

# CILP2 overexpression predicts poor prognosis in colorectal cancer

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## Research

**Keywords:** CILP2; Colorectal cancer; TCGA; Immunohistochemistry; Prognosis

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# Abstract

**Background:** Cartilage Intermediate Layer Protein 2 (CILP2), a glycoprotein with mutations associated with abnormal blood lipid concentrations in normal and cardiovascular diseases patients, was barely reported with clinical features of tumors. We evaluated the role of CILP2 among all stages and histology in colorectal cancer (CRC) in the Cancer Genome Atlas (TCGA), and furtherly verified using immunohistochemistry assay within human CRC tissues. **Materials and methods :** Clinical information and RNA-seq data were derived from TCGA colorectal carcinoma cohort. CILP2 expression at mRNA level was estimated by bioinformatical analysis of TCGA cases. Tissue microarray (TMA) was constructed containing paraffin-embedded 64 pairs of CRC and matched adjacent normal tissues. The expression at protein level was detected in 64 pairs of CRC and matched adjacent normal tissues by immunohistochemical analysis. CILP2 expression level and its clinical value were estimated by bioinformatical analysis with linear and logistic regression. Survival analysis was performed between high and low groups of CILP2 expression by Cox regression analysis, and P value was calculated by log-rank test. Kaplan-Meier curves were tested by log-rank test. **Results :** CILP2 was significantly higher expressed in the colorectal cancer tissues when compared with paired adjacent normal tissues in TCGA cohort (  $P < 0.001$ ) and in TMA cohort (  $P = 0.001$ ). In addition, CILP2 high-expression was strongly correlated with T3/4 stage (  $P = 0.001$ ), N1/2/3 stage (  $P = 0.005$ ), M1 stage (  $P = 0.048$ ), and higher clinical stage (UICC 2010 stage) (  $P < 0.001$ ) in TCGA cohort, and also positively associated with T3/4 stage (  $P = 0.022$ ) and higher clinical stage (UICC 2010 stage) (  $P = 0.03$ ) in TMA cohort. Furthermore, CILP2 overexpression predicted poor prognosis and could be as an independent prognostic factor (  $P = 0.003$ ). **Conclusion :** We revealed that CILP2 is associated with advanced stages and could play a role as an independent predictor of poor survival in colorectal cancer. **Key words:** CILP2; Colorectal cancer; TCGA; Immunohistochemistry; Prognosis

## Introduction

Colorectal cancer (CRC) is one of the most common cancers that ranks second in cancer-associated mortality among the world, with increasing morbidity in recent years. It was estimated that in 2018, more than 1.8 million new colorectal cancer cases occurred with 881,000 deaths <sup>[1]</sup>. Colorectal cancer is caused by a variety of factors and is involved successive accumulation of genetic and epigenetic alternations <sup>[2]</sup>. Surgical resection is the mainstay for treatment of CRC patients, but tumor recurrence is common. A large cohort study indicated that the median survival time was 13.3 months before recurrence <sup>[3]</sup>. Numerous works have been done to reveal the underlying mechanisms of CRC, and encouraging progresses have been made <sup>[4-7]</sup>. However, further investigating works are still needed to deeply understand the molecular mechanisms, and molecular biomarkers for both early detection and prognosis are to be developed for better therapeutic uses in CRC patients.

Cartilage Intermediate Layer Protein 2 (CILP2) is a secreted glycoprotein that has been first isolated from human articular cartilage. The *CILP* gene located on chromosome 19p13. It was reported that through genome-wide association studies (GWAS), the *Neurocan-cartilage intermediate layer protein 2- pre-B-cell*

*leukemia homeobox 4 (NCAN-CILP2-PBX4)* region, an intergenic region spanning 300 kb, is associated with concentrations of low-density lipoprotein cholesterol and triglycerides in sera [8]. Eleven genes and one miRNA are encoded in this region. This region has been shown consistent and deep association with serum lipid levels in subsequent studies for individuals of European and Chinese descents [9–11]. In addition to plasma lipid levels, the genome region around *CILP2* was identified as a non-alcoholic fatty liver disease (NAFLD)-associated locus by GWAS in individuals of European descent [12], but not in Japanese individuals [13]. A significant association was also highlighted between polymorphisms in the *CILP* gene and osteoarthritis progression [14]. But to our best knowledge, few reports have described the relationship between *CILP2* and cancers, except one that reported an expression quantitative trait locus, namely rs8103992, was significantly associated with osteosarcoma risk [15].

