Effect of CDX2 on proliferation, invasion, migration, and apoptosis of duodenal cancer cells

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ABSTRACT

This study investigates the expression and biological role of caudal homologous transcription factor 2 (CDX2) in duodenal carcinoma. Paraffin-embedded samples from 40 duodenal carcinoma cases were analyzed using immunohistochemistry on a tissue microarray to assess CDX2 expression and its prognostic significance. CDX2 overexpression plasmids and CDX2-siRNA were transfected into colon and duodenal cancer cells. Transfection efficiency was confirmed by RT-PCR and Western blotting. Cell proliferation was assessed using CCK8 assay, migration *via* scratch assay, and cell cycle and apoptosis by flow cytometry. CDX2 staining was primarily nuclear, with a positive rate of 65% in duodenal carcinoma tissues, significantly lower than in adjacent non-tumor tissues ($p<0.05$). CDX2-positive patients had better prognoses than negative patients ($p<0.05$). Reduced CDX2 expression significantly enhanced the proliferation of $CaCO_2$ and HuTu-80 cells ($p<0.001$), whereas CDX2 overexpression suppressed proliferation ($p<0.001$). CDX2 knockdown increased migration, while its overexpression reduced migration ($p<0.05$). CDX2 overexpression led to a significant increase in G_0/G_1 phase cells and a decrease in S phase cells ($p<0.05$), whereas knockdown reduced G_0/G_1 phase cells and increased S and G_2/M phase cells ($p<0.05$). Apoptosis was significantly increased following CDX2 overexpression ($p<0.001$) and decreased after CDX2 knockdown ($p<0.001$). CDX2 expression is downregulated or lost in duodenal carcinoma, acting as a tumor suppressor. CDX2 may serve as a crucial biomarker for predicting the onset, progression, and treatment of duodenal carcinoma.

Key words: duodenal carcinoma; caudal homologous transcription factor 2; cell apoptosis; tumor suppressor gene.

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Contributions: all authors made a substantive intellectual contribution, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no conflict of interest.

Ethical approval: the study was approved by the Jinling Hospital Medical Ethics Committee (approval no. DZGZRDW2400112).

Availability of data and materials: the data used to support the findings of this study are available from the corresponding author upon reasonable request.

Funding: this work was supported by the Chinese Society of Clinical Oncology-Haoseng Oncology Research Foundation (Y-HS2017-066) and the Wu Jieping Medical Foundation (320.6750.2022-18-51).

Introduction

One of duodenal carcinoma is a rare malignant tumor. The global number of new cases of small bowel cancer in 2021 is 11,390, 60% of which are duodenal cancers. Its incidence accounts for 3% of all digestive tract tumors, and is only 1/100-1/50 of colorectal cancer.[1] If the disease can be surgically resected, the 5-year survival rate is 46%,[2] but most patients are diagnosed in the advanced stage and can only receive palliative care, and the 5-year survival rate is only 14-33%.[3] Due to the low incidence of duodenal carcinoma, the pathogenesis and treatment of this disease have not been fully studied. Therefore, its treatment is still dominated by traditional chemotherapy and lacks targeted molecular targeted therapy. By means of molecular biology, it is of great clinical significance to explore new markers to predict the occurrence and development of duodenal cancer and further discover new therapeutic targets for the prevention and treatment of duodenal cancer. The caudal homologous transcription factor 2 (CDX2) is a gut-specific transcription factor mainly expressed in the small intestine and colorectal, and is a key regulatory protein for intestinal epithelial formation and differentiation. In patients with colorectal cancer, loss of CDX2 expression is a poor prognostic factor, while positive CDX2 is associated with better prognosis.[4] However, the study of CDX2 in duodenal carcinoma is rarely reported. Studies have shown that small intestine and colorectal cancer are not only structurally adjacent, but also have similarities in tissue origin, and their chemotherapy is often referred to colorectal cancer.[5] Considering that CDX2 plays an important role in epithelial formation and differentiation of small intestine and colorectal cancer, and CDX2 has been shown to be closely related to the occurrence and development of colorectal cancer, we speculate that CDX2 may also play a role in the occurrence and development of duodenal cancer. Therefore, in this study, the expression of CDX2 protein in duodenal carcinoma tissues was detected by immunohistochemistry, and endogenous CDX2 expression was regulated in tumor cells to observe its effects on their proliferation, invasion, migration and apoptosis.

Materials and Methods

Tissue sample

Forty paraffin specimens of duodenal carcinoma excised, which were archived in the pathology department of our hospital from 2012 to 2016, were selected. The pathological diagnosis results were all adenocarcinoma after review by two pathologists, including 25 males and 15 females. There were 24 cases with age ≤60 years old, 16 cases with age >60 years old, and the median age was 60 years old. There were 30 cases of differentiation and 10 cases of low differentiation. There were 25 cases of adjacent non-tumor tissue. Among these patients, there were only one case having the primary lesion located in the bulb, thirty-six cases in the descending part, two cases in the horizontal part, and one case in the ascending part. Due to the significant disparity in the distribution of cases across the four anatomical regions, where the majority of patients exhibited primary lesions concentrated in the descending part and fewer in the other regions, it was deemed inappropriate to conduct further subgroup analyses. Consequently, the relationship between the expression differences of CDX2 in the aforementioned regions and prognosis was not analyzed. Inclusion criteria for this study include individuals aged 18 to 80 years with an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0 or 1, and those with histologically or cytologically confirmed duodenal adenocarcinoma. Exclusion criteria encompass patients with non-adenocarcinoma duodenal tumors, those with pancreatobiliary-type adenocarcinoma of the duodenum, individuals with a history of severe bleeding tendencies or coagulation dysfunctions, and those with a history of immunodeficiency. The study was approved by the the Jinling Hospital Medical Ethics Committee (DZGZRDW2400112).

