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Research article

CALD1 is a prognostic biomarker and correlated with immune infiltrates in gastric cancers

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HIGHLIGHTS

- CALD1 plays an important role in immune infiltration in gastric cancer.
- CALD1 can affect the prognosis in gastric cancer patients with lymph node metastasis.
- CALD1 plays a vital role in immune escape in the gastric cancer microenvironment.

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ABSTRACT

Background: Caldesmon gene (CALD1) plays an important role in many cellular functions. Some researchers have found the correlation between CALD1 expression and prognosis of gastrointestinal cancer (GI), but the association with tumor-infiltrating lymphocytes (TILs) still unclear.

Methods: The expression of CALD1 in different human tumor was analyzed by Oncomine and Tumor Immune Estimation Resource (TIMER) databases. The correlations between CALD1 and prognosis in types cancer were explored by Kaplan–Meier plotter and Gene Expression Profiling Interactive Analysis (GEPIA) databases. The association between CALD1 expression and tumor immune cell infiltration was further analyzed via TIMER and GEPIA databases.

Results: The CALD1 expressions in types cancer between tumor tissues and adjacent normal tissues were significantly different. The high expression of CALD1 was related with poor overall survival (OS) of patients with gastric cancer, especially in gastric cancer patients at N1, N2 and N3 stages. The expression of CALD1 was positively associated with immune-infiltrated, such as CD8+T cells, CD4+T cells, macrophages, neutrophils, and dendritic cells (DCs) in gastric cancer.

Conclusions: CALD1 was considerably a key role in prognosis of patients with gastric cancer. The expression level of CALD1 is significantly associated with immune-infiltrated in gastric cancer. Furthermore, CALD1 expression may be involved in regulating tumor-associated macrophages (TAMs), dendritic cells, exhausted T cells and regulatory T cells in gastric cancer. These findings suggest that CALD1 could be utilized as a marker of prognosis and immune infiltration in gastric cancer.

1. Introduction

Gastric cancer, the second leading cause of cancer-related mortality, is the fourth common malignancies among both male and female around the world [1]. There are more than 950,000 new cases reported worldwide every year [2]. The limited prognosis of gastric cancer partly attributed to tumor metastasis [3]. Immunotherapy, including cytotoxic T lymphocyte associated antigen 4 (CTLA4), programmed death-1 (PD-1) and programmed death ligand-1(PD-L1) inhibitors, now has shown remarkable promise in some cancers, such as non-small cell lung cancer (NSCLC), melanoma, renal cancer and others [4, 5]. Immunological mechanisms are important factors that impact the development and prognosis of gastric cancer, and various immunotherapies have been used as a means of effectively treating gastric cancer [6]. Clinically, immune cells infiltration

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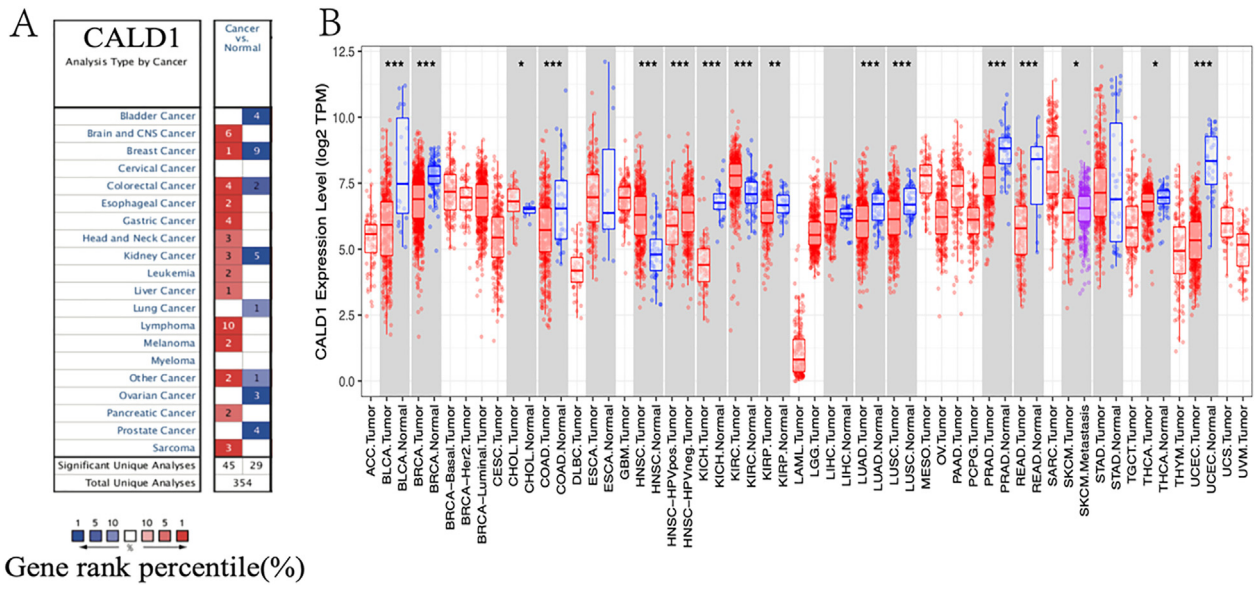

Figure 1. The expression levels of CALD1 in different types of tumor in databases. (A)Expression levels of CALD1 in different types of tumor and normal tissues in the Oncomine database. The color depth is related to the differential sequence of gene expression, the red saturated color block represents the Top 1%, medium saturation color block, the Top 5%, white color block represents Top 10%, blue, and so on. (P value is 1E-4, fold change is 2, and gene ranking of TOP 10%.) (B) Compare the expression data of CALD1 in different tumor types from TCGA using TIMER (The statistical significance computed by the Wilcoxon test is annotated by the number of stars *: p-value < 0.05; **: p-value <0.01; ***: p-value <0.001). "Diff Exp (differential expression)" was used to explore the differential expression between tumor and adjacent normal tissues for interesting genes across all TCGA tumors. Box plots are used to show the distributions of gene expression levels.

is most likely to be utilized as drug targets in tumors to improve survival. However, patients with gastric cancer can rarely get benefit from immunotherapy [7]. Anti-CTLA4 shows a poor clinical efficacy in metastatic gastric and colon cancer, and anti-PD-1 and anti-PD-L1 show partial responses in advanced gastric and colon cancers [8, 9, 10]. Some studies believed that the tumor-associated macrophages (TAMs), tumor-infiltrating neutrophils (TINs) and other tumor-infiltrating lymphocytes have affections on the prognosis and efficacy of chemotherapy and immunotherapy in cancer patients [11, 12]. Clarifying the biomarkers of immune interaction with gastric cancer and identifying new targets for immunotherapy are necessary.

