ZCCHC24 is a Prognostic-related Biomarker and Correlated with Immune Infiltrates in Gastric Cancer and Lung Squamous Cell Carcinoma

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Abstract

Background: ZCCHC24 is one of alternative splicing factors which affect immune cell infiltration, but the mechanisms whereby it drives immune infiltration in cancer remain uncertain.

Methods: ZCCHC24 expression was analyzed via the Oncomine database and Tumor Immune Estimation Resource (TIMER) site. We evaluated the influence of ZCCHC24 on clinical prognosis using Kaplan-Meier plotter, the Prognoscan database and Gene Expression Profiling Interactive Analysis (GEPIA). The correlations between ZCCHC24 and cancer immune infiltrates was investigated via TIMER. In addition, correlations between ZCCHC24 expression and gene marker sets of immune infiltrates were analyzed by TIMER and GEPIA.

Results: ZCCHC24 significantly impacts the prognosis of diverse cancers via The Cancer Genome Atlas (TCGA). elevated ZCCHC24 expression was significantly correlated with poor OS and progression-free survival (PFS) in gastric cancers (OS HR = 2.11, P = 6.3e-12; PFS HR = 2.03, P = 3.1e-11). Moreover, ZCCHC24 significantly impacts the prognosis of diverse cancers via The Cancer Genome Atlas (TCGA). Specifically, high ZCCHC24 expression was correlated with worse OS and PFS in Stage, T stage, N stage, M stage, Lauren classification and differentiation of gastric cancer patients. ZCCHC24 expression was positively correlated with infiltrating levels of CD4+ T_HCD8+ T cells, Macrophages, Neutrophils and dendritic cells (DCs) in stomach adenocarcinoma (STAD) and lung squamous cell carcinoma (LUSC). ZCCHC24 expression showed strong correlations with diverse immune marker sets in STAD and LUSC.

Conclusions: These findings suggest that ZCCHC24 is a key factor which governs immune cell recruitment to gastric cancer and lung squamous cell carcinoma, potentially playing a vital role in governing immune cell infiltration and thus representing a valuable prognostic biomarker in gastric cancer and lung squamous cell carcinoma patients.

Introduction

Gastric cancer (GC) is the most common malignancy in worldwide, and it is particularly prevalent in East Asia(1). Despite advances in diagnostic imaging, surgical techniques and systemic therapy, GC remains to be the third leading cause of cancer-related deaths worldwide, and its prognosis is still poor(2). The interactions between the external environment and a series of factors concerning genetics and epigenetics cause the onset of GC and lead to the deregulation of the signalling pathways that may induce the onset of cancer(3, 4). Lung squamous cell carcinoma (LUSC) is one of the most common histological subtypes of non-small-cell lung cancer (NSCLC), it remain leading cause of cancer related death worldwide for a long period(5). The development and progression of GC and LUSC are regulated by immunological mechanism(5, 6). The immune infiltration of tumours is correlated with poor prognosis, which involves macrophages, neutrophils and regulatory T cells(7–9). The cytotoxic T lymphocytes, CD8 + and DCs of tumour infiltration are generally associated with favourable Gastric and Lung cancer outcomes (5, 12–13). Although inverse prognosis prediction values are available for several subsets of these immune cells, high ratios of Foxp3+/CD4 + and Foxp3+/CD8 + provide a poor prognosis in tumours(14, 15), whereas high Th1/Th2 ratios in tumours are associated with good prognosis(16). Many different immunotherapies have been proposed to treat this cancer type. Inhibitors of Cytotoxic programmed death ligand-1(PD-L1), programmed death-1(PD-1) and T lymphocyte-associated antigen 4 (CTLA4) show promising antitumor effects in malignant melanoma and NSCLC, However, anti-CTLA4, anti-PD-1 and anti-PD-L1 did not show great efficacy in GC (17, 18).

Zinc finger CCHC-type 24 (ZCCHC24) is a member of the ZCCHC superfamily of proteins, which includes two zinc finger domains: one associated with the CCHC-type superfamily and the other to the zf-3CxxC superfamily(19). ZCCHC superfamily proteins are involved in biological functions, such as cell growth, differentiation, DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly and lipid binding (20). Each member plays diverse roles in cellular physiological function and disease aetiology. 2. ZCCHC2 regulates retinoblastoma tumorigenesis by inhibiting the activity of c-My (21); ZCCHC7 is disrupted by the 3' sequences of FSHD region gene 1 family, member B, whereas LOC1499503 is a potential genetic target in acute lymphoblastic leukaemia(22); ZCCHC8 is identified as a fusion partner of ROS1 in congenital glioblastoma multiforme(23), ZCCHC12 is referred to as a novel oncogene of papillary thyroid carcinoma(24, 25). ZCCHC1, ZCCHC5, ZCCHC9, ZCCHC13, ZCCHC20 and ZCCHC21 are related to various solid tumours, such as breast, lung, liver, colorectal and renal cancers(21). ZCCHC24 is also involved in the regulation of apoptosis. However, the research on ZCCHC24 in cancer is limited.