Immunohistochemistry

Tissue sections of 4 μm obtained from tissue array blocks underwent deparaffinization using xylene, followed by alcohol rehydration and antigen retrieval in citrate buffer at 96°C for 15 min. After cooling to room temperature, the slides were treated to block endogenous peroxidase activity with a solution of 0.3% H_2O_2 and 1% methanol. Subsequently, non-specific binding was blocked using 1% bovine serum albumin in Phosphate Buffered Saline (PBS). The sections were then incubated overnight at 4°C with a primary antibody recognizing CDX2 protein (ABclonal Technology, Wuhan, China, A19030, dilution 1:200) diluted in 1% PBS. Following this, the sections were incubated for 30 min at room temperature with a secondary antibody (Abcam, Cambridge, UK, Anti-Rabbit IgG, ab99697, dilution 1:2000). After slight drying of the sections, DAB solution was applied. Under a microscope, the color development time was monitored (generally within 2 min), resulting in a positive color of brownish yellow. Finally, the sections were counterstained with hematoxylin and dehydrated. Positively stained cells were identified as those appearing brownish yellow or yellowish brown under the microscope. Histomorphological analysis was performed using a light microscope (Olympus, Tokyo, Japan, CX41) along with the Pathology and Information Management System (Jieda Technology, Nanjing, China, V5.1) using 20x and 40x objectives for image acquisition. Semi-quantitative analysis of the stained cells was conducted using the immunoreactive score (IRS) classification by analyzing five random fields in each slide. The scoring was evaluated by two independent reviewers analyzing images at 200x magnification, with results categorized as negative, weak, intermediate, or strong staining. Each test was performed a minimum of three times. Positive controls were utilized according to the manufacturer's specifications, while negative controls were established by omitting the primary antibody (Figure 1).

Cell transfection

Human colorectal adenocarcinoma cell line CaCO2 and human duodenal adenocarcinoma cell line HuTu-80 (donated by Shanghai Shengbo Biomedical Technology Co., LTD., Shanghai, China) were selected and cultured in an incubator with 10% fetal bovine serum (Gibco, Rockville, MD, USA) in DMEM medium (Hyclone, South Logan, UT, USA) at 37°C and 5% CO_2. The CDX2 overexpression plasmid and CDX2 knockdown plasmid were obtained from Shanghai Jikai Gene Technology Co., Ltd. (Shanghai, China). Prior to the transfection of CDX2-siRNA and the overexpression plasmid, bowel cancer cells were digested, counted, and seeded in 6 cm petri dishes. When cell growth reached 70-80%, siRNA and the overexpression plasmid were transfected into the bowel cancer cells using FuGENE® 6 transfection reagent (Promega, Madison, WI, USA), following the manufacturer's instructions. A control group was established, and after 48 h of culture, the supernatant was discarded and the cells were collected. The efficiency of interference and overexpression was assessed using qPCR and Western blotting. The siRNA sequences utilized in the experiment were: agCCCTTGAGTCCGGTGTCTT (C98), ccGCAGAGCAAAGGAGAGGAA (C99), and agAC AAATATCGAGTGGTGTA (C96).

RT-PCR RNA

Total RNA was extracted from CaCO2 and HuTu-80 cells before and after transfection using the TRIzol kit (Invitrogen, Carlsbad, CA, USA). RNA concentration and quality were assessed using the Nano1000, and reverse transcription was performed to synthesize cDNA with a reverse transcription kit (TaKaRa, Tokyo, Japan). The SYBR Green PCR kit (TaKaRa) was employed for the PCR amplification of CDX2 and internal reference genes. The primer sequences used were as follows: CDX2 upstream primer: 5'-GAACCTGTGCGAGTGGATG-3' and downstream primer: 5'-CGGATGGTGATGTAGCGAC-3'. The internal reference gene β-actin was amplified using the upstream primer 5'-CATGTACGTTGCTATCCAGGC-3' and the downstream primer 5'-CTCCTTAATGTCACGCAGAT-3'. The PCR reaction conditions were as follows: pre-denaturation at 94°C for 5 min, followed by 50 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s; finally, an extension at 72°C for 5 min and cooling at 4°C for 5 min. The ΔΔCt and RQ values were calculated, and the expression level of CDX2 was determined based on these values.

Western blotting

In this study, the PIAP protein cleavage reagent was applied across all experimental groups. Protein supernatants were obtained through ultrasonic disruption, and their concentrations were quantified using the BCA method. The mass concentration of the SDS-PAGE gel was set at 100 g/L. Protein samples were combined with loading buffer and subjected to a boiling water bath for 5 min. Electrophoresis was conducted at a constant voltage of 90 V until bromophenol blue reached the bottom edge of the glass plate. The gel was subsequently removed and placed in transfer buffer. A PVDF membrane was cut to an appropriate size, and proteins were transferred at a current of 300 mA for 45 min. Following transfer, the PVDF membrane was incubated in a solution of 50 g/L skim milk powder for 1 h, then stored at 4 °C overnight with primary antibodies targeting the following proteins: CDX2 (ABclonal Technology, A19030, dilution 1:500) and GAPDH (Abcam, ab9482, dilution 1:1000). After incubation, the membranes were washed three times with washing solution for 10 min each and then incubated with a secondary antibody (Abcam, Anti-Rabbit IgG, ab99697, dilution 1:2000) for 1 h at room temperature. The membranes were washed again with washing solution for three 10-min intervals before color development using ECL (Beyotime Biotechnology, Beijing, China). Finally, the gray values of the target band CDX2 and the internal reference GAPDH were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA).