Caldesmon gene (CALD1), a protein found in the cytoskeleton [12], is an important role in many cellular functions, such as proliferation, apoptosis, cell motility, adhesion, receptor function, and second messenger pathways [13, 14]. Some research have suggested that CALD1 is related with prognosis and metastasis of colon cancer [15, 16], but the relationship between CALD1 and gastric cancer still unclear. Considering immune-related mechanisms play an important role in gastrointestinal cancer (GI), and immunotherapeutic strategies are urgent need for the treatment of GI. We explored the role of CALD1 in tumor progression and immunology in gastric cancer.

We analyzed the expression of CALD1 and its relationship with the prognosis of cancers by Oncomine, Kaplan-Meier plotter and GEPIA databases. We further explored the correlation between CALD1 and immune cells infiltration in cancers using Tumor Immunoassay Resource (TIMER). These findings reveal the important role of CALD1 in gastric cancer, and highlight the relationship between CALD1 and tumor-immune interactions.

2. Materials and methods

2.1. Oncomine database analysis

The expression level of CALD1 gene in multiple cancers was analysis in the Oncomine database (https://www.oncomine.org/resource/login.html) [17]. Oncomine is a large tumor gene chip database, covering 65 gene chip data sets, 4700 chips and 480 million gene expression data. It can be used to analyze gene expression differences, find outliers and predict co-expression genes. It can also be classified according to clinical information such as tumor stage, grade and tissue type. It can also find possible molecular markers and therapeutic targets based on known gene-drug analysis. The threshold was determined according to the following values: P-value of 1E-4, fold change of 2, and gene ranking of 10%.

TCGA abbreviations list can be found in https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations.

2.2. GEPIA database analysis

The online database Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) [18] is a communal network server that includes a transcriptome sequencing dataset of 9,736 tumors together with 8587 adjacent normal tissues from TCGA and Genotype-Tissue Expression (GTEx) data sets. We analyzed the relationship between CALD1 expression and patient prognosis in various tumor types. We further explored the link between CALD1 expression and expression of some particular markers associated with immune cell infiltration tumors by Spearman's correlation with statistical significance. $P < 0.05$ were considered significant.

2.3. Kaplan-Meier plotter database analysis

The Kaplan–Meier plotter (http://kmplot.com/analysis/) [19] is an online database contains microarray gene expression and survival information from TCGA and Cancer Biomedical informatics Grid. We observed the relationship between overall survival (OS) and CALD1 expression in cancer patients. Hazard ratios (HR) of 95% confidence intervals (CIs) were calculated. The log-rank P-value was also determined.

2.4. TIMER database analysis

The TIMER database (https://cistrome.shinyapps.io/timer/) [20] is a website for the analyzing the molecular characterization of tumor-immune interactions in multiple cancers. This database has incorporated 10,897 samples ranging from 32 types cancer from

Table 1. Detailed information of particular tumor types.

Cancer	Cancer type	P-value	Fold change	Over-expression Gene Rank	Sample	Reference (PMID)
Brain and CNS Cancer	Glioblastoma	7.81E-29	3.954	12 (in top 1%)	81	16616334
	Anaplastic Astrocytoma	1.18E-07	2.409	441 (in top 3%)	19	16616334
Breast Cancer	Invasive Breast Carcinoma Stroma	1.8E-30	6.403	7 (in top 1%)	53	18438415
Colorectal Cancer	Rectal Adenocarcinoma	3.75E-07	2.007	21 (in top 1%)	8	17615082
	Rectosigmoid Adenocarcinoma	0.00000612	2.054	421 (in top 3%)	10	17615082
Esophageal Cancer	Esophageal Adenocarcinoma	4.52E-19	4.105	70 (in top 1%)	75	21152079
	Barrett's Esophagus	1.55E-10	3.322	242 (in top 2%)	15	21152079
Gastric Cancer	Diffuse Gastric Adenocarcinoma	6.81E-08	3.236	75 (in top 1%)	13	12925757
	Gastric Intestinal Type Adenocarcinoma	2.43E-13	2.372	214 (in top 2%)	66	12925757
Head and Neck Cancer	Head and Neck Cancer	6.25E-17	4.466	54 (in top 1%)	41	22460905
Kidney Cancer	Kidney Cancer	3.72E-07	6.261	502 (in top 3%)	21	22460905
Leukemia	B-Cell Acute Lymphoblastic Leukemia	4.09E-17	3.514	149 (in top 2%)	238	21487112
Liver Cancer	Hepatocellular Carcinoma	0.0000946	2.372	90 (in top 1%)	70	20395200
Lymphoma	Unspecified Peripheral T-Cell Lymphoma	1.58E-27	73.971	2 (in top 1%)	28	17304354
	Angioimmunoblastic T-Cell Lymphoma	2.83E-07	113.858	481 (in top 3%)	6	17304354
	Anaplastic Large Cell Lymphoma	0.0000011	65.851	704 (in top 4%)	6	17304354
Pancreatic Cancer	Pancreatic Ductal Adenocarcinoma	5.84E-13	2.482	224 (in top 2%)	39	19260470
sarcoma	Leiomyosarcoma	1.83E-17	3.336	1 (in top 1%)	85	20581836

Table 2. Interrelation Between CALD1 expression level and clinical features.

