Egr-1 inhibits colon cancer cell proliferation, migration and invasion via regulating CDKL1 at the transcriptional level

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Abstract. Colon cancer is one of the most common malignant tumors worldwide, and the molecular mechanisms involved in the oncogenesis and progression of colon cancer remain unclear. Early growth response 1 (Egr-1) is a transcription factor that is closely associated with several tumor processes; however, its role in colon cancer is unknown. The present study aimed to explore the function and mechanism of transcription factor Egr-1 in colon cancer progression. The association between Egr-1 expression and the survival of patients with colon cancer was analyzed. Transwell assay was used to measure the migration and invasion of colon cancer cells. Cell Counting Kit-8 assay was used to evaluate the cell proliferative ability. Reverse transcription-quantitative PCR and western blot assays were used to identify whether Egr-1 could regulate cyclin-dependent kinase-like 1 (CDKL1). Luciferase and chromatin immunoprecipitation assays were used to detect the mechanism by which Egr-1 regulated CDKL1. Based on The Cancer Genome Atlas database, it was found that low Egr-1 expression was associated with a poor prognosis in patients with colon cancer. Furthermore, overexpression of Egr-1 inhibited colon cancer cell proliferation, migration, and invasion, whereas knockdown of Egr-1 increased colon cancer cell proliferation, migration and invasion. Additionally, overexpression of Egr-1-induced cell proliferation, migration and invasion were reversed by overexpression of CDKL1. Furthermore, it was demonstrated that Egr-1 regulated CDKL1 expression at the transcriptional level. The present study illustrated the mechanism of Egr-1 regulating CDKL1, by which Egr-1 affected colon cancer cell proliferation, migration and invasion. The current findings suggested that Egr-1/CDKL1 may be a new promising target for the treatment of colon cancer.

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Introduction

Colon cancer is one of the most common malignant tumors (1-3). Every year, there are >945,000 new cases of colon cancer worldwide, and ~492,000 deaths (1-4). Although there have been numerous improvements in tumor therapy, colon cancer remains one of the leading causes of cancer-associated deaths in the world (1,2). In patients with colon cancer, metastasis is the main cause of mortality (5). It is a huge challenge for both clinicians and researchers to find novel therapeutic approaches for colon cancer (6).

Early growth response gene 1 (Egr-1) is a transcription factor that serves important roles in tumor processes, including cell proliferation, migration, invasion, differentiation and apoptosis (7-10). Egr-1 expression is dysregulated in several types of tumor, such as leukemia (11), pancreatic tumor (12), gastric tumor (9), thyroid tumor (13), liver cancer (14) and glioma (15), and is a positive prognostic factor for the survival of patients with several types of cancer, such as gastric cancer (16); therefore, Egr-1 is suggested to be a new biomarker and a promising therapeutic target (17). However, the function of Egr-1 in the progression of colon cancer is not fully understood.

Egr-1 is a ubiquitous transcription factor and regulates various target genes by recognizing and binding GC-rich regions in the promoter region of target genes (18-20). Egr-1 can exert both positive and negative transcription roles in regulating different target genes (21). The dual characters of Egr-1 in regulating target genes may be due to the co-factors

recruited by Egr-1. Egr-1 interacts with transcriptional coactivators, such as cyclic AMP response element-binding protein/p300, to trigger the transcription of several genes (22,23), while by binding with histone deacetylases, Egr-1 also serves an inhibitory role in regulating target genes (24).

Cyclin-dependent kinase-like 1 (CDKL1), located on chromosome 14q21.3, is a member of the CDKL kinase family (25,26). CDKL1 expression is upregulated in breast cancer (27), gastric cancer (28), melanoma (29) and colon cancer (30). Moreover, knockdown of CDKL1 in these types of cancer induces apoptosis and/or inhibits cell proliferation, migration and invasion (27-30). Therefore, CDKL1 may serve as a promising therapeutic target; however, the mechanism by which CDKL1 expression is regulated is unknown. To the best of our knowledge, there is no report on the role of CDKL1 in colon cancer progression. Therefore, the present study aimed to investigate whether Egr-1 regulated CDKL1 expression and the role of Egr-1-regulated CDKL1 in colon cancer progression.

The present study aimed to illustrate the function and the underlying mechanism of Egr-1-mediated CDKL1 regulation in colon cancer cell proliferation, migration and invasion, and to verify whether Egr-1/CDKL1 may be promising prognostic biomarkers and therapeutic targets for colon cancer.

Materials and methods

Survival analysis. The association of Egr-1 expression and the survival of 279 patients with colon adenocarcinoma was analyzed using The Cancer Genome Atlas (TCGA) in the UALCAN website (http://ualcan.path.uab.edu/), which is a comprehensive, user-friendly and interactive web resource for analyzing cancer OMICS data. 'TCGA Gene Analysis' was chosen and 'Egr-1' was scanned in TCGA dataset of colon adenocarcinoma. Samples were categorized into two groups: High expression (with TPM values above upper quartile) and Low/Medium expression (with TPM values below upper quartile) (31). The survival curve of the effect of Egr-1 expression on the survival of patients with colon adenocarcinoma was then presented. The Kaplan-Meier method was used for survival analysis.

Cell lines. Since colon adenocarcinoma and colon carcinoma are the two main types of colon cancer, the present study used two cell lines, namely SW480 (a colon adenocarcinoma cell line) and HCT-116 (a colon carcinoma cell line). SW480 and HCT-116 cells were purchased from the American Type Culture Collection (ATCC) and were cultured in DMEM containing 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in an incubator with 5% CO_2.

Egr-1-knockdown in colon cancer cells. The lentivirus (Lv)-shEgr1 was constructed by Shanghai GeneChem Co., Ltd., using a 3rd generation packing system. A total of 2 μg of the packing vector containing short hairpin RNA with the sequence 5'-GGCATACCAAGATCCACTT-3' targeting Egr-1 and 4 μg envelope plasmids mix were transfected into 293 cells (ATCC) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After the cells were cultured at 37˚C for 48 h, the lentivirus particles were collected and concentrated using ultracentrifugation at 100,000 x g at 4˚C for 30 min. Egr-1-knockdown was performed by infecting the Lv-shEgr1 at MOI of 10 into SW480 and HCT-116 cells. Briefly, cells were cultured in a 6-well plate, until the confluence of the cells reached 80%. Lv-shEgr1 was added into the medium. After 24 h of transduction, the medium containing Lv-shEgr1 was replaced with a medium without lentivirus and cultured for 48 h at 37˚C. Cells infected with Lv-shEgr1 were selected with 10 μg/μl puromycin.

