The Role of M6A-related CBLL1 in the Prognosis and Immune Infiltration of Pan-cancer

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Research

Keywords: CBLL1, m6A, pan-carcinoma, prognosis, immunity

Posted Date: July 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-40873/v1

Abstract

Background

The modification of N6-methyladenosine (m6A) plays an important role in physiology and disease progression. The relationship between the role of m6a-related gene CBLL1 in pan-carcinoma and tumor immune infiltrates has remained unknown.

Methods

To explore the expression level of CBLL1 methylation in pan-cancer in SMART database, and download mRNA expression, mutation and clinical data in UCSC database, to analyze the expression level of CBLL1, and the relationship between CBLL1 expression and clinicopathological features, prognosis, mutation and immune microenvironment in pan-cancer. CIBERSORT was used to analyze the relationship between the expression of CBLL1 and the infiltration of pan-carcinoma immune cells. The mRNA expression data of UCSC database were used to analyze the correlation between CBLL1 expression and pan-cancer immunomodulations, checkpoints and receptor molecules. Gene Set enrichment analysis (GSEA) revealed the possible mechanism of CBLL1 in the regulation of pan-cancer progression.

Results

The levels of CBLL1 methylation and mRNA expression in pan-cancer tissues were abnormal. The level of CBLL1 is related to the age, race, clinical stage and treatment effect of patients with pan-carcinoma and associated with the prognosis of patients with KIRC, LUSC, THCA, THYM, MESO, PRAD, STAD, and UVM. Univariate COX regression analysis showed that expression of CBLL1 was a risk factor for poor prognosis in patients with KICH, KIRC, LAML, THYM, KIRC, PCPG, OV, PRAD, STAD, GBM and UVM.The expression level of CBLL1 was correlated with BLCA, BRCA, COAD, LAML, LGG, LUAD, LUSC, SARC, STAD, THCA, THYM and UVM tumor mutational burden (TMB), and with ACC, BRCA, CESC, COAD, DLBC, HNSC, PRAD, READ, SARC, STAD, TGCT, THCA and UCEC microsatellite instability (MSI). The expression level of CBLL1 was correlated with pan-cancer stromal cells and immune cells. The expression of CBLL1 is related to pan-cancer immunomodulators, checkpoints and receptor molecules. GSEA found that CBLL1 may participate in the progression of pan-cancer through B cell receptor singaling pathway, mRNA binding, immunoglobulin receptor binding, Positive Regulation of cell cycle phase transition and other mechanisms.

Conclusions

CBLL1 is abnormally expressed in patients with pan-carcinoma, which is expected to be a biomarker for prognosis, mutation and immune infiltration in patients with pan-carcinoma.

Background

There will be 18.1 million new cancers and 9.6 million cancer deaths worldwide in 2018. Lung cancer is the most common malignant tumor in both male and female patients, accounting for 11.6% of the total incidence of cancer. It is also the main cause of cancer death, accounting for 18.4% of the total number of cancer deaths. The second to 10th highest new cancer incidence rates were Prostate cancer (7.1%), Colon cancer (6.1%), Nonmelanoma of skin (5.8%), Stomach cancer (5.7%), Liver cancer (4.7%), Rectum cancer (3.9%), Esophagus cancer (3.2%), Cervix uteri cancer (3.2%) and Thyroidcancer (3.1%). The 2th to 10th highest cancer mortality rates were Stomach cancer (8.2%), Liver cancer (8.2%), Breast cancer (6.6%), Colon cancer (5.8%), Esophagus cancer (5.3%), Prostate cancer (3.8%), Cervix uter cancer (3.3%), Rectum cancer (3.2%) and Leukemia (3.2%). Overall, the top 10 cancer cases account for about 65% of new cancer cases and deaths [1]. The main treatment methods of cancer patients are surgery, radiotherapy, chemotherapy, targeted therapy and so on, but cancer patients will develop drug resistance and metastasis, resulting in poor prognosis and high mortality.

Modification of N6-methyladenosine (m6A) mRNA is involved in a variety of biological processes. For example, it is involved in mRNA stability, translation, RNA splicing, etc [2–6]. Under physiological conditions, m6A modification was associated with methyltransferase -like 3 (METTL3), methyltransferase-like 14 (METTL14), WT1 associated protein (WTAP), RNA binding motif protein

15 (RBM15), vir like m6A methyltransferase associated (KIAA1429), Zinc finger CCCH-type containing 13 (ZC3H13), ALKB homologue 5 (ALKBH5), Fat weight and obesity related proteins (FTO), YTH domain containing 1 (YTHDC1), YTH domain containing 2 (YTHDC2), YTH domain containing 3 (YTHDF3), etc [7,8]. Under pathological conditions, the abnormal modification of m6A is closely related to diseased progression. For example, Guo et al. reported lower ALKBH5 m6A related gene expression and patients with pancreatic cancer (PC) associated with poor prognosis. Increasing ALKBH5 expression reduced the proliferation, migration and invasion of PC cells in vitro and inhibited tumor growth in vivo. Interfering with the expression of ALKBH5 promotes the progress of PC. ALKBH5 can activate PER1, in a m6A-YTHDF2 -dependent manner and increase the expression of PER1, which can lead to the activation of ATM-CHK2-P53/CDC25C signal pathway, which in turn inhibits the progress of PC [9]. Meng et al. found that the expression of METTL3 in nasopharyngeal carcinoma (NPC) tissues was significantly higher than that in the adjacent normal tissues. METTL3 expression was associated with T staging and lymph node metastasis in NPC patients, and the Overall survival (OS) of NPC patients with elevated METTL3 expression was poorer. Knocking down the expression of METTL3 in SUNE-1 cells can inhibit cell viability and migration ability, and reduce the expression of EZH2 protein. M6A modification plays a role in the process of METTL3 binding to EZH2 [10]. Zhang et al. found that there was a negative correlation between the expression of YTHDF2 and the prognosis of patients with hepatocellular carcinoma (HCC). Interfering with the expression of YTHDF2 can damage the stemness of HCC cells. On the contrary, increasing the expression of YTHDF2 increased the phenotype of cancer stem cell (CSC). The increase or decrease of YTHDF2 expression leads to the change of m6A level and OCT4 protein expression in OCT4 mRNA 5'- untranslated region (UTR), and affects OCT4 translation. Interfering with the expression of YTHDF2 reduced the ability of transplanted tumor in vivo in nude mice [11]. Studies have reported that the m6a-related gene cbl proto-oncogene like 1 (CBLL1) is highly expressed in the tissues of non-small cell lung cancer (NSCLC).