Cancer Tpye	OS				RFS			
	N	logrank P	HR	P(HR)	N	logrank P	HR	P(HR)
ACC	38	0.67	0.85	0.67	38	0.45	1.3	0.45
BLCA	201	**0.0022**	1.6	**0.0024**	201	**0.012**	1.5	**0.013**
BRCA	535	0.69	0.94	0.69	535	0.85	0.97	0.85
CESC	146	0.16	1.4	0.16	146	0.38	1.3	0.38
CHOL	18	0.37	0.64	0.37	18	0.69	0.83	0.68
COAD	135	0.095	1.5	0.098	125	**0.039**	1.7	**0.041**
DLBC	23	0.95	0.95	0.95	23	0.83	1.1	0.83
ESCA	91	0.71	1.1	0.71	91	**0.034**	1.7	**0.036**
GBM	81	0.14	1.3	0.12	81	0.35	1.2	0.36
HNSC	259	0.48	1.1	0.47	259	0.72	1.1	0.72
KICH	32	0.43	1.8	0.44	32	0.26	2.2	0.28
KIRC	258	**4.20E-05**	0.53	**5.70E-05**	258	0.36	0.85	0.36
KIRP	141	0.51	1.2	0.51	141	0.64	0.87	0.63
LAML	53	0.48	1.2	0.49	53	1	1	N
LGG	257	**0.00034**	2	**0.00044**	257	**0.00033**	1.8	**0.00039**
LIHC	182	0.38	1.2	0.28	182	0.6	1.1	0.61
LUAD	239	0.14	1.3	0.14	239	0.91	1	0.9
LUSC	241	**0.03**	1.4	**0.03**	241	0.21	1.3	0.21
MESO	41	**0.018**	1.8	**0.019**	41	**0.02**	1.9	**0.022**
OV	212	0.87	0.98	0.86	212	0.83	0.98	0.85
PAAD	89	0.75	1.1	0.75	89	0.34	1.3	0.34
PCPG	91	0.4	0.49	0.41	91	0.5	0.72	0.5
PRAD	246	0.47	0.6	0.48	246	0.063	0.67	0.064
READ	46	0.84	1.1	0.84	46	0.38	1.5	0.39
SARC	131	0.17	0.76	0.17	131	0.75	0.95	0.75
SKCM	229	0.34	0.88	0.34	229	0.35	0.89	0.35
STAD	192	**0.05**	1.4	0.051	192	0.11	1.4	0.11
TGCT	68	0.077	5.10E+08	1	68	0.093	1.9	0.098
THCA	255	0.55	1.4	0.55	255	0.99	1	0.99
THYM	59	0.98	1	0.98	59	0.42	0.69	0.42
UCEC	86	0.75	0.89	0.75	86	0.67	0.87	0.67
UCS	28	0.96	1	0.95	28	0.23	0.64	0.23
UVM	39	0.93	1	0.94	39	0.24	1.7	0.25