Overexpression of Egr-1 or CDKL1 in colon cancer cells. Lv-oeEgr1 was constructed by Shanghai GeneChem Co., Ltd., using a 3rd generation packing system. A total of 2 μg of the packing vector containing the Egr-1 coding sequence and 4 μg envelope plasmids mix were transfected into 293 cells using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After the cells were cultured at 37˚C for 48 h, the lentivirus particles were collected and concentrated using ultracentrifugation at 100,000 x g at 4˚C for 30 min. Overexpression of Egr-1 was performed by infecting the lentivirus Lv-oeEgr1 at MOI of 10 into SW480 and HCT-116 cells. Briefly, cells were cultured in a 6-well plate, until the confluence of the cells reached 80%, Lv-oeEgr1 was added into the medium. After 24 h of transduction, the medium containing Lv-oeEgr1 was replaced with a medium without lentivirus, and cultured for 48 h culture at 37˚C. Cells infected with Lv-oeEgr1 were selected with 10 μg/μl puromycin.

Overexpression of CDKL1 was performed with the same procedure as overexpression of Egr-1, except that the lentivirus was Lv-CDKL1 containing the coding region of CDKL1.

Western blot assay. Proteins were extracted from SW480 and HCT-116 cells using a RIPA lysis buffer kit (EMD Millipore). The protein concentration was measured using a Pierce Rapid Gold BCA kit (Pierce; Thermo Fisher Scientific, Inc.). A total of 20 μg protein/lane was subjected to 10% SDS-PAGE to separate the proteins according to their molecular weight, and the proteins were then transferred to a nitrocellulose membrane (EMD Millipore). After blocking with 5% skimmed milk at 20˚C for 30 min, the membrane was incubated with primary antibody for 16 h at 4˚C, followed by incubation with peroxidase-conjugated secondary antibody (1:10,000; cat. no. A0208; Beyotime Institute of Biotechnology) at 20˚C for 1 h. The membrane was finally incubated in ECL reagents (EMD Millipore) and visualized using a chemiluminescence imager (Bio-Rad Laboratories, Inc.). The Egr-1 primary antibody was purchased from Cell Signaling Technology, Inc. (cat. no. 4154), while the CDKL1 (cat. no. ab136129) and β-actin (cat. no. ab8227) primary antibodies were purchased from Abcam. All the primary antibodies were diluted at 1:1,000 in 5% skimmed milk.

Reverse transcription-quantitative (RT-q)PCR. RNA was extracted from cells using TRzol reagent (cat. no. RA101-01; Beijing Biomed Gene Technology Co., Ltd.) according to the manufacturer's protocol. An ultraviolet-spectrophotometer (Thermo Fisher Scientific, Inc.) was used to measure the mRNA concentration. mRNA (2 μg) was reverse transcribed into cDNA using a First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was performed in a PCR

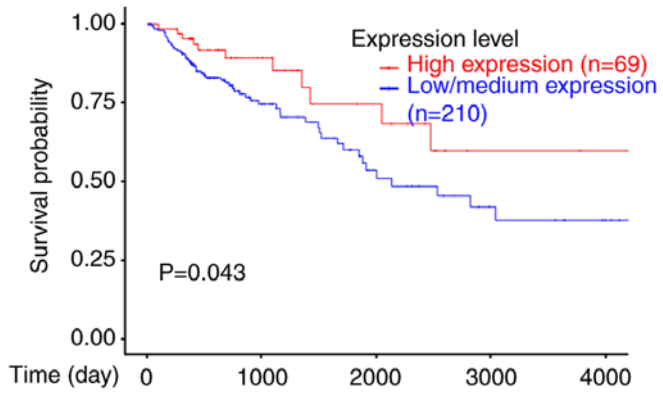

Figure 1. Association of Egr-1 expression with the survival of patients with colon adenocarcinoma. Egr-1, early growth response 1.

instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using a SYBR Green Master Mix Reagent (Thermo Fisher Scientific, Inc.). β-actin was used as the reference gene. The thermocycling conditions were as follows: 30 sec denaturation at 95˚C, followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing and extension at 60˚C for 1 min. The primers used were as follows: Egr-1 sense, 5'-GAACAACCC TACGAGCACCTG-3' and antisense, 5'-GCCACAAGTGT TGCCACTG-3'; CDKL1 sense, 5'-CGAATGCTCAAGCAA CTCAAGC-3' and antisense, 5'-GCCAAGTTATGCTCTTCA CGAG-3'; and β-actin sense, 5'-GTGGACATCCGCAAA GAC-3' and antisense, 5'-AAAGGGTGTAACGCAACTA-3'. The data were analyzed using the $2^{-\Delta\Delta Cq}$ relative expression method (32).

Construction of CDKL1 promoter-luciferase reporter. The human CDKL1 promoter sequence was obtained from GenBank (www.ncbi.nlm.nih.gov/gene/). CDKL1 promoter (-1,500 bp to +100 bp, the translational starting site as +1) was amplified from the genomic DNA of SW480 cells using a high-fidelity DNA polymerase (New England BioLabs, Inc.) using the following conditions: 30 sec denaturation at 95˚C, followed by 35 cycles of denaturation at 95˚C for 15 sec and annealing and extension at 65˚C for 1.5 min. The primers used were as follows: Sense, 5'-CCAAGCTTGTAGAGG AAAGTGCTGACTTT-3' and antisense, 5'-CCTCGAGGT GTCCCTGTTTCTACATTTGA-3. After PCR amplification, the product was cut with *Hin*dIII and *Xho*I restriction enzymes (New England BioLabs, Inc.) and inserted into the pGL3-Basic plasmid (Promega Corporation) between the *Hin*dIII and *Xho*I sites. The promoter-luciferase reporter was named pGL3-CDKL1.

Luciferase assay. SW480 or HCT-116 cells transfected with shCon, shEgr-1, oeCon or oeEgr-1 were seeded into a 12-well plate at 20,000 cells/well. After 24 h of culture, the pGL3-CDKL1 plasmid (1 μg) was transfected into cells using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After another 24 h of culture following transfection, cells were lysed and mixed with the luciferin substrate of a Luciferase Assay System kit (cat. no. E1500; Promega Corporation); luciferase activity was determined with a luminometer (Thermo Fisher Scientific, Inc.).

