Original Article
CELF1 promotes colorectal cancer proliferation and chemoresistance via activating the MAPK signaling pathway

Hongbin Wang[2], Yuekun Zhu[1], Tao Jiang[1], Hong Chen[3], Anlong Zhu[1], Daxun Piao[1]

[1]Department of Colorectal Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, China; [2]Department of Breast Surgery, The Harbin Medical University Cancer Hospital, Harbin, China; [3]Department of Anesthesiology, The Harbin Medical University Cancer Hospital, Harbin, China

Abstract: Colorectal cancer is the third most commonly diagnosed type of cancer worldwide with a high incidence and mortality rate. The long-term post-operational survival of colorectal cancer is limited by metastasis and chemoresistance. In this study, we demonstrated that CELF1 expression was upregulated in colorectal cancer. Knockdown of CELF1 expression in colorectal cancer cells inhibited cell proliferation and chemoresistance. Conversely, overexpression of CELF1 promoted colorectal cancer cell proliferation and chemoresistance. We also determined that loss of CELF1 expression downregulated the MAPK signaling pathway, and that CELF1 regulated colorectal cancer cell proliferation via activating the MAPK signaling pathway.

Keywords: Colorectal cancer, CELF1, MAPK, cell proliferation, chemoresistance

Introduction

Colorectal cancer is the third most commonly diagnosed type of cancer worldwide with a high incidence and mortality rate [1]. Each year, more than 600,000 individuals die from colon cancer [2]. The tumorigenesis of colorectal cancer is a multistep process that involves accumulation of genetic and epigenetic changes [3]. It has been shown that the occurrence and progression of colorectal cancer are driven by a variety of signaling pathways, including p53 pathway, EGFR-MAPK pathway, Wnt-β-catenin pathway, PI3K-Akt pathway, and NF-κB pathway [4]. Despite the improvement in the available treatment regimens, the long-term post-operational colorectal cancer survival is still hindered by metastasis and chemoresistance. Therefore, targeting metastasis and chemoresistance is an important strategy for treating colorectal cancer, and this would require better understanding of the precise molecular mechanisms of colorectal cancer. CELF1 is a member of the CELF family (CUGBP and embryonic lethal abnormal vision-like factor) that belongs to the ELAV-like family of RNA binding proteins. CELF1 was found to preferentially bind to GU-rich elements predominantly located in the 5' and 3' UTRs of mRNAs [5-7]. The functional consequences of CELF1 association with its targets include altered mRNA splicing, translation and turnover [8, 9]. Most previous research on CELF1 was focused on its function during embryonic development process, including myotonic development [10, 11]. However, emerging evidence has suggested that CELF1 is also a potential regulator of cancer development. CELF1 expression correlates with poor patient survival in non-small cell lung cancer. Reduction of CELF1 in lung cancer cells inhibited proliferation and colony formation [12, 13]. CELF1 is also overexpressed in human squamous cell carcinoma. Knockdown of CELF1 expression in oral cancer cells promoted apoptosis and suppressed cell growth [14]. A series of studies have demonstrated that CELF1 is overexpressed in many other human malignant tumors, including hepatic carcinoma [15], breast cancer [16], glioma [17], acute B cell lymphoma [18] and acute myeloid leukemia [18]. The role of CELF1 in colorectal cancer, however, has not been studied before. In this study, we reported that CELF1 expression was upregulated in colorectal cancer. We also found

that knockdown of CELF1 inhibited colorectal cancer cell proliferation and chemoresistance. Conversely, overexpression of CELF1 promoted colorectal cancer cell proliferation and chemo-resistance. In addition, we determined that loss of CELF1 expression downregulated the MAPK signaling pathway, and that CELF1 regulated colorectal cancer cell proliferation via activating the MAPK signaling pathway.

Materials and methods

Human samples

Freshly frozen 10 human colorectal cancer tissues and adjacent non-cancerous normal colorectal tissues were obtained from surgical resection specimens of pathologically confirmed colorectal cancer patients at Cancer Hospital of Harbin Medical Hospital. The informed consents were obtained from all participants before this work. This study was reviewed and approved by the Ethics Committee of Cancer Hospital of Harbin Medical Hospital.

