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Combination of CDX2 expression and T stage improves prognostic prediction of colorectal cancer

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Abstract

Objective: Prognostic prediction of colorectal cancer (CRC) remains challenging because of its heterogeneity. Aberrant expression of caudal-type homeobox transcription factor 2 (CDX2) is strongly correlated with the prognosis of CRC.

Methods: Tissue samples of patients with CRC who underwent surgery in Xinhua Hospital (Shanghai, China) from January 2010 to January 2013 were collected. CDX2 expression was semiquantitatively evaluated via immunohistochemistry.

Results: In total, 138 patients were enrolled in this study from a prospectively maintained institutional cancer database. The median follow-up duration was 57.5 months (interquartile range, 17.0–71.0 months). In the Cox proportional hazards model, low CDX2 expression combined with stage T4 CRC was significantly the worst prognostic factor for disease-free survival (hazard ratio = 7.020, 95% confidence interval = 3.922–12.564) and overall survival (hazard ratio = 5.176, 95% CI = 3.237–10.091). In the Kaplan–Meier survival analysis, patients with low CDX2 expression and stage T4 CRC showed significantly worse disease-free survival and overall survival than those with low CDX2 expression alone.

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Conclusion: CDX2 expression combined with the T stage was more accurate for predicting the prognosis of CRC. Determining the prognosis of CRC using more than one variable is valuable in developing appropriate treatment and follow-up strategies.

Keywords
Colorectal cancer, caudal-type homeobox transcription factor 2, T stage, prognosis, disease-free survival, overall survival

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Introduction

Colorectal cancer (CRC) is the third most common malignancy, with approximately 134,490 new cases diagnosed and 49,190 related deaths reported in 2016.[1] The high incidence and mortality rates of CRC make this disease a major therapeutic challenge. Although CRC is potentially curable by surgical resection of the primary lesion,[2] approximately 30% of patients with stage I to III CRC and nearly 65% of patients with stage IV CRC are at risk of developing recurrence.[3] Patients' overall survival (OS) and disease-free survival (DFS) remain poor despite advances in CRC treatment, including surgery and adjuvant chemotherapy, which are particularly targeted to patients with stage III CRC.[4,5] The prognosis of CRC is usually determined through clinical staging using the unified guidelines of the Union for International Cancer Control and American Joint Committee on Cancer (AJCC) Tumor Node Metastasis (TNM) system.[6] However, these guidelines do not accurately reflect patient survival, possibly because of the heterogeneity of both the patients and the disease. The most widely used tumor marker for CRC is carcinoembryonic antigen (CEA).[7] Although the CEA level is related to poor patient outcomes,[8] its clinical value as an independent prognostic marker is controversial. Thus, the identification of new prognostic markers to categorize patients into high- and low-risk categories is crucial for developing more effective and individualized treatment strategies.

Caudal-type homeobox transcription factor 2 (CDX2), an essential intestine-specific regulator, is involved in the development and differentiation of intestinal epithelial cells[9–11] and is associated with cell proliferation, migration, and tumorigenesis.[12] Decreased CDX2 expression may indicate advanced CRC and a poor prognosis.[13–16] A recent study evaluated CDX2 expression in 2115 tumor samples and concluded that those lacking CDX2 expression were at high risk for poor outcomes.[17] However, the study mainly focused on the identification of patients who might benefit from adjuvant chemotherapy; the potential of CDX2 as a prognostic biomarker for other stages of CRC was not clarified. Thus, the clinical value of CDX2 as an essential biomarker for CRC remains unclear.

The present study was performed to explore the value of the combination of the T stage and postoperative CDX2 expression for predicting the prognosis in surgically treated patients with CRC. The aim was to provide data that help to better stratify patients for more appropriate and individualized therapy and ultimately prolong OS.

Materials and methods

Patients and tissue samples

From January 2010 to January 2013, 192 patients underwent curative surgery in Xinhua Hospital (Shanghai, China). This retrospective study used the tissue microarray (TMA) data obtained in a previous study, and the immunohistochemistry results were obtained from a database in our department. Briefly, formalin-fixed, paraffin-embedded CRC tissues were collected and converted into TMA for further immunohistochemistry analysis. Patients lost to follow-up were excluded from the study. The Ethics Committee of Xinhua Hospital approved this study (approval No. XHEC-D-2018-044) and allowed exemption from the need for informed consent because of the retrospective nature of the research. Details can be found in the review document.