Tumors have been considered as high demands of energy and abnormal anabolism for their rapid growth, in which lipid metabolism plays a key role in tumorigenesis. But subtle mechanisms underlying over-reacted lipid metabolism remain poorly understood. *CILP2* was barely reported with clinical features of tumors. In this study, we evaluated *CILP2* expression and its correlations with clinicopathological characteristics, such as tumor stages, and overall survival of CRC patients in the Cancer Genome Atlas (TCGA), and furtherly verified using immunohistochemistry assay within human CRC tissues, which may provide a new potential molecular marker for prognostic use of CRC patients.

## Materials And Methods

### TCGA Data mining and gene expression datasets.

The CRC cohort in TCGA was downloaded and level 3 RNA-seq V2 datasets was used, which was based on Illumina HiSeq 2000. Matched clinical data from CRC patients were also downloaded (<https://portal.gdc.cancer.gov/>). In the cohort, 621 CRC patients were included, and 609 among them had intact survival data recorded. So, 609 patients were included in the survival analysis in the study. For each gene, the transcript with highest expression was selected for the following process. Meanwhile, the data of one gene was considered invalid when raw counts of the gene in all samples were less than 50. All filtered genes expressions had been processed and been normalized by Trimmed Mean of M-values analysis.

### Tissue microarray construction.

Sixty-four pairs of CRC and matched adjacent normal tissues were obtained from patients undergoing CRC surgery between January 2016 and October 2019 at the Department of Gastrointestinal Tumor Surgery, Fujian Cancer Hospital. Patients received chemotherapy or radiotherapy before surgery were excluded. Tissue microarray recipient block was constructed containing paraffin-embedded 64 pairs of CRC and matched adjacent normal tissues previously fixed in 10% formaldehyde. The most representative tumor or normal areas were carefully selected and marked based on the matched haematoxylin-eosin-stained slides. Altogether, 128 cores (diameter 1.8 mm) of test tissue were taken from

the donor blocks with the tissue microarrayer (Beecher Instruments, Silver Spring, MD, USA) and inserted into the recipient block.

### **Immunohistochemistry analysis.**

Unstained 4 mm sections were cut from the tissue microarray recipient block and deparaffinized in xylene, and the slides were bathed in 0.01 mol/l sodium citrate and heated in a microwave oven for 12 min. The sections were incubated with anti-CILP2 antibody (Santa Cruz, CA, USA) and kept at 4°C overnight. Negative control slides were treated with only non-immunized mouse immunoglobulin fraction under equivalent conditions. For the secondary developing reagents, a labeled streptavidin-biotin kit (Dako, CA, USA) was used. Slides were developed with diaminobenzaminidine and counterstained with hematoxylin.

### **Evaluation of immunostaining results**

Immunohistochemistry staining was scored blindly by two independent pathologists without knowledge of the patient's clinicopathology and clinical outcome. According to the antibody specification, cytoplasm and nucleus staining with red/brown color in epithelial cells were positive. The expression level of CILP2 in each sample was evaluated based on the proportion of positively stained tumour cells for CILP2 and the intensity of the staining. The immunoscores ranged between 0 and 3 as follows: I) 0, no recognizable staining, referred to as negative (-); II) 1, slight staining, referred to as weak positive (+); III) 2, moderate staining, referred to as moderate positive (++); and IV) 3, distinct staining, referred to as strong positive (+++). Positive expression of CILP2 protein was defined as moderate positive staining (++) and strong positive staining (+++) for CILP2, whereas Negative expression of CILP2 protein was defined as negative (0) and weak positive (+) staining.

### **Statistical Analysis.**

Statistical analyses were performed using spss 22.0 software. CILP2 gene expression in different groups (divided by each parameter) was compared using Mann-Whitney U test. Correlation between CILP2 gene expression and different TNM stages were analyzed by Spearman's test, and Spearman rank correlation coefficient ( $r_s$ ) was used to evaluate the strength of association. CILP2 gene expression in different groups was analyzed by one-way ANOVA followed by Welch's t-test. CILP2 protein expression in different groups was analyzed by Fisher's exact test. Survival analysis was performed between high and low groups of CILP2 expression (defined by median value of CILP2 expression) by Cox regression analysis, and *P* value was calculated by log-rank test. Kaplan-Meier curves were tested by log-rank test.