Chromatin immunoprecipitation (ChIP) assay. The ChIP assay was performed using a ChIP assay kit (Beyotime Institute of Biotechnology) following the manufacturer's protocol. Briefly, SW480 or HCT-116 cells were cross-linked by being incubated at 25˚C for 10 min in a thermostatic incubator (Thermo Fisher Scientific, Inc.), and then the cross-link was stopped by glycin. The genomic DNA was sheared into fragments of 200-1,000 bp by 300 W ultra-sonication at 4˚C for 5 min. A total of 500 μl cell lysate per IP reaction was incubated with 10 μg anti-Egr-1 antibody (cat. no. 4154; Cell Signaling Technology, Inc.) or anti-Flag antibody (cat. no. 14793; Cell Signaling Technology, Inc.) for 16 h at -4˚C, followed by incubation with Protein A/G MagBeads (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After the cross-linking was unfastened with 0.2 μM NaCl at 65˚C for 6 h, the DNA was subjected to PCR with a Taq DNA Polymerase (Beyotime Institute of Biotechnology) using the following conditions: 30 sec denaturation at 95˚C, followed by 35 cycles of denaturation at 95˚C for 15 sec, annealing at 55˚C for 1 min and extension at 72˚C for 1 min. The primers for amplifying the fragments of CDKL1 promoter were: Sense, 5'-GCAAATCTAGCAGGTCTGC-3' and antisense, 5'-GTTCTAATTTCGGGTCTCA-3'.

Transwell assay. For migration, 50,000 cells were seeded into the upper chambers containing serum-free DMEM. The lower reservoir contained DMEM with 15% FBS. After 10 h of culture at 37˚C, the cells in the upper chambers were removed, and the cells that crossed the membrane were stained with crystal violet reagent (Beyotime Institute of Biotechnology) at 25˚C for 5 min. The stained cells were observed under a light microscope (Olympus Corporation) at x200 magnification and counted in six randomly selected visual fields.

The invasion assays were performed using the same procedures as the migration assays, except that the upper surface of the membrane was precoated with 20 μg Matrigel (Sigma-Aldrich; Merck KGaA) at 37˚C for 2 h and the number of seeded cells was 100,000 cells/chamber.

Cell Counting Kit-8 (CCK-8) cell proliferation assay. Cells were seeded into 96-well plates at 5,000 cells/well. After 0, 24 and 48 h of culture at 37˚C, 10 μl CCK-8 reagent (Dojindo Molecular Technologies, Inc.) was added into each well, and the plate was incubated at 37˚C for 2 h. The absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Statistical analysis. Data were analyzed with SPSS software version 19.0 (IBM Corp.). The log-rank test was used to analyze the association between Egr-1 expression and patient survival. Data of qPCR, Transwell, CCK-8 and luciferase assays were repeated three times and presented as the mean ± SD, and differences were analyzed by one-way ANOVA followed by Tukey's post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association of Egr-1 expression and the survival of patients with colon adenocarcinoma. UALCAN is a comprehensive, user-friendly and interactive web resource for analyzing