The increased expression of CBLL1 (Hakai) is related to the size of tumor in patients with NSCLC. The overexpression of CBLL1 promotes the cell cycle transition of G1-S, which leads to cell proliferation and invasion. Interfering with the expression of CBLL1 gene inhibits cell invasion by increasing the expression of adhesion proteins, and reduces the expression of MMP2 and MMP9 in cells [12,13]. Compared with healthy human colon tissue, Hakai is highly expressed in adenoma and colon adenocarcinoma, and is related to patient stage. The overexpression of Hakai can enhance cell transformation and invasion, inhibit the expression of E-cadherin and promote the expression of N-cadherin. Hakai can significantly induce tumor growth and local invasion in nude mice [14]. Overexpression of Hakai inhibits the proliferation and migration of breast cancer cells [15]. However, the role of CBLL1 in prognosis, mutation and immune infiltration in pan-cancer is still unknown. In this study, we aimed to use SMART, UCSC and CIBERSORT to explore the level of CBLL1 methylation and mRNA expression in pan-cancer, and to analyze the relationship between CBLL1 mRNA expression and clinicopathological features, prognosis, mutation, immune microenvironment, immune cell infiltration in pan-cancer patients. The mRNA expression data of UCSC database were used to analyze the correlation between CBLL1 expression level and pan-cancer immunomodulators, checkpoints and receptor molecules, as well as the possible mechanism of regulating pan-cancer progression.

Materials And Methods

SMART database

SMART (http://www.bioinfo-zs.com/smartapp/) database is an online database website for analysis based on the Cancer Genome Atlas (TCGA) data [16]. The methylation expression levels of CBLL1 in 33 cancers were analyzed by using SMART database to explore whether the methylation expression levels of CBLL1 in pan-carcinoma showed differential expression changes.

UCSC database

The pan-cancer mRNA expression data, clinical data and mutation data were downloaded from UCSC database [17]. The expression data of CBLL1 mRNA in 33 kinds of cancer tissues were extracted and analyzed.The clinicopathological features of 33 cancer patients were extracted and sorted out to analyze the correlation between CBLL1 expression level and age, race, clinical stage, treatment effect and prognosis (OS, Disease-specific survival (DSS), Disease-free interval (DFI) and Progression-free interval (PFI)) of patients with pan-cancer. Univariate COX analyzed the correlation between the expression of CBLL1 and OS, DSS, DFI and PFI in patients with pan-cancer. The tumor mutational burden (TMB) in each tumor sample was calculated, and the MSI score of tumor microsatellite instability (MSI) was downloaded [18]. The correlation between CBLL1 expression level and TMB and MSI was analyzed. The contents of stromal cells and immune cells in 33 kinds of cancers were scored, and the relationship between the

expression of CBLL1 mRNA and the microenvironment of pan-cancerous tumors was analyzed. The mRNA expression data of UCSC database was used to analyze the correlation between CBLL1 expression level and pan-cancer immunomodulators, checkpoints and receptor molecules. The median value of CBLL1 expression was used to analyze the gene expression data, and GSEA was used to analyze the possible mechanism of CBLL1 regulating the progression of pan-cancer [19].

CIBERSORT

CIBERSORT (https://cibersort.stanford.edu/) is a kind of bioinformatics algorithms which can calculate according to the gene expression profiles of immune cells. We used CIBERSORT to evaluate the relative proportion of 22 immune cell types in 33 cancers. The scores of immune cells were downloaded from The Cancer Imaging Archive (TCIA) (https://tcia.at/home) database, and the correlation between the expression level of CBLL1 and tumor immune cells was analyzed. Screening criteria: $P < 0.05$ [20].

Results

Abnormal CBLL1 methylation and mRNA expression in pan-cancerous tissues

In SMART database, we found 17 CBLL1 gene probes and abnormal CBLL1 methylation levels in pan-cancerous tissues (Fig. 1 and Fig. S1-13). Probe cg10861925 organizes abnormalities in BLCA, BRCA, HNSC, KIRC, LUSC, PAAD, PGAD, READ, SARC and THCA (Fig. 1A); probe cg02952735 organizes abnormalities in BLCA, BRCA, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PRAD, READ, SARC and THCA (Fig. 1B); probe cg03443922 organizes abnormalities in BLCA, BRCA, COAD, ESCA, HNSC, KIRC, LIHC, LUSC, PRAD, READ and READ (Fig. 1C). Probe cg19318403 organizes abnormalities in BLCA, BRCA, ESCA, KIRC, KIRP, LIHC, LUAD, LUSC, PCPG, PRAD and THCA (Fig. 1D); probe cg06724108 organizes abnormalities in KIRC, LUSC, SARC and SKCM (Fig. S1); probe cg07614064 organizes abnormalities in BLCA, COAD, KIRC, LUAD, LUSC, READ, THCA and UCEC (Fig. S2); probes cg01331191 organizes abnormalities in BLCA, COAD, HNSC, KIRC, KIRP, LIHC, LUAD, PRAD and UCEC (Fig. S3). Probe cg03147542 organizes abnormalities in BRCA, CHOL, COAD, ESCA, HNSC, PAAD and SARC (Fig. S4); probe cg26385438 organizes abnormalities in BRCA, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD and SARC (Fig. S5); probe cg25183958 organizes abnormalities in BLCA, LIHC, LUSC, PAAD, PRAD, SARC, THCA and UCEC (Fig. S6); probe cg15056119 organizes abnormalities in LIHC, LUSC, PAAD, PRAD, READ, SARC and UCEC (Fig. S7). Probe cg04433990 is abnormal in BLCA, BRCA, HNSC, KIRC, LUSC, and SARC (Fig. S8); probe cg20263098 is abnormal in BRCA, HNSC, KIRC, LIHC, LUSC, PAAD, PRAD, READ and SARC (Fig. S9); probe cg21810311 is abnormal in BLCA, COAD, KIRC, KIRP, LUSC, SARC and SKCM (Fig. S10); probe cg00449210 organizes abnormalities in KIRP, LIHC, SARC and THCA (Fig. S11); probe cg27396403 organizes abnormalities in BRCA, COAD, KIRC, LIHC, LUSC, LUSC and PRAD (Fig. S12). Probe cg17158101 was abnormal in BLCA, KIRP, LIHC, LUSC, PAAD, PCPG, PRAD, READ, SARC and STAD (Fig. S13). In addition, in the UCSC database, we found abnormal expression of CBLL1 in a variety of tumors (Fig. S14 and Fig. 2). In detail, CBLL1 is highly expressed in CHOL, COAD, ESCA, GBM, KICH, LIHC, LUSC, HNSC, READ and STAD tissues (Fig. 2A-J), and poorly expressed in THCA and UCEC tissues (Fig. 2K-L).

The expression of CBLL1 is related to the age, race, clinical stage and therapeutic effect of patients with pan-cancer

After collating and analyzing the clinicopathological features of 33 cancer patients, it was found that the expression level of CBLL1 was correlated with the age of BRCA, CHOL, KIRP, LIHC and PAAD patients (Fig. S15), the expression level of CBLL1 mRNA was correlated with the race of patients with BLCA, BRCA, GBM, KIRC, LIHC and THYM (Fig. S16), and the expression level of CBLL1 mRNA was correlated with the clinical stage of patients with ACC, BRCA, KIRC, LIHC, PAAD, STAD, TGCT and THCA (Fig. 3). The expression level of CBLL1 mRNA was related to the therapeutic effect of patients with BLCA, KIRC, KIRP, LGG, PRAD and UCEC (Fig. S17).