Cell proliferation experiment

The proliferative capacity of CaCO2 and HuTu-80 cells was assessed before and after transfection with CDX2-siRNA and a CDX2 overexpression plasmid using the CCK8 reagent (Tontonique, Japan). The transfected CaCO2 and HuTu-80 cells were digested, suspended, and counted using pancreatic enzymes, adjusting the cell density to 5000 cells/mL. A total of 100 μL of the cell suspension was added to each well of a 96-well plate, followed by the addition of 10 μL of CCK8 reagent to each well. Measurements were taken at 24, 48, and 72 h post-incubation, with a 4 h incubation period and subsequent mixing by shaking. The optical density (OD) at a wavelength of 450 nm was measured using an enzyme reader. This experiment was repeated three times, and the mean value was calculated.

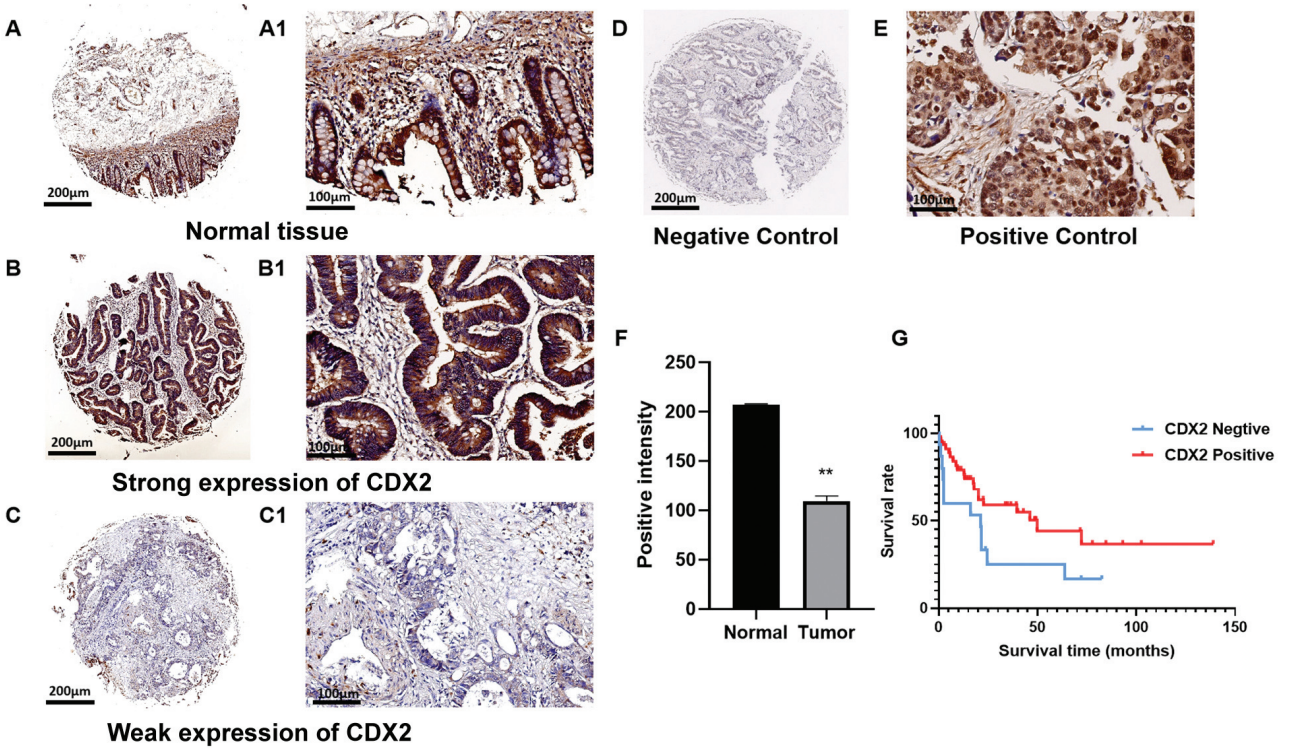

Figure 1. Expression spectrum of CDX2 in duodenal carcinoma and its relationship with prognosis. **A**) Expression of CDX2 in adjacent non-tumor tissues. **B**) Strong expression of CDX2 in duodenal carcinoma tissues. **C**) Weak expression of CDX2 in duodenal carcinoma. **D**) Negative control. **E**) Positive control. **F**) CDX2 expression intensity in duodenal carcinoma and adjacent normal tissues. **G**) Relationship between CDX2 expression and prognosis in duodenal carcinoma. $^{**}p<0.01$.

Cell scratch experiment

Before and after the transfection of CDX2-siRNA and the overexpression plasmid, CaCO2 and HuTu-80 cells were digested using trypsin, suspended in serum-free medium, and counted. The cell concentration was adjusted to 5×10^5 cells/mL, and the cells were plated in 6-well plates. The following day, a scratch was made with a pipette tip perpendicular to the center line of each well. The cells were washed three times with PBS to remove any detached cells, serum-free medium was then added, and the samples were incubated at 37 °C in a 5% CO_2 incubator.

Cell cycle detection

Before and after the transfection of CDX2-siRNA and overexpressed plasmid, the supernatants of CaCO2 and HuTu-80 cells were digested, collected, and discarded using pancreatic enzymes, followed by two washes with pre-cooled PBS and, after centrifugation, 70% ethanol, pre-cooled to -20°C, was slowly added while shaking. The cell suspension was then incubated at -20°C for over 4 h and then the samples were centrifuged at 350 g for 5 min, and the supernatant was discarded. The pellets were washed twice with PBS, and 500 µL of PBS containing 50 µg/mL of propidium iodide and 50 µg/mL of RNase was added. The samples were incubated at 37°C for 30 min in the dark, with mixing once during the staining process. Red fluorescence (PE channel) was detected using flow cytometry (Zhongsheng Biotech, Suzhou, China, model ZSAE7s) at an excitation wavelength of 488 nm. The DNA content of the cells was analyzed using Modifit software (Verity Software House, ME, USA). The experiment was repeated three times.