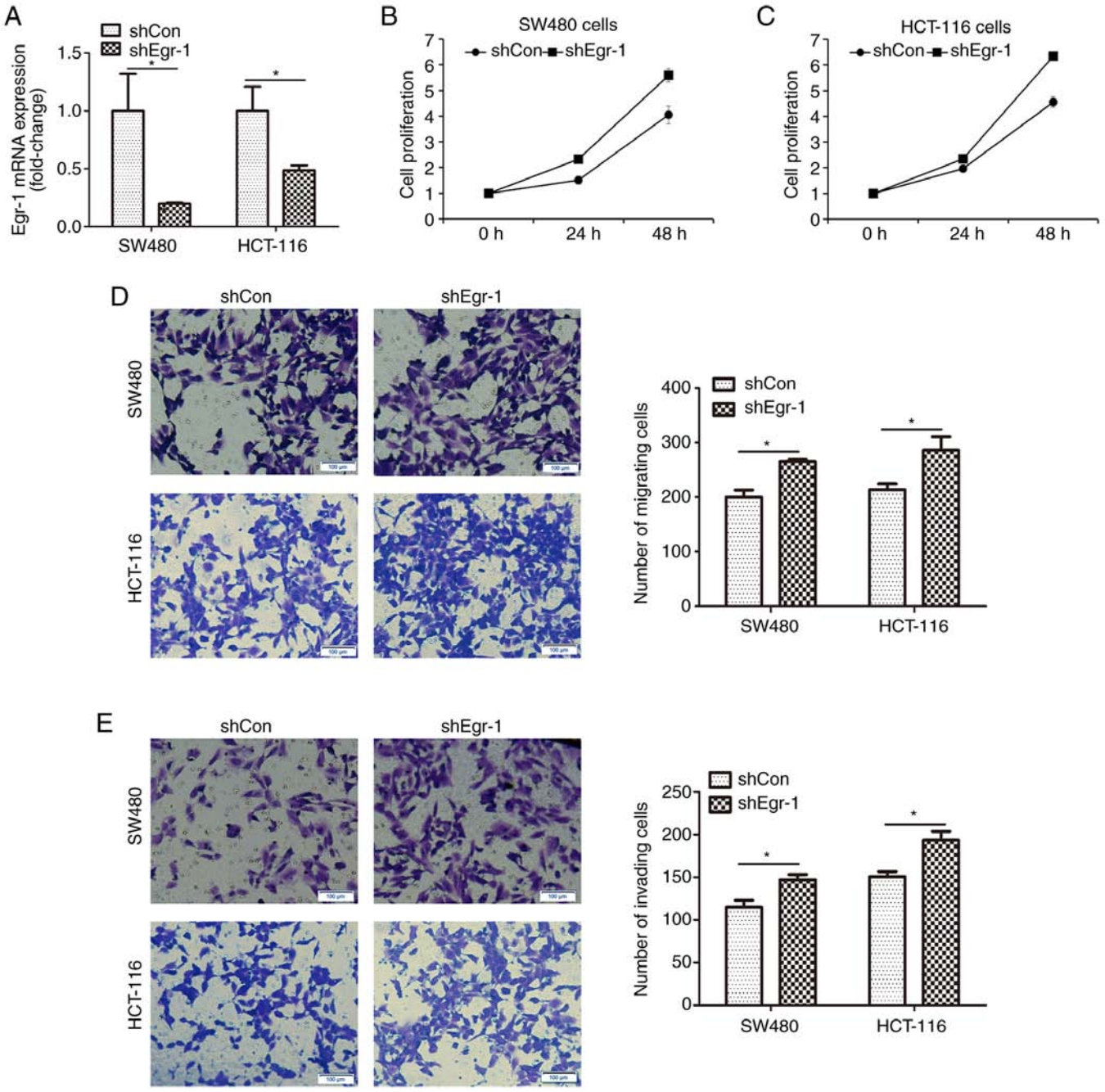

Figure 2. Knockdown of Egr-1 induces colon cancer cell proliferation, migration and invasion. (A) Egr-1 shCon and shEgr-1 lentiviruses were transfected into SW480 and HCT-116 cells, and Egr-1 mRNA expression was detected via reverse transcription-quantitative PCR. Proliferation of (B) SW480 and (C) HCT-116 cells with or without Egr-1-knockdown was detected via Cell Counting Kit-8 assay. (D) Migration and (E) invasion of cells were determined via Transwell assay (scale bar, 100 μm). *P<0.05. Egr-1, early growth response 1; sh, short hairpin; Con, control.

cancer OMICS data (31). Based on UALCAN, the present study analyzed the association of Egr-1 expression with the survival of patients with colon adenocarcinoma, which is one of the main types of colon cancer. As shown in Fig. 1, patients with higher Egr-1 expression exhibited an improved survival rate than patients with lower Egr-1 expression. These data indicated that Egr-1 may function as a prognostic biomarker for improved survival of patients with colon adenocarcinoma. Since there is no data about colon carcinoma, another main type of colon cancer, the association of Egr-1 expression with the survival of patients with colon carcinoma was not analyzed.

Knockdown of Egr-1 induces colon cancer cell proliferation, migration and invasion. Egr-1-knockdown was achieved by infecting SW480 and HCT-116 cells with shEgr-1 lentivirus or shCon lentivirus used as the negative control. Fig. 2A indicates that after infection, Egr-1 mRNA expression was significantly decreased in both cell lines, indicating that Egr-1 was successfully knocked down. Cells with or without Egr-1-knockdown were subjected to CCK-8 assay. As shown in Fig. 2B and C, knockdown of Egr-1 increased the proliferation of SW480 and HCT-116 cells. Furthermore, cell migration and invasion were measured by Transwell assay. As shown in Fig. 2D, the migratory capacity of both SW480 and HCT-116 cells was