Immunohistochemistry

Tumor tissue specimens were fixed in neutral formalin and embedded in paraffin. Paraffin-embedded sections (4 μm) were dewaxed in xylene and rehydrated in a gradient alcohol. Sections were incubated in 3% hydrogen peroxide for 10 min to deactivate the endogenous peroxidase. Then, the sections were heated at 100°C for 15 min in 0.01 M natriumcitricum for antigen. The sections were blocked with normal goat serum at 37°C for 10 min to prevent nonspecific binding and then incubated overnight at 4°C with anti-CELF1 primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The EliVision plus kit (Maixin-Bio, Fuzhou, China) was used to examine the antibody binding. The slide was stained with diaminobenzidinetetrahydrochloride (DAB, Sigma, St. Louis, MO, USA), washed in flowing water, re-stained with hematoxylin, dehydrated, cleared, dried and mounted.

Quantity real-time PCR (qRT-PCR)

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase I (TaKaRa, Dalian, China) to remove residual genomic DNA. For qRT-PCR, RNA was reverse transcribed to cDNA by using a reverse transcription with PrimeScript RT Master Mix (TaKaRa). Real-time PCR analyses were performed with SYBR PCR Master Mix (TaKaRa). The qRT-PCR assays were conducted on Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Forster City, CA, USA). The relative expression of CELF1 was calculated using the 2-ΔΔCt method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal control. The primers used for the study are as follows: CELF1 forward 5'-ACCTGTTCATCTACCACCTG-3' and reverse 5'-GGCTTGCTGTCATTCTTCG-3'; CCNA1 forward 5'-GGGCTCCCAGATTTCGTCTT-3' and reverse 5'-GACCTCGGGCCACTGTAGC-3'; CCND1 forward 5'-CGTGGCCTCTAAGATGAAGG-3' and reverse 5'-CTGGCATTTTGGAGAGGAAG-3'; c-myc forward 5'-GCCACGTCTCCACACATCAG-3' and reverse 5'-TCTTGGCAGCAGGATAGTCCTT-3'; FOXM1 forward 5'-AACCGCTACTTGACATTGG-3' and reverse 5'-GCAGTGGCTTCATCTTCC-3'; MAPK3 forward 5'-CTTCCCTGGCAAGCACTACC-3' and reverse 5'-GTTTCGGGCTTCATGTTGA-3'; GAPDH forward 5'-GACCCCTTCATTGACCTCAAC-3' and reverse 5'-CTTCTCCATGGTGGTGAA-GA-3'.

Cell culture

Two human colorectal cell lines (SW-480 and Caco2) were cultured in RPMI 1640 (Gibco Lab., Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco Lab.), 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA, USA) in humidified air at 37°C with 5% CO_2.

Transfection

Colorectal cancer cells were transfected with siRNAs and plasmid vectors using Lipofectamine 2000 transfection reagent (Invitrogen) following the manufacturer's protocol. Design of CELF1 siRNA as described previously [13]. The full-length of CELF1 complementary DNA was amplified from SW-480 cells and subcloned into the pcDNA3 (+) vector (Invitrogen) according to the manufacturer's instructions. At 48 h post-transfection, cells were subjected to next analysis.

Western blot

Colorectal cells were lysed with RIPA lysis buffer [50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, and 2 mM EDTA] containing protease inhibitors for 30 min, followed by centrifugation
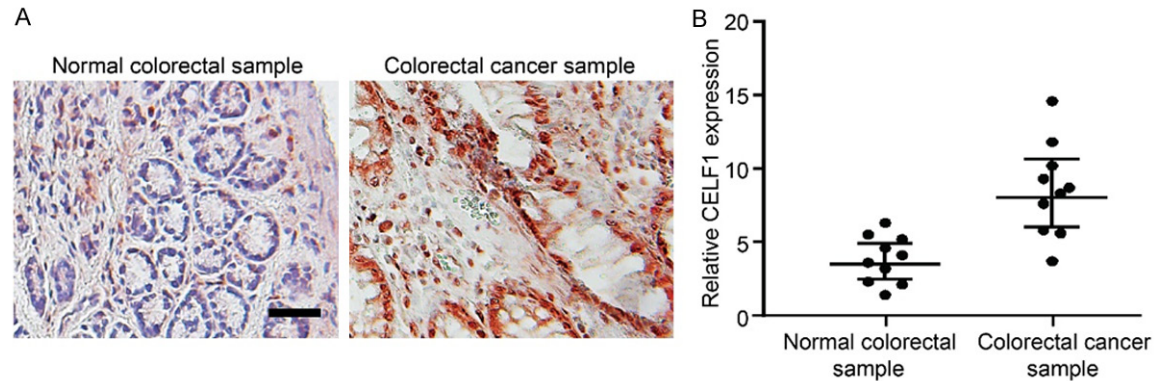
Figure 1. CELF1 expression is upregulated in colorectal cancer. A. Representative immunohistochemistry staining of CELF1 in normal and cancerous colorectal tissues. B. The mRNA levels of CELF1 in 10 colorectal cancer tissues compared with those of adjacent normal colorectal tissues, as determined by qRT-PCR (P<0.001).