Clinical and survival data evaluation

Clinical data were obtained from a prospectively maintained, institutional review board-approved database. Clinical characteristics including sex, age, tumor location, tumor differentiation, TNM stage, and AJCC stage as well as serological findings were collected from the electronic medical records. At the end of surgical treatment (curative surgery), the patients were routinely followed up every 3 months for the first 2 years and every 6 months thereafter. Follow-up visits consisted of cancer-focused history taking and physical examination and were conducted via telephone interviews and outpatient examinations. OS was measured from the date of surgery until the date of death of any cause, and DFS was measured from the date of surgery until the date of local or metastatic recurrence. Patients who were alive at the date of last follow-up were censored.

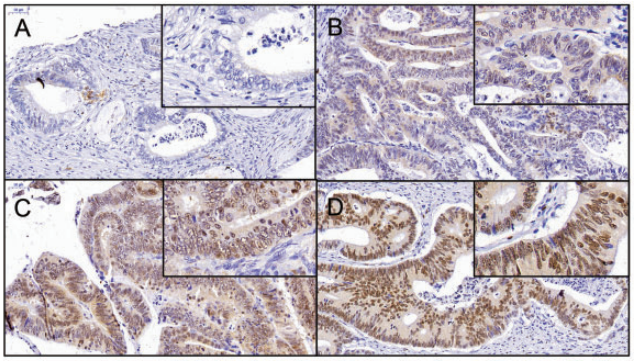
Evaluation of the TNM stage in the present study specifically referred to the pathological stage. Laboratory evaluation involved measurement of the serum CEA, hemoglobin, and albumin levels at cutoff values of 10 ng/mL, 110 g/L, and 35 g/L, respectively.

Immunohistochemistry

TMA comprised 192 primary CRC tissues that were then immunohistochemically stained to determine the CDX2 expression. Core tissue biopsy samples of 2 mm in diameter were obtained from two different regions (the tumor tissue and the area surrounding the same tumor) of individual formalin-fixed, paraffin-embedded tissue specimens for each patient. TMAs were incubated in the oven at 67°C for 90 minutes, routinely deparaffinized in xylene and ethanol of descending concentrations, and treated with citrate buffer (pH 6.0) in a pressure cooker for 20 min at 98°C for antigen retrieval. Endogenous peroxidases and nonspecific antigens were blocked by applying 3% hydrogen peroxide and 5% goat serum at room temperature for 30 minutes each. The primary antibody to CDX2 was incubated overnight at 4°C (A1629; ABclonal, Woburn, MA, USA), while the secondary antibody was incubated for 30 minutes at room temperature followed by the addition of diaminobenzidine chromogen (Beyotime, Haimen, China). Finally, the samples were counterstained with hematoxylin, dehydrated, and cover-slipped.

Histopathologic evaluation of CDX2 expression

The CDX2 expression in TMA was evaluated and semiquantitatively scored based on the immunohistochemistry results by two independent pathologists (Figure 1). Both the staining proportion (the



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	CDX2 high expression	CDX2 low expression
Gender		
male	33	41
female	33	31
T stage		
T1	2	0
T2	7	6
T3	29	21
T4	28	45
N stage		
N0	37	31
N1	19	25
N2	10	16
M stage		
M0	63	62
M1	3	10
Total	66	72

F

Score	Intensity	Proportion
0	no staining	0
1	weak positive	0-25%
2	moderate positive	25%-50%
3	strong positive	50%-75%
4	/	75%-100%

Final score=intensity sorce x proportion score

Figure 1. Distribution of CDX2 expression in patients with colorectal cancer and immunohistochemical scoring method. (a–d) Negative, weakly positive, moderately positive, and strongly positive expression of CDX2 in patients with colorectal cancer (e) Distribution of high and low CDX2 expression by sex, T stage, N stage, and M stage. (f) Detailed immunohistochemical scoring method of CDX2 expression. CDX2, caudal-type homeobox transcription factor 2.

percentage of cells stained) and the staining intensity were considered, as suggested by Remmele and Stegner.[18] The proportion was scored as 0 (0%), 1 (>0% to 25%), 2 (>25% to 50%), 3 (>50% to 75%), or 4 (>75%), while the intensity was scored as 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The final staining score was calculated by multiplying the proportion score by the intensity score. Samples with a staining score of ≤4 comprised the low-expression group, and those with a score of >4 comprised the high-expression group.