## **Results**

### **CILP2 was overexpressed in Colorectal cancer.**

Aiming at searching for potential novel prognostic markers of CRC, we firstly analyze expression data of TCGA CRC cohort from Illumina HiSeq 2000 platform, which contains 621 samples and correlating clinical and demographic information. We found that CILP2 was strongly correlated with clinical features

of CRC samples in TCGA cohort, and CILP2 has not been reported in CRC before. So we focused on CILP2 and furtherly analyzed the association between CILP2 expression and CRC prognosis. To determine the role of CILP2 in colorectal cancer, we first analyzed CILP2 gene expression in 50 patients samples with paired adjacent normal tissues in TCGA cohort, and the results suggested that CILP2 gene was overexpressed significantly in tumor samples compared to paired adjacent normal tissues (Fig. 1A-1B, Fold change = 3.412,  $P < 0.001$ , Table 1). Additionally, CILP2 gene expression was upregulated in total amount of colorectal cancer samples compared with adjacent normal tissue samples in TCGA cohort (Fig. 1C, Fold change = 8.6161,  $P < 0.001$ , Table 2), indicating that CILP2 might be an potential biomarker.

Table 1

CILP2 gene expression in 50 paired tumor and matched adjacent normal tissue samples in TCGA cohort

ID	Gene symbol	FC	<i>P</i> -value	Total sample	Sample unchanged	Sample up	Sample down
148113	CILP2	3.412	2.56E-07	50	15	31	4

Table 2

CILP2 gene expression in all normal and cancer samples in TCGA cohort

ID	Gene symbol	FC	<i>P</i> -value
148113	CILP2	8.6161	1.28E-19

To further testify upregulation of CILP2 in CRC, we detected CILP2 protein expression in a Tissue microarray (TMA) ( $n = 64$ ) by immunohistochemical staining (IHC). We found that 68.75% (44/64) tumor tissues positively expressed CILP2 protein, whereas only 39.06% (25/64) of matched adjacent normal tissues positively expressed CILP2 protein. The staining result showed that CILP2 protein expression was significantly more prevalent in tumors than in matched adjacent normal tissues (Fig. 1D,  $P = 0.001$ ). The representative images of CILP2 immunostaining were shown in Fig. 1E-1F.

### Correlations between CILP2 expression and clinicopathological parameters in Colorectal cancer.

Furthermore, to dissect the role of CILP2 in CRC carcinogenesis, correlations between CILP2 expression and clinicopathological parameters were analyzed based on TCGA cohort (mRNA) and TMA cohort (protein), presenting in Table 3. And Table 4 showed correlation analysis results of TCGA cohort using Spearman's test. Among 621 samples from TCGA cohort, part of clinicopathological data were missed in some cases. Median expression of CILP2 of all CRC samples was chosen as a cutoff to divide CRC samples into CILP2-high ( $n = 310$ ) group and CILP2-low ( $n = 311$ ) group. We observed that in TCGA cohort tumors of high CILP2 expression were positively associated with T3/4 stage (T1/2, 36.51%; T3/4, 53.35%;  $P = 0.001$ , Table 3), and with N1/2/3 stage (N0, 44.89%; N1/2/3, 74.29%;  $P = 0.005$ , Table 3). Similarly, the percentage of tumors with high CILP2 expression increased with grading of clinical stage (UICC 2010

stage) (stage I, 34.29%; stage II, 49.35%; stage III, 54.19%; stage IV, 60.00%,  $P < 0.001$ , Table 3), and distant metastasis (M0, 48.69%; M1, 60.23%,  $P = 0.048$ , Table 3). It was suggested that CILP2 gene expression was strongly correlated with T stage ( $P = 0.001$ ), N stage ( $P = 0.005$ ), M stage ( $P = 0.048$ ), and higher clinical stage ( $P < 0.001$ ), respectively in TCGA cohort (Fig. 2). However, there was no significant correlation of CILP2 expression with patients' age or gender ( $P > 0.05$ , Table 3). In TMA cohort, tumors of positive CILP2 protein expression was significantly associated with T3/4 stage (T1/2 44.83%; T3/4, 74.29%;  $P = 0.022$ , Table 3), and higher clinical stage (UICC 2010 stage) (stage I, 35.71%; stage II, 50.00%; stage III, 70.83%; stage IV, 90.00%,  $P = 0.03$ , Table 3). However, there was no significant correlation of CILP2 protein expression with N stage ( $P = 0.2$ , Table 3), it may be due to limited set of data. Although the significant correlation was also not reached, there was a tendency that tumors with positive CILP2 protein expression were more likely to distantly metastasize compared with CILP2-negative tumors ( $P = 0.074$ , Table 3).