at 12,000×g for 30 min. The protein concentration of each sample was quantified with the BCA method (Beyotime, Beijing, China). Then, equal amounts of proteins were electrophoresed on a 10% gradient gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% BSA in Tris-buffered saline and 0.2% Tween (TBST) at room temperature for 1 h and then incubated overnight at 4°C with the following specific primary antibodies: anti-CELF1, anti-p-ERK1/2, anti-ERK1/2 and GAPDH. All primary antibodies were purchased from Santa Cruz Biotechnology. The blots were washed three times with TBST and incubated with a horseradish peroxidase (HRP) conjugated secondary antibody (Beyotime) for 1 h at room temperature. The expression signals were detected with an enhanced chemiluminescence (ECL) reagent (Beyotime).

MTT assay

Colorectal cell proliferation and survival was assessed by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. SW-480 or Caco2 HCC cells (3000/well) were seeded into 96-well plates to allow adhering to the plates. Fresh medium containing 50 μL of MTT solution (0.5 mg/mL) was then added to each well at different time points. After incubation for another 3 hours, the MTT formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and viable cells were detected by measuring the absorbance at 570 nm wavelength using a microplate reader.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) of at least 3 replicates. Analysis was conducted with GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). Significance between two groups was analyzed using the unpaired two-tailed t test (*P<0.05, **P<0.01, ***P<0.001).

Results

CELF1 expression is upregulated in colorectal cancer

In order to investigate the involvement of CELF1 in colorectal cancer, we first analyzed the protein levels of CELF1 in human colorectal tissues by immunohistochemistry staining. We found that the protein levels of CELF1 were substantially increased in colorectal cancer tissues, compared with those of adjacent normal tissues (**Figure 1A**). Furthermore, we used qRT-PCR analysis to determine the mRNA levels of CELF1 in 10 paired colorectal cancer tissue samples and adjacent histologically normal tissue samples. We found that the mRNA levels of CELF1 were significantly higher in colorectal cancer tissues than those in matched normal tissues (**Figure 1B**). Taken together, these results indicated that CELF1 expression was upregulated in colorectal cancer.