Most ZCCHC superfamily proteins participate in RNA metabolism, including transcriptional elongation, polyadenylation, translation, pre-messenger RNA splicing, RNA export, RNA degradation, microRNA and ribosomal RNA biogenesis and post-transcriptional gene silencing(27). Alternative splicing (AS) is one of the most important mechanisms of post-transcriptional regulation. This mechanism is a regulated process by which RNA precursors are effectively spliced and joined and can generate great biodiversity (28). Studies demonstrated the physiological contribution of AS to tissue-identity acquisition, organ development and tissue physiology; meanwhile, studies of AS demonstrated its involvement in multiple pathologies, including cancer(28). Growing evidence demonstrates that AS plays an important role in immune microenvironment formation (30, 31). AS alterations may not only affect immune cell infiltration but also regulate tumor-associated immune cytolytic activity. Additionally, cancer-specific AS changes have been recognised as important signatures to predict the treatment efficacy in recent years(32). Research showed that changes in the expression of AS factor ZCCHC24 contribute to phase-specific AS during reprogramming(33). These findings suggest that ZCCHC24 has multifaceted functional roles in immune cell infiltration.

Materials And Methods

Oncomine Database Analysis

The expression level of the ZCCHC24 gene and prognostic outcome in various types of cancers was identified in the Oncomine database (<https://www.oncomine.org/resource/login.html>)(34). The threshold was determined according to the following values: P-value of 0.001, fold change of 1.5, and gene ranking of all.

PrognoScan Database Analysis

The PrognoScan database is designed to facilitate meta-analyses of gene prognostic value by comparing the relationship between gene expression and relevant outcome including overall survival (OS) in a wide range of published cancer microarray data sets(35). We therefore used this database to assess the relationship between ZCCHC24 expression and patient outcome(<http://www.abren.net/Progn.oScan/>).

Kaplan-Meier Plotter Database Analysis

The Kaplan-Meier plotter offers a means of readily exploring the impact of a wide array of genes on patient survival in 21 different types of cancer, with large sample sizes for the breast (n = 6,234), ovarian (n = 2,190), lung (n = 3,452) and gastric (n = 1,440) cancer cohorts. The correlation between ZCCHC24 expression and survival in bladder, ovarian, lung and gastric cancers was analyzed by Kaplan-Meier plotter (<http://kmplot.com/analysis/>)(36). The hazard ratio (HR) with 95% confidence intervals and log-rank P-value were also computed.

TIMER Database Analysis

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types (<https://cistrome.shinyapps.io/timer/>).³⁷ TIMER applies a deconvolution previously published statistical method to infer the abundance of tumor-infiltrating immune cells (TIICs) from gene expression profiles(38). The TIMER database includes 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA) to estimate the abundance of immune infiltrates. We analyzed ZCCHC24 expression in different types of cancer and the correlation of ZCCHC24 expression with the abundance of immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, Neutrophils, Macrophages, and Dendritic cells, via gene modules. Gene expression levels against tumor purity is displayed on the left-most panel (39). In addition, correlations between ZCCHC24 expression and gene markers of tumor-infiltrating immune cells were explored via correlation modules. The gene markers of tumor-infiltrating immune cells included markers of CD8+ T cells, T cells (general), B cells, Monocytes, TAMs, M1 macrophages, M2 macrophages, Neutrophils, Natural killer (NK) cells, Dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, Follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs, and exhausted T cells. These gene markers are referenced in prior studies (40–42). The correlation module generated the expression scatter plots between a pair of user defined genes in a given cancer type, together with the Spearman's correlation and the estimated statistical significance. ZCCHC24 was used for the x-axis with gene symbols, and related marker genes are represented on the y-axis as gene symbols. The gene expression level was displayed with log2 RSEM.

Gene Correlation Analysis in GEPIA

GEPIA is an online database which facilitates the standardized analysis of RNA-seq data from 9,736 tumour samples and 8,587 normal control samples in the TCGA and GTEx data sets (<http://gepia.cancer-pku.cn/index.html>) (43). We therefore employed this database to assess the link between ZCCHC24 expression and patient prognosis in multiple tumour types, and we further assessed the link between ZCCHC24 expression and the expression of particular markers associated with immune cell infiltration of tumours.

Statistical Analysis

The PrognoScan, Kaplan-Meier plotter, TIMER and GEPIA databases were used for generating Survival curves. The results generated in Oncomine are displayed with P-values, fold changes, and ranks. The results of Kaplan-Meier plots, PrognoScan, and GEPIA are displayed with HR and P or Cox P-values from a log-rank test. Spearman's correlation analyses were used to gauge the degree of correlation between particular variables, with the following values being used to judge the strength of correlation: 0.00–0.19 “very weak,” 0.20–0.39 “weak,” 0.40–0.59 “moderate,” 0.60–0.79 “strong,” 0.80–1.0 “very strong.” P-values <0.05 were considered statistically significant.

