190	*	0.018	0.890	0.310	0.130
	CD163	0.490	***	0.620	***	0.550	***	0.550	*	0.095	0.450	0.690	***
	VSIG4	0.530	***	0.350	*	0.560	***	0.720	***	0.110	0.380	0.580	**
	MS4A4A	0.550	***	0.470	**	0.570	***	0.750	***	0.071	0.570	0.520	**
Th2	IL13	0.230	***	0.410	*					0.130	0.290	0.290	0.150
	STAT5A	0.500	***	0.830	***					0.038	0.760	0.740	***
	STAT6	0.210	***	0.610	***					0.061	0.630	0.810	***
	GATA3	0.360	***	-0.260	0.130					-0.300	*	-0.059	0.780
Treg	CCR8	0.430	***	-0.250	0.140					0.230	0.059	0.110	0.580
	FOXP3	0.300	***	-0.490	**					0.240	0.052	0.520	**
	STAT5B	0.660	***	0.930	***					0.092	0.460	0.770	***
	TGFβ(TGFB1)	0.670	***	0.540	***					0.580	***	0.750	***
*P < 0.05,**P < 0.01 ***P < 0.001; STAD, stomach adenocarcinoma; BLCA, bladder urothelial carcinoma; KICH, kidney chromophobe; TAM, tumor-associated macrophage; pDC, plasmacytoid dendritic cell; Th, T helper cell; Treg, regulatory T cell; R, R value of Spearman's correlation.													

5.1. Macrophages Infiltrations

Rough evaluation by the Gene module suggested high TSHZ3 expression was correlated with high infiltration level of macrophages. Refined analyses by the Correlation module revealed high TSHZ3 expression was associated with high infiltration level of M2 macrophages but had no correlations with the infiltration level of M1 Macrophages in STAD and BLCA (Fig. 4A, Table 2). Positive correlation between TAMs infiltration level and TSHZ3 expression in STAD was also revealed (Fig. 4B). These findings imply that TSHZ3 plays a critical role in regulating macrophage polarization in STAD and BLCA.

5.2. DCs Infiltrations

Rough evaluation in TIMER showed a high TSHZ3 expression was associated with high infiltration level of DCs. Further refined analyses revealed high TSHZ3 expression was correlated to high infiltration level of pDCs but had no associations with the infiltration level of cDCs1 in STAD and BLCA

(Fig. 4C, Table 2). These results further suggest that there is a correlation between TSHZ3 and pDCs infiltration in STAD and BLCA.

5.3. TILs Infiltrations

Refined analyses revealed high infiltration level of Th2 cells and Tregs correlate with high TSHZ3 expression in STAD (Fig. 4D, Fig. 4E and Table 2), those two subsets are well documented to be pro-tumorigenic. As for the anti-tumorigenic TILs, such as Th1 cells and Tfh cells, their infiltration levels had no significant correlation with TSHZ3 expression in STAD and BLCA (Table 2). In addition, PD-1(PDCD1) and TIM-3(HAVCR2), the crucial genes that regulate T cell exhaustion, had correlation with TSHZ3 expression in STAD (Table 2). Therefore, these findings further confirm that TSHZ3 plays an important role in immune escape in the GC microenvironment.

5.4. Other TIICs infiltrations

Other TIICs with pro-tumorigenic effect, including monocytes and B cells, have high infiltration level in STAD and BLCA which present high TSHZ3 expression (Fig. 4F, Fig. 4G). Our results also suggest that infiltrations of other TIICs, such as NK cells, Th 17 cells, Tfh cells and neutrophils has no association with TSHZ3 expression level (Table 2).

Discussion

TSHZ3 is a driver of the 19q12 amplicon and the silencing of TSHZ3 is selectively lethal in breast cancer cells harbouring its amplification [30]. Additionally, high expression level of TSHZ3 predicts poor prognosis in tongue squamous cell carcinomas by slight correlation with immune infiltration [20]. Here, we report that TSHZ3 expression is up-regulated in many cancer types, such as STAD and BLCA. Furthermore, high expression of TSHZ3 predicts poor prognosis in those two cancers. Our findings are in consistence with the previous studies [20]. Since the TSHZ3 expression levels in different cancer types remain controversial, we also conducted meta-analysis with Oncomine to provide more convinced evidence on the expression levels of TSHZ3 in different cancer types.