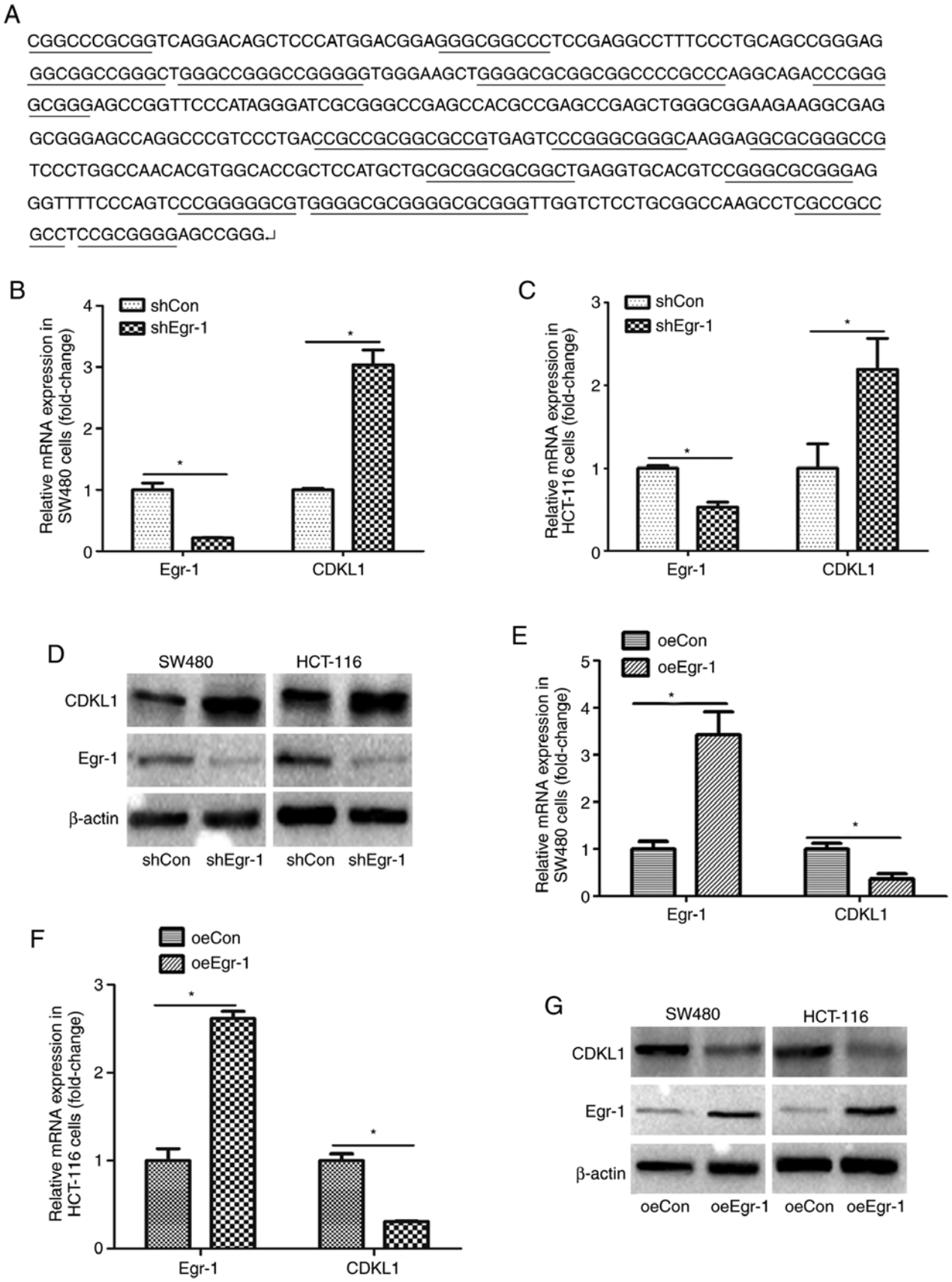

Figure 3. Egr-1 negatively regulates CDKL1. (A) Sequence of -1195/-793 (translational starting site as +1) region of Egr-1 promoter. The CDKL1 promoter sequence was taken from GenBank (www.ncbi.nlm.nih.gov/gene/). The underlined sites are segments containing continuous GC. Egr-1 and CDKL1 mRNA expression was detected via RT-qPCR in (B) SW480 and (C) HCT-116 cells with Egr-1-knockdown. (D) Egr-1 and CDKL1 protein expression was detected via western blotting in SW480 and HCT-116 cells with Egr-1-knockdown. Egr-1 and CDKL1 mRNA expression was detected via RT-qPCR in (E) SW480 and (F) HCT-116 cells with Egr-1 overexpression. (G) Egr-1 and CDKL1 protein expression was detected via western blotting in SW480 and HCT-116 cells with Egr-1 overexpression. *P<0.05. CDKL1, cyclin-dependent kinase-like 1; RT-qPCR, reverse transcription-quantitative PCR; Egr-1, early growth response 1; sh, short hairpin; Con, control; oe, overexpression.

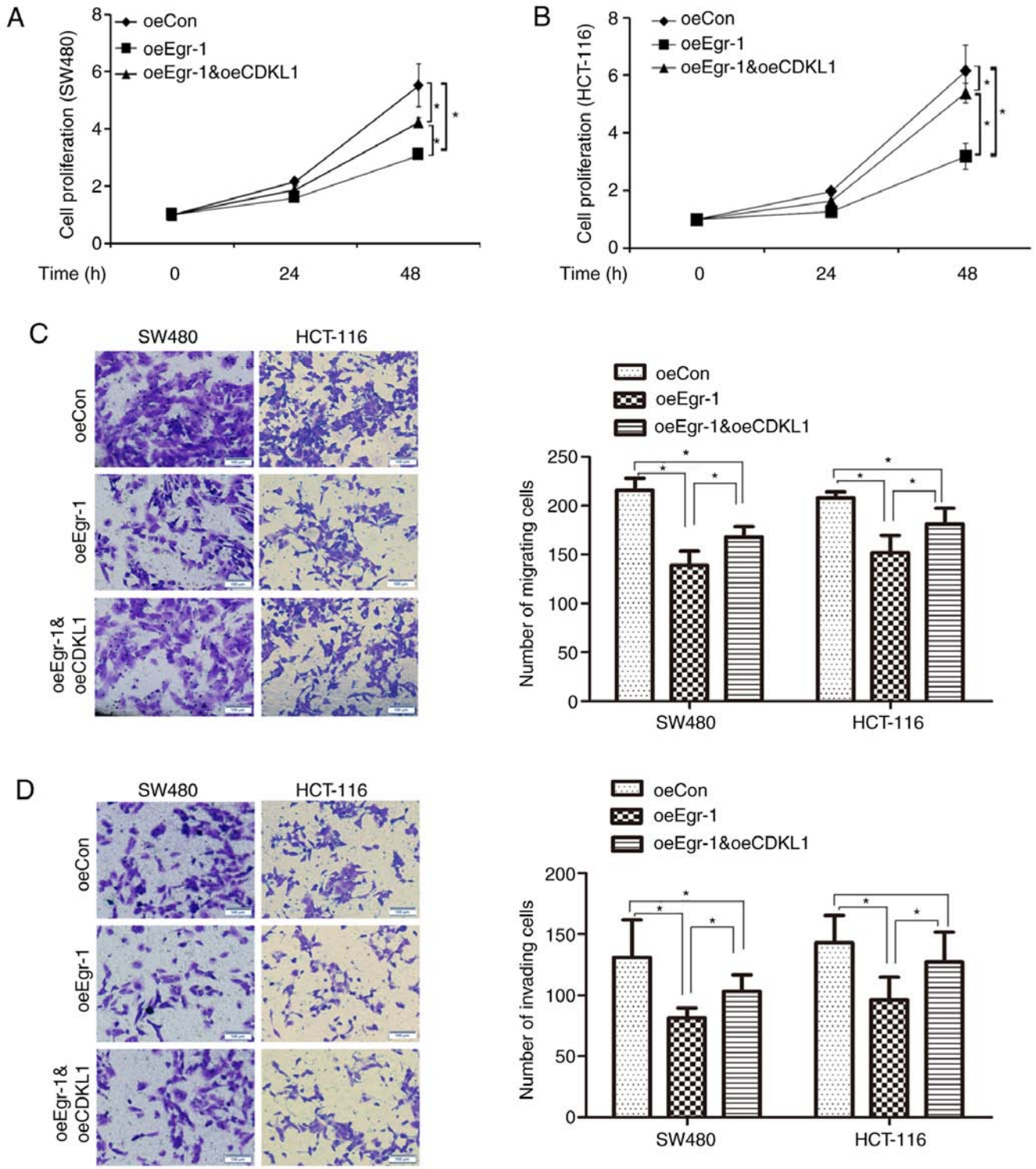

Figure 4. Egr-1 regulates colon cancer cell proliferation, migration and invasion through CDKL1. Proliferation of (A) SW480 and (B) HCT-116 cells with Egr-1 overexpression or with Egr-1 and CDKL1 overexpression was detected via Cell Counting Kit-8 assay. (C) Migration and (D) invasion of SW480 and HCT-116 cells with Egr-1 overexpression or with Egr-1 and CDKL1 overexpression were evaluated via Transwell assays (scale bar, 100 μm). *P<0.05. CDKL1, cyclin-dependent kinase-like 1; Egr-1, early growth response 1; Con, control; oe, overexpression.

significantly improved by Egr-1-knockdown; similarly, the invasive capacity of both cell lines was significantly enhanced (Fig. 2E). These results indicated the inhibitory role of Egr-1 in colon cancer progression.