The expression of CBLL1 is related to the prognosis of patients with pan-cancer

Survival analysis of clinical information of 33 kinds of cancer patients showed that the expression level of CBLL1 mRNA was correlated with OS in patients with KIRC (Fig. 4A), the expression level of CBLL1 mRNA was correlated with DSS in patients with KIRC, LUSC, THCA and THYM (Fig. 4B-E), the expression level of CBLL1 mRNA was correlated with DFI in patients with MESO, PRAD and STAD (Fig. 4F-H), and the expression level of CBLL1 mRNA was correlated with PFI in patients with KIRC, PRAD and UVM (Fig. 7I-K). Univariate COX regression analysis found that CBLL1 mRNA expression level was a risk factor for OS in patients with KICH, KIRC, LAML and THYM (Fig. S18A);DSS in patients with KIRC, PCPG and THYM (Fig. S18B); DFI in patients with OV, PRAD and STAD (Fig. S18C); PFI in patients with GBM, KIRC, OV, THYM and UVM (Fig. S18D).

The expression level of CBLL1 is associated with TMB and MSI in patients with pan-cancer

The mutation load of pan-cancer tumor was calculated based on the expression data of pan-cancer mRNA in UCSC database, and the correlation between the expression level of CBLL1 mRNA and TMB of pan-cancer patients was analyzed. In other words, the expression level of CBLL1 mRNA was correlated with TMB in patients with BLCA, BRCA, COAD, LAML, LGG, LUAD, LUSC, SARC, STAD, THCA, THYM and UVM (Fig. S19A and Table 1); The expression level of CBLL1 mRNA was correlated with MSI in patients with pan-cancer. In detail, the expression level of CBLL1 mRNA was associated with ACC, BRCA, CESC, COAD, DLBC, HNSC, PRAD, READ, SARC, STAD, TGCT, THCA and MSI in patients with UCEC (Fig. S19B and Table 2).

Table 1
The expression level of CCBL1 is related to TMB in patients with pan-cancer.

Cancer	cor	P	Cancer	cor	P	Cancer	cor	P
ACC	0.054142902	0.635564064	KIRC	-0.102232312	0.062798785	PRAD	-0.008189613	0.857674661
BLCA	0.148662078	0.002609396	KIRP	-0.029322985	0.626390391	READ	0.034712864	0.692734917
BRCA	-0.109007953	0.000654847	LAML	0.270586236	0.031961519	SARC	0.140875647	0.030862194
CESC	0.095841162	0.105781572	LGG	0.234959513	1.03E-07	SKCM	0.038816735	0.403661151
CHOL	0.021641127	0.900302869	LIHC	-0.008575317	0.871372482	STAD	0.218241875	2.41E-05
COAD	-0.228484562	4.36E-06	LUAD	0.231940191	1.43E-07	TGCT	-0.039723537	0.635227514
DLBC	-0.199146515	0.236406449	LUSC	0.103243922	0.022549494	THCA	-0.147243369	0.001187156
ESCA	-0.070773267	0.373833529	MESO	0.071290726	0.532403545	THYM	-0.203247682	0.027959796
GBM	0.096803132	0.241829324	OV	0.069487861	0.253401475	UCEC	0.052933772	0.225963196
HNSC	0.088138064	0.050721582	PAAD	0.064774996	0.429420079	UCS	-0.116530763	0.392392087
KICH	0.082648833	0.512771489	PCPG	0.05657479	0.454493292	UVM	-0.236577168	0.03461792

Note: cor, correlation coefficient.

Table 2
The expression level of CCBL1 is related to MSI in patients with pan-cancer.

Cancer	cor	P	Cancer	cor	P	Cancer	cor	P
ACC	0.331783493	0.002816794	KIRC	-0.071313799	0.192903946	PRAD	-0.14023156	0.001762789
BLCA	0.052697193	0.288279558	KIRP	-0.043347334	0.466054376	READ	0.196212708	0.015405561
BRCA	-0.080131522	0.010053698	LAML	0.102531803	0.405397976	SARC	0.151246453	0.01605443
CESC	0.128669674	0.025347158	LGG	-0.067684434	0.127628875	SKCM	-0.006347044	0.891076845
CHOL	0.107850708	0.529849386	LIHC	-0.00963497	0.853654122	STAD	0.115846826	0.025063683
COAD	-0.205064265	1.95E-05	LUAD	0.057166114	0.196999196	TGCT	-0.199563168	0.014350286
DLBC	-0.677595334	1.23E-07	LUSC	-0.028070043	0.534075678	THCA	-0.094575424	0.036170301
ESCA	0.024476121	0.75867615	MESO	0.210823638	0.057274624	THYM	-0.033674092	0.717349781
GBM	0.079716687	0.330561054	OV	0.099127277	0.102818564	UCEC	0.102287651	0.017632145
HNSC	-0.106990995	0.017142546	PAAD	-0.033739987	0.657573586	UCS	0.095697051	0.482933666
KICH	0.105036553	0.405009768	PCPG	0.043598971	0.563358916	UVM	0.179750916	0.110608901

Note: cor, correlation coefficient.

The expression level of CBLL1 mRNA is related to the microenvironment of pan-cancer

The expression level of CBLL1 mRNA was correlated with tumor microenvironment (Fig. 5, Fig. S20 and Table 3). Figure 5 shows the correlation between CBLL1 mRNA expression level and tumor stromal cells in the top 9 P values. In detail, the expression level of CBLL1 was correlated with tumor stromal cells such as TGCT, LGG, SARC, GBM, LUSC, BLCA, BRCA, THCA, PCPG and so on (Fig. 5 and Table 3). Figure S20 shows the correlation between CBLL1 mRNA expression level and tumor immune cells in the top 9 P values. In detail, the expression level of CBLL1 was correlated with tumor immune cells such as BRCA, LGG, THCA, GBM, LUSC, SARC, UCEC, PCPG, LIHC and so on (Fig. S20 and Table 3).

Table 3
The expression level of CBLL1 mRNA is related to the immune infiltration of pan-cancerous tumors.

Cancer type	StromalScore	ImmuneScore
ACC	0.124603384	0.010137825
BLCA	3.84E-05	0.001271417
BRCA	3.89E-05	9.56E-11
CESC	0.393921421	0.002109213
CHOL	0.614788213	0.40869677
COAD	0.963871374	0.001587956
DLBC	0.234894177	0.952807531
ESCA	0.627477696	0.515859015
GBM	3.19E-08	1.60E-07
HNSC	0.749378092	0.262083978
KICH	0.248132174	0.134449688
KIRC	0.083590093	0.001019052
KIRP	0.512345762	0.002132654
LAML	0.664580379	0.027041677
LGG	2.10E-11	5.00E-09
LIHC	0.137828166	8.44E-06
LUAD	0.187731048	0.500395871
LUSC	3.08E-05	0.00021417
MESO	0.03062979	0.055479795
OV	0.024490708	0.004501272
PAAD	0.516894301	0.99423637
PCPG	0.000883697	5.41E-06
PRAD	0.62106203	0.021780091
READ	0.320999231	0.089231316
SARC	3.89E-11	6.05E-07
SKCM	0.048128866	0.001705958
STAD	0.016515899	0.074448303
TGCT	0	0.002831903
THCA	0.000341586	3.17E-08
THYM	0.000961122	0.16042945
UCEC	0.202244891	8.63E-07
UCS	0.716896263	0.208104708
UVM	0.524138205	0.511113834

The expression level of CBLL1 is related to the immune cells of pan-cancerous tumors