The survival analysis in relation to the TSHZ3 expression differs in different databases, we chose the databases with a larger sample size to conduct the survival analyses and we also selected the cancer types whose prognosis have significant correlation with TSHZ3 expression both revealed by the two methods in TIMER to further exploration. Our study demonstrated that TSHZ3 is a potential biomarker to predict poor prognosis in STAD and BLCA. Interestingly, increased TSHZ3 expression levels is able to influence the prognosis of GC patients with lymph node metastasis or distant metastasis, indicating that the TSHZ3 expression can be also used as a predictor for cancer metastasis. Immune infiltration analysis by the Gene module roughly presents the immune infiltrates. Gene makers listed is the sufficient evidence of relevant TIICs infiltration, furthermore, different subsets of TIICs have different effects on the progression of cancers. Thus, we eventually identified immune infiltrates by immune infiltration analysis with the Correlation module of TIMER. In our present study, we found M2 macrophages infiltration, instead of M1 macrophages, is directly correlated with TSHZ3 expression in STAD and BLCA. It is well-documented that M2 macrophages function as pro-tumorigenic by producing Th2 cytokines. In contrast, M1 macrophages are anti-tumorigenic as they generate Th1 cytokines and express tumoricidal phenotype molecules, such as iNOS [13]. In addition, our data show that TAMs infiltration has positive correlation with TSHZ3 expression in STAD. Recent studies report that TAMs is similar to M2 macrophages in phenotypic characteristics and they promote angiogenesis and invasion in tumors [31, 32]. Our data also show that monocytes infiltrations are positively correlated with TSHZ3 expressions in STAD and BLCA. It has been reported that monocytes present pro-tumorigenic effect by promoting tumorigenesis and angiogenesis, inhibiting the antitumor immune response *in vivo*, and differentiate into TAMs [33, 34].

TILs play key roles in the regulation of tumor microenvironment. As subsets of CD4 + T cells, Th1 cells are anti-tumorigenic, Th2 cells are pro-tumorigenic, and Treg cells usually exert pro-tumorigenic effects by suppressing immune-surveillance [13]. Our results reveal that the infiltrations of Th2 cells and Treg cells are directly correlated with TSHZ3 expression in STAD but the infiltrations of Th1 cells are not. We suggest that high expression of TSHZ3 leads to poor prognosis by promoting Th2 cells and Treg cells infiltrations. B cells promote cancer growth by producing pro-tumorigenic cytokines and altering ratios of Th1 cells: Th2 cells. B cells support tumor growth in B cell-deficient mice by present resistance to engraftment of syngeneic cancers [13, 35]. Moreover, B cells are positively associated with immunotherapy response in some tumors [36, 37]. Our study shows that B cells infiltration is positively correlated with TSHZ3 expression in STAD and BLCA. We also show that the expressions of some key marker for T cell exhaustion [38–40], such as PD-1(PDCD1) and TIM-3(HAVCR2), have positive correlations with TSHZ3 expression in STAD, suggesting that TSHZ3 plays a key role in T cell exhaustions mediated by PD-1 and TIM-3.

As for DCs infiltration, recent studies documented the biological functions of pDCs and cDC1s in tumors. pDCs, present the phenotypes as CD303(CLEC4C), CD304(NRP1), CD123(IL3RA), CCR2 and CXCR3, which predict poor prognosis of cancers by promoting the expansion of Treg cell populations [12, 41]. cDC1s, present the phenotypes as HLA-DR, CD141, XCR1, CLEC9A and DEC-205(LY75), are often related to superior cross-presentation of antigens, which results in stronger CD8 + T cell immunity, and can additionally support Th1 cell polarization of CD4 + T cells [42–46]. These findings support our findings that pDCs infiltration has positive correlation with TSHZ3 expression level in STAD and BLCA.

NK cells are anti-tumorigenic TIICs [47]. However, our data show that NK cell infiltration has no significant correlation with TSHZ3 expression in STAD and BLCA. The infiltrations of Th17 cells, Tfh cells and neutrophils also have no significant correlations with TSHZ3 expression in STAD and BLCA. The effects of Th17 cells on cancers are bidirectional. On the one hand, Th17 cells promote tumor growth by secreting angiogenic factors (*via* IL-17) and exhibiting their immunosuppressive functions. On the other hand, Th17 cells also drive antitumor immune responses *via* recruiting immune cells, activating effector T cells (CD8+), or directly converting their phenotypes toward Th1 cells [48]. It could be a good explanation for our finding that the

secretion of IL-17A is negatively correlated with TSHZ3 expression in STAD and BLCA. High Tfh cells infiltrations have been reported to be related to good prognosis of some cancers [49]. Our study does not find a significant correlation between Tfh cells infiltration and TSHZ3 expression. Some studies reported that neutrophils are existed in different subsets which have diverse effects on cancers, but their phenotypic characteristics are less studied [50]. The correlation between the phenotypic characteristics of different neutrophil subsets and the TSHZ3 expression deserves further investigation. There are some limitations in our study. Firstly, we failed to divide some TILs into different subsets for further evaluations because there is a lack of well-recognized phenotypic characteristics for these TILs subsets. Secondly, though we enlarge the sample size in the meta-analysis with the Oncomine database, heterogeneity among the included studies may exist.