Cell apoptosis detection

An apoptosis kit (Beyotime Biotechnology, C1052) was utilized to evaluate apoptosis. Cells cultured in 96-well plates at a density of 1.5×10^6 cells/mL were treated according to the experimental design outlined above. Following the harvesting, washing, and centrifugation of the cells, their concentration was adjusted to 1.5×10^6 cells/mL. The precipitated cells from each well were then resuspended in 100 µL of binding buffer. Subsequently, each sample tube was treated with 5 µL of Annexin V-FITC and 10 µL of propidium iodide, and incubated in the dark for 15 min at 37 °C. After the addition of 1 × binding buffer, flow cytometry (Zhongsheng Biotech, Suzhou, China, ZSAE7s) was employed to analyze apoptosis. The experiment was conducted in triplicate.

Follow-up visit

The follow-up was conducted by telephone until March 2024. Overall survival (OS) was defined as the time from diagnosis of duodenal cancer to death or last follow-up.

Statistical methods

Data processing was conducted using the Statistical Package for Social Science (SPSS) version 22.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 7 (La Jolla, CA, USA). All data are presented as mean ± SD, and comparisons between groups were performed using the *t*-test. The Kaplan-Meier method was employed to construct the survival curve, and the Log-rank test was applied to assess differences in survival. A *p*-value of less than 0.05 was considered statistically significant.

Results

Expression lineage of CDX2 in duodenal carcinoma

CDX2 positive staining was mainly located in the nucleus, showing a light yellow or dark brown color. 84% (21/25) of adjacent non-tumor tissues showed strong diffuse staining of CDX2,

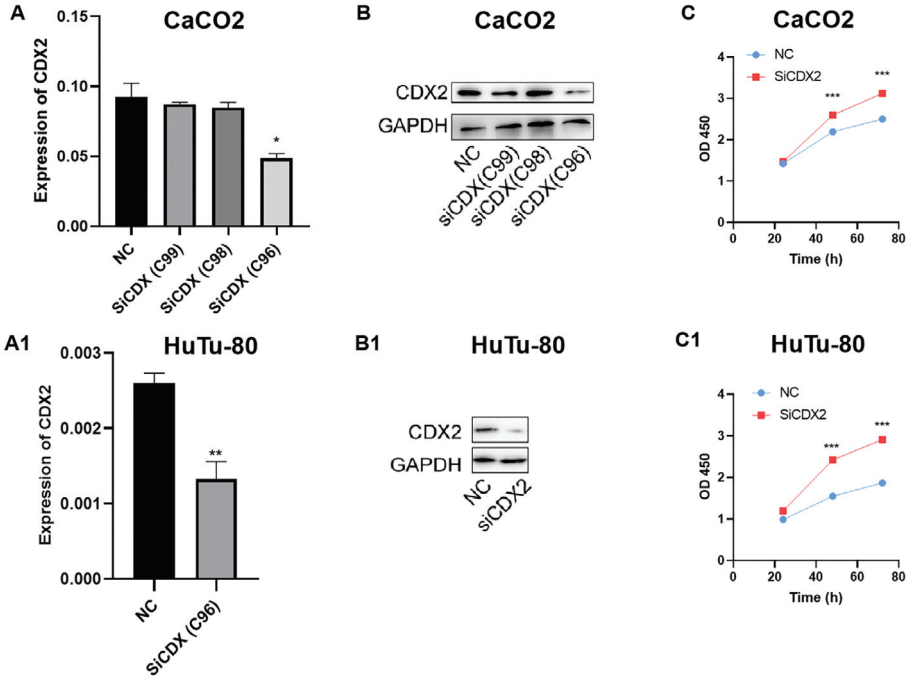

Figure 2. Knockdown of endogenous CDX2 expression promotes the proliferation of colorectal cancer cells. **A, A1**) Endogenous CDX2 mRNA is down-expressed by CDX2-siRNA. **B, B1**) The endogenous protein level of colorectal cancer cells was knocked down by CDX2-siRNA. **C**) Cell proliferation capacity was detected by CCK8 method. ***$p<0.001$.

with a staining intensity of 207.2±134.5. The positive rate of CDX2 in duodenal carcinoma tissues was 65% (26/40), and the positive staining was diffuse or diffuse, with a staining intensity of 110.5±112.0. The staining intensity of CDX2 in duodenal cancer cells was significantly weaker than that in adjacent non-tumor tissues ($p<0.01$). CDX2 expression was associated with prognosis, and the median OS of CDX2-positive and negative patients was 49.8 months and 21.6 months, respectively ($p<0.05$), suggesting a better prognosis for CDX2-positive patients (Figure 1).

Knockdown the expression of endogenous CDX2 inhibits the proliferation of colorectal cancer cells

CDX2-sirna was used to knock down endogenous CDX2 expression in CaCO2 and HuTu-80 cells, and the mRNA and protein levels showed that C96 interfered better (Figure 2 A,A1,B,B1). Then C96 was used to carry out follow-up experiments. CCK8 was used to detect cell proliferation. Figure 2 C,C1 showed that the proliferation capacity of CaCO2 and HuTu-80 cells was significantly enhanced at 24 h and 48 h after CDX2 expression decreased ($p<0.001$).

Overexpression of CDX2 inhibits the proliferation of colorectal cancer cells

CaCO2 and HuTu-80 cells were transfected with CDX2 overexpression plasmid, and the proliferation ability of the cells was detected. After transfection with CDX2 overexpression plasmid, both mRNA and protein levels of CDX2 were significantly increased (Figure 3 A,A1,B,B1). Exogenous overexpression of CDX2 significantly inhibited the proliferation of bowel cancer cells (Figure 3 C,C1) ($p<0.001$).