Results

The mRNA Expression Levels of ZCCHC24 in Different Types of Human Cancers

We first used Oncomine database to assess the expression of ZCCHC24 in multiple tumour and normal tissue types. This analysis revealed that the ZCCHC24 expression was higher in Colorectal Cancer, Esophageal Cancer, Gastric cancer, lymphoma tumors, Pancreatic Cancer and Sacorma compared to the normal tissues. In addition, we also found that relative to normal tissue controls, ZCCHC24 expression was lower in Bladder, Breast, Cervical, Colorectal, Head and neck, Kidney, Leukemia, Liver, Lung, Ovarian and Prostate cancers (Figure 1A). The detailed results of ZCCHC24 expression in different cancer types are summarized in Supplementary Table 1. We examined ZCCHC24 expression using the RNA-seq data of multiple malignancies in TCGA to further evaluate ZCCHC24 expression in human cancers. We found that the expression of ZCCHC24 was significantly elevated relative to normal controls in HNSC (cholangiocarcinoma) and SKCM metastasis (Skin Cutaneous Melanoma). In constrast, ZCCHC24 expression was significantly lower in BLCA (bladder urothelial carcinoma), BRCA (breast invasive carcinoma), CHOL(cholangiocarcinoma),COAD (colon adenocarcinoma), KICH (kidney chromophobe), LICHLiver hepatocellular carcinoma,LUAD (lung adenocarcinoma), LUSC(lung squamous cell carcinoma), PRAD(prostatic cancer),READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), THCA (thyroid carcinoma),and UCEC (uterine corpus endometrial carcinoma) compared with adjacent normal tissues(Figure 1B).

Prognostic Potential of ZCCHC24 in Cancers

We investigated whether ZCCHC24 expression was correlated with prognosis in cancer patients. The relationships between the expression of ZCCHC24 and cancer patient outcome using the PrognScan database (Supplementary Table 2).Notably, We found that multiple cancer types exhibited a significant association between patient prognosis and ZCCHC24 expression including Blood,Breast, Head and neck, Ovarian, Brain and Colon cancer (Figure 2A-I)44-51. We additionally employed the Kaplan-Meier plotter database in order to assess how ZCCHC24 expression relates to prognosis in a range of cancer types. The poor prognosis in gastric and ovarian cancer (OS HR= 2.11, 95% CI = 1.7 to 2.63, P = 6.3e-12; OS HR= 1.26, 95% CI = 1.09 to 1.44, P = 0.003;) was shown to correlate with higher ZCCHC24 expression (Figures 2). However, ZCCHC24 expression shows a better OS in lung cancer and breast cancer (Figures 2). In addition, We further used GEPIA database to assess how ZCCHC24 expression relates to patient prognosis ,analysing 33 TCGA cancer types (Supplementary Figure 1). High ZCCHC24 expression levels were associated with poorer prognosis of DFS in COAD and BLCA, OS in STAD, OV, LUSC, KIRP, TGCT and THCA. Meanwhile, high ZCCHC24 expression was correlated with better prognosis of OS and DFS in LGG and SKCM, DFS in USC. These results confirmed the prognostic value of ZCCHC24 in some specific types of cancers and that increased and decreased ZCCHC24 expression have different prognostic value depending on the type of cancer.

Elevated ZCCHC24 expression is linked to prognosis in gastric cancer patients exhibiting lymphatic metastasis

As we found ZCCHC24 expression to be linked with poor gastric cancer patient prognosis, we next investigated the relationship between the ZCCHC24 expression and clinical characteristics of gastric cancer patients via Kaplan-Meier plotter databases. Overexpression of ZCCHC24 was associated with worse OS and PFS in male and female patients as well as two types of Lauren classification and differentiation (P < 0.05). Specifically, high ZCCHC24 mRNA expression was correlated with worse OS and PFS in stage 1 to 4,each N and M0-1 stage of gastric cancer patients (Table 1). In addition, high ZCCHC24 expression has the highest HR values of N1 of OS and PFS in the four N categories. The most common type of metastasis is lymph node metastasis and is directly linked with patients' prognosis in gastric cancer (44). These results suggest that ZCCHC24 expression level can impact the prognosis in gastric cancer patients with lymph node metastasis.

TABLE 1

Kaplan-Meier plotter to determine the effect of different clinicopathological factors on the expression of ZCCHC24 gene and clinical prognosis in gastric cancer