CELF1 enhances colorectal cancer cell proliferation and chemoresistance

To investigate the functional role of CELF1 in colorectal cancer progression, we first knocked
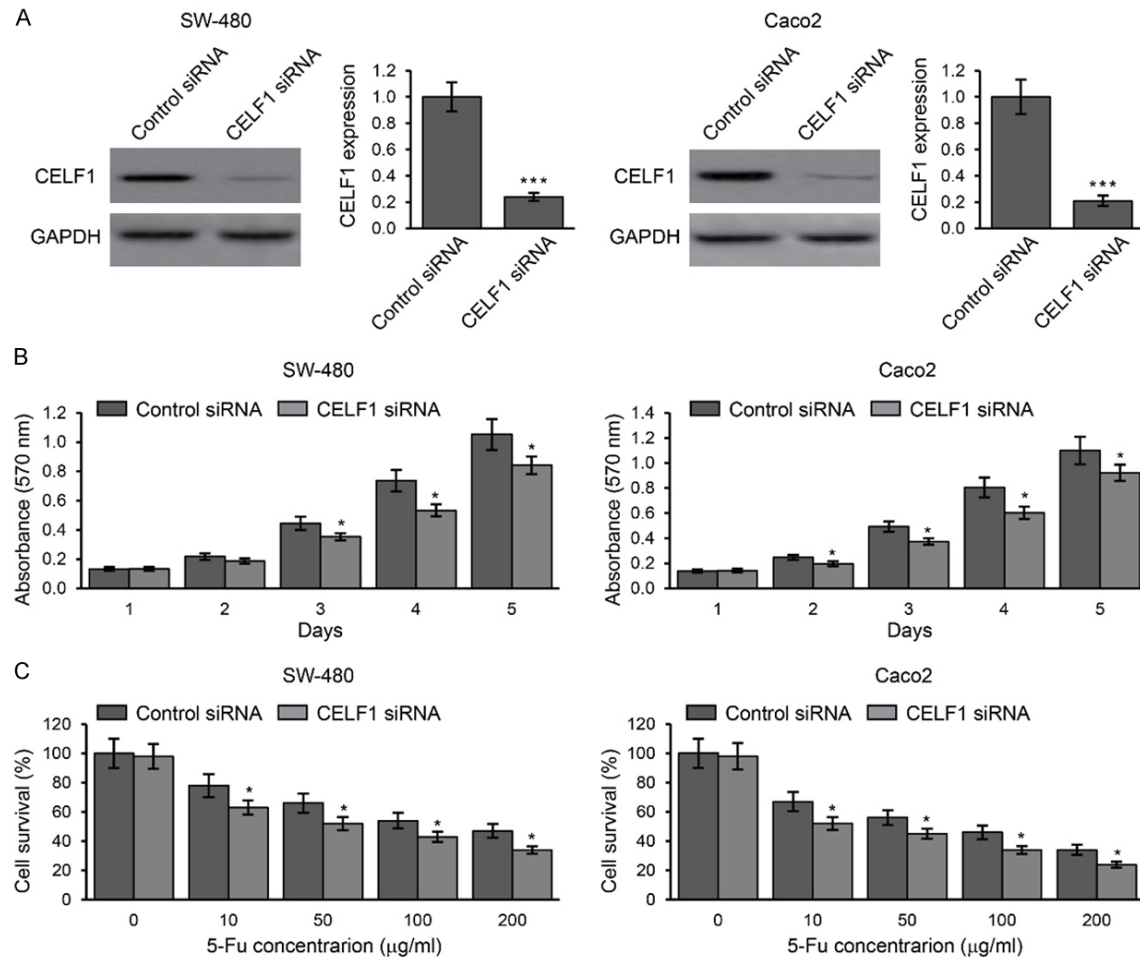
Figure 2. Knockdown of CELF1 inhibits colorectal cancer cell proliferation and chemoresistance. A. Protein levels of CELF1 as determined by Western blot analysis and relative mRNA levels of CELF1 as determined by qRT-PCR in SW-480 and Caco2 cells transfected with control siRNA or CELF1 siRNA. GAPDH protein was used as an internal control in the Western blot analysis. B. MTT assay in SW-480 and Caco2 cells transfected with control siRNA or CELF1 siRNA for the indicated times. C. MTT assay in SW-480 and Caco2 cells transfected with control siRNA or CELF1 siRNA treated with the indicated concentrations of 5-Fu (0, 10, 50, 100 and 200 µg/ml). *P<0.05, ***P<0.001.

down CELF1 expression in two independent colorectal cancer cell lines (SW-480 and Caco2). The efficacy of knockdown was confirmed in both protein and mRNA levels (**Figure 2A**). We carried out MTT assays on these cells and found that knockdown of CELF1 expression significantly inhibited colorectal cell proliferation in both SW-480 and Caco2 cells (**Figure 2B**). To examine whether CELF1 is involved in the chemoresistance of colorectal cancer cells, we treated CELF1 knockdown and control colorectal cancer cells with different concentration of 5-Fu (0, 10, 50, 100 and 200 µg/ml) and performed MTT assay on these cells. We found that knockdown of CELF1 significantly reduced the chemoresistanceability of both SW-480 and Caco2 cells (**Figure 2C**). Con-

versely, we overexpressed CELF1 in colorectal cancer cells by transfecting vector or CELF1 expressing plasmid into SW-480 and Caco2 cells (**Figure 3A**). We found that in contrast to the results in CELF1 knock down cells, CELF1 overexpression led to significantly enhanced cell proliferation (**Figure 3B**) and chemoresistance (**Figure 3C**) in both SW-480 and Caco2 cells.

CELF1 regulates colorectal cancer cell proliferation via the MAPK signaling pathway