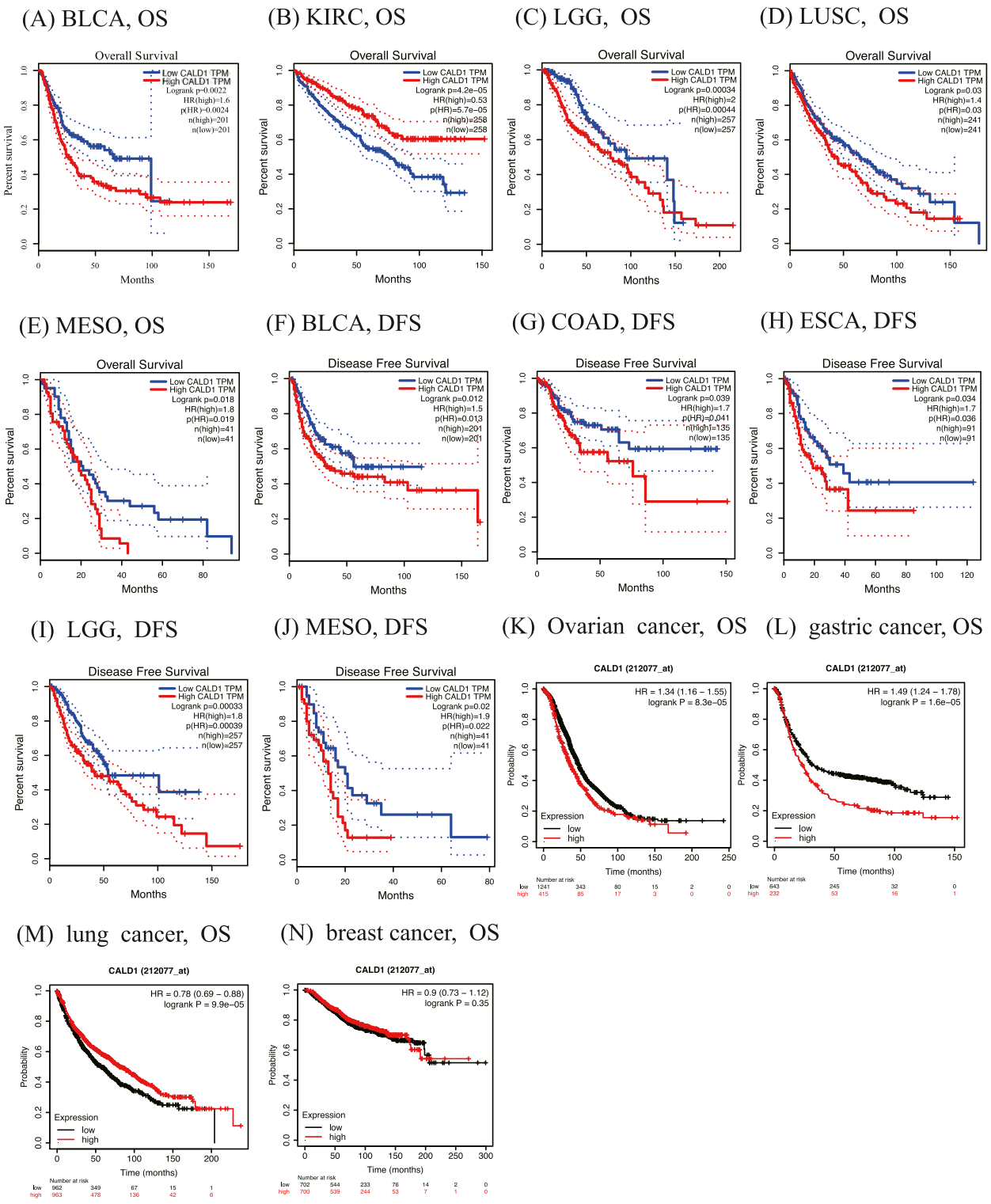

Figure 2. The expression of CALD1 in different types cancer in GEPAI (A–J) and Kaplan-Meier plotter database (K–N). (A–E) GEPIA database shows overall survival in BLCA, KIRC, LGG, LUSC, MESO. (F–J) GEPIA database shows disease free survival in BLCA, COAD, ESCA, LGG, MOSO. (K–N) Kaplan-Meier plotter database overall survival in ovarian cancer, gastric cancer, lung cancer and breast.

TCGA in order to estimate tumor immune infiltration by neutrophils, macrophages, dendritic cells, B cells and CD4/CD8 T cells. We first explored differences in CALD1 expression in particular types cancer using TIMER database, and then observed the relationship between CALD1 expression and degree of infiltration by some immune cell subsets. Furthermore, we used Kaplan-Meier to analyze gene expression and immune cell infiltration compared with the results of TIMER. Finally, we found that the expression of CALD1 is correlated with the expression of particular immune-infiltrated cell subset markers.

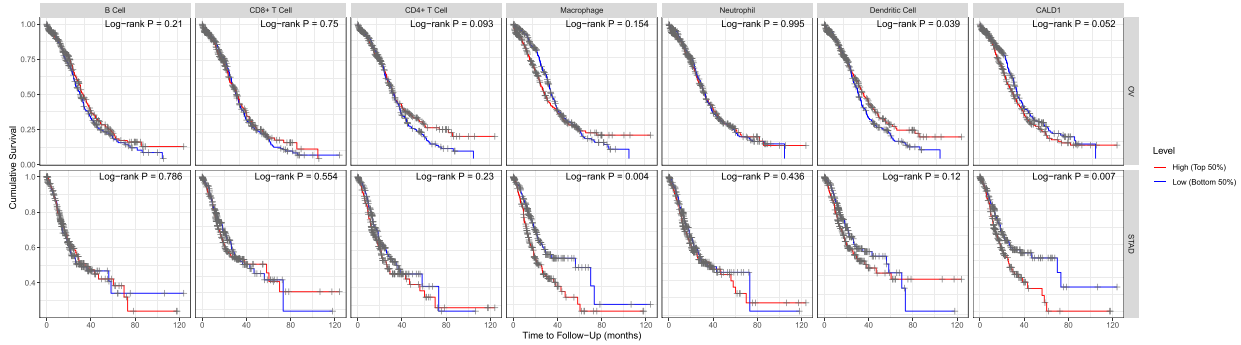

Figure 3. The relationship between patients' prognosis and immune cells infiltration. Kaplan-Meier plots of immune infiltration and CALD1 expression levels in OV and STAD.

Table 3. Correlation of CALD1 mRNA expression and clinicopathological information in gastric cancer by Kaplan-Meier plotter.

	gastric cancer		
	overall survival (n = 875)		
	N	Hazard Ratio	P-value
Sex			
female	236	1.85 (1.29–2.64)	*0.00061*
male	566	1.34 (1.08–1.67)	*0.0086*
Stage			
1	69	0.52 (0.16–1.62)	0.25
2	145	2.05 (1.07–3.93)	*0.027*
3	319	1.63 (1.21–2.21)	*0.0013*
4	152	1.89 (1.28–2.8)	*0.0012*
Stage T			
1	14	NA	NA
2	253	1.96 (1.25–3.08)	*0.003*
3	208	1.89 (1.34–2.67)	*0.00023*
4	39	3.48 (1.41–8.56)	*0.0041*
Stage N			
0	76	2.03 (0.89–4.66)	0.088
1+2+3	437	2.59 (1.98–3.39)	*6.80E-13*
1	232	2.73 (1.8–4.15)	*8.50E-07*
2	129	2.2 (1.39–3.48)	*0.0006*
3	76	2.36 (1.38–4.04)	*0.0013*
Stage M			
0	459	2.35 (1.77–3.11)	*8.50E-10*
1	58	1.87 (1.02–3.42)	*0.041*
Lauren classification			
intestinal	320	1.73 (1.25–2.4)	*0.00076*
diffuse	248	2.28 (1.62–3.21)	*1.20E-06*
mixed	33	2.62 (0.83–8.26)	0.088
Differentiation			
poor differentiated	166	1.53 (0.98–2.4)	0.061
moderate differentiated	67	2 (1.02–3.96)	*0.041*
well differentiated	32	3.99 (1.53–10.36)	*0.0023*

Italic values indicate $P < 0.05$.

2.5. Statistical analysis

The differences of CALD1 expression in various cancers were observed in Oncomine (P-value of 1E-4, fold change of 2, and gene ranking of 10%) and TIMER databases. Survival curves were analyzed by Kaplan-Meier plotter, TIMER and GEPIA databases, with data including HR and P-value. Spearman's correlation was used to analyze the degree of association between particular variables. $P < 0.05$ were considered significant.