	OS(881)			RFP(645)		
	N	Hazard ratio	P-value	N	Hazard ratio	P-value
SEX						
Female	236	2.21(1.5-3.24)	3.4e-05	201	2.02(1.35-3.02)	0.00049
male	544	2.32(1.77-3.03)	3.4e-10	437	2.05(1.56-2.7)	1.7e-07
STAGE						
1	67	277852890.27(0-Inf)	0.043	60	301452716.19(0-Inf)	0.052
2	140	2.4(1.31-4.51)	0.0037	131	1.65(0.9-3.03)	0.1
3	305	2.24(1.63-3.06)	2.7e-07	186	2.11(1.46-3.07)	5.4e-05
4	148	1.81(1.22-2.7)	0.0029	141	1.59(1.07-2.35)	0.02
STAGE T						
2	241	2.11(1.38-3.22)	0.00043	239	1.85(1.22-2.79)	0.003
3	204	1.7(1.19-2.52)	0.0029	204	1.47(1.04-2.06)	0.026
4	38	3.52(1.43-8.68)	0.0039	39	3.42 (1.48-7.88)	0.0024
STAGE N						
0	74	2.71(1.05-6.98)	0.032	72	2.57(1-6.61)	0.043
1	225	2.47(1.64-3.72)	7.9e-06	222	2.22(1.49-3.25)	4.8e-05
2	121	2.55 (1.61-4.02)	3.2e-05	125	2.18(1.41-3.37)	0.00037
3	76	2.25(1.3-3.89)	0.0021	76	1.79(1.04-3.07)	0.033
1+2+3	422	2.34(1.8-3.05)	6.8e-11	423	2.02 (1.57-2.6)	2.9e-08
STAGE M						
0	444	2.21(1.65-2.95)	5.1e-08	443	1.87(1.42-2.47)	6.2e-06
1	56	2.02(1.12-3.64)	0.018	56	1.53(0.84-2.82)	0.16
LAUREN CLASSIFICATION						
Intestinal	320	2.71(1.95-3.76)	5.3e-10	263	2.25(1.58-3.2)	3.4e-06
Diffuse	241	2.01 (1.4-2.88)	0.00012	231	1.9(1.34-2.7)	0.00027
DIFFERENTIATION						
Poor	165	1.42 (0.86-2.35)	0.17	121	1.31(0.78-2.19)	0.3
Moderate	67	1.9 (0.93-3.87)	0.073	67	2.49(1.03-6.03)	0.037

ZCCHC24 Expression is correlated With Immune Infiltration Level in Gastric Cancers

Survival and lymph node metastasis are independently predicted in cancer patients by the frequency of lymphocytes infiltrating into the tumour (52,53). We assessed the correlations of ZCCHC24 expression with immune infiltration levels in 39 cancer types from TIMER. The results show that ZCCHC24 expression has significant correlations with tumor purity in 26 types of cancer and significant correlations with B cell infiltration levels in 21 types of cancers. In addition, ZCCHC24 expression has significant correlations with infiltrating levels of CD8+ T cells in 24 types of cancer, CD4+ T cells in 20 types of cancer, Macrophages in 33 types of cancer, Neutrophils in 26 types of cancer, and Dendritic cells in 19 types of cancer (Figure 3 and Supplementary Figure 2). Given the association of ZCCHC24 expression with immune infiltration level in diverse types of cancer, we next determined the distinct types of cancers in which ZCCHC24 was associated with prognosis and immune infiltration. Tumor purity is an important factor that influences the analysis of immune infiltration in clinical tumor samples by genomic approaches (54), and TIMER and GEPIA have most of the homologous data from TCGA (37, 43). Therefore, we selected the cancer types in which ZCCHC24 expression levels have a significant negative correlation with tumor purity in TIMER and a significant correlation with prognosis in GEPIA. Interestingly, we found that ZCCHC24 expression level correlate with poorer prognosis and high immune infiltration in STAD and LUSC, using liver hepatocellular carcinoma (LIHC) as the control. ZCCHC24 expression level has significant correlations with infiltrating levels of B cell ($R = 0.118$, $P = 2.29e-02$), CD8+ T cells ($R = 0.213$, $P = 3.62e-05$), CD4+ T cells $R = 0.561$, $P = 1.31e-31$), Macrophages ($R = 0.654$, $P = 1.94e-46$), Neutrophils ($R = 0.25$, $P = 1.04e-06$) and DCs ($R = 0.462$, $P = 5.32e-21$) in STAD (Figure 3A). Similarly, there were correlations with infiltrating levels of B cell ($r = 0.218$, $P = 1.76e-06$), CD8+ T cells ($R = 0.222$, $P = 1.01e-06$), CD4+ T

cells ($r = 0.508$, $P = 1.83 \times 10^{-32}$), Macrophages ($r = 0.573$, $P = 6.78 \times 10^{-43}$), Neutrophils ($r = 0.456$, $P = 7.82 \times 10^{-26}$) and DCs ($R = 0.535$, $P = 1.61 \times 10^{-36}$) in LUSC(Figure 3B). ZCCHC24 expression to significantly correlate with STAD prognosis (Figure 3C), whereas no significant correlation between prognosis and immune cell infiltration or ZCCHC24 expression was observed in LIHC (Figure 3D).These findings strongly suggest that ZCCHC24 plays a specific role in immune infiltration in STAD and LUSC especially those of Macrophages, CD4+ T cells and DCs.