Conclusion

Overall, our study demonstrates that TSHZ3 expression is elevated in STAD and BLCA, which predicts the poor prognosis of the STAD and BLCA patients. Furthermore, TSHZ3 expression potentially contributes to the increased tumor infiltrations of M2 macrophages, pDCs, monocytes and B cells in STAD and BLCA, and TAMs, Th2 cells, Tregs and T cell exhaustion in STAD. Our findings strongly suggest that TSHZ3 plays a critical role in the immune infiltration and can be used as a prognostic biomarker in patients with STAD and BLCA.

Declarations

Competing interests

All authors declare that no conflict of interest exists regarding the submission of this work.

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Availability of data and materials

All the dataset supporting our findings was retrieved by using the Oncomine database, [<https://www.oncomine.org/resource/login.html>], GEPIA, [<http://gepia.cancer-pku.cn/>], Kaplan-Meier plotter, [<http://kmplot.com/analysis/>] and TIMER, [<https://cistrome.shinyapps.io/timer/>].

Author's contributions

Xi Zhang and Xiao-shan Zhao designed the study and interpreted data. Xi Zhang collected and analysed data, formed figures and wrote the manuscript. Min Wang, Han-qi Lu, Qiu-xing He, Yan-ting You, Xing-hong Zhou, Xiu-qiong Fu, Dong-hui Liang, Hiu Yee Kwan and Lin Zhou revised the manuscript.

Ethics approval

This study is not concerned with any clinical trials or animal experiments.

Consent for publication

Not applicable.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: E359-86.
2. Cumberbatch M, Jubber I, Black PC, Esperto F, Figueroa JD, Kamat AM, et al. Epidemiology of Bladder Cancer: A Systematic Review and Contemporary Update of Risk Factors in 2018. *Eur Urol* 2018;7 4: 784-95.