3. Results

3.1. The mRNA level of CALD1 in multiple cancer types of human

The CALD1 mRNA expression levels in different tumors and normal were analyzed using the Oncomine database. The analysis revealed that the CALD1 expression was higher in brain and CNS, breast, colorectal, esophageal, gastric, head and neck, kidney, leukemia, liver, lymphoma, pancreatic cancer and sarcoma compared to the normal tissues

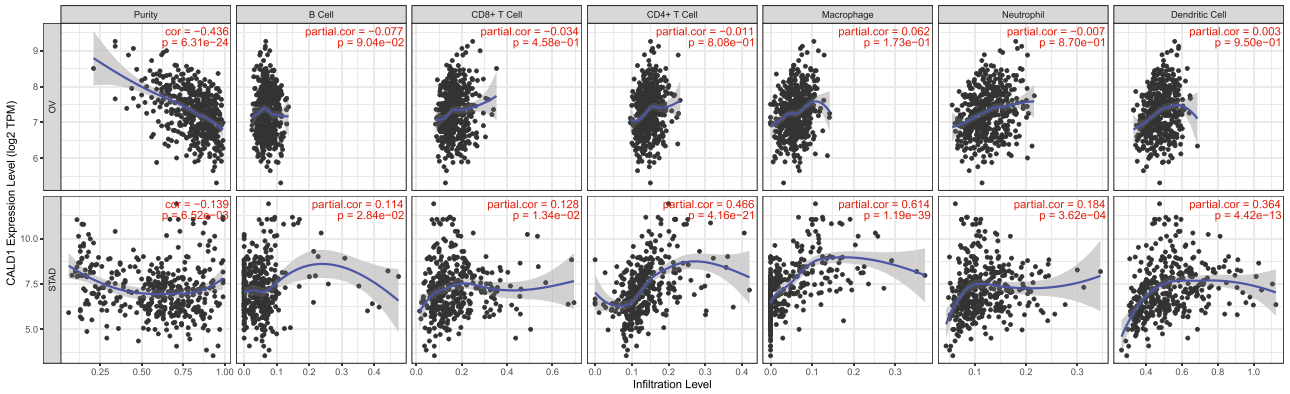

Figure 4. The relationship between immune infiltration level and CALD1 in STAD and OV via TIMER.

(Figure 1A). Specifically, CALD1 has been found with only 4 out of 23 analyses meeting the threshold in 3 out of 7 datasets in gastric cancer compared to normal tissues. Detailed information of particular tumor types is compiled in Table 1.

To further evaluate CALD1 expression in multiple human cancers, we examined CALD1 expression through Transcriptome-seq data of various malignancies via TCGA. The CALD1 expression, across all TCGA tumors, between differential tumor and adjacent normal tissues is shown in Figure 1B. CALD1 expression was significantly lower in BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma), COAD (Colon adenocarcinoma), KICH (Kidney Chromophobe), KIRP (Kidney renal papillary cell carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), THCA (Thyroid carcinoma), UCEC (Uterine Corpus Endometrial Carcinoma) compared with adjacent normal tissues. However, CALD1 expression was significantly higher in CHOL (Cholangiocarcinoma), HNSC (Head and Neck squamous cell carcinoma), KIRC (Kidney renal clear cell carcinoma) compared with adjacent normal tissues.

3.2. Prognostic potential of CALD1 in human tumors

To investigated whether CALD1 expression was correlated to prognosis of cancer patients, we explored the interrelation between CALD1 expression level and clinical information of patients with 33 TCGA cancers types in the GEPIA database (Table 2). We found that the CALD1 expression level of multiple cancer types have a significant association with patient prognosis. The expression of CALD1 significantly impacts overall survival in 5 types cancers ($P < 0.05$), including BLCA (OS HR = 1.6 P = 0.0022), KIRC (OS HR = 0.53 P = 4.2E-5), LGG (OS HR = 2 P = 0.00034), LUSC (OS HR = 1.4 P = 0.03) and MESO (OS HR = 1.8 P = 0.018) (Figure2 A, B, C, D, E). As shown in the figure, the high expression of CALD1 is always associated with poor prognosis except in KIRC. And the CALD1 expression level significantly impacts disease free survival in 5 types cancers, including BLCA (DFS HR = 1.5 P = 0.013), COAD (DFS HR = 1.7 P = 0.039), ESCA (DFS HR = 1.7 P = 0.034), LGG (DFS HR = 1.8 P = 0.00039) and MESO (DFS HR = 1.9 P = 0.02) (Figure2 F,I, JJ). Thus, the higher expression of CALD1 was both associated with poorer prognosis in OS and RFS of LGG and MESO. It shown that high or low CALD1 expression can impact prognosis of patients in a range of cancer types.

We further analyze the prognostic value of CALD1 using Kaplan-Meierplotter based on Affymetrix microarrays in order to assess how CALD1 expression relates to prognosis in cancer. We observed overall survival (OS) in a range of cancer types. Surprisingly, we found that high expression of CALD1 was associated with poorer prognosis in ovarian cancer (OS HR = 1.34, 95% CI = 1.16 to 1.55, P = 8.3E-05) and gastric cancer (OS HR = 1.49, 95% CI = 1.24 to 1.78, P = 1.6E-05) (Figure2 K, L), but low expression of CALD1 was associated with poorer prognosis in lung cancer (OS HR = 0.78 95% CI = 0.69 to 0.88, P = 9.9E-05) (Figure 2 M). However, the expression of CALD1 has no significant association with breast cancer (Figure 2 N). These results suggest that increased and decreased CALD1 expression have different prognosis value in different cancers.

3.3. The association between CALD1 expression and prognosis of cancers in patients with lymphatic metastasis

As we found high CALD1 expression to be linked with poorer prognosis in patients of LGG, MESO, OV and STAD, we first explore the clinical relevance of the four tumor immune subsets using TIMER. Kaplan-Meier plots show survival differences with immune cells infiltration and CALD1 expression in 39 tumor types. Prognosis of STAD patients was significantly associated with Macrophage infiltration (P = 0.004) and CALD1 expression (P = 0.007). However, there was no significant correlation between prognosis and immune cell infiltration or CALD1 expression was observed in OV (Figure 3).