Correlation Analysis Between ZCCHC24 Expression and Immune Marker Sets

We next further explored the link between ZCCHC24 expression and levels of immune cell infiltration based on sets of immunological markers in STAD and LUSC using the TIMER and GEPIA databases. We analyzed the correlations between ZCCHC24 expression and immune marker genes of different immune cells, included CD8+ T cells, T cells(general), B cells, monocytes, TAMs, M1 and M2 macrophages, Neutrophils, NK cells and DCs in STAD and LUSC, using LIHC as the control. We also analyzed the different functional T cells, such as Th1 cells, Th2 cells, Tfh cells, Th17 cells, and Tregs, as well as exhausted T cells. We also analyzed the different functional T cells, such as Th1 cells, Th2 cells, Tfh cells, Th17 cells, and Tregs, as well as exhausted T cells. ZCCHC24 expression level was significantly correlated with most immune marker sets of various immune cells and different T cells in STAD and LUSC.(Table 2 and Figures 4). In contrast, ZCCHC24 expression correlated with just 17 of these markers in LIHC (Table 2).We therefore further assessed the relationship between ZCCHC24 expression and these markers in STAD and LUSC using the GEPIA database revealing similar correlations between ZCCHC24 and markers of monocytes, TAMs, and M1 and M2 macrophages to those in TIMER (Table 3). These findings suggest that ZCCHC24 may regulate Macrophage polarisation in STAD and LUSC. High ZCCHC24 expression relates to the high infiltration level of DCs in STAD and LUSC. DC markers also showed significant correlations with the ZCCHC24 expression. These results further reveal the strong relationship between ZCCHC24 and DC infiltration. DCs can increase the levels of tumour metastasis via enhancing regulatory T cell (Treg) responses and suppressing CD8+ T cell cytotoxicity.(55) Further work will be necessary to establish whether ZCCHC24 plays a key role in regulating DC infiltration and tumour metastasis. A significant correlation exists between ZCCHC24 and markers of Neutrophil. Neutrophils can also promote tumour progression, metastasis and angiogenesis directly (56). Further work will be necessary to establish whether ZCCHC24 plays a key role in regulating Neutrophil infiltration. We further observed a significant correlation between ZCCHC24 and markers of Tregs and exhausted T cells, indicating that ZCCHC24 may play a role in immune escape in gastric cancer and lung squamous cell carcinoma. However, further work will be needed to confirm the mechanisms underlying such escape. We also observed a significant correlation between ZCCHC24 and markers of multiple T cell markers (Th1, Th2, Tfh and Th17), including STAD and LUSC. These findings can be indicative of a potential mechanism in which ZCCHC24 regulates T cell functions in STAD and LUSC. Altogether, these results highlight the capability of ZCCHC24 to potentially regulate immune cell recruitment and activation in STAD and LUSC. Therefore, the findings of this study further confirm that ZCCHC24 is specifically correlated with immune infiltrating cells in STAD and LUSC and thus plays a vital role in immune escape in gastric cancer and lung squamous cell carcinoma microenvironment.

TABLE 2

Correlation analysis between ZCCHC24 and relate genes and markers of immune cells in TIMER

Description	Gene markers	STAD				LUSC				LICH			
		None		Purity		None		Purity		None		Purity	
		Cor	P	Cor	P	Cor	P	Cor	P	Cor	P	Cor	P
CD8+T cell	CD8A	0.31	***	0.298	***	0.317	***	0.199	***	-0.006	9.01E-01	0.024	6.56E-01
	CD8B	0.202	***	0.198	**	0.264	***	0.206	***	-0.0093	7.39E-02	0.024	6.56E-01
T cell (general)	CD3D	0.224	***	0.202	***	0.348	***	0.183	***	0.14	*	-0.128	1.78E-02
	CD3E	0.269	***	0.282	***	0.447	***	0.302	***	0.013	8.02E-01	-0.046	3.99E-01
	CD2	0.296	***	0.284	***	0.406	***	0.26	***	-0.012	8.13E-01	0.018	7.35E-01
B cell	CD19	0.387	***	0.379	***	0.376	***	0.196	***	-0.038	4.67E-01	-0.038	4.86E-01
	CD79A	0.383	***	0.361	***	0.449	***	0.289	***	0.031	5.48e-01	0.064	2.38e-01
Monocyte	CD86	0.351	***	0.341	***	0.542	***	0.418	***	-0.018	7.29e-01	0.009	8.72e-01
	CD115 (CSF1R)	0.383	***	0.361	***	0.449	***	0.289	***	0.077	1.41e-01	0.112	3.76e-02
TAM	CCL2	0.52	***	0.507	***	0.552	***	0.48	***	0.117	2.43e-02	0.163	*
	CD68	0.217	***	0.203	***	0.32	***	0.311	***	-0.018	7.89e-01	0.009	8.72e-01
	IL10	0.405	***	0.401	***	0.525	***	0.444	***	0.013	8.11e-01	0.001	9.78e-01
M1 Macrophage	INOS (NOS2)	-0.097	4.86E-02	-0.108	3.63E-02	0.035	4.39E-0	0.044	3.34E-01	0.181	**	0.177	**
	IRF5	0.325	***	0.327	***	0.142	*	0.112	1.41E-02	0.088	9.16e-02	0.088	1.04e-01
	COX2(PTGS2)	0.149	***	0.151	*	0.424	**	0.428	***	0.162	*	0.199	*
M2 Macrophage	CD163	0.424	***	0.418	***	0.613	***	0.53	***	0.152	*	0.191	**
	VSIG4	0.456	***	0.46	***	0.542	***	0.446	***	0.098	6.03e-02	0.133	1.33e-02
	MS4A4A	0.489	***	0.482	***	0.546	***	0.44	***	0.102	5.06e-02	0.13	1.55e-02
Neutrophil markers	CD66b (CEACAM8)	-0.009	8.63E-01	0.002	9.74E-01	0.145	***	0.125	*	-0.004	9.45e-01	-0.003	9.55e-01
	CD11b (ITGAM)	0.474	***	0.474	***	0.613	***	0.525	***	0.01	8.54e-01	0.024	6.57e-01
	CCR7	0.485	***	0.476	***	0.435	***	0.294	***	0.179	*	0.229	***
Natural killer cell	KIR2DL1	0.102	3.84E-02	0.094	6.74E-02	0.15	**	0.082	7.41E-02	0.039	4.59e-01	0.056	2.96e-01
	KIR2DL3	0.026	5.98E-01	-0.012	8.18E-01	0.188	***	0.131	*	0.01	8.48e-01	0.024	6.56e-01
	KIR2DL4	-0.145	*	-0.162	*	0.105	1.82E-02	0.009	8.51E-01	-0.135	*	-0.111	3.98e-02
	KIR3DL1	0.063	1.98E-01	0.044	3.93E-01	0.267	***	0.185	***	0.076	1.43e-01	0.12	2.58e-02
	KIR3DL2	0.123	1.24E-02	0.107	3.69E-02	0.162	**	0.069	1.31E-01	-0.011	8.3e-01	0.01	8.58e-01