CDX2 affects the migration of colorectal cancer cells

The migration ability of CaCO2 and HuTu-80 cells after CDX2 overexpression or knockdown was detected by scratch assay. After CDX2 knockdown, the migration ability of bowel cancer cells was significantly increased, while after CDX2 upregulation, the migration ability of bowel cancer cells was significantly decreased (Figure 4 A,B), and the difference was statistically significant compared with the control group (Figure 4 C,D) (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

CDX2 affects the cell cycle changes of colorectal cancer cells

The cell cycle changes of CaCO2 cells after CDX2 overexpression or knockdown were detected by flow cytometry (Figure 5A). After overexpression of CDX2, the number of G_0/G_1 phase cells was significantly increased ($p<0.05$), and the number of S phase cells was significantly decreased ($p<0.01$). After CDX2 knockdown, cells in G_0/G_1 phase were significantly decreased ($p<0.05$), cells in G_2/M phase were significantly increased ($p<0.01$), and cells in S phase were also increased (Figure 5 B-D).

CDX2 affects the apoptosis of colorectal cancer cells

Apoptosis of CaCO2 cells after CDX2 overexpression or knockdown was detected using Annexin V-FITC/PI apoptosis detection kit and flow cytometry (Figure 6A). Compared with the control group, the apoptotic cells were significantly increased after overexpression of CDX2 ($p<0.001$). After CDX2 was knocked down, apoptotic cells were significantly reduced ($p<0.001$) (Figure 6 B,C).

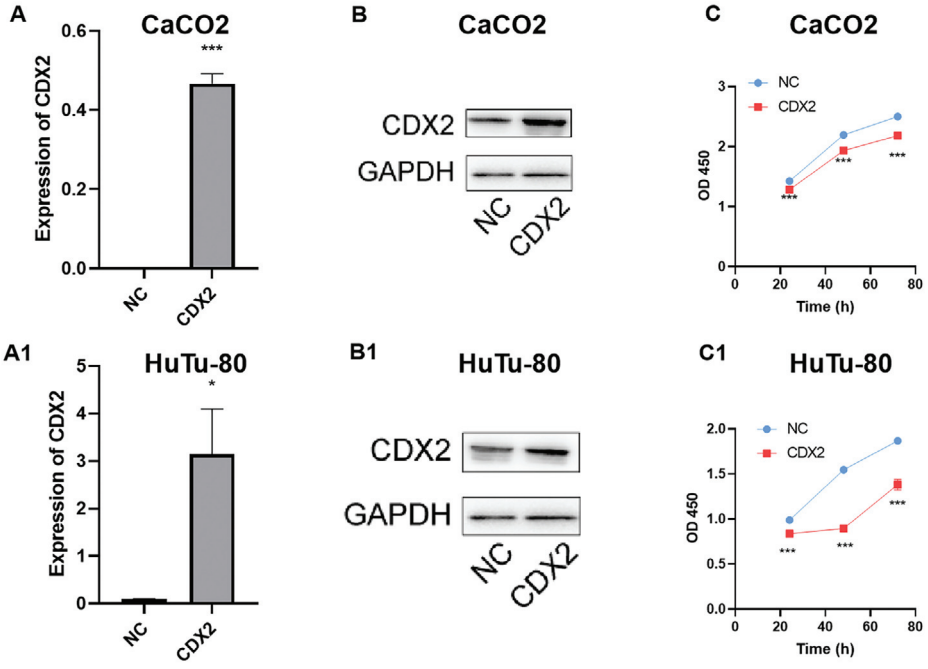

Figure 3. Overexpression of CDX2 promotes the proliferation of colorectal cancer cells. **A,A1**) overexpression of CDX2 mRNA in colorectal cancer cells. **B,B1**) CDX2 protein was overexpressed in colorectal cancer cells. **C**) Cell proliferation capacity was detected by CCK8 method. NC, control group; CDX2, transfection group. ***$p<0.001$.