To better understand the correlation and underlying mechanisms of the CALD1 expression level in cancers, we analyzed the relationship between CALD1 and gastric cancer patients in the Kaplan-Meier plotter database (Table 3). The high expression of CALD1 was associated with worse OS in female and male ($P < 0.05$). We also found that high CALD1 mRNA expression impacts OS in stage 2–4 of gastric but not stage 1. Specifically, high CALD1 expression has high HR value of OS in the N1–N3 categories. Here, N refers to lymph node developed into cancer. N0 means no regional lymph node metastasis, N1–N3 indicate the number and location of regional lymph node metastasis. Meanwhile, CALD1 was associated with two types of Lauren classification and differentiation ($P < 0.05$). It is indicated thatcCALD1 expression level can affect the prognosis in gastric cancer patients with lymph node metastasis.

3.4. The expression of CALD1 is correlated with immune infiltration in gastric cancers

In cancer patients, tumor-infiltrating lymphocytes are independent predictor of lymph node metastasis and survival [21]. We next investigated whether CALD1 expression was associated with the degree of immune cell infiltration. We explored the relationship into 39 tumor types from TIMER. We observed that CALD1 expression has correlations with tumor purity in 28 types of cancer and positive correlations with B cell infiltration level in 17 types of cancer (cor >0.1). Furthermore, the expression of CALD1 level obviously has positive correlations with CD8+T cell infiltration in 22 types of cancer (cor >0.1), CD4+T cell infiltration in 24 cancer types, with macrophages infiltration in 33 cancer

Table 4. The correlation between CALD1 and immune cells in STAD and OV explored by TIMER database.

Description	Gene markers	STAD				OV			
		None		Purity		None		Purity	
		Cor	P-value	Cor	P-value	Cor	P-value	Cor	P-value
CD8+T cell	CD8A	0.232	***	0.203	***	0.178	*	-0.049	4.44E-01
	CD8B	0.123	1.25E-02	0.108	3.49E-02	0.08	1.65E-01	-0.133	3.65E-02
T cell (general)	CD3D	0.157	*	0.118	2.19E-02	0.157	*	-0.108	8.81E-02
	CD3E	0.197	***	0.161	*	0.213	**	-0.04	5.35E-01
	CD2	0.229	***	0.198	**	0.213	**	-0.03	6.30E-01
B cell	CD19	0.265	***	0.253	***	-0.078	1.76E-01	-0.078	2.21E-01
	CD79A	0.246	***	0.211	***	0.195	**	0.031	6.28E-01
Monocyte	CD86	0.309	***	0.289	***	0.235	***	-0.014	8.22E-01
	CD115(CSF1R)	0.417	***	0.397	***	0.346	***	0.117	6.59E-02
TAM	CCL2	0.478	***	0.463	***	0.224	***	-0.006	9.23E-01
	CD68	0.091	6.33E-02	0.076	1.41E-01	0.247	***	-0.01	8.71E-01
	IL10	0.305	***	0.297	***	0.335	***	0.191	*
M1 Macrophage	INOS(NOS2)	-0.055	2.68E-01	-0.058	2.59E-01	0.234	***	0.162	1.04E-02
	IRF5	0.147	*	0.139	*	0.042	4.61E-01	-0.079	2.13E-01
	COX2(PTGS2)	0.277	***	0.273	***	0.332	***	0.228	**
M2 Macrophage	CD163	0.349	***	0.329	***	0.348	***	0.146	2.14E-02
	VSIG4	0.397	***	0.393	***	0.313	***	0.072	2.54E-01
	MS4A4A	0.417	***	0.405	***	0.339	***	0.111	8.05E-02
Neutrophils	CD66b (CEACAM8)	0.018	7.12E-01	0.023	6.56E-01	0.128	2.62E-02	0.147	2.00E-02
	CD11b (ITGAM)	0.37	***	0.364	***	0.324	***	0.1	1.15E-01
	CCR7	0.309	***	0.278	***	0.22	**	0.023	7.16E-01
Natural killer cell	KIR2DL1	0.175	**	0.152	*	0.12	3.75E-02	0.059	3.55E-01
	KIR2DL3	0.101	3.99E-02	0.067	1.91E-01	-0.011	8.50E-01	-0.08	2.10E-01
	KIR2DL4	-0.094	5.58E-02	-0.131	1.08E-02	-0.015	7.94E-01	-0.133	3.53E-02
	KIR3DL1	0.0093	5.94E-02	0.064	2.15E-01	0.144	1.21E-02	0.008	8.95E-01
	KIR3DL2	0.135	*	0.107	3.67E-02	0.069	2.33E-01	-0.049	4.40E-01
	KIR3DL3	-0.138	*	-0.145	*	-0.032	5.77E-01	-0.073	2.53E-01
	KIR2DS4	0.054	2.69E-01	0.018	7.20E-01	0.122	3.39E-02	0.064	3.16E-01
Dendritic cell	HLA-DPB1	0.2	***	0.163	*	0.09	1.18E-01	-0.134	3.42E-02
	HLA-DQB1	0.06	2.19E-01	0.018	7.22E-01	0.042	4.61E-01	-0.106	9.60E-02
	HLA-DRA	0.101	3.99E-02	0.065	2.05E-01	0.033	5.66E-01	-0.147	2.02E-02
	HLA-DPA1	0.129	*	0.089	8.19E-02	0.083	1.51E-01	-0.11	8.23E-02
	BDCA-1(CD1C)	0.373	***	0.366	***	0.185	*	-0.005	9.40E-01
	BDCA-4(NRP1)	0.624	***	0.619	***	0.457	***	0.333	***
	CD11c (ITGAX)	0.351	***	0.328	***	0.285	***	0.083	1.94E-01
Th1	T-bet (TBX21)	0.201	***	0.181	**	0.202	**	-0.053	4.07E-01
	STAT4	0.318	***	0.292	***	0.224	8.64–05	0.028	6.57E-01
	STAT1	0.024	6.32E-01	0.001	9.80E-01	0.046	4.20E-01	0.03	6.39E-01
	IFN-γ (IFNG)	-0.055	2.65E-01	-0.081	1.18E-01	0.054	3.50E-01	-0.13	4.01E-02
	TNF-α (TNF)	0.018	7.13E-01	-0.021	6.91E-01	0.072	2.14E-01	0.036	5.71E-01
Th2	GATA3	0.364	***	0.353	***	0.293	***	0.163	1.02E-02
	STAT6	0.139	*	0.143	*	0.147	1.06E-02	0.187	*
	STAT5A	0.332	***	0.327	***	0.208	**	0.144	2.28E-02
	IL13	0.124	1.16E-02	0.137	*	0.028	6.27E-01	0.011	8.67E-01
Tfh	BCL6	0.504	***	0.482	***	0.212	**	0.291	***
	IL21	0.034	4.93E-01	0.024	6.35E-01	-0.094	1.03E-01	-0.138	2.90E-02
Th17	STAT3	0.367	***	0.357	***	0.311	***	0.244	***
	IL17A	-0.276	***	-0.284	***	0.083	1.52E-01	-0.009	8.93E-01
Treg	FOXP3	0.181	**	0.15	*	0.183	*	0.017	7.92E-01
	CCR8	0.297	***	0.29	***	0.134	1.94E-02	0.043	4.97E-01
	STAT5B	0.515	***	0.503	***	0.341	***	0.343	***
	TGFβ (TGFB1)	0.535	***	0.521	***	0.414	***	0.199	*
T cell exhaustion	PD-1 (PDCD1)	0.096	5.17E-02	0.062	2.28E-01	0.067	2.46E-01	-0.162	1.03E-02
	CTLA4	0.088	7.17E-02	0.06	2.48E-01	0.152	*	-0.05	3.68E-01
	LAG3	0.092	6.23E-02	0.057	2.65E-01	0.038	5.09E-01	-0.127	4.48E-02
	TIM-3 (HAVCR2)	0.308	***	0.292	***	0.272	***	0.009	8.88E-01
	GZMB	0.003	9.50E-01	-0.044	3.94E-01	0.073	2.05E-01	-0.135	3.28E-02