	KIR3DL3	-0.137	*	-0.138	*	0.075	9.27E-02	0.046	3.12E-01	-0.066	2.07e-01	-0.076	1.58e-01
	KIR2DS4	-0.003	9.53E-1	-0.029	5.68E-01	0.185	***	0.134	*	0.063	2.29e-01	-0.064	2.36e-01
Dendritic cell	HLA-DPB1	0.307	***	0.286	***	0.571	***	0.456	***	0.04	4.433-01	0.073	1.77e-01
	HLA-DQB1	0.137	*	0.111	3.15E-02	0.449	***	0.346	***	-0.034	5.17e-01	-0.011	8.38e-01
	HLA-DRA	0.181	**	0.159	*	0.498	***	0.375	***	0.064	2.19e-01	0.102	5.85e-02
	HLA-DPA1	0.241	***	0.22	***	0.541	***	0.43	***	0.098	6.03e-02	0.135	1.21e-02
	BDCA-1(CD11c)	0.55	***	0.55	***	0.451	***	0.287	***	0.154	*	0.175	*
	BDCA-4(NRP1)	0.646	***	0.638	***	0.686	***	0.632	***	0.342	***	0.373	***
	CD11c (ITGAX)	0.402	***	0.388	***	0.6	***	0.48	***	-0.011	8.39e-01	-0.002	9.70e-01
Th1	T-bet (TBX21)	0.274	***	0.266	***	0.417	***	0.293	***	0.059	2.59e-01	0.081	1.35e-01
	STAT4	0.357	***	0.344	***	0.542	***	0.437	***	0.026	6.23e-01	-0.049	3.68e-01
	STAT1	0.003	9.49E-01	-0.002	9.6E-01	0.217	***	0.138*		0.082	1.16e-01	0.101	9.96e-01
	IFN-g (IFNG)	-0.095	5.25E-02	-0.101	5.03E-02	0.099	2.74E-02	0	9.99E-01	-0.095	6.81e-02	-0.068	2.07e-01
	TNF-a (TNF)	0.03	4.41E-01	0.009	8.63E-01	0.367	***	0.252	***	0.06	2.51e-01	0.075	1.65e-01
Th2	GATA3	0.429	***	0.429	***	0.473	***	0.414	***	0.044	4.02e-01	-0.082	1.28e-01
	STAT6	0.237	***	0.24	***	0.156	**	0.144	*	0.251	***	-0.264	***
	STAT5A	0.428	***	0.432	***	0.516	***	0.42	***	0.219	***	0.263	***
	IL13	0.163	**	0.177	**	0.168	**	0.09	4.87E-02	-0.04	4.43e-01	-0.033	5.38e-01
Tfh	BCL6	0.537	***	0.529	***	0.037	4.04E-01	0.091	4.69E-02	0.31	***	0.296	***
	IL21	0.063	2.01E-01	0.054	2.98E-01	0.148	**	0.045	3.28E-01	0.015	7.71e-01	-0.001	9.78e-01
Th17	STAT3	0.417	***	0.413	***	0.345	***	0.332	***	0.373	***	0.412	***
	IL17A	-0.297	***	-0.304	***	-0.002	9.71E-01	-0.079	8.50E-02	0.094	7.09e-02	0.105	5.13e-02
Treg	FOXP3	0.27	***	0.252	***	0.56	***	0.452	***	0.114	2.78e-02	0.108	4.56e-02
	CCR8	0.363	***	0.359	***	0.545	***	0.438	***	0.111	3.21e-02	0.132	1.38e-02
	STAT5B	0.633	***	0.619	***	0.301	***	0.339	***	0.439	***	0.444	***
	TGFb (TGFB1)	0.631	***	0.618	***	0.507	***	0.449	***	0.068	1.89e-01	0.1	6.37e-02
T cell exhaustion	CTLA4	0.119	1.51E-02	0.106	3.84E-02	0.429	***	0.285	***	-0.168	*	-0.154	*
	LAG3	0.1	4.15E-02	0.089	8.37E-02	0.243	***	0.134	*	-0.135	*	-0.107	4.70e-02
	TIM-3 (HAVCR2)	0.35	***	0.346	***	0.524	***	0.405	***	-0.049	3.43e-01	-0.024	6.55e-01