Table 3

Association between CILP2 expression level and clinicopathological parameters in CRC patients

Clinicopathological parameters	Expression of CILP2 mRNA in TGCA		Expression of CILP2 protein in TMA cohort			
	High (n = 310)	Low (n = 311)	<i>P</i> value	Positive(n = 39)	Negative(n = 25)	<i>P</i> value
Age (years)			0.059			0.37
≤68 (n = 331)	177 (53.47%)	154 (46.53%)		20(66.67%)	10(33.33%)	
≥68 (n = 290)	133 (45.86%)	157 (54.14%)		19(55.88%)	15(44.12%)	
Gender			0.400			0.8
Male (n = 331)	160 (48.34%)	171 (51.66%)		22(62.86%)	13(37.14%)	
Female (n = 290)	150 (51.72%)	140 (48.28%)		17(58.62%)	12(41.38%)	
Pathological T stage <sup>a</sup>			<b>0.001</b>			<b>0.022</b>
T1/2 (n = 126)	46 (36.51%)	80 (63.49%)		13(44.83%)	16(55.17%)	
T3/4 (n = 493)	263 (53.35%)	230 (46.65%)		26(74.29%)	9(25.71%)	
N stage <sup>a</sup>			<b>0.005</b>			0.2
N0 (n = 352)	158 (44.89%)	194 (55.11%)		16(51.61%)	15(48.39%)	
N1/2/3 (n = 265)	149 (56.23%)	116 (43.77%)		23(69.70%)	10(30.30%)	
M stage <sup>a</sup>			<b>0.048</b>			0.074
M0 (n = 458)	223 (48.69%)	235 (51.31%)		30(55.56%)	24(44.44%)	
M1 (n = 88)	53 (60.23%)	35 (39.77%)		9(90.00%)	1(10.00%)	
Clinical stage <sup>a</sup>			<b>&lt; 0.001</b>			<b>0.03</b>
Stage I (n = 105)	36 (34.29%)	69 (65.71%)		5(35.71%)	9(64.29%)	

Clinicopathological parameters	Expression of CILP2 mRNA in TGCA			Expression of CILP2 protein in TMA cohort		
	High (n = 310)	Low (n = 311)	<i>P</i> value	Positive(n = 39)	Negative(n = 25)	<i>P</i> value
Stage II (n = 229)	113 (49.35%)	116 (50.65%)		8(50.00%)	8(50.00%)	
Stage III (n = 179)	97 (54.19%)	82 (45.81%)		17(70.83%)	7(29.17%)	
Stage IV (n = 90)	54 (60.00%)	36 (40.00%)		9(90.00%)	1(10.00%)	
<p><sup>a</sup>:some missing data for parameter.<i>P</i> value &lt; 0.05 was considered statistically significant (in bold).  T: tumor; N: Regional lymph node; M: metastasis.</p>						

Table 4

Correlation between CILP2 expression and TNM stages in CRC patients in TCGA cohort

		<b>CILP2 Expression</b>	<b>T Stage</b>	<b>N Stage</b>	<b>M Stage</b>	<b>Clinical stage</b>
CILP2 Expression	$r_s^2$	1.000	0.136	0.112	0.085	0.149
	$P$ (Two-tailed)	N.A	<b>0.001</b>	<b>0.005</b>	<b>0.048</b>	<b>&lt; 0.001</b>
T Stage	$r_s^2$	0.136	1.000	0.308	0.197	0.589
	$P$ (Two-tailed)	<b>0.001</b>	N.A	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
N Stage	$r_s^2$	0.112	0.308	1.000	0.418	0.846
	$P$ (Two-tailed)	<b>0.005</b>	<b>&lt; 0.001</b>	N.A	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
M Stage	$r_s^2$	0.085	0.197	0.418	1.000	0.665
	$P$ (Two-tailed)	<b>0.048</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	N.A	<b>&lt; 0.001</b>
Clinical stage	$r_s^2$	0.149	0.589	0.846	0.665	1.000
	$P$ (Two-tailed)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	N.A
$P$ value < 0.05 was considered statistically significant (in bold).						
T: tumor; N: Regional lymph node; M: metastasis.						

### High CILP2 expression is associated with poor outcome of colorectal cancer patients.

Kaplan-Meier analysis was performed to investigate relationship between CILP2 expression and overall survival (OS) in TCGA cohort. There were 609 CRC samples available of prognostic information. Median expression of CILP2 of all CRC samples was chosen as a cutoff to divide CRC samples into CILP2-high (n = 305) group and CILP2-low (n = 304) group. As shown in Fig. 3, Table 5, CRC patients with high CILP2 expression exhibited a poorer OS rate compared with the lowexpression group ( $P = 0.003$ ). Moreover, the univariate Cox regression analysis indicated that high CILP2 expression was strongly associated with a poor prognosis ( $P = 0.003$ ). Other clinical variables, such as age ( $P < 0.0001$ ), T stage ( $P = 0.005$ ), N stage ( $P < 0.0001$ ), M stage ( $P < 0.0001$ ), and clinical stage (UICC 2010 stage) ( $P < 0.0001$ ) were all associated with OS (Table 6). Moreover, the multivariate analysis revealed that high CILP2 expression ( $P = 0.034$ ), age ( $P < 0.0001$ ), M stage ( $P < 0.0001$ ) and clinical stage (UICC 2010 stage) ( $P = 0.017$ ) were

independently associated with a poor prognosis (Table 6). These results suggested that CILP2 could be used as an independent prognostic predictor for colorectal cancer patients in the dataset.