Since deregulation of the MAPK signaling pathway has been shown to be involved in the development of multiple cancer types (ref), we hypothesized that CELF1 could regulate
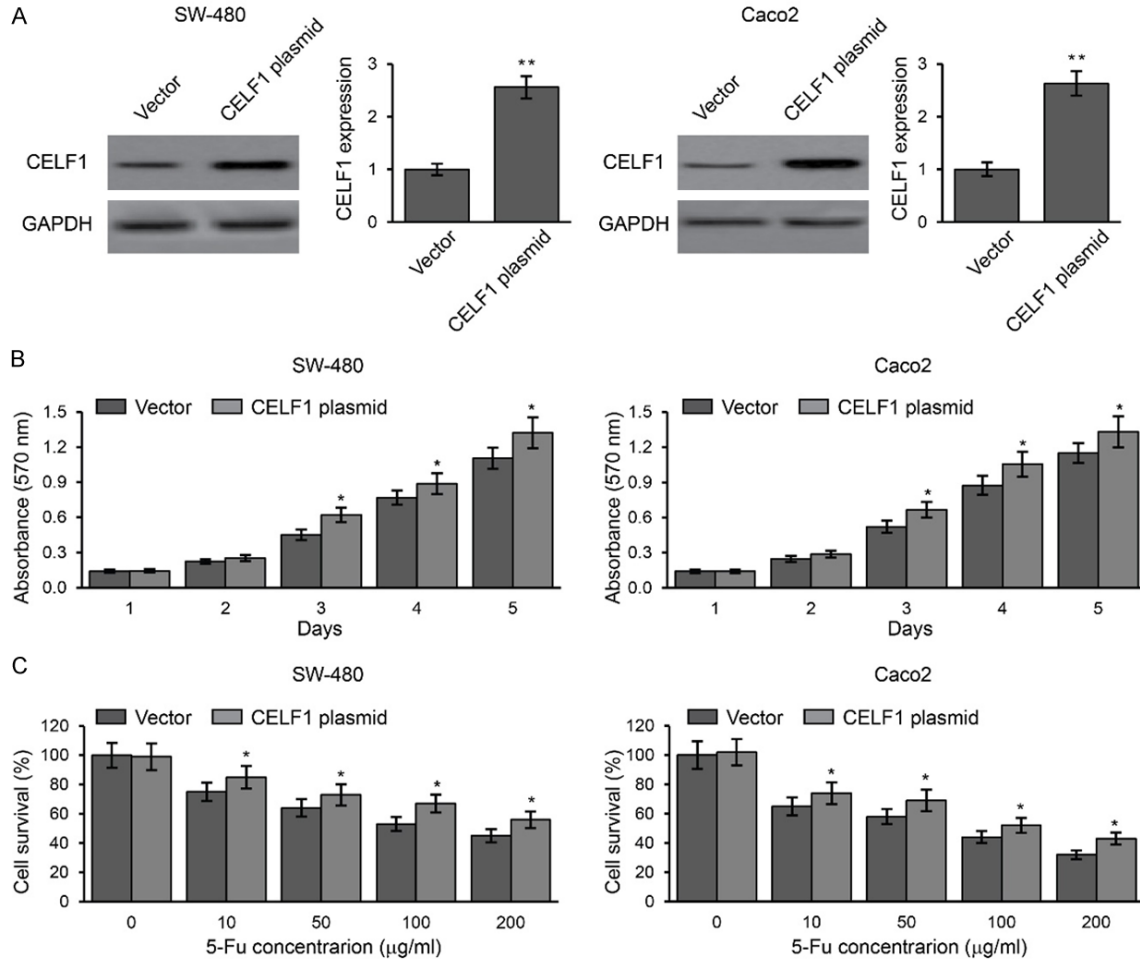
Figure 3. Overexpression of CELF1 promotes colorectal cancer cell proliferation and chemoresistance. A. Protein levels of CELF1 as determined by Western blot analysis and relative mRNA levels of CELF1 as determined by qRT-PCR in SW-480 and Caco2 cells transfected with empty vector or CELF1 expressing plasmid. GAPDH protein was used as an internal control in the Western blot analysis. B. MTT assay in SW-480 and Caco2 cells transfected with empty vector or CELF1 expressing plasmid for the indicated times. C. MTT assay in SW-480 and Caco2 cells transfected with empty vector or CELF1 expressing plasmid treated with the indicated concentrations of 5-Fu (0, 10, 50, 100 and 200 µg/ml). $^*P<0.05$, $^{**}P<0.01$.

colorectal cancer via the MAPK signaling pathway. To test this hypothesis, we knocked down CELF1 expression in SW-480 and Caco2 cells and examined the levels of phosphorylated ERK. We found that loss of CELF1 expression significantly decreased the levels of phosphorylated ERK1/2 (**Figure 4A**), indicating reduced MAPK signaling activity. In addition, we examined the expression of a series of downstream target genes of the MAPK signaling pathway, including CCNA1, CCND1, c-myc, FOXM1 and MAPK3. Consistently, knockdown of CELF1 led to suppressed expression of all these genes in both SW-480 and Caco2 cells (**Figure 4B**). Taken together, these results demonstrated

that loss of CELF1 expression downregulated the MAPK signaling pathway.