Figure 6 and figure S21 show the correlation between the expression level of CBLL1 mRNA and the tumor immune infiltrating cells with the lowest P value. The expression level of CBLL1 is correlated with BLCA (T cells follicular helper), BRCA (Tcells CD4 memory resting), COAD (B cells memory), GBM (T cells gamma delta), HNSC (B cell snaive), KICH (T cells CD4 memory resting), KIRC (T cells regulatory,Tregs), LAML (Monocytes), LIHC (T cells CD8), LUAD (T cells CD4 memory activated), LUSC (Neutrophils), OV (T cells CD8.), PRAD (T cells CD4 memory resting), READ (Macrophages M1), SARC (Macrophages M2), SKCM (Tregs), STAD (Tregs), TGCT (B cells naive), THCA (Dendritic cells activated), THYM (Macrophages M0), UCEC (T cells CD4 memory resting), UVM (Monocytes) and KIPR (T cells CD8)(Fig. 6 and Fig. S21; Supplementary table S1). In addition, the expression level of CBLL1 was associated with various immune infiltrating cells in pan-cancer. For example, the expression level of CBLL1 was significantly correlated with immune cells such as BRCA B cells naive, B cells memory, T cells CD8, T cells CD4 memory activated, Tregs; HNSC B cells naive, B cells memory, T cells CD4 memory resting, NK cells activated; PRAD B cells naive, B cells memory, Plasma cells, T cells CD8, T cells CD4 memory resting ($P < 0.05$, Supplementary table S1).

The expression level of CBLL1 is related to immunomodulators, checkpoints and receptor molecules in pan-cancerous tumors

To further explore the relationship between CBLL1 mRNA expression and tumor immune markers, in order to understand the role of CBLL1 in pan-cancer immune escape. immunomodulators include immunostimulators, immunoinhibitors and MHC molecules. We found that the expression level of CBLL1 was associated with pan-cancer immunomodulators (Fig. 7A-C). For example, the expression level of CBLL1 was correlated with BLCA immunostimulators CD160, ADORA2A, TGFBR1, IL10RB and BTLA, with BRCA immunostimulators LGALS9, IL10RB, CD160, KDR, TGFBR1, etc., and with CESC immunostimulators KDR, ADORA2A, LAG3, LGALS9 and IL10RB ($P < 0.05$, Fig. 7A). The expression level of CBLL1 was correlated with BLCA immunoinhibitors TNFRSF4, TNFRSF14, CD276TNFRSF18, TNFSF9, TNFRSF25, BRCA immunoinhibitors TNFRSF4, TNFRSF14, ULBP1, MICB, TNFSF18, and CESC immunoinhibitors TNFSF9, TNFSF18, NT5ETNFRSF4, TNFSF13 ($P < 0.05$, Fig. 7B). The expression level of CBLL1 was significantly correlated with BLCA MHC molecules HLA-A, HLA-E, HLA-F, TAPBP and HLA-DPB1, BRCA MHC molecules HLA-A, HLA-F, HLA-EHLA-C, HLA-G, HLA-B, and CESC MHC molecules HLA-F, HLA-C, HLA-B, HLA-A, TAPBP ($P < 0.05$, Fig. 7C). In addition, the expression level of CBLL1 was associated with pancancerous immune checkpoint molecules (Fig. 7D). For example, the expression level of CBLL1 was correlated with BLCA checkpoint molecules CCL26, CCL14, CCL23, CCL21, CCL28; BRCA checkpoint molecules CCL26, CCL3, CCL17, CCL23, CX3CL1, CXCL2; CESC checkpoint molecules CXCL2, CXCL3, CCL3, CXCL16, CCL13 ($P < 0.05$, Fig. 7D). In addition, the expression level of CBLL1 was associated with receptor molecules (Fig. S22 and Table 4). For example, the expression level of CBLL1 is related to BLCA receptor molecules CCR9, CCR8, CCR4, CCR7 and CXCR5; BRCA receptor molecules CCR10, CCR8, CCR9, CCR4, CXCR3, etc; CESC molecules CCR8, XCR1, CCR2, CCR6, CCR4, etc (Table 4).

Table 4
The expression level of CBLL1 is related to tumor immune receptor molecules.

Receptor	BLCA	BRCA	CESC
CCR1	0.937418488	0.298791645	0.054925253
CCR2	0.606569164	0.002536219	0.009550921
CCR3	0.12648539	0.648938001	0.123018037
CCR4	0.010496864	0.001147277	0.014677045
CCR5	0.819332104	0.243023525	0.406713675
CCR6	0.053292813	0.003573498	0.010188994
CCR7	0.033661703	0.421512886	0.894509139
CCR8	0.000136705	3.35E-07	4.25E-05
CCR9	4.94E-06	0.000473587	0.866908606
CCR10	0.102926437	2.24E-21	0.149478695
CXCR1	0.219670706	0.923072873	0.021085763
CXCR2	0.129164084	0.114858105	0.514219098
CXCR3	0.510329501	0.00186531	0.77737012
CXCR5	0.044729072	0.576671819	0.018422254
CXCR6	0.132770807	0.25368142	0.954407026
XCR1	0.677307888	0.21604114	0.001885185
CX3CR1	0.233819871	0.002982067	0.507290776

The possible mechanism of the involvement of CBLL1 in the progression of pan-cancer

The expression of CBLL1 mRNA is related to the prognosis and immunity of patients with pan-cancer. Therefore, we use GSEA to analyze the possible mechanism of CBLL1 involved in tumor progression. The results showed that CBLL1 was involved in the development of pan-cancer through a variety of signal pathways (Fig. 8, Fig. S23 and Supplementary table S2-S34). For example, CBLL1 may participate in ACC progress through B cell receptor singaling pathway, mRNA cis splicing via spliceosome, immunoglobulin receptor binding, olfactory receptor activity, membrane disruption in other organism, tRNA threonylcarbamoyladenosine metabolic process, etc. (Fig. 8A and Supplementary table S2). CBLL1 may participate in the progress of BLCA through gene singaling, mRNA binding, regulation of cellualr amide metabolic process, endothelial cell migration, regulation of epithelial cell migration, etc. (Fig. 8B and Supplementary table S3). CBLL1 may participate in the progress of BRCA through positive regulation of cell cycle phase transition, RNA binding involve dinposttranscriptional gene silencing, positive regulation of cell cycle G1-S phase transition, positive regulation of G1-S transition of mitotic cell cycle, trophoblast cell migration, T cell receptor complex, etc (Fig. 8C and Supplementary table S4). CBLL1 may participate in the progress of CHOL through methyl CPG binding, methylation dependent chromatin silencing, CIS trans isomerase activity, T cell receptor complex, positivere gulation of translational initiation, mRNA binding, etc (Fig. 8D and Supplementary table S5). CBLL1 may participate in the progress of DLBC through RNA binding involved in posttranscriptional gene silencing, negative regulation of tumor necrosis factor mediated signaling pathway, positive regulation of lipoprotein particle clearance, gene silencing, mRNA binding, positive regulation of cell aging, regulation of microglial cell activation, etc (Fig. 8F and Supplementary table S6). These results suggest that m6A related gene CBLL1 is strongly related to cancer.