3. Matuszewski M, Szymanska B, Dlugosz A, Malkiewicz B, Dembowski J, Piwowar A. Preliminary Evaluation of the Diagnostic Usefulness of Uroplakin 2 with an Assessment of the Antioxidant Potential of Patients with Bladder Cancer. *Biomed Res Int* 2018; 2018: 8693297.
4. Angell HK, Lee J, Kim KM, Kim K, Kim ST, Park SH, et al. PD-L1 and immune infiltrates are differentially expressed in distinct subgroups of gastric cancer. *Oncoimmunology* 2019; 8: e1544442.
5. Pan S, Zhan Y, Chen X, Wu B, Liu B. Bladder Cancer Exhibiting High Immune Infiltration Shows the Lowest Response Rate to Immune Checkpoint Inhibitors. *Front Oncol* 2019; 9: 1101.
6. Annels NE, Mansfield D, Arif M, Ballesteros-Merino C, Simpson GR, Denyer M, et al. Phase I Trial of an ICAM-1-Targeted Immunotherapeutic-Coxsackievirus A21 (CVA21) as an Oncolytic Agent Against Non Muscle-Invasive Bladder Cancer. *Clin Cancer Res* 2019; 25: 5818-31.
7. Solinas C, Pusole G, Demurtas L, Puzzone M, Mascia R, Morgan G, et al. Tumor infiltrating lymphocytes in gastrointestinal tumors: Controversies and future clinical implications. *Crit Rev Oncol Hematol* 2017; 110: 106-16.
8. Lu J, Xu Y, Wu Y, Huang XY, Xie JW, Wang JB, et al. Tumor-infiltrating CD8⁺ T cells combined with tumor-associated CD68⁺ macrophages predict postoperative prognosis and adjuvant chemotherapy benefit in resected gastric cancer. *Bmc Cancer* 2019; 19: 920.
9. Qi Y, Chang Y, Wang Z, Chen L, Kong Y, Zhang P, et al. Tumor-associated macrophages expressing galectin-9 identify immunoevasive subtype muscle-invasive bladder cancer with poor prognosis but favorable adjuvant chemotherapeutic response. *Cancer Immunol Immunother* 2019; 68: 2067-80.
10. Noh BJ, Kim JH, Eom DW. Prognostic Significance of Categorizing Gastric Carcinoma by PD-L1 Expression and Tumor Infiltrating Lymphocytes. *Ann Clin Lab Sci* 2018; 48: 695-706.
11. Ajili F, Kourda N, Darouiche A, Chebil M, Boubaker S. Prognostic value of tumor-associated macrophages count in human non-muscle-invasive bladder cancer treated by BCG immunotherapy. *Ultrastruct Pathol* 2013; 37: 56-61.
12. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol* 2020; 20: 7-24.
13. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* 2013; 19: 1423-37.
14. Chabbert D, Caubit X, Roubertoux PL, Carlier M, Habermann B, Jacq B, et al. Postnatal Tshz3 Deletion Drives Altered Corticostriatal Function and Autism Spectrum Disorder-like Behavior. *Biol Psychiatry* 2019; 86: 274-85.
15. Caubit X, Gubellini P, Andrieux J, Roubertoux PL, Metwaly M, Jacq B, et al. TSHZ3 deletion causes an autism syndrome and defects in cortical projection neurons. *Nat Genet* 2016; 48: 1359-69.
16. Caubit X, Lye CM, Martin E, Core N, Long DA, Vola C, et al. Teashirt 3 is necessary for ureteral smooth muscle differentiation downstream of SHH and BMP4. *Development* 2008; 135: 3301-10.
17. Li Y, Huang Y, Qi Z, Sun T, Zhou Y. MiR-338-5p Promotes Glioma Cell Invasion by Regulating TSHZ3 and MMP2. *Cell Mol Neurobiol* 2018; 38: 669-77.
18. McBride DJ, Etemadmoghadam D, Cooke SL, Alsop K, George J, Butler A, et al. Tandem duplication of chromosomal segments is common in ovarian and breast cancer genomes. *J Pathol* 2012; 227: 446-55.
19. Natrajan R, Mackay A, Wilkerson PM, Lambros MB, Wetterskog D, Arnedos M, et al. Functional characterization of the 19q12 amplicon in grade III breast cancers. *Breast Cancer Res* 2012; 14: R53.
20. Zeng H, Li H, Zhao Y, Chen L, Ma X. Transcripto-based network analysis reveals a model of gene activation in tongue squamous cell carcinomas. *Head Neck* 2019.
21. Elemeery MN, Badr AN, Mohamed MA, Ghareeb DA. Validation of a serum microRNA panel as biomarkers for early diagnosis of hepatocellular carcinoma post-hepatitis C infection in Egyptian patients. *World J Gastroenterol* 2017; 23: 3864-75.
22. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 2017; 45: W98-102.
23. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res* 2017; 77: e108-10.
24. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007; 9: 166-80.
25. Li B, Severson E, Pignon JC, Zhao H, Li T, Novak J, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol* 2016; 17: 174.
26. Pan JH, Zhou H, Cooper L, Huang JL, Zhu SB, Zhao XX, et al. LAYN Is a Prognostic Biomarker and Correlated With Immune Infiltrates in Gastric and Colon Cancers. *Front Immunol* 2019; 10: 6.
27. Danaher P, Warren S, Dennis L, D'Amico L, White A, Disis ML, et al. Gene expression markers of Tumor Infiltrating Leukocytes. *J Immunother Cancer* 2017; 5: 18.
28. Aran D, Sirota M, Butte AJ. Systematic pan-cancer analysis of tumour purity. *Nat Commun* 2015; 6: 8971.
29. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; 20: 1-19.