Table 5  
Survival analysis was performed by Kaplan-Meier method

Variables		N	Means for Survival time (Month)	Survival time (Month, 95% CI)		P value
CILP2 Expression	Low	304	83.770	74.181	93.359	<b>0.003</b>
	High	305	70.805	61.802	79.807	
	Total	609	77.193	70.583	83.803	
Gender	Male	329	74.017	64.871	83.163	0.941
	Female	280	80.242	70.918	89.566	
	Total	609	77.193	70.583	83.803	
Age	≤ 68	324	86.777	77.219	96.334	<b>&lt; 0.0001</b>
	> 68	285	68.173	59.326	77.021	
	Total	609	77.193	70.583	83.803	
T stage	T1/2	126	97.409	81.150	113.667	<b>0.003</b>
	T3/4	481	74.489	67.519	81.460	
	Total	607	77.572	70.950	84.194	
N stage	N0	348	88.348	79.810	96.887	<b>&lt; 0.0001</b>
	N1/2/3	257	63.017	53.234	72.799	
	Total	605	77.530	70.908	84.152	
M stage	M0	450	87.130	79.689	94.571	<b>&lt; 0.0001</b>
	M1	86	37.131	29.468	44.794	
	Total	536	78.778	71.809	85.747	
Clinical stage	StageI/II	330	89.089	80.335	97.843	<b>&lt; 0.0001</b>
	StageIII/IV	262	64.632	54.851	74.413	
	Total	592	78.325	71.646	85.005	
<i>P</i> value < 0.05 was considered statistically significant (in bold).						
T: tumor; N: Regional lymph node; M: metastasis.						

Table 6

Survival analysis was performed by univariate and multivariate Cox regression analysis

Variables	Univariate analysis			Multivariate analysis		
	P-value	HR	95%CI	P-value	HR	95%CI
CILP2 Expression (High vs. Low)	0.003	1.713	1.194–2.457	<b>0.034</b>	1.547	1.033–2.317
Gender (Male vs. Female)	0.941	0.987	0.694–1.404	0.132	1.351	0.913–1.999
Age (> 68 vs. ≤68)	<b>&lt; 0.0001</b>	1.923	1.337–2.767	<b>&lt; 0.0001</b>	2.626	1.743–3.957
T stage (T3/4 vs. T1/2)	<b>0.005</b>	2.457	1.320–4.572	0.143	1.817	0.816–4.044
N stage (N1/2/3 vs. N0)	<b>&lt; 0.0001</b>	2.734	1.897–3.942	0.264	0.589	0.232–1.491
M stage (M1 vs. M0)	<b>&lt; 0.0001</b>	4.126	2.776–6.133	<b>&lt; 0.0001</b>	2.854	1.769–4.604
Clinical stage (III/IV vs. I/II)	<b>&lt; 0.0001</b>	3.001	2.046–4.401	<b>0.017</b>	3.408	1.244–9.337
P value < 0.05 was considered statistically significant (in bold).						
T: tumor; N: Regional lymph node; M: metastasis; HR, hazard ratio; CI, confidence interval						

## Discussion

The incidence of colorectal cancer has risen sharply in recent years<sup>[1]</sup>, with limited diagnostic and prognostic tools for early detection and patients' survival prediction. There are many researches focusing on the issue, and numerous advances have been achieved to reveal the underlying mechanisms of cancer development<sup>[4–7]</sup>. For example, lots of studies have shown that microsatellite instability (MSI) in genome could act as an exclusive prognostic marker in the early stages of CRC<sup>[4, 16]</sup>. Another useful tool, Septin9 hypermethylation detection in blood samples has received researchers' attention and was the first-approved serum test for CRC screening by FDA. But further estimation on Septin9 serum assay for CRC screening turned out that it was weakly recommended because of low sensitivity for cancer, and inability to detect advanced adenomas<sup>[17]</sup>. Extensive works are still needed to provide new insights into the tumor.