Discussion

M6A-related genes are closely associated to cancer [3–6,21,22]. For example, m6A methyltransferase METTL14 is significantly up-regulated in breast cancer (BC) tissues compared with normal tissues. The expression of m6A was up-regulated or down-regulated by METTL14 in MCF-7 and MDA-MB-231 cells. The overexpression of METTL14 enhanced the migration and invasion ability of BC cells.

M6A inhibitor therapy inhibited this migration and invasion [21]. WTAP is highly expressed in (GC) tissues of gastric cancer, and the patients with increased expression of WTAP suggest a poor prognosis, and WTAP is an independent risk factor for the prognosis of patients with GC [22]. However, there are few reports on the value of CBLL1 in cancer. In this study, we found that the level of CBLL1 methylation was abnormally expressed in a variety of cancers in the SMART database. Abnormal methylation rate often leads to changes in the level of its gene, which in turn affects the prognosis of tumor patients [23]. Therefore, we analyzed the transcriptome data, clinical and mutation data of TCGA database and found that CBLL1 was highly expressed in CHOL, COAD, ESCA and other cancer tissues, while low expression in THCA and UCEC tissues. The expression level of CBLL1 is related to the age, race, clinical stage and therapeutic effect of patients with pan-cancer. In addition, the expression of CBLL1 was correlated with the prognosis of patients with KIRC, LUSC, THCA, THYM, MESO, PRAD, STAD and UVM. Univariate COX regression analysis found that the expression of CBLL1 mRNA was a prognostic risk factor in patients with KICH, KIRC, LAML, THYM, PCPG, OV, PRAD, STAD, GBM and UVM. This suggests that CBLL1 is related to the occurrence and progression of tumor and is expected to become a target molecule for cancer therapy.

Programmed death inhibitor-1 (PD-1) protein or its has achieved significant clinical efficacy in the treatment of a variety of tumors. TMB and MSI are used as biomarkers to evaluate the therapeutic effect of PD-1 antibody and microsatellite instability is also one of the tumor progression[24,25]. We found that the expression level of CBLL1 was correlated with TMB in patients with BLCA, BRCA, COAD, LAML, LGG, LUAD, LUSC, SARC, STAD, THCA, THYM and UVM, and with MSI in patients with ACC, BRCA, CESC, COAD, DLBC, HNSC, PRAD, READ, SARC, STAD, TGCT, THCA and UCEC, which indicates that CBLL1 may be a biomarker of treatment and prognosis in patients with pan-cancer.

Cancer is considered to be a disease of tumor microenvironment [26]. Tumor microenvironment is a complex and dynamic cell population, which is composed of tumor epithelial cells, tumor immune cells, fibroblasts, immunosuppressive cells, adipocytes, endothelial cells and so on. The interaction between tumor microenvironment and tumor cells is a key factor in immune escape, physiological tolerance and local and systemic invasiveness of malignant cells [27]. The expression level of CBLL1 was correlated with TGCT, LGG, SARC, GBM, LUSC, BLCA, BRCA, THCA, PCPG and other tumor stromal cells. The expression level of CBLL1 was correlated with tumor immune cells such as BRCA, LGG, THCA, GBM, LUSC, SARC, UCEC, PCPG, LIHC, LUSC and so on. Furthermore, The expression level of CBLL1 in BLCA (T cells follicular helper cells), BRCA (T cells CD4 memory resting), COAD (B cells memory), GBM (T cells gamma delta), HNSC (B cells naive), KICH (T cells CD4 memory resting), KIRC (Tregs), LAML (Monocytes), LIHC (T cells CD8), LUAD (T cells CD4 memory activated), LUSC (Neutrophils), OV (T cells CD8.), PRAD (T cells CD4 memory resting), READ (Macrophages M1), SARC (Macrophages M2), SKCM (Tregs), STAD (Tregs), TGCT (B cells naive), THCA (Dendritic cells activated), THYM (Macrophages M0), UCEC (T cells CD4 memory resting), There was a significant correlation between UVM (Monocytes) and KIPR (T cells CD8) and other immune cells. In addition, immunomodulators, checkpoints and receptors are associated with the progression and prognosis of cancer patients [28–30]. For example, cyclic actin promotes gastric cancer progression through sponge miRNA-331-3p and regulation of TGFBR1 mRNA expression [28]. The expression of TNFSF9 was down- regulated in liver cancer tissues and cells. Overexpression of TNFSF9 can inhibit the proliferation, migration and invasion of Huh7 and SMMC-7721 cells in vitro, and inhibit the growth and metastasis of HCC in vivo [29]. We found that the expression level of CBLL1 was associated with pan-cancer immunomodulators, checkpoints and receptor molecules. For example, CBLL1 expression level is significantly correlated with BRCA immunostimulators (LGALS9, IL10RB, CD160, KDR, TGFBR1, etc), immunoinhibitors (TNFRSF4, TNFRSF14, ULBP1, MICB, TNFSF18, etc), MHC molecules (HLA-A, HLA-F, HLA-EHLA-C, HLA-G, HLA-B, etc), checkpoint molecules (CCL26, CCL3, CCL17, CCL23, CX3CL1, CXCL2, etc) and receptor molecules (CCR10, CCR8, CCR9, CCR4, CXCR3, etc). It is suggested that CBLL1 can interact with tumor microenvironment and affect tumor immune escape, and then participate in tumor progression.

At present, CBLL1 is mainly involved in cancer progression by regulating EMT-related E-cadherin and N-cadherin pathways [13,14,31,32]. For example, interfering with CBLL1 gene expression can inhibit cell migration and invasion by increasing E-cadherin protein expression and reducing MMP2 and MMP9, and regulate cell sensitivity to cisplatin through AKT pathway. Overexpression of Hakai can enhance cell transformation and invasion, inhibit the expression of E-cadherin and promote the expression of N-cadherin [14]. Through GSEA, we found that CBLL1 can participate in tumor progression through mRNA binding, B cell receptor singaling pathway, Endothelial cell migration, Regulation of epithelial cell migration, Positive regulation of cell cycle phase transition, RNA binding involved in posttranscriptional gene silencing, Methyl CPG binding, Methylation dependent chromatin silencing, CIS trans isomerase activity, T cell receptor complex, Positive regulation of translational initiation, Negative regulation of tumor necrosis factor mediated signaling pathway, Positive regulation of lipoprotein particle clearance, gene silencing, mRNA binding, Positive regulation of

cell aging, Regulation of microglial cell activation and so on. These mechanisms, especially tumor-related mechanisms, are worthy of our in-depth study.

Conclusions

This study found that CBLL1 methylation and mRNA expression were abnormally expressed in pan-cancer, which is expected to be a marker of prognosis, mutation and tumor immune infiltration in cancer patients.