STAD: stomach adenocarcinoma. TAM: tumor-associated macrophage. Th: T helper cell. Tfh: Follicular helper T cell. Treg: regulatory T cell. Cor: R value of Spearman's correlation. none: correlation without adjustment. purity: correlation adjusted by purity.
*p < 0.01; **p < .001; ***p < 0.0001.

types (cor >0.1), neutrophil infiltration in 31 cancer types (cor >0.1), and dendritic cells in 29 cancer types (cor >0.1).

As we can observe in Figure 4, there was significant relationship between CALD1 and immune infiltration in STAD, but none in OV. In STAD, CALD1 expression was associated with B cell infiltration, CD8+T cell infiltration, CD4+T cell infiltration, macrophage infiltration, neutrophil infiltration and dendritic cell infiltration (cor >0.1), especially in macrophage (cor = 0.614, P = 1.19E-39), CD4+T cell (cor = 0.466, P = 4.16E-21) and dendritic cell (cor = 0.364, P = 4.42E-13). These results demonstrated that CALD1 expression plays an important role in immune infiltration in gastric cancer.

3.5. Relationship analysis between CALD1 expression and immune markers

We explored the correlation between CALD1 and several types of infiltrated immune cells in STAD compared with OV using TIMER and GEPIA databases, including CD8+T cells, T cells (general), B cells, monocytes, TAMs, M1/M2 macrophages, neutrophils, natural killer cells, and dendritic cells (Table 4). We further explored the different functions of T cells, such as CD8+T cells, T cells (general), B cells, monocytes, TAMs, M1/M2 macrophages, neutrophils, natural killer cells, and dendritic cells. Tumor purity represents a significant confounding factor in molecular taxonomy based on gene expression [22]. After the purity-related adjustments, the CALD1 expression level was significantly correlated with most immune markers in divergent immune cell types and different T cells in STAD. Interesting, we found that there were strong correlations between CALD1 expression and most marker sets expression of T cell, B cell, monocytes, M2 macrophages, dendritic cell, Th17 and Treg in STAD (Table 4).

We showed CD86 and CSF1R of monocyte, CCL2, IL10 of TAM, IRF5, COX2 of M1 macrophage, CD163, VSIG4 and MS4A4A of M2 macrophage are significantly correlate with CALD1 expression in STAD (Figure 5). To confirm the correlation between CALD1 expression and the above markers of monocytes, we further analyzed in the GEPIA database (Table 5). Surprisingly, the results in GEPIA are similar to those in TIMER. These suggested that CALD1 may regulate macrophage polarization in STAD.

We can find the high expression of CALD1 is related with the dense dendritic cell infiltration in STAD. The expression of dendritic cell markers HLA-DPB1, HLA-DPA1, BDCA-1 (CD1C), BDCA-4 (NRP1) and CD11C (ITGAX) significantly correlated with CALD1 expression. The result shows a close relationship between CALD1 and infiltration by dendritic cells. We further explored the correlation between CALD1 and markers of Treg and T cell exhaustion (Table 4, Table 5). We found there are strong correlations between CALD1 and those gene markers,

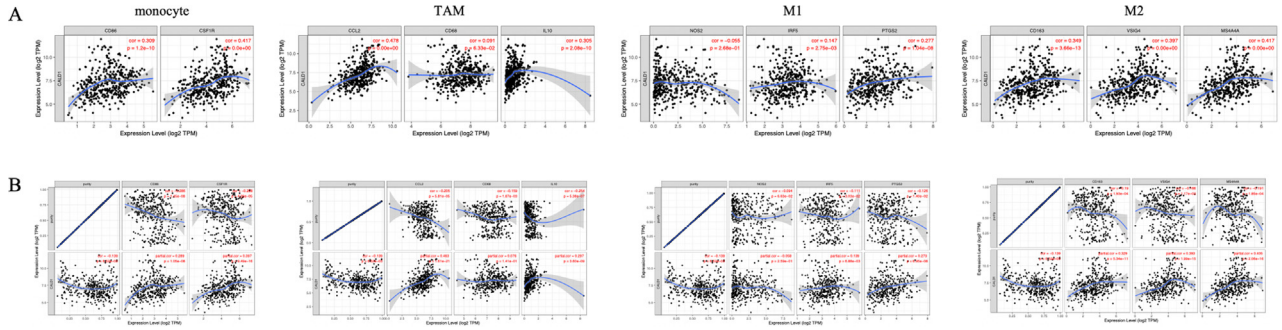

Figure 5. The correlation between CALD1 expression and macrophage in STAD. (A) shows CD86 and CSF1R of monocyte, CCL2, IL10 of TAM, IRF5, COX2 of M1 macrophage, CD163, VSIG4 and MS4A4A of M2 macrophage are significantly correlate with CALD1 expression in STAD. (B) shows the correlation adjusted by tumor purity.