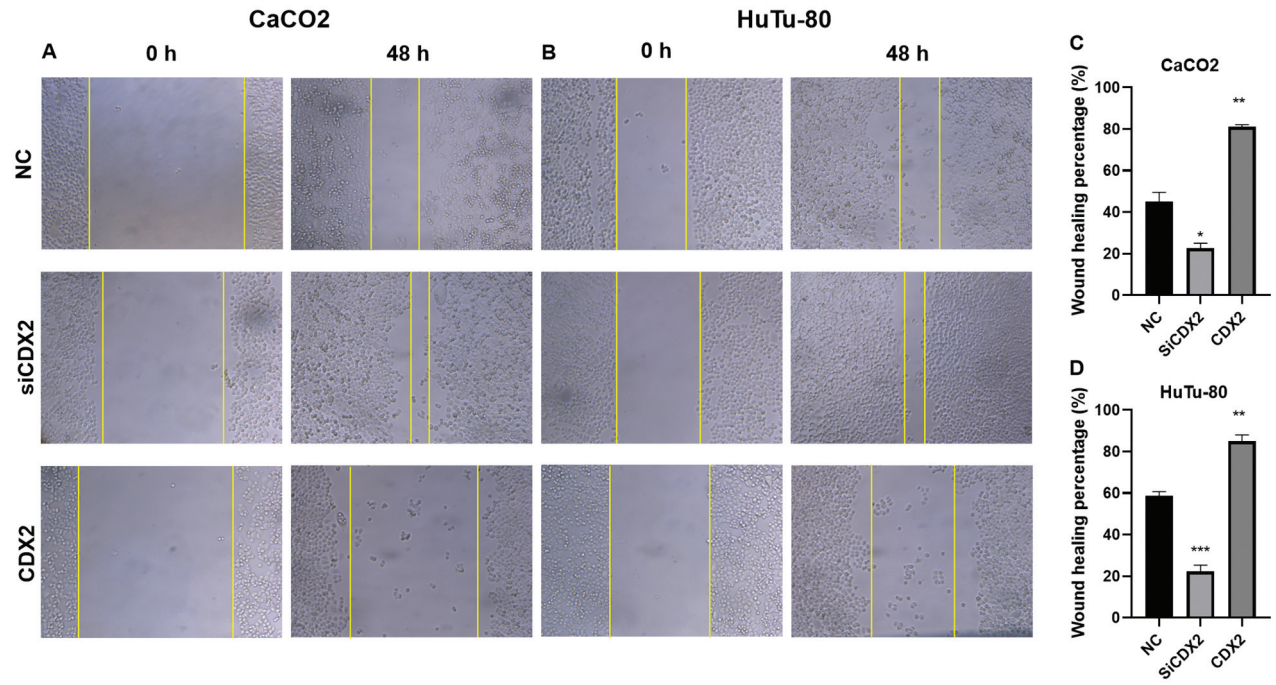

Figure 4. CDX2 inhibits the migration of colorectal cancer cells. **A**) CaCO2 cell scratch test record (×100). **B**) HuTu-80 cell scratch test record (×100). **C,D**) Compared with the control group, the statistical significance was analyzed. NC, control group; CDX2, transfection group. *p<0.05, **p<0.01, ***p<0.001.

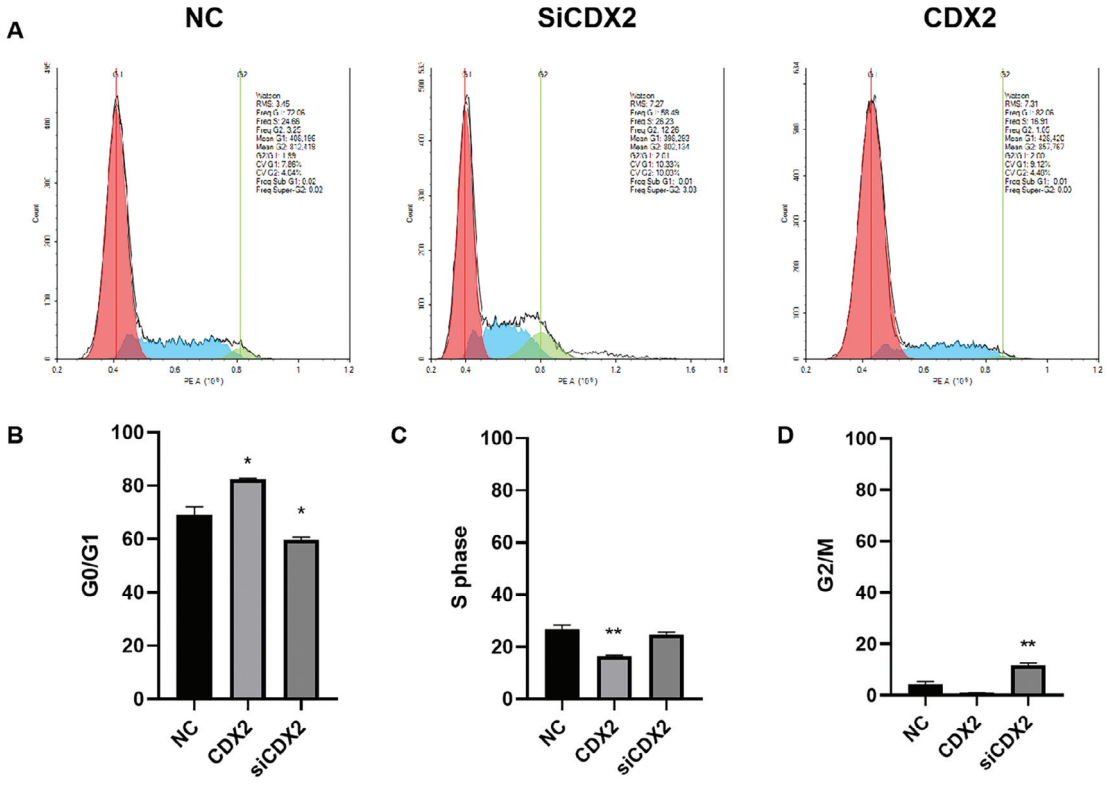

Figure 5. Changes of cell cycle after CDX2 overexpression and CDX2 knockdown in colorectal cancer cells detected by flow cytometry. **A**) Flow cytometry. **B**) Proportion of G_0/G_1 phase cells. **C**) S phase cell proportion. **D**) G_2/M phase cell ratio. NC, control group; CDX2, transfection group. *p<0.05, **p<0.01.

Discussion

Although the treatment of small intestine cancer is recommended by the NCCN guidelines, the low incidence of this disease complicates the execution of large-scale randomized controlled clinical studies. Consequently, the recommendations in the NCCN guidelines largely stem from small-sample retrospective studies, resulting in a lower level of evidence-based medical support. Therefore, further clinical research is essential to provide more robust evidence for the treatment of small intestine cancer. Additionally, current treatments predominantly rely on traditional chemotherapy, with insufficient evidence supporting the efficacy of emerging targeted therapies and immunotherapies. Notably, there is a significant lack of prospective research regarding the application of CDX2 in targeted therapy. Thus, employing molecular biology techniques to identify new biomarkers for predicting the occurrence and progression of duodenal cancer, as well as to discover novel therapeutic targets, is of considerable clinical importance for the prevention and treatment of this malignancy. As previously mentioned, small intestine cancer is a rare tumor characterized by a low incidence rate, and there is a scarcity of prospective randomized controlled clinical studies. To date, there have been no reports of prospective studies investigating the role of CDX2 in small intestine cancer.