Abbreviations

m6A, N6-methyladenosine; CBLL1, Cbl proto-oncogene like 1; GSEA, Gene Set enrichment analysis; MSI, microsatellite instability; TMB, Tumor mutational burden; METTL3, methyltransferase-like 3; METTL4, methyltransferase-like 14; WTAP, WT1 associated protein; RBM15, RNA binding motif protein 15; KIAA1429, vir like m6A methyltransferase associated; ZC3H13, Zinc finger CCCH-type containing 13; ALKBH5, ALKB homologue 5; FTO, Fat weight and obesity related proteins; YTHDC1, YTH domain containing 1 ; YTHDC2, YTH domain containing 2; YTHDC3, YTH domain containing 3; PC, pancreatic cance; NPC, nasopharyngeal carcinoma; OS, Overall survival; HCC, hepatocellular carcinoma; CSC, cancer stem cell; UTR, untranslated region; NSCLC, non-small cell lung cancer; DSS, Disease-specific survival; DFI, Disease-free interval; PFI, Progression-free interval.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors agree to publish the manuscript

Availability of data and material

The datasets generated for this study are available on request to the corresponding author.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding

Not Applicable.

Authors' contributions

GJL and JYM designed the experiment and explained the data; GQ processed and analyzed the TCGA data; GQ and LD wrote the manuscript; GJL provided general guidance. The author read and approved the final manuscript.

Acknowledgements

Not applicable.

Declarations

All authors read and approved the final manuscript. The authors declare that there is no conflict of interest regarding the publication of this paper.

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Figures

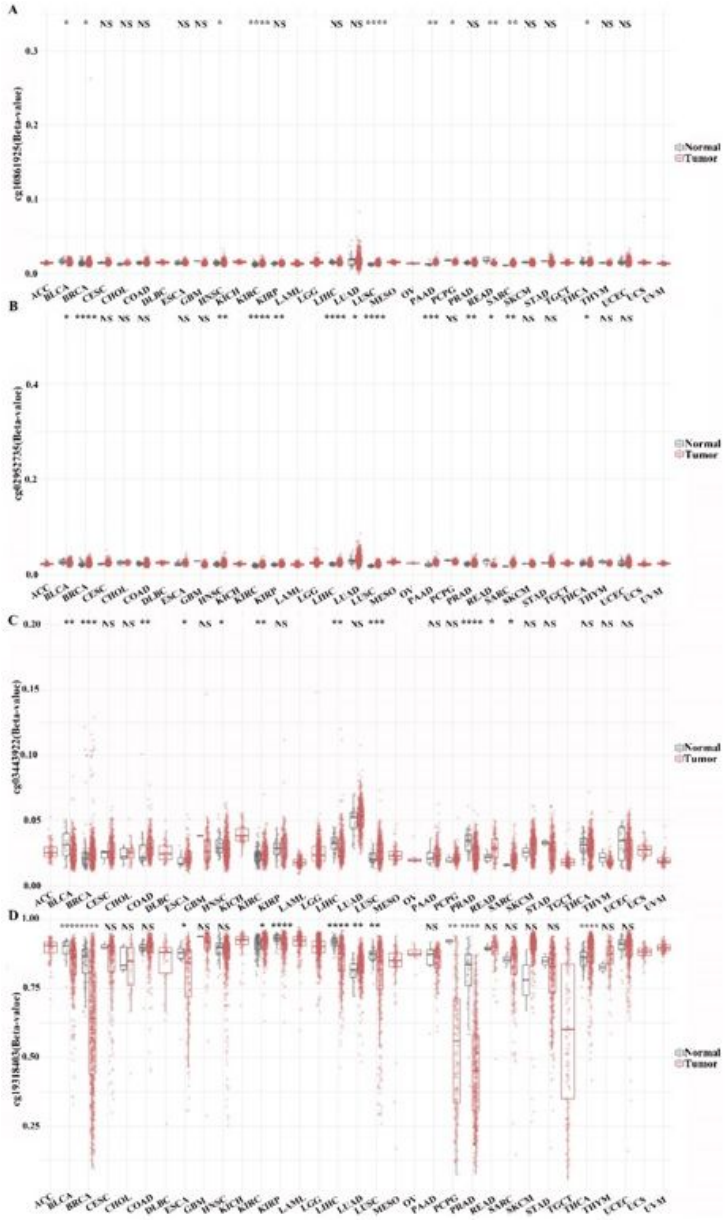

Figure 1

Abnormal expression of CBLL1 methylation in pan-cancerous tissues. (A)cg10861925; (B)cg02952735; (C)cg03443922; (D)cg19318403.

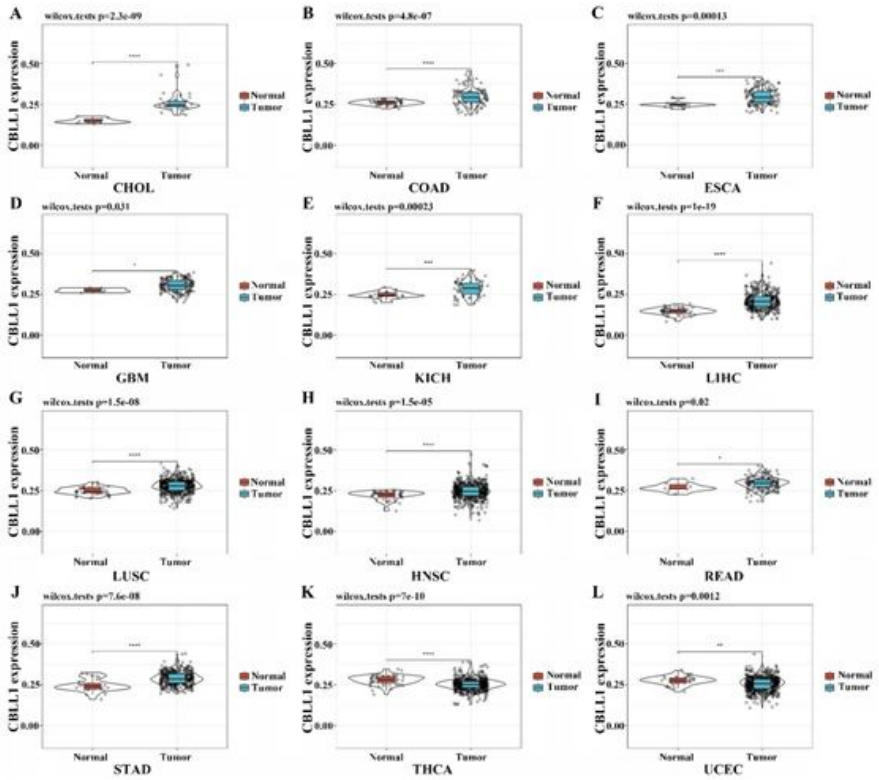

Figure 2

Abnormal expression of CBLL1 mRNA in pan-cancerous tissues. (A)CHOL; (B)COAD; (C)ESCA; (D)GBM; (E)KICH; (F)LIHC; (G)LUSC; (H)HNSC; (I)READ; (J)STAD; (K)THCA; (L)UCEC.