30. Yoshihara K, Shahmoradgoli M, Martinez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun* 2013; 4: 2612.
31. Qian B, Deng Y, Im JH, Muschel RJ, Zou Y, Li J, et al. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *Plos One* 2009; 4: e6562.
32. Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006; 66: 11238-46.
33. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; 9: 162-74.
34. Sasaki A, Iwashita Y, Shibata K, Matsumoto T, Ohta M, Kitano S. Prognostic value of preoperative peripheral blood monocyte count in patients with hepatocellular carcinoma. *Surgery* 2006; 139: 755-64.
35. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* 2005; 7: 411-23.
36. Petitprez F, de Reynies A, Keung EZ, Chen TW, Sun CM, Calderaro J, et al. B cells are associated with survival and immunotherapy response in sarcoma. *Nature* 2020; 577: 556-60.
37. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* 2020; 577: 549-55.
38. Huang YH, Zhu C, Kondo Y, Anderson AC, Gandhi A, Russell A, et al. CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. *Nature* 2015; 517: 386-90.
39. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood* 2011; 117: 4501-10.
40. Wherry EJ. T cell exhaustion. *Nat Immunol* 2011; 12: 492-9.
41. Aspod C, Leccia MT, Charles J, Plumas J. Plasmacytoid dendritic cells support melanoma progression by promoting Th2 and regulatory immunity through OX40L and ICOSL. *Cancer Immunol Res* 2013; 1: 402-15.
42. Wculek SK, Amores-Iniesta J, Conde-Garrosa R, Khouili SC, Melero I, Sancho D. Effective cancer immunotherapy by natural mouse conventional type-1 dendritic cells bearing dead tumor antigen. *J Immunother Cancer* 2019; 7: 100.
43. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, et al. Expansion and Activation of CD103(+) Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. *Immunity* 2016; 44: 924-38.
44. Sanchez-Paulete AR, Cueto FJ, Martinez-Lopez M, Labiano S, Morales-Kastresana A, Rodriguez-Ruiz ME, et al. Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti-PD-1 Monoclonal Antibodies Requires BATF3-Dependent Dendritic Cells. *Cancer Discov* 2016; 6: 71-9.
45. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* 2010; 207: 1247-60.
46. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science* 2008; 322: 1097-100.
47. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol* 2008; 9: 503-10.
48. Guery L, Hugues S. Th17 Cell Plasticity and Functions in Cancer Immunity. *Biomed Res Int* 2015; 2015: 314620.
49. Gu-Trantien C, Migliori E, Buisseret L, de Wind A, Brohee S, Garaud S, et al. CXCL13-producing TFH cells link immune suppression and adaptive memory in human breast cancer. *JCI Insight* 2017; 2.
50. Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? *Carcinogenesis* 2012;33: 949-55.

Figures

Figure 1

Figure 1

TSHZ3 expression in BLCA and STAD. (A) TSHZ3 expression in BLCA. (B) TSHZ3 expression in STAD.

Figure 2 Kaplan-Meier plots comparing the high and low expression of TSHZ3 in cancers with TSHZ3 differential expression by Kaplan-Meier plotter (A2, A3, B1, B2, B4 and C1) and TIMER (A1, A4, B3, C2 and C3). (A1) Kaplan-Meier plot of OS in BLCA (bladder urothelial carcinoma) (n=406). (A2-A3) Kaplan-Meier plot of OS and PFS in gastric cancer (n=631, n=522). (A4) Kaplan-Meier plot of OS in STAD (stomach adenocarcinoma) (n=408). (B1-B2) Kaplan-Meier plot of OS and PFS in lung cancer (n=1145, n=596). (B3) Kaplan-Meier plot of OS in LUSC (lung squamous cell carcinoma) (n=495). (B4-C1) Kaplan-Meier plot of OS and PFS in ovary cancer (n=655, n=614). (C2) Kaplan-Meier plot of OS in OV (ovarian serous cystadenocarcinoma) (n=561). (C3) Kaplan-Meier plot of OS in KICH (Kidney Chromophobe) (n=65). OS, overall survival; PFS, progression-free survival.

Figure 3

Rough evaluation reveal the correlation of immune infiltration level with TSHZ3 expression in BLCA, STAD and KICH. (A) TSHZ3 expression is significantly negatively correlated to tumor purity and has significant positive correlations with infiltrating levels of CD4+ T cells, macrophages, neutrophils, and dendritic cells but not B cells and CD8+ T cells in BLCA. (n = 408). (B) TSHZ3 expression has no significant correlations with tumor purity and infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in KICH (n = 66). (C) TSHZ3 expression is significantly negatively related to tumor purity and has significant positive correlations with infiltrating levels of CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells but not B cells in STAD (n = 415). BLCA (colon adenocarcinoma), STAD (stomach adenocarcinoma) and KICH (kidney chromophobe).

Figure 4

Figure 4

Refined analysis on the correlation between TSHZ3 Expression and Immune Infiltrates in BLCA, STAD and KICH. (A) M2 macrophages infiltration in STAD, BLCA and KICH. (B) TAMs infiltration in STAD and KICH. (C) pDCs infiltration in STAD, BLCA and KICH. (D) Th2 cells infiltration in STAD and KICH. (E) Tregs infiltration in STAD and KICH. (F) Monocytes infiltration in STAD, BLCA and KICH. (G) B cells infiltration in STAD, BLCA and KICH. STAD (stomach adenocarcinoma), BLCA (colon adenocarcinoma), KICH (kidney chromophobe).

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