CILP2 (Cartilage Intermediate Layer Protein 2) protein is a noncollagenous protein in human articular cartilage. In last few years, correlations between CILP2 and plasma lipid concentration in different populations have been studied in some GWAS researches. According to Kathiresan et al.<sup>[8]</sup>, in Caucasian individuals analyzed, rs16996148 variant of *CILP2* gene had a reducing role in triglyceride and LDL-C level. While in other reports, the relationship between *CILP2* polymorphism and lipid metabolism was not

yet discovered <sup>[18]</sup>, nor in Japanese population <sup>[19]</sup> or in Slovak Midlife women <sup>[20]</sup>. However, Lenka et al. indicated that the minor T allele in *CILP2* gene was associated with lower LDL-C, apoB, and atherogenic indices and higher HDL-C levels <sup>[20]</sup>. This result was in accordance with the study in Singaporean population ranging from 40 to 80 years of age <sup>[21]</sup>. On the other hand, it have been reported that SNPs in *CILP2* gene was associated with adult height attainment <sup>[22]</sup>, and CpGs in *CILP2* were significantly associated with both body mass index and fat-free mass index in preschool children <sup>[23]</sup>.

According to Chenan Zhang et al., an expression quantitative trait locus for *CILP2* gene, rs8103992, was significantly associated with adult height attainment and osteosarcoma risk after adjustment for multiple comparisons in 864 osteosarcoma cases and 1879 controls of European ancestry <sup>[15]</sup>. To our best acknowledgement, there were no more reports describing relationship between *CILP2* and cancers.

Our work presented here has evaluated the prognostic value of *CILP2* in CRC by analyzing dataset of TCGA cohort and TMA cohort. For the first time, we found out that *CILP2* was upregulated in colorectal cancer tissues compared to normal tissues. In addition, we observed that *CILP2* expression was significantly correlated with clinicopathological parameters of CRC patients in TCGA cohort and TMA cohort. In high-stage CRC samples, *CILP2* was upregulated compared to low-stage CRC samples. To evaluate prognostic value of *CILP2* on overall survival of CRC patients in TCGA cohort, Kaplan-Meier and Cox regression analysis were performed. We found out that higher *CILP2* expression was correlated with much poorer prognosis in CRC patients. These results indicated that *CILP2* could act as an independent prognostic marker in colorectal cancer.

Recently, many reports have shown that obesity represents a common risk factor for several types of cancer <sup>[24, 25]</sup>, especially for hormone dependent cancers, such as breast cancer <sup>[26, 27]</sup> and advanced prostate cancer <sup>[28]</sup>. The biological association between obesity and cancer might relate to tissue lipid metabolism. It is well known that cancer cells, including CRC cells, show alterations in lipid metabolism of synthesis, desaturation, elongation and mitochondrial oxidation of fatty acids <sup>[29–31]</sup>. A population-based study has revealed incidences of colorectal cancer to be associated with circulating levels of apolipoproteins <sup>[30]</sup>. Sophisticated correlation and therapeutic use of lipid metabolism-related alternations remain further investigations.

## Conclusions

Our study has raised that *CILP2* might serve as a potential prognostic marker in CRC patients. Further studies would be needed to detect *CILP2* expression in serum of CRC patients, and confirm the prognostic value and feasibility in larger and multi-center cohorts of CRC patients, as well as to further elucidate molecular mechanisms underlying correlations between *CILP2* and colorectal cancer development.

## Abbreviations

CILP2: Cartilage Intermediate Layer Protein 2; TCGA: The Cancer Genome Atlas; OS: Overall survival; CRC: Colorectal cancer; GWAS: Genome-wide association studies; NCAN-CILP2-PBX4: Neurocan-cartilage intermediate layer protein 2- pre-B-cell leukemia homeobox 4; NAFLD: Non-alcoholic fatty liver disease; MSI: Microsatellite instability; SNPs: Single nucleotide polymorphisms; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol

## **Declarations**

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### **Authors' contributions**

Qing Ye designed the study. Feng Huang and Yuanfei Peng collected the data and carried out the experiment. Jinhu Chen, Yangming Li and Shengyuan Liu performed the statistical analyses. Yangmei Xu and Lijie Huang took responsibility of histology and immunohistochemistry. Feng Huang and Qing Ye wrote the manuscript. Qing Ye took responsibility for obtaining permission from all coauthors for the submission of any version of the paper and for any changes in authorship. All authors read and approved the final manuscript.