Aa a member of the caudal type homeobox gene family, originally discovered while studying homologous mutations in *Drosophila melanogaster*, the CDX2 gene is 22~23 KB long, located in 13q12-13, and consists of 3 exons and 2 introns. The CDX2 protein contains 311 amino acids that are recognized and bound to specific DNA corresponding regions in a helix-loop-helix manner. CDX2 is expressed in all three derm layers in early embryo, and gradually concentrated in endoderm and mesoderm, and basically located in small intestine and colorectal during neonatal period, and is a key regulatory protein in intestinal epithelium formation and differentiation.[6,7] Due to the relatively specific and sensitive expression of CDX2 in intestinal epithelial tissues, it is commonly used in clinical immunohistochemical staining to assist in identifying whether tumors are of intestinal origin.[8] In recent years, some studies have found that the expression rate of CDX2 in colorectal cancer tumor tissues is significantly lower than that in adjacent tissues.[9,10] Asgari-Karchekani *et al*.[11] further found that the expression level of CDX2 was related to the location of the patient's primary lesion and lymph node metastasis, among which those with low expression of CDX2 were more common in the right half colon and more prone to lymph node metastasis. The results suggest that the low expression of CDX2 is associated with poor biological behavior, and CDX2 may play a role as a tumor suppressor gene in colorectal cancer. Similar to the results of the above study, we used tissue chip and immunohistochemistry to detect the expression of CDX2 protein in duodenal cancer. The positive rate of CDX2 in tumor tissues was 65%, and the staining intensity was 110.5±112.0, which was much lower than 84% and 207.2±134.5 in adjacent non-tumor tissues. In particular, in the same sample, the adjacent tissue was strongly positive, while the tumor cells were not colored. These results indicate that CDX2 plays a role as a tumor suppressor gene in the development of duodenal carcinoma. Survival analysis further demonstrated that the median OS of CDX2-positive and negative patients was 49.8 months and 21.6 months, respectively, and the survival difference was statistically significant, suggesting that CDX2-positive patients had a better prognosis. Similar to the results of this study,

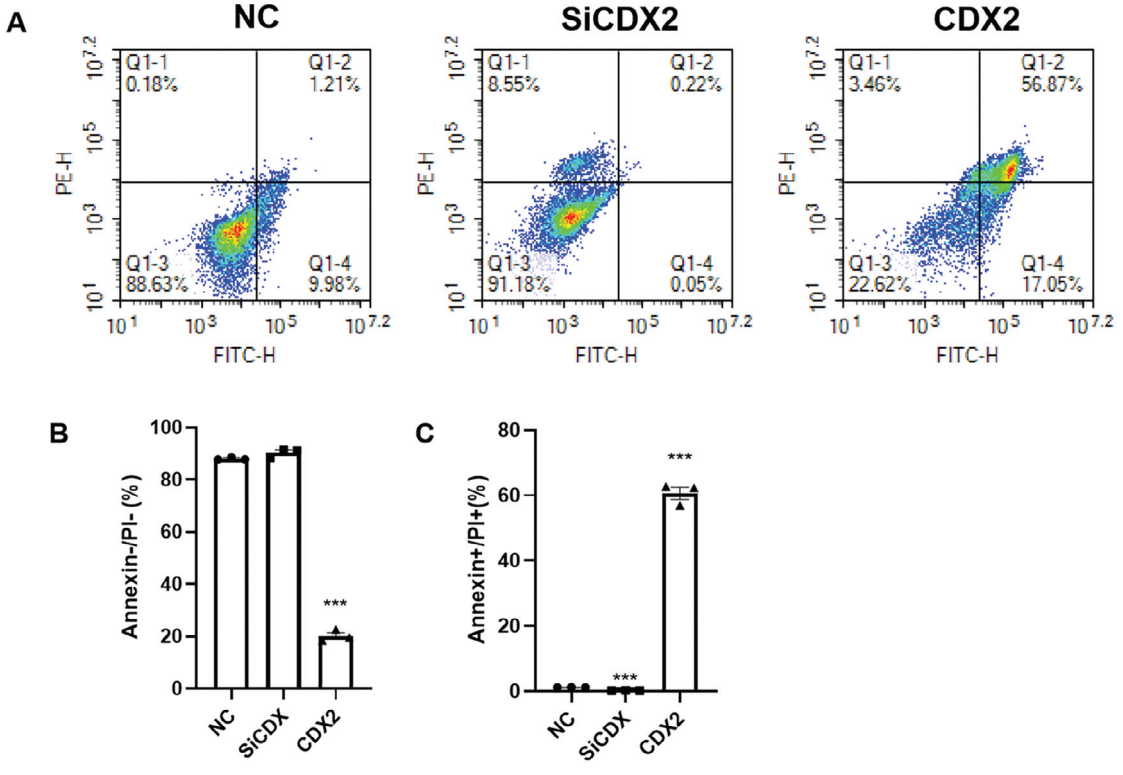

Figure 6. Apoptosis of colorectal cancer cells after CDX2 overexpression and CDX2 knockdown was detected by Annexin V-FITC/PI. **A**) Apoptosis chart. **B**) Annexin-/PI- cell ratio. **C**) Annexin+/PI+ cell ratio. NC, control group; CDX2, transfection group. ***p<0.001.