In order to further confirm whether or not CELF1 regulates colorectal cancer cell proliferation via the MAPK signaling pathway, we treated CELF1 overexpressing SW-480 and Caco2 cells with MAPK signaling pathway inhibitor PD98059 (20 µM). Importantly, we found that when MAPK signaling is inhibited, overexpression of CELF1 was no longer able to enhance the proliferation of colorectal cancer cells (**Figure 5**). These results collectively suggested that CELF1 promoted colorectal cancer cell proliferation via activating the MAPK signaling pathway.
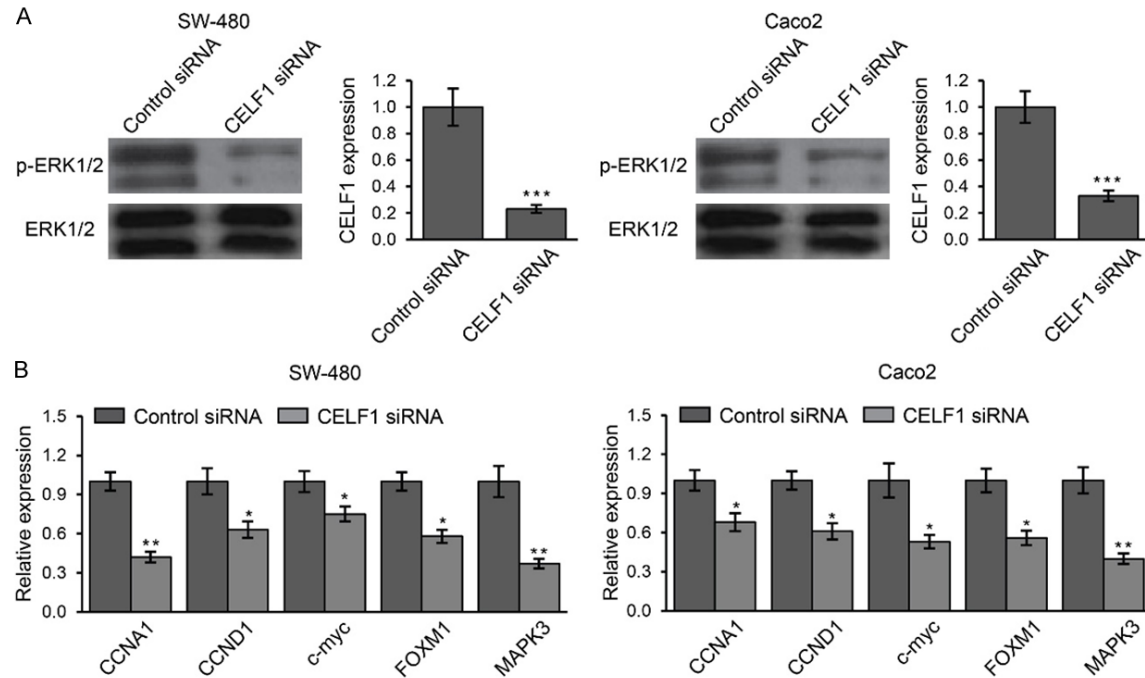
Figure 4. Loss of CELF1 expression downregulates the MAPK signaling pathway. A. Levels of phosphorylated and total ERK1/2 in SW-480 and Caco2 cells transfected with control siRNA or CELF1 siRNA, as determined by Western blot analysis. B. mRNA levels of downstream target genes of the MAPK signaling pathway (CCNA1, CCND1, c-myc, FOXM1 and MAPK3), as determined by qRT-PCR analysis. *P<0.05, **P<0.01, ***P<0.001.
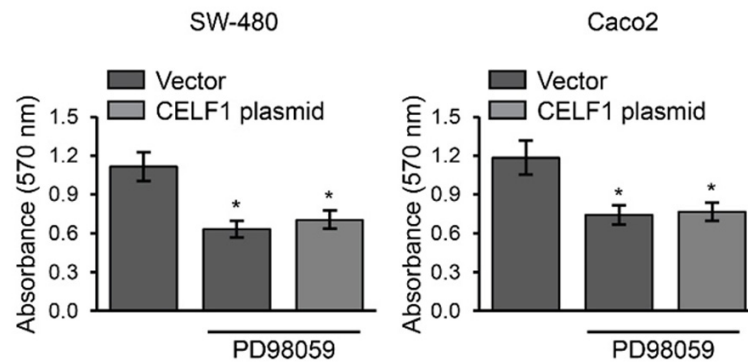

Figure 5. CELF1 regulates colorectal cancer cell proliferation via the MAPK signaling pathway. MTT assay in SW-480 and Caco2 cells transfected with empty vector or CELF1 expressing plasmid treated with PD98059. *P<0.05.