Egr-1 negatively regulates CDKL1 expression. The CDKL1 promoter sequence was obtained from GenBank (www.ncbi.nlm.nih.gov/gene/). In some regions of the CDKL1 promoter, the content of G and C reached >80%, and there were several segments containing continuous GC, indicating that CDKL1 possessed a GC-rich promoter (Fig. 3A). Therefore, it was hypothesized that CDKL1 may be a target gene of Egr-1. CDKL1 expression was first analyzed following Egr-1-knockdown. The mRNA of SW480 and HCT-116 cells was extracted, and

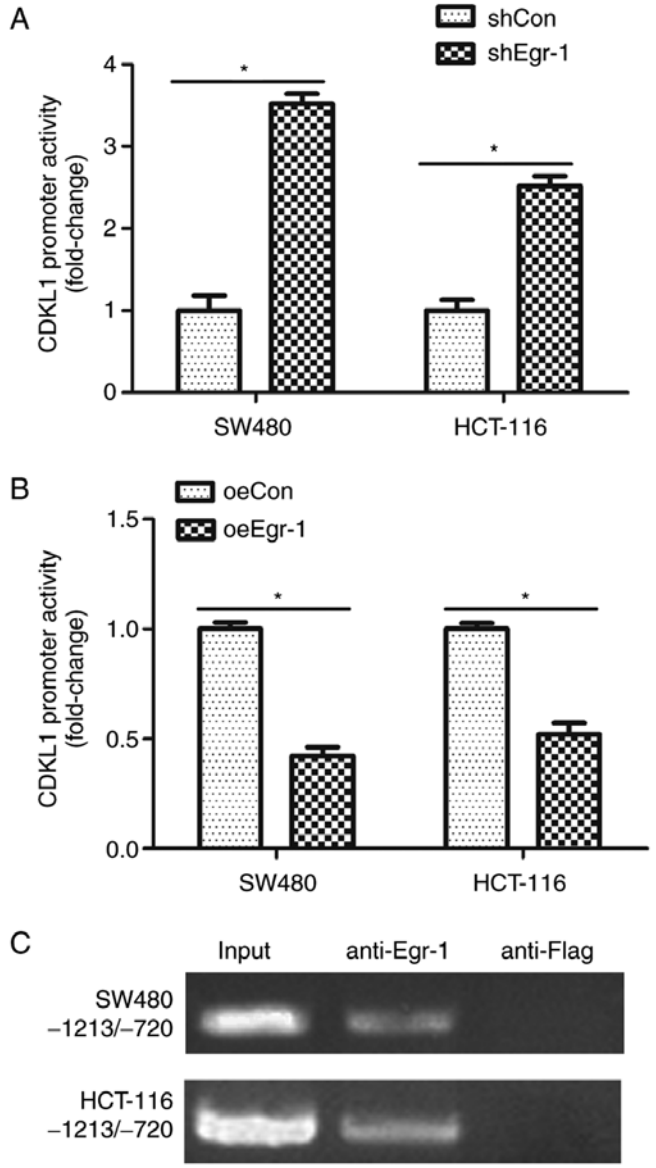

Figure 5. Egr-1 negatively regulates CDKL1 expression by modulating the promoter activity. pGL3-CDKL1 was transfected into SW480 cells with (A) Egr-1-knockdown or (B) Egr-1 overexpression, and luciferase activity was detected. *P<0.05. (C) Chromatin immunoprecipitation assay was performed to determine whether Egr-1 was bound to the -1213/-720 region of the CDKL1 promoter. CDKL1, cyclin-dependent kinase-like 1; Egr-1, early growth response 1; sh, short hairpin; Con, control; oe, overexpression.

CDKL1 expression was measured using RT-qPCR. As shown in Fig. 3B and C, CDKL1 mRNA expression was significantly higher in cells with Egr-1-knockdown. Additionally, western blot analysis indicated that CDKL1 protein expression was upregulated following Egr-1-knockdown (Fig. 3D). Furthermore, CDKL1 expression was detected under Egr-1 overexpression in SW480 and HCT-116 cells. As shown in Fig. 3E-G, both CDKL1 mRNA and protein expression was decreased when Egr-1 was overexpressed. These results indicated that CDKL1 was negatively regulated by Egr-1.

Egr-1 regulates colon cancer cell proliferation, migration and invasion through CDKL1. As CDKL1 was identified as a target gene of Egr-1, whether Egr-1 regulated colon cancer cell proliferation, migration and invasion through CDKL1 was further analyzed. CDKL1 was overexpressed in colon cancer cells with Egr-1 overexpression, and cell proliferation, migration and invasion were measured. As shown in Fig. 4A and B, Egr-1 overexpression significantly inhibited the proliferation of SW480 and HCT-116 cells, while the inhibitory role of Egr-1 was reversed by CDKL1 overexpression. As shown in Fig. 4C and D, Egr-1 overexpression inhibited the migration and invasion of SW480 and HCT-116 cells, while the inhibitory role of Egr-1 was reversed by CDKL1 overexpression. Therefore, Egr-1 may inhibit colon cancer progression at least partially through downregulating CDKL1.

Egr-1 negatively regulates CDKL1 expression through modulating the promoter activity. As Egr-1 overexpression downregulated CDKL1 expression and correspondingly inhibited colon cancer cell proliferation, migration and invasion, it was necessary to reveal the mechanism by which Egr-1 regulated CDKL1. A CDKL1 promoter-reporter, pGL3-CDKL1, was constructed in the present study. The luciferase assay showed that knockdown of Egr-1 significantly increased CDKL1 promoter activity in both SW480 and HCT-116 cells (Fig. 5A); however, overexpression of Egr-1 significantly inhibited CDKL1 promoter activity in both SW480 and HCT-116 cells (Fig. 5B). A ChIP assay was also performed, revealing that Egr-1 could bind to the CDKL1 promoter region in both SW480 and HCT-116 cells (Fig. 5C). These results indicated that Egr-1 regulated CDKL1 at the transcriptional level.

Discussion

The present study demonstrated that Egr-1 regulated colon cancer cell proliferation, migration and invasion through CDKL1. Additionally, the current results indicated that Egr-1 modulated CDKL1 expression at the transcriptional level.