Xu *et al*.[4] observed the expression of CDX2 in 138 patients with colorectal cancer. We found that low CDX2 expression was significantly associated with poor median Progression Free Survival (PFS) and median OS compared to high CDX2 expression. The results showed that the prognosis of low CDX2 expression was poor, and the prognosis of high CDX2 expression was good. A retrospective study from Japan[12] analyzed the significance of CDX2 expression in patients with advanced colorectal cancer liver metastases. The study included 396 patients with liver metastases of colorectal cancer, all of whom underwent primary and liver metastases resection. The PFS of those with low and high CDX2 expression were 245 and 420 days, respectively. The OS is 1024 days and 3145 days respectively. The results indicated that even after surgical resection of primary and metastatic sites and R0 radical treatment, the expression of CDX2 was still closely related to the prognosis, and those with low expression had a poor prognosis, while those with high expression had a good prognosis. At the same time, the study further found that 65.7% of the patients had recurrence and metastasis after surgery, and the common metastatic sites included liver, lung, peritoneum and adrenal gland, etc. The proportion of two or more organ metastases in the patients with low expression of CDX2 was much higher than that in the patients with high expression of CDX2, suggesting that the patients with low expression of CDX2 were more malignant and aggressive. Metastasis of multiple organs is more likely to occur, affecting the prognosis of patients. The poor clinical manifestations of patients with low CDX2 expression may be related to multiple signaling pathways in the occurrence and development of colorectal cancer.[13] Wang et al.[14] found that low expression of CDX2 can activate the epithelial mesenchymal transition (EMT) pathway. By downregulating the expression of E-cadherin and upregulating the expression of N-cadherin, zinc finger transcription inhibitor protein (Snail) and Vimentin, the polarity of tumor cells is reduced, which makes tumor cells lose the characteristic pebble-shaped appearance of epithelial cells, and obtain mesenchymal shape, which becomes rounder in shape and easier to connect with neighboring cells. Enhanced cell infiltration and migration ability. In addition, the upregulated Snail protein can enhance the expression of matrix metalloproteinase (MMPs), and MMPs can promote the breakdown of the basement membrane of cells, further improving the ability of cell invasion.CDX2 is also closely related to the Wingless or Int1 (Wnt) signaling pathway mediated by β-Catenin. Wnt signaling pathway plays an important role in the occurrence and development of multiple tumors.[15,16] When the Wnt signaling pathway is activated, Wnt binds with the cell membrane receptor Frizzled protein (FZD), activates the Dishevelled protein (Dvl/Dsh) in the cytoplasm, inhibits Glycogen synthase kinase 3β (GSK-3β), The degradation activity of β-Catenin degradation complex formed by GSK-3β and other proteins stabilized the free β-Catenin protein in the cytoplasm. After stable accumulation in the cytoplasm, β-Catenin enters the nucleus, binds to the Lymphoid enhancer factor/T-cell factor (LEF/TCF) family, initiates the transcription of downstream target genes, and promotes the occurrence and development of tumors. Yu *et al*.[17] demonstrated that in colorectal cancer cells with CDX2 gene knockdown or overexpression, the expression of downstream targets of Wnt signaling pathway, such as β-Catenin, was upregulated or downregulated accordingly. Dual luciferin reporter gene and quantitative chromatin immunoprecipitation assay further confirmed that CDX2 activated the expression of GSK-3β and Axis inhibition protein 2 (Axin2) by directly binding to the promoter of GSK-3β and upstream activator of Axin2. Axin2, GSK-3β, and Adenomatous polyposis coli (APC) genes in the cytoplasm formed destruction complexes. This destructive complex binds to and phosphorylates β-Catenin, and finally degrades by ubiquitination modification, so as to inhibit the proliferation of colorectal cancer cells and tumor formation by inhibiting the Wnt /β-catenin signaling pathway. Previous studies have extensively demonstrated that CDX2 functions as a tumor suppressor gene in colorectal cancer; however, there is a paucity of literature reporting its role in duodenal cancer. In this study, CDX2 expression was knocked down in duodenal cancer cells, which promoted cell proliferation and cell migration in vitro, induced cell cycle transition from G_0/G1 phase to S phase and G_2/M phase, and reduced cell apoptosis, while overexpression of CDX2 inhibited cell proliferation and migration, and blocked cells in G_0/G_1 phase. S and G_2/M phase cells were decreased, and apoptosis cells were significantly increased. Combined with the literature and our experimental results, CDX2 can block cell cycle progression and induce apoptosis in duodenal carcinoma, and then inhibit the proliferation and migration of tumor cells. The specific mechanism remains to be further explored.

Targeted therapy and immunotherapy have been extensively utilized across various tumor types, yielding favorable therapeutic outcomes. However, the low incidence and limited patient population of small intestinal cancer pose challenges for conducting large-scale clinical studies: as a result, there are relatively few reports detailing advancements in targeted therapy and immunotherapy specifically for this cancer. Therefore, employing molecular biology techniques to identify new biomarkers for predicting the occurrence and progression of duodenal cancer, as well as discovering novel therapeutic targets, is of significant clinical importance for the prevention and treatment of this malignancy. This study confirmed that the expression of CDX2 is decreased or absent in duodenal cancer, and the low expression of CDX2 is closely related to the proliferation, invasion and migration of duodenal cancer cells, and promotes the progression of tumors. CDX2 may be a potential marker and therapeutic target for the occurrence, development and prognosis of duodenal carcinoma, which is worthy of further clinical exploration and application. In addition to this, low expression of CDX2 may also reduce the expression of multi-drug resistance genes and improve the benefit of chemotherapy in patients. So, patients with low CDX2 expression may be more in need of enhanced postoperative adjuvant chemotherapy management. Therefore, CDX2 expression level may be closely related to patient treatment. The specific mechanism of its action still needs further exploration. Certainly, the patients who underwent immunohistochemical analysis in this study were derived from a single-center dataset, which may introduce certain biases. In future research, we will conduct a multicenter retrospective analysis to expand the sample size and provide more robust evidence for the clinical application of CDX2 in duodenal cancer.

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Received: 10 March 2025. Accepted: 4 April 2025.

European Journal of Histochemistry 2025; 69:4203
doi:10.4081/ejh.2025.4203