Discussion

Our study primarily focused on the effect of CELF1 on the development of colorectal cancer. As shown in the results, the expression of CELF1 was higher in colorectal cancer tissues compared with normal tissues. Moreover, knockdown of CELF1 expression in colorectal cancer cells inhibited cell proliferation and chemoresistance, and overexpression of CELF1 promoted cell proliferation and chemoresistance. Significantly, we provided data supporting the hypothesis that CELF1 regulated colorectal cancer cell proliferation via activating the MAPK signaling pathway.

The findings of our study is in consistent with a series of recent studies concerning the roles of CELF1 in various cancer types, including lung cancer [12, 13], squamous cell carcinoma [14], hepatic carcinoma [15], breast cancer [16], glioma [17], acute B cell lymphoma [18] and acute myeloid leukemia [18]. Although CELF1 levels are upregulated in all of these reports, the mechanism by which CELF1 regulates cancer development appears to be cell type specific. For example, CELF1 was shown to affect E-cadherin levels in A549 lung cancer cells, but the mechanism is not well understood [12]. CELF1 was also reported to regulate the proliferation of intestine epithelial cells via

modulating the translation of oncogene Myc [19]. In addition, CELF1 promoted glioma cell proliferation by inhibiting the expression of cyclin dependent kinase inhibitor CDKN1B expression [17].

In this study, we showed that CELF1 regulated colorectal cancer cell proliferation via activating the MAPK signaling pathway. However, the detailed mechanism underlying this regulation remains to be investigated. It is important to notice that CELF1 is an RNA binding protein (RBP). There are more than 400 predicted RBPs in human genome, but only a few have been extensively characterized for their roles in cancer development [20]. RBPs are critical regulators transcriptional and translational gene expression and are capable of associating with both mRNAs and non-coding RNAs [21]. RBPs interact with their targets by recognizing specific RNA sequence motifs or secondary structures [22]. Consequently, RBPs play major roles in the metabolism of mRNAs [23, 24]. In response to extracellular stimuli, the RNA-binding activity and the expression level of RBPs can be rapidly modulated [25]. As a result, deregulation of RBPs can lead to cancer progression. In this context, it is intriguing that a recent study reported that the overexpression of CELF1 could alter the expression levels of EGF receptor, causing activation of downstream targets of EGFR signaling in oral cancer [26]. Since MAPK signaling is one of the downstream targets of EGFR signaling. It is therefore possible that in colorectal cancer, CELF1 activates the MAPK signaling pathway by increasing the expression of EGFR.

Since suppression of CELF1 expression inhibits the proliferation and chemoresistance of colorectal cancer cells, it is likely that the CELF1 mediated gene expression program, including the expression of EGFR, could act as stimulators in the development of colorectal cancer. Therefore, a promising direction in future study would include pharmacological intervention disrupting the CELF1-mRNA interfaces, which could potentially inhibit the development of colorectal cancer.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Daxun Piao, Department of Colorectal Surgery, The First Affiliated Hospital of Harbin Medical, 23 Youzheng Street, 150001, Heilongjiang, China. Tel: 86-451-8555-6000; Fax: 86-451-85556000; E-mail: piaodaxun@sina.com

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917.

[3] Pritchard CC and Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut 2011; 60: 116-29.

[4] Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009; 9: 489-99.

[5] Lee JE, Lee JY, Wilusz J, Tian B, Wilusz CJ. Systematic analysis of cis-elements in unstable mRNAs demonstrates that CUGBP1 is a key regulator of mRNA decay in muscle cells. PLoS One 2010; 5: e11201.

[6] Marquis J, Paillard L, Audic Y, Cosson B, Danos O, Le Bec C, Osborne HB. CUG-BP1/CELF1 requires UGU-rich sequences for high-affinity binding. Biochem J 2006; 400: 291-301.

[7] Mori D, Sasagawa N, Kino Y, Ishiura S. Quantitative analysis of CUG-BP1 binding to RNA repeats. J Biochem 2008; 143: 377-83.

[8] Dasgupta T and Ladd AN. The importance of CELF control: molecular and biological roles of the CUG-BP, Elav-like family of RNA-binding proteins. Wiley Interdiscip Rev RNA 2012; 3: 104-21.

[9] Vlasova-St Louis I, Dickson AM, Bohjanen PR, Wilusz CJ. CELFish ways to modulate mRNA decay. Biochim Biophys Acta 2013; 1829: 695-707.