GZMB	-0.051	2.96E-01	-0.085	9.98E-02	0.221	***	-0.069	1.35E-1	-0.068	1.89e-01	-0.061	2.57e-01
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TABLE 3

Correlation analysis between ZCCHC24 and relate genes and markers of monocyte, TAM and macrophages in GEPIA.

Description	Gene markers	STAD				LUSC				LIHC			
		Tumor		Normal		Tumor		Normal		Tumor		Normal	
		R	P	R	P	R	P	R	P	R	P	R	P
Monocyte	CD86	0.34	***	-0.14	0.43	0.51	***	-0.1	0.47	0.031	0.55	0.2	0.17
	CD115 (CSF1R)	0.56	***	0.26	0.12	0.68	***	0.43	*	0.14	*	0.094	0.52
TAM	CCL2	0.51	***	0.54	**	0.56	***	0.0043	0.98	0.14	*	0.1	0.47
	CD68	0.22	***	-0.48	*	0.42	***	-0.022	0.88	0.11	0.043	0.29	0.043
	IL10	0.42	***	0.07	0.69	0.5	***	0.19	0.18	0.13	0.013	0.11	0.46
M1 Macrophage	INOS (NOS2)	-0.096	0.053	0.13	0.46	0.35	***	0.26	0.067	0.26	***	0.022	0.88
	IRF5	0.33	***	-0.34	0.045	0.13	0.0045	0.037	0.8	0.11	0.028	0.28	0.05
	COX2 (PTGS2)	0.18	**	0.85	***	0.29	***	0.12	0.41	0.23	***	0.18	0.22
M2 Macrophage	CD163	0.38	***	0.69	***	0.57	***	0.13	0.35	0.019	0.71	0.072	0.62
	VSIG4	0.45	***	0.49	*	0.54	***	-0.16	0.27	0.12	0.024	0.15	0.3
	MS4A4A	0.49	***	0.57	**	0.54	***	-0.041	0.78	0.14	*	0.16	0.25

Discussion

ZCCHC24, which is characterised with the consensus sequence CX2-C-X4-H-X4-C, has a high-affinity binding to single-stranded nucleic acids, especially single-stranded RNAs(18). increasing literatures have implicated the ZCCHC superfamily in the regulation of RNA metabolism. AS is a ubiquitous process by which a single pre-mRNA can generate diverse mature mRNAs and expand the protein diversity, providing the potential for functional and regulatory complexity in cells(57).The importance of AS on tumour immunity increasingly gains support, and the increased analysis of AS events has demonstrated the independent oncogenic effects that may be relevant to the suppressive immune microenvironment in cancers (58). The current study reports that ZCCHC24 contributes to the phase-specific patterns of AS during reprogramming (59).

In this study, we found that ZCCHC24 expression correlated with patient prognosis in several types of cancer and a particularly strong correlation between high ZCCHC24 expression and a poor STAD and LUSC prognosis. This elevated ZCCHC24 expression was also a reliable predictor of the presence of lymph node metastasis in GC patients, indicating that ZCCHC24 may be a valuable prognostic indicator of metastatic progression in GC tumour types. We further found that the degree of ZCCHC24 expression correlated with the expression of several different markers of immune cell subsets within tumours, thus highlighting a possible role for ZCCHC24 in the immunological interactions in GC and LUSC, making it a valuable biomarker worthy of further research in this type of cancer.