Egr-1 has been previously reported to be overexpressed in colon cancer (33). Egr-1 is involved in tumor progression via regulating cell proliferation, apoptosis, cell migration and invasion (8-10). Considering the important roles of Egr-1 in tumor progression, it has been suggested as a promising target for tumor therapy. The present study demonstrated that high Egr-1 expression was associated with improved patient survival and inhibited colon cancer cell proliferation, migration and invasion. However, there are several controversial reports about the function of Egr-1 in tumor progression. A study by Sun *et al* (9) has reported that Egr-1 promotes the proliferation and invasion of gastric cancer cells. Additionally, Scharnhorst *et al* (34) reported the tumor-promoting role of Egr-1 in Wilms' tumor. In colon cancer, it is reported that Egr-1 is associated with lymphovascular invasion, lymph node and distant metastasis, indicating a positive role in tumor progression (35). However, in another study, Egr-1 exerted a tumor-suppressive role and participated in tolfenamic acid-induced apoptosis in colon cancer cells (33). The conclusions of the present study are in agreement with the latter study and suggested the tumor-suppressive role of Egr-1 in colon cancer. The dual functions of Egr-1 in the regulation of tumor progression may due to the individual difference of cell types or patients.

Egr-1 participates in tumor progression by regulating several targets. For example, Egr-1 suppresses breast cancer cell proliferation via downregulating Cyclin Ds (36). However, the mechanisms by which Egr-1 regulates colon cancer cell progression remain unclear. In the current study, it was revealed that the inhibitory roles of Egr-1 in cell proliferation, migration and invasion were partially abolished by CDKL1, indicating that Egr-1 participates in colon cancer progression via regulating CDKL1.

In the present study, Egr-1-knockdown increased CDKL1 mRNA and protein expression, while overexpression of Egr-1 decreased CDKL1 mRNA and protein expression, indicating that Egr-1 may be an inhibitor of CDKL1 expression. CDKL1 was identified as a novel Egr-1 target gene. Additionally, the present study revealed that Egr-1 inhibited CDKL1 expression by blocking CDKL1 transcription. Egr-1 binds to GC-rich regions at the promoter of target genes (18-20). There are several GC-rich regions at the CDKL1 promoter, and the current study has demonstrated that Egr-1 could bind to the promoter of CDKL1; however, a limitation of the present study is that the accurate binding site was not determined. To the best of our knowledge, the current study is the first to suggest that Egr-1 may regulate CDKL1 transcription. In addition to Egr-1, there are other transcription factors that can bind to GC-rich region of promoters, such as specific protein 1 (Sp1), GC binding factor 2 and Kruppel-like factors (37-39); therefore, these transcription factors may also regulate CDKL1 at the transcriptional level. The binding sites of some transcription factors may overlap in a promoter, such as the binding sites of Sp1 and Egr-1 overlapping in the promoter of nasopharyngeal carcinoma-associated gene 6, inducing competitive binding of the related transcription factors (40). Whether the binding sites of the aforementioned transcription factors are overlapping should be further investigated in future studies.

In conclusion, the present study indicated that Egr-1 bound to the promoter of CDKL1 and inhibited its expression, and correspondingly inhibited colon cancer cell proliferation, migration and invasion. The current study suggested that Egr-1/CDKL1 may be used as promising prognostic biomarkers and therapeutic targets for colon cancer.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Figshare repository, https://figshare.com/s/1720cea2192d08d21b5f.

Authors' contributions

SS performed the cell biological experiments, such as CCK-8, Transwell and cell infection assays. MJ performed the western blot and RT-qPCR assays. JL constructed the pGL3-CDKL1 plasmid. XL performed the luciferase activity assay. HL analyzed the association of Egr-1 expression with the survival of patients with colon adenocarcinoma. DW performed the ChIP assay and confirmed the authenticity of all the raw data. CX performed statistical analysis and confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA and Jemal A: Colorectal cancer statistics, 2020. CA Cancer J Clin 70: 145-164, 2020.
2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70: 7-30, 2020.
3. Brody H: Colorectal cancer. Nature 521: S1, 2015.
4. Weitz J, Koch M, Debus J, Hohler T, Galle PR and Buchler MW: Colorectal cancer. Lancet 365: 153-165, 2005.
5. Vatandoust S, Price TJ and Karapetis CS: Colorectal cancer: Metastases to a single organ. World J Gastroenterol 21: 11767-11776, 2015.
6. Wrobel P and Ahmed S: Current status of immunotherapy in metastatic colorectal cancer. Int J Colorectal Dis 34: 13-25, 2019.
7. Park YS, Kim HS, Kim JH, Choi SH, Kim DS, Ryoo ZY, Kim JY and Lee S: NAB2-STAT6 fusion protein mediates cell proliferation and oncogenic progression via EGR-1 regulation. Biochem Biophys Res Commun 526: 287-292, 2020.
8. Yi L, Lyn YJ, Peng C, Zhu RL, Bai SS, Liu L, Wang PX, Zhou H and Dong Y: Sinomenine inhibits fibroblast-like synoviocyte proliferation by regulating α7nAChR expression via ERK/Egr-1 pathway. Int Immunopharmacol 56: 65-70, 2018.
9. Sun T, Tian H, Feng YG, Zhu YQ and Zhang WQ: Egr-1 promotes cell proliferation and invasion by increasing β-catenin expression in gastric cancer. Dig Dis Sci 58: 423-430, 2013.
10. Lee BS, Kang S, Kim KA, Song YJ, Cheong KH, Cha HY and Kim CH: Met degradation by SAIT301, a Met monoclonal antibody, reduces the invasion and migration of nasopharyngeal cancer cells via inhibition of EGR-1 expression. Cell Death Dis 5: e1159, 2014.
11. Liu P, Li J, Lu H and Xu B: Thalidomide inhibits leukemia cell invasion and migration by upregulation of early growth response gene 1. Leuke Lymphoma 50: 109-113, 2009.
12. Skorokhod A, Bachmann J, Giese NA, Martignoni ME and Krakowski-Roosen H: Real-imaging cDNA-AFLP transcript profiling of pancreatic cancer patients: Egr-1 as a potential key regulator of muscle cachexia. BMC Cancer 12: 265, 2012.
13. Kong Y, Yin J, Fu Y, Chen Y, Zhou Y and Geng X: Suppression of Elk1 inhibits thyroid cancer progression by mediating PTEN expression. Oncol Rep 40: 1769-1776, 2018.
14. Kim SO, Kwon JI, Jeong YK, Kim GY, Kim ND and Choi YH: Induction of Egr-1 is associated with anti-metastatic and anti-invasive ability of beta-lapachone in human hepatocarcinoma cells. Biosci Biotechnol Biochem 71: 2169-2176, 2007.
15. Calogero A, Porcellini A, Lombari V, Fabbiano C, Arcella A, Miscusi M, Ponti D and Ragona G: Sensitivity to cisplatin in primary cell lines derived from human glioma correlates with levels of EGR-1 expression. Cancer Cell Int 11: 5, 2011.
16. Myung E, Park YL, Kim N, Chung CY, Park HB, Park HC, Myung DS, Kim JS, Cho SB, Lee WS and Joo YE: Expression of early growth response-1 in human gastric cancer and its relationship with tumor cell behaviors and prognosis. Pathol Res Pract 209: 692-699, 2013.