[10] Koshelev M, Sarma S, Price RE, Wehrens XH, Cooper TA. Heart-specific overexpression of CUGBP1 reproduces functional and molecular abnormalities of myotonic dystrophy type 1. Hum Mol Genet 2010; 19: 1066-75.

[11] Zhang L, Lee JE, Wilusz J, Wilusz CJ. The RNA-binding protein CUGBP1 regulates stability of tumor necrosis factor mRNA in muscle cells: implications for myotonic dystrophy. J Biol Chem 2008; 283: 22457-63.

[12] Gao C, Yu Z, Liu S, Xin H, Li X. Overexpression of CUGBP1 is associated with the progression of non-small cell lung cancer. Tumour Biol 2015; 36: 4583-9.

[13] Wu LN, Xue YJ, Zhang LJ, Ma XM, Chen JF. Si-RNA mediated knockdown of CELF1 gene suppressed the proliferation of human lung cancer cells. Cancer Cell Int 2013; 13: 115.

[14] Talwar S, Balasubramanian S, Sundaramurthy S, House R, Wilusz CJ, Kuppuswamy D, D'Silva N, Gillespie MB, Hill EG, Palanisamy V. Overexpression of RNA-binding protein CELF1 prevents apoptosis and destabilizes pro-apoptotic mRNAs in oral cancer cells. RNA Biol 2013; 10: 277-86.

[15] Liu Y, Huang H, Yuan B, Luo T, Li J, Qin X. Suppression of CUGBP1 inhibits growth of hepatocellular carcinoma cells. Clin Invest Med 2014; 37: E10-8.

[16] Mukhopadhyay D, Jung J, Murmu N, Houchen CW, Dieckgraefe BK, Anant S. CUGBP2 plays a critical role in apoptosis of breast cancer cells in response to genotoxic injury. Ann N Y Acad Sci 2003; 1010: 504-9.

[17] Xia L, Sun C, Li Q, Feng F, Qiao E, Jiang L, Wu B, Ge M. CELF1 is Up-Regulated in Glioma and Promotes Glioma Cell Proliferation by Suppression of CDKN1B. Int J Biol Sci 2015; 11: 1314-24.

[18] Guerzoni C, Bardini M, Mariani SA, Ferrari-Amorotti G, Neviani P, Panno ML, Zhang Y, Martinez R, Perrotti D, Calabretta B. Inducible activation of CEBPB, a gene negatively regulated by BCR/ABL, inhibits proliferation and promotes differentiation of BCR/ABL-expressing cells. Blood 2006; 107: 4080-9.

[19] Liu L, Ouyang M, Rao JN, Zou T, Xiao L, Chung HK, Wu J, Donahue JM, Gorospe M, Wang JY. Competition between RNA-binding proteins CELF1 and HuR modulates MYC translation and intestinal epithelium renewal. Mol Biol Cell 2015; 26: 1797-810.

[20] Cook KB, Kazan H, Zuberi K, Morris Q, Hughes TR. RBPDB: a database of RNA-binding specificities. Nucleic Acids Res 2011; 39: D301-8.

[21] Fujimura K, Kano F and Murata M. Dual localization of the RNA binding protein CUGBP-1 to stress granule and perinucleolar compartment. Exp Cell Res 2008; 314: 543-53.

[22] Lunde BM, Moore C and Varani G. RNA-binding proteins: modular design for efficient function. Nat Rev Mol Cell Biol 2007; 8: 479-90.

[23] Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volker SE, Pambuccian SE, Ondrey FG, Adams GL, Gaffney PM. Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. Cancer Res 2004; 64: 55-63.

[24] Peng CH, Liao CT, Peng SC, Chen YJ, Cheng AJ, Juang JL, Tsai CY, Chen TC, Chuang YJ, Tang CY, Hsieh WP, Yen TC. A novel molecular signature identified by systems genetics approach predicts prognosis in oral squamous cell carcinoma. PLoS One 2011; 6: e23452.

[25] Vizcaíno JA, Côté RG, Csordas A, Dianes JA, Fabregat A, Foster JM, Griss J, Alpi E, Birim M, Contell J, O'Kelly G, Schoenegger A, Ovelleiro D, Pérez-Riverol Y, Reisinger F, Ríos D, Wang R, Hermjakob H. The PRoteomics IDEntifications (PRIDE) database and associated tools: status in 2013. Nucleic Acids Res 2013; 41: D1063-9.

[26] House RP, Talwar S, Hazard ES, Hill EG, Palanisamy V. RNA-binding protein CELF1 promotes tumor growth and alters gene expression in oral squamous cell carcinoma. Oncotarget 2015; 6: 43620-34.