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## **Availability of data and materials**

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### **Ethics approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

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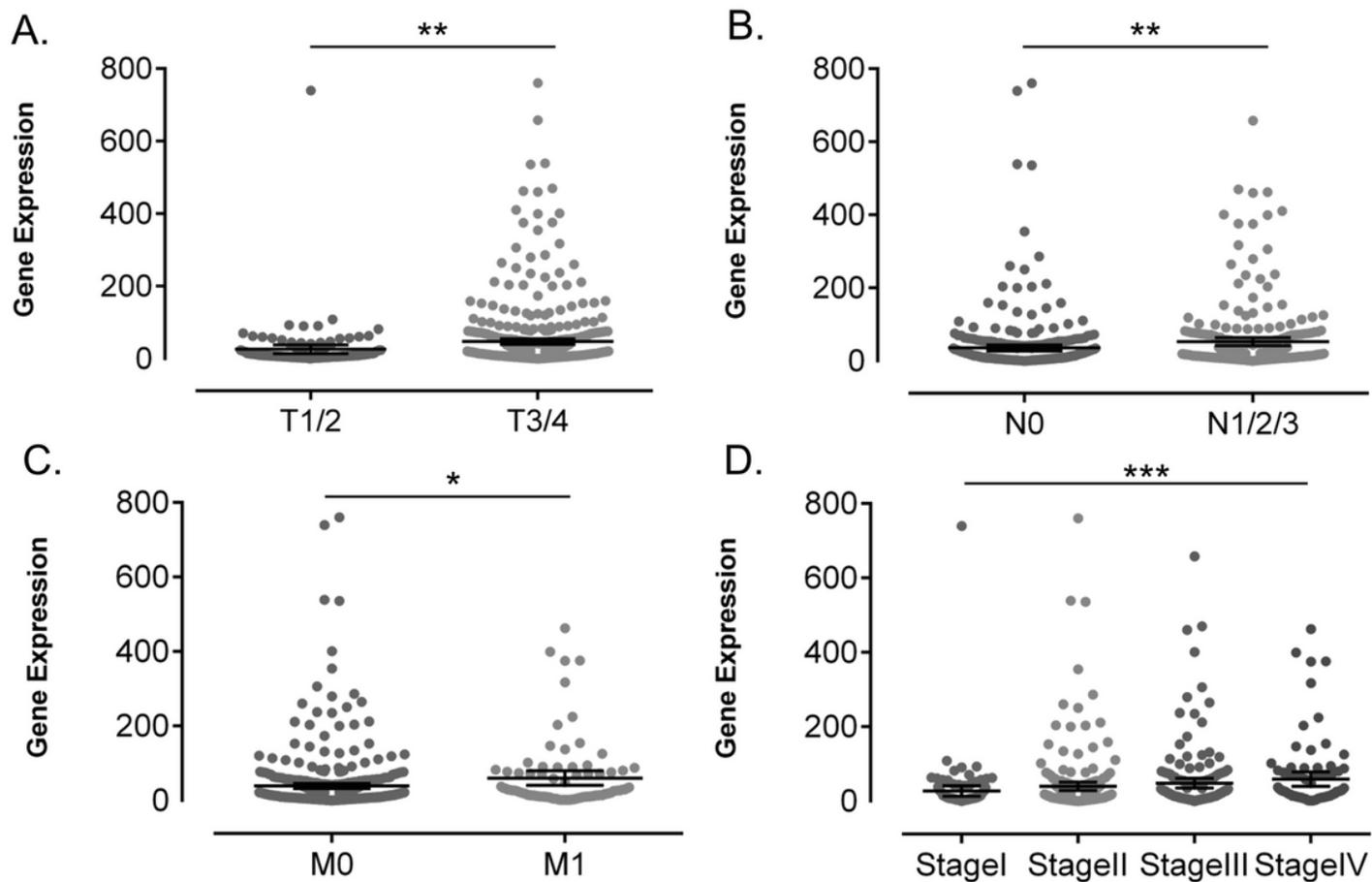
## Figures



### Figure 2

CILP2 was upregulated in colorectal cancer samples. A, B. The line chart and Histogram of CILP2 gene expression in 50 paired tumor and matched adjacent normal tissue samples in TCGA cohort. FC, fold change. C. Scatter plot of CILP2 expression in all normal and cancer samples in TCGA cohort. \*\*\*:  $P < 0.001$ . D. Positive or negative expression of CILP2 protein in matched adjacent normal tissues (normal) or cancer tissues in TMA cohort, \*\*:  $P = 0.001$ . E. Representative image of normal tissues immunohistochemical staining in TMA cohort. Left: Original magnification 100 X; Right: Original

magnification 400 X. F. Representative image of cancer tissues immunohistochemical staining in TMA cohort. Left: Original magnification 100 X; Right: Original magnification 400 X.