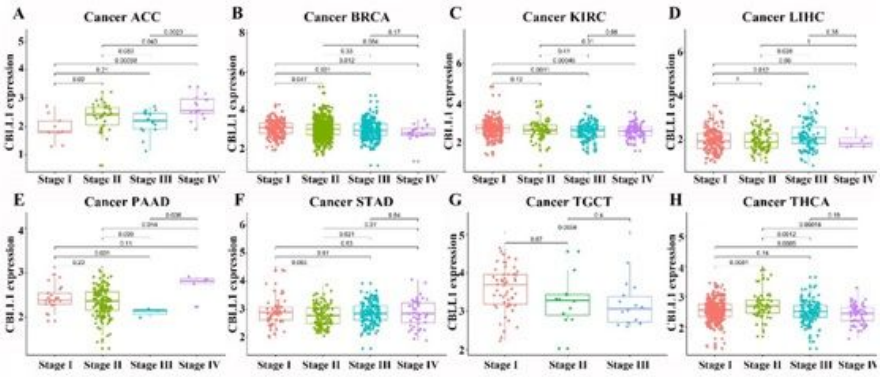

Figure 3

The expression of CBLL1 mRNA is related to the clinical stage of patients with pan-cancer. (A)ACC;(B)BRCA;(C)KIRC;(D)LIHC;(E)PAAD; (F)STAD;(G)TGCT;(H)THCA.

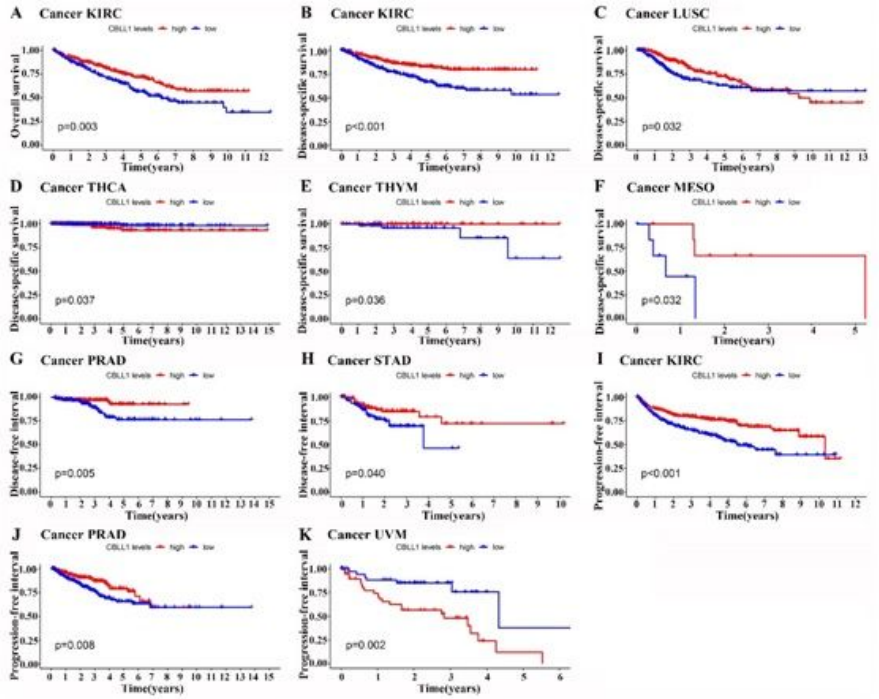

Figure 4

Survival analysis showed that the expression of CBLL1 mRNA was related to the prognosis of patients with pan-cancer. (A)KIRC OS; (B)KIRC DSS;(C)LUSC DSS;(D)THCA DSS;(E)THYM DSS;(F)MESO DFI;(G)PRAD DFI;(H)STAD DFI;(I)KIRC PFI;(J)PRAD PFI;(K)UVM PFI.

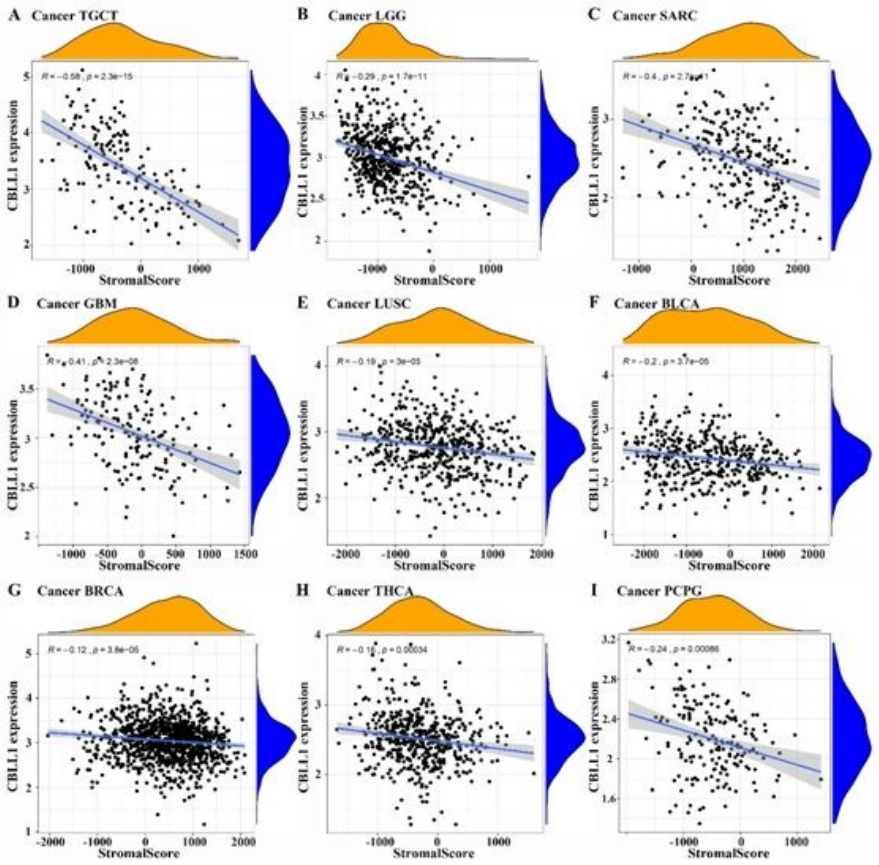

Figure 5

The expression level of CBLL1 mRNA is related to pan-cancerous tumor stromal cells. (A)TGCT;(B)LGG;(C)SARC;(D)GBM;(E)LUSC; (F)BLCA;(G)BRCA;(H)THCA;(I)PCPG.