In this report, we examined the expression levels of ZCCHC24 and systematic prognostic landscape in different types of cancers using independent datasets in Oncomine and 33 types of TCGA data in GEPIA. The differential expression of ZCCHC24 between cancer and normal tissues was observed in many types of cancers. Based on the Oncomine database, we found that ZCCHC24 was highly expressed in Colorectal Cancer, Esophageal Cancer, Gastric cancer, lymphoma tumors, Pancreatic Cancer and Sacoma compared with normal tissues, while some data sets showed that ZCCHC24 has a lower level of expression in Bladder, Breast, Cervical, Colorectal, Head and neck, Kidney, Leukemia, Liver, Lung, Ovarian and Prostate cancers. However, ZCCHC24 expression was higher in HNSC and SKCM metastasis, but lower expression in BLCA, BRCA, CHOL,COAD, KICH, LICH, LUAD, LUSC, PRAD,READ, STAD, THCA and UCEC compared with normal adjacent tissues in analysis of the TCGA data. Altered ZCCHC24 expression in a range of different cancers may be due to the different means of data collection in different studies, or it may relate to differences in the underlying biological mechanisms. Nevertheless, in these databases we found consistent prognostic correlations between ZCCHC24 expression in Bladder,Blood, Head and neck,ovarian, brain and colon cancer. In the TCGA database, elevated ZCCHC24 levels were correlated with a poorer outcome for patients with STAD,OV,LUSC,KIRPTGCT and THCA, But high levels of ZCCHC24 expression showed a better prognosis in LGG and SKCM. Similarly, the Kaplan-Meier plotter database found elevated ZCCHC24 to correlate with poor GC and ovarian cancer outcome. In addition, a high level of ZCCHC24 expression was

shown to be correlated with poor prognosis of gastric cancer in stage, T stage, N stage, M stage, Lauren classification and differentiation. Elevated ZCCHC2 expression in GC correlated with a higher T stage HR in OS. These results together thus suggest that ZCCHC24 may have value as a GC prognostic biomarker.

In this study, The expression of ZCCHC24 correlated with the degree of immune infiltration in multiple cancer types, and particularly in GC and LUSC. We found that ZCCHC24 expression was moderately positively correlated with the degree of Macrophages, CD4+ T cells and DCs infiltration, and weakly positively correlated with the degree of CD8+ T cells, Neutrophil infiltration in STAD and LUSC. Moreover, the correlation between ZCCHC24 expression and the marker genes of immune cells implicate that ZCCHC24 can control immune cell infiltration and interactions within the tumour microenvironment in STAD and LUSC. First, gene markers of M1 macrophages showed weak and gene markers of M2 macrophages showed positive correlations with ZCCHC24 expression. These results reveal the potential regulating role of ZCCHC24 play a role in regulating TAM polarization. In addition, our results indicated that ZCCHC24 levels in STAD and LUSC to correlate with markers of Treg cells and T cell exhaustion, This suggests that ZCCHC24 has the potential to activate Tregs. Moreover, ZCCH24 expression also show significant correlations with DC markers and Neutrophil markers. These results reveal the potential regulating role of ZCCHC24 in tumour metastasis. Furthermore, we found that expression of ZCCHC24 correlated with that of multiple T cell markers (Th1, Th2, Tfh and Th17) in STAD and LUSC. These correlations could be indicative of a potential mechanism where ZCCHC24 regulates T cell functions in STAD and LUSC. Together, these results highlight the ability of ZCCHC24 to potentially regulate immune cell recruitment and activation in STAD and LUSC.

In summary, ZCCHC24 may be an important regulator of immune cell infiltration and a valuable prognostic biomarker in gastric cancer and lung adenocarcinoma patients.

Declarations

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Not applicable.

Authors' contributions

Jiahuang, Huang carried out data collection, and measurement, statistical analysis, interpretation of results and wrote the manuscript. Qinhui, Zhang and Wei, Li participated in collecting data. Jianchang, Shu provided critical appraisal of the manuscript. All authors read and approved the final version prior to submission.

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- ## Figures

Figure 3

ZCCHC24 expression is correlated with the level of immune infiltration in Stomach adenocarcinoma (STAD), lung adenocarcinoma (LUSC) and liver hepatocellular carcinoma (LIHC). (A) ZCCHC24 expression is correlated with the level of immune infiltration in liver hepatocellular carcinoma (LIHC). (B) ZCCHC24 expression is correlated with the level of immune infiltration in lung adenocarcinoma (LUSC). (C) ZCCHC24 expression is correlated with the level of immune infiltration in Stomach adenocarcinoma (STAD). (D) Kaplan-Meier plots of immune infiltration and ZCCHC24 expression levels in lung adenocarcinoma (LUSC). (E) Kaplan-Meier plots of immune infiltration and ZCCHC24 expression levels in liver hepatocellular carcinoma (LIHC). (F) Kaplan-Meier plots of immune infiltration and ZCCHC24 expression levels in Stomach adenocarcinoma (STAD).

Figure 4

Correlation analysis between ZCCHC24 expression and immunological marker set in adenocarcinoma (STAD), lung adenocarcinoma (LUSC) and liver hepatocellular carcinoma (LIHC). (A–D) Scatterplots of correlations between ZCCHC24 expression and gene markers of monocytes (A), TAMs (B), and M1 (C) and M2 macrophages (D) in LUSC. (E–H) Scatterplots of correlations between ZCCHC24 expression and gene markers of monocytes (E), TAMs (F), and M1 (G) and M2 macrophages (H) in STAD. (I–L) Scatterplots of correlations between ZCCHC24 expression and gene markers of monocytes (I), TAMs (J), and M1 (K) and M2 macrophages (L) in LIHC.

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