Table 5. The correlation between CALD1 and immune cells in gastric cancer and normal explored by GEPIA database.

Description	Description	STAD			
		Tumor		Normal	
		R	P-value	R	P-value
Monocyte	CD86	0.31	***	-0.36	2.90E-02
	CD115(CSF1R)	0.43	***	0.0064	***
TAM	CCL2	0.48	***	0.56	**
	CD68	0.097	0.05	-0.53	**
	IL10	0.32	***	-0.18	0.28
M2 Macrophage	CD163	0.4	***	0.6	**
	VSIG4	0.41	***	0.29	0.083
	MS4A4A	0.43	***	0.42	0.01
Treg	FOXP3	0.2	***	-0.57	**
	CCR8	0.32	***	-0.3	0.073
	STAT5B	0.53	***	0.91	***
	TGFβ (TGFB1)	0.55	***	0.5	*

STAD: stomach adenocarcinoma. Tumor: correlation analysis in tumor tissue of TCGA. Normal: correlation analysis in normal tissue of TCGA.
*p < 0.01; **p < .001; ***p < 0.0001.

including FOXP3, CCR8, STAT5B, TGFB1 and TIM-3. FOXP3 plays a critical role in Treg cells, which can influence cytotoxic T cells targeting tumor cells [23]. TIM-3, a key gene that regulates T cell exhaustion, has a strong correlation with CALD1 expression, suggesting that the high CALD1expression is indispensable in TIM-3-mediated T cell failure. These findings further confirm that the CALD1 expression is specifically related to immune infiltration in STAD, and CALD1 plays a vital role in immune escape in the gastric cancer microenvironment.

4. Discussion

CALD1 plays an important role in many cellular functions, such as proliferation, apoptosis, cell motility, adhesion, receptor function, and second messenger pathways. Some researchers have found the correlation between CALD1 expression and prognosis of gastrointestinal cancer. No relationship between CALD1 and immune-infiltrated has been reported so far. In this article, we report that differences of CALD1 expression is associated with different prognosis of human tumors. The high expression of CALD1 mRNA is related with worse prognosis of gastric cancer and better prognosis of lung cancer. The expression of CAL1 can affect the prognosis of patients with lymph node metastasis of gastric cancer, demonstrating that the CALD1 expression level could be used as a predictive marker of tumor metastasis. Furthermore, we showed that CALD1 expression was connected with both immune-infiltrated degree and expressions of the various immunological markers. Our study offers new ideas into the functions of CADL1 in tumor immunity and cancer marker.

In this study, we analyzed the mRNA expression level of CALD1 in Oncomine and TIMER databases in multiple cancers. CALD1 expression levels in types cancer, compared to adjacent normal tissues, have been observed. Unexpectedly, the results in different databases are slightly different. The differences may reflect data collection approaches and fundamental mechanisms are different in separate databases. We further investigated the correlation between CALD1 expression and prognosis of cancers via GEPIA and Kaplan-Meier plotter databases. The high or low expression of CALD1 has different significance in prognosis of patients with different cancers. According to the results of Kaplan-Meier plotter, we found that overall survival of gastric cancer and ovarian cancer was significantly associated with CALD1 expression level. We next investigated the association between CALD1 and N stages. These results suggest that CALD1 could be further as a prognostic biomarker in gastric cancer.

Our results also reveal a correlation between CALD1 expression and diverse immune infiltration levels in gastric cancer. We demonstrated that there are positive correlations from moderated to strong between CALD1 expression level and infiltration level of CD8+ T, CD4+ T cells, neutrophils and DCs, and a significantly positive relationship between infiltration level of macrophages and CALD1 expression in gastric cancer (Figure 4). In addition, there is a strong correlation between CALD1 expression and molecular markers of immune cells in gastric cancer. Gene markers of M1 macrophages (including IRF5 and PTGS2) showed weak correlations with CALD1 expression but gene markers of M2 (including CD163, VSIG4, and MS4A) showed strong correlations with CALD1 (Table 4). There results above suggested that CALD1 might be a potential regulating gene in polarization of tumor-associated macrophages (TAM). The high expression of CALD1 positively correlates with the expression of Treg and T cell exhaustion markers (including FOXP3, CCR8, STATA5B, TGFB1 and TIM-3) in gastric cancer (Table 4). Several markers of T helper cells (such as Th1, Th2, Tfh, and Th17), including TBX21, STAT4, GATA3, STAT6, STAT5A, BCL6 and STAT3, also have significant correlations in gastric cancer. These findings suggest that CALD1 plays an important role in regulating immune-infiltrated cells in gastric cancer.

In summary, the high expression level of CALD1 was correlated with poor prognosis and increased immune infiltration levels in CD8+ T cells, CD4+ T cells, macrophages, neutrophils and DCs of various tumors. In gastric cancer, especially, CALD1 expression contributes to the regulation of tumor-associated macrophages (TAMs), DCs, T cell exhaustion, and Tregs. Accordingly, CALD1 was related with both prognosis and immune infiltration as an important role in gastric cancer.

Declarations

Author contribution statement

Yixuan Liu, Suhong Xie: Analyzed and interpreted the data; Wrote the paper.

Keyu Zhu, Xiaolin Guan: Analyzed and interpreted the data.

Lin Guo, Renquan Lu: Conceived and designed the experiments.

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Data availability statement

The authors are unable or have chosen not to specify which data has been used.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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