**Figure 3**

CILP2 expression was correlated with different clinicopathological parameters of CRC patients in TCGA cohort, as in A. T stages; B, Regional lymph node metastatic patients; C, Distant metastatic patients ; and D, UICC clinical stages. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

# Survival proportions: Survival of Two Groups

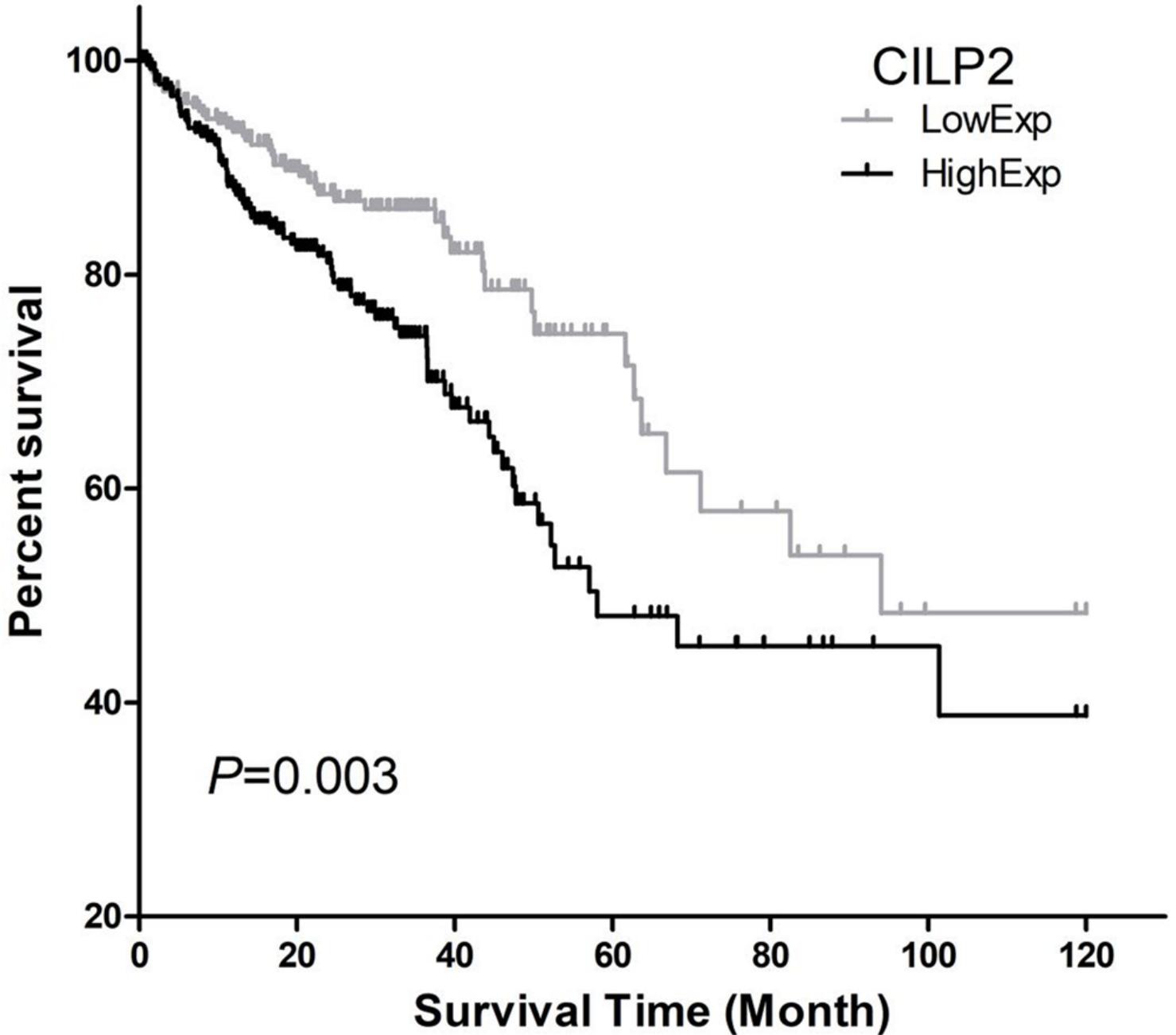


Figure 5

Kaplan-Meier analysis of CILP2 expression and overall survival in total CRC samples of 10 years in TCGA cohort. Higher CILP2 expression group had a poorer overall survival than low CILP2 expression group. (P=0.003)

## Supplementary Files

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