17. Li TT, Liu MR and Pei DS: Friend or foe, the role of EGR-1 in cancer. Med Oncol 37: 7, 2019.
18. Sukhatme VP, Cao XM, Chang LC, Tsai-Morris CH, Stamenkovich D, Ferreira PC, Cohen DR, Edwards SA, Shows TB, Curran T, *et al*: A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. Cell 53: 37-43, 1988.
19. Thiel G, Muller I and Rossler OG: Expression, signaling and function of Egr transcription factors in pancreatic β-cells and insulin-responsive tissues. Mol Cell Endocrinol 388: 10-19, 2014.
20. Kubosaki A, Tomaru Y, Tagami M, Arner E, Miura H, Suzuki T, Suzuki M, Suzuki H and Hayashizaki Y: Genome-wide investigation of in vivo EGR-1 binding sites in monocytic differentiation. Genome Biol 10: R41, 2009.
21. Thiel G and Cibelli G: Regulation of life and death by the zinc finger transcription factor Egr-1. J Cell Physiol 193: 287-292, 2002.
22. Silverman ES, Du J, Williams AJ, Wadgaonkar R, Drazen JM and Collins T: cAMP-response-element-binding-protein-binding protein (CBP) and p300 are transcriptional co-activators of early growth response factor-1 (Egr-1). Biochem J 336: 183-189, 1998.
23. Vo N and Goodman RH: CREB-binding protein and p300 in transcriptional regulation. J Biol Chem 276: 13505-13508, 2001.
24. Cartron PF, Blanquart C, Hervouet E, Gregoire M and Vallette FM: HDAC1-mSin3a-NCOR1, Dnmt3b-HDAC1-Egr1 and Dnmt1-PCNA-UHRF1-G9a regulate the NY-ESO1 gene expression. Mol Oncol 7: 452-463, 2013.
25. Meyerson M, Enders GH, Wu CL, Su LK, Gorka C, Nelson C, Harlow E and Tsai LH: A family of human cdc2-related protein kinases. EMBO J 11: 2909-2917, 1992.
26. Yen SH, Kenessey A, Lee SC and Dickson DW: The distribution and biochemical properties of a Cdc2-related kinase, KKIALRE, in normal and Alzheimer brains. J Neurochem 65: 2577-2584, 1995.
27. Tang L, Gao Y, Yan F and Tang J: Evaluation of cyclin-dependent kinase-like 1 expression in breast cancer tissues and its regulation in cancer cell growth. Cancer Biother Radiopharm 27: 392-398, 2012.
28. Sun W, Yao L, Jiang B, Shao H, Zhao Y and Wang Q: A role for Cdkl1 in the development of gastric cancer. Acta Oncol 51: 790-796, 2012.
29. Song Z, Lin J, Sun Z, Ni J and Sha Y: RNAi-mediated downregulation of CDKL1 inhibits growth and colony-formation ability, promotes apoptosis of human melanoma cells. J Dermatol Sci 79: 57-63, 2015.
30. Qin C, Ren L, Ji M, Lv S, Wei Y, Zhu D, Lin Q, Xu P, Chang W and Xu J: CDKL1 promotes tumor proliferation and invasion in colorectal cancer. Onco Targets Ther 10: 1613-1624, 2017.
31. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S: UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 19: 649-658, 2017.
32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
33. Lee SH, Bahn JH, Choi CK, Whitlock NC, English AE, Safe S and Baek SJ: ESE-1/EGR-1 pathway plays a role in tolfenamic acid-induced apoptosis in colorectal cancer cells. Mol Cancer Ther 7: 3739-3750, 2008.
34. Scharnhorst V, Menke AL, Attema J, Haneveld JK, Riteco N, van Steenbrugge GJ, van der Eb AJ and Jochemsen AG: EGR-1 enhances tumor growth and modulates the effect of the Wilms' tumor 1 gene products on tumorigenicity. Oncogene 19: 791-800, 2000.
35. Myung DS, Park YL, Kim N, Chung CY, Park HC, Kim JS, Cho SB, Lee WS, Lee JH and Joo YE: Expression of early growth response-1 in colorectal cancer and its relation to tumor cell proliferation and apoptosis. Oncol Rep 31: 788-794, 2014.
36. Wei LL, Wu XJ, Gong CC and Pei DS: Egr-1 suppresses breast cancer cells proliferation by arresting cell cycle progression via down-regulating CyclinDs. Int J Clin Exp Pathol 10: 10212-10222, 2017.
37. Ma Y, Ren Y and Guan J: Knockdown of GC binding factor 2 by RNA interference inhibits invasion and migration of vascular smooth muscle cells. Mol Med Rep 20: 1781-1789, 2019.
38. Kou XX, Hao T, Meng Z, Zhou YH and Gan YH: Acetylated Sp1 inhibits PTEN expression through binding to PTEN core promoter and recruitment of HDAC1 and promotes cancer cell migration and invasion. Carcinogenesis 34: 58-67, 2013.
39. Buttar NS, DeMars CJ, Lomberk G, Rizvi S, Bonilla-Velez J, Achra S, Rashtak S, Wang KK, Fernandez-Zapico ME and Urrutia R: Distinct role of Kruppel-like factor 11 in the regulation of prostaglandin E2 biosynthesis. J Biol Chem 285: 11433-11444, 2010.
40. Liu M, Wang X, Peng Y, Shen S and Li G: Egr-1 regulates the transcription of NGX6 gene through a Sp1/Egr-1 overlapping site in the promoter. BMC Mol Biol 15: 14, 2014.