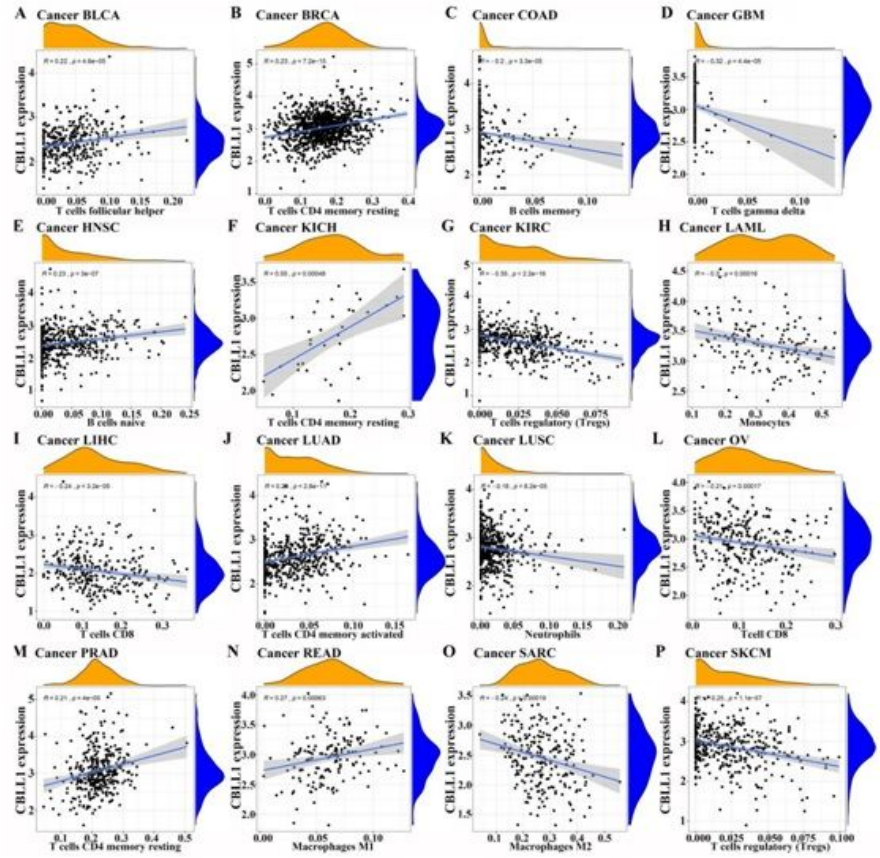

Figure 6

Correlation between CBLL1 mRNA expression level and pan-cancerous immune infiltrating cells. (A)BLCA; (B)BRCA; (C)COAD; (D) GBM; (E)HNSC; (F)KICH; (G)KIRC; (H)LAML; (I)LIHC; (J)LUAD; (K)LUSC; (L)OV; (M)PRAD; (N)READ; (O)SARC; (P)SKCM.

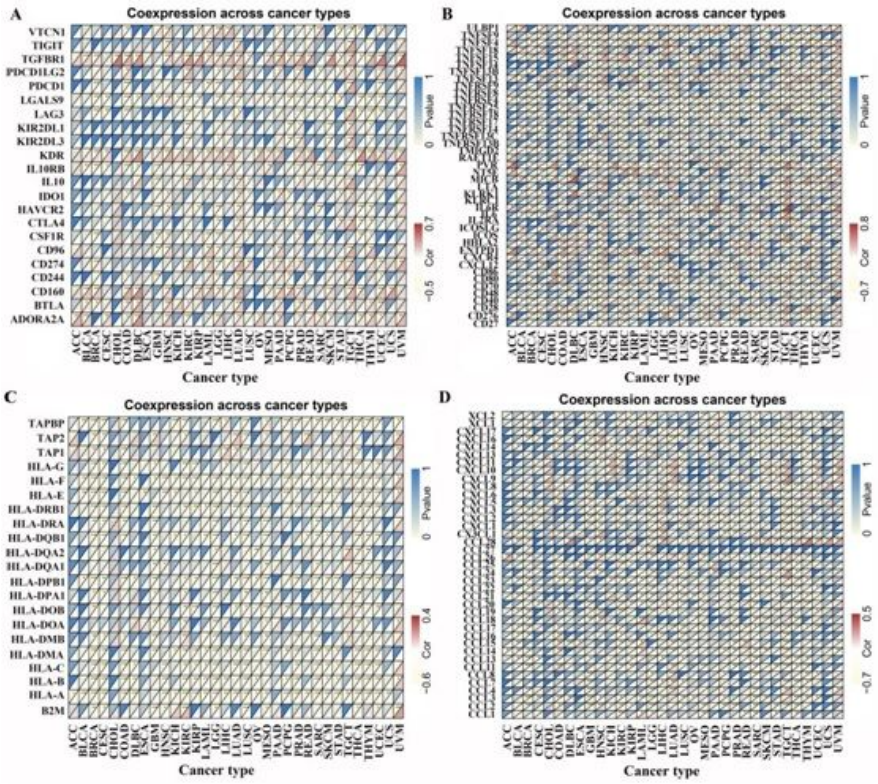

Figure 7

The expression level of CBLL1 is related to tumor immune markers. (A)immunostimulators; (B) immunoinhibitors; (C)MHC molecules; (D) checkpoints.

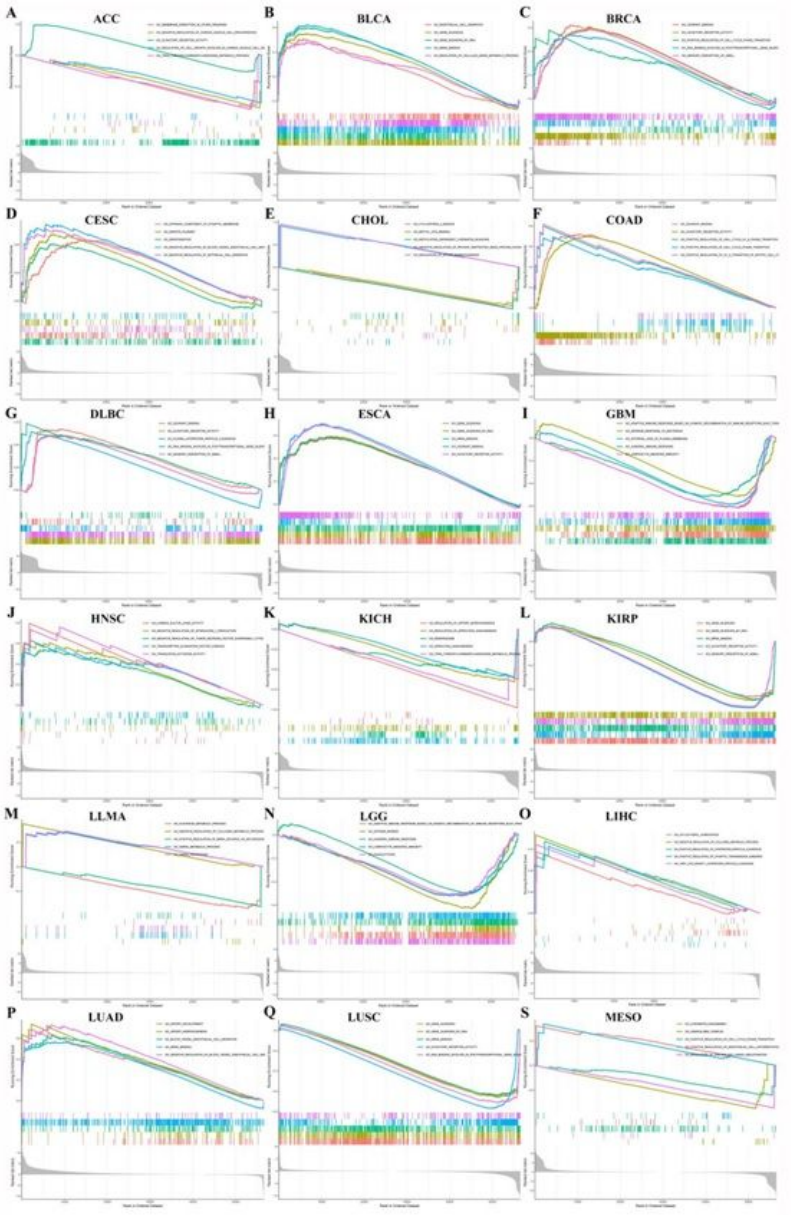

Figure 8

GSEA analysis of CBLL1 may be involved in the regulation of pan-cancer progression.

Supplementary Files

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