Statistical analysis

GraphPad Prism 5 software (GraphPad, San Diego, CA, USA) and SPSS version 19.0 software (IBM Corp., Armonk, NY, USA) were used for the statistical analyses. For the survival analysis, the Kaplan–Meier method was used to assess the survival time distribution according to different prognoses, and the log-rank test was performed to test the significance of a deviation from the null hypothesis in DFS and OS among the different prognostic groups. A Cox proportional hazard model was used to compute the hazard ratio (HR) for the multivariate

survival analyses, with adjustments for variables that may be significant prognostic factors according to the univariate analyses. The confidence intervals (CIs) were set at 95%, all statistical tests were two-sided, and a p-value of <0.05 was considered significant.

Results

Demographic and clinical characteristics

From January 2010 to January 2013, 138 patients with CRC who underwent surgical resection at our institution were enrolled in this retrospective study (54 of the original 192 patients were lost to follow-up). Of these, 74 were men and 64 were women. The median patient age was 66 years (interquartile range, 55.0–76.3 years), and the median follow-up time was 57.5 months (interquartile range, 17.0–71.0 months). The tumor was located in the rectum, left colon, and right colon in 47.1%, 33.3%, and 19.6% of the patients, respectively. Ten (7.2%) patients had stage I CRC, 56 (40.6%) had stage II, 59 (42.8%) had stage III, and 13 (9.4%) had stage IV. The detailed demographic, clinical, and laboratory characteristics are listed in Table 1.

Table 1. Patients' baseline characteristics.

Variable	Value
Sex, male/female	74/64
Age, years	66.0 (55.0–76.3)
Follow-up, months	57.5 (17.0–71.0)
Tumor site	
Rectum	65 (47.1)
Left side	46 (33.3)
Right side	27 (19.6)
Histology	
Well-differentiated	24 (17.4)
Moderately differentiated	99 (71.7)
Poorly differentiated	15 (10.9)
T stage	
T1	2 (1.4)
T2	13 (9.4)
T3	50 (36.2)
T4	73 (52.9)
N stage	
N0	68 (49.3)
N1	44 (31.9)
N2	26 (18.8)
Cancer stage	
I	10 (7.2)
II	56 (40.6)
III	59 (42.8)
IV	13 (9.4)
Serum albumin	
$\geq$35 g/L	113 (81.9)
$<$35 g/L	25 (18.1)
Hemoglobin	
$\geq$110 g/L	97 (70.3)
$<$110 g/L	41 (29.7)
Serum carcinoembryonic antigen	
$<$10 ng/mL	85 (61.6)
$\geq$10 ng/mL	53 (38.4)
CDX2 expression status	
High expression	66 (47.8)
Low expression	72 (52.2)

Data are presented as n, median (interquartile range), or n (%).
CDX2, caudal-type homeobox transcription factor 2.

CDX2 expression status in CRC

In total, 72 (52.2%) and 66 (47.8%) samples were categorized into the low- and high-expression groups, respectively. A total of 73 patients had stage T4 disease; of these, 45 (61.6%) had low CDX2 expression. The detailed distribution of CDX2 expression by T stage, N stage, M stage, and sex is shown in Figure 1.

Univariate and multivariate analysis of OS and DFS in patients with CRC

Cox proportional hazards regression analysis was performed to determine the risk factors for survival and the potential prognostic value of clinicopathological factors. Factors that may have significant prognostic value ($p < 0.05$) in the univariate analysis

Table 2. Univariate and multivariate analyses of overall survival and disease-free survival.

	Disease-free survival				Overall survival			
Variable	Univariate HR (95% CI)	p value	Multivariate HR (95% CI)	p value	Univariate HR (95% CI)	p value	Multivariate HR (95% CI)	p value
Age, years								
<65	1				1			
≥65	1.244 (0.742–2.085)	0.408			1.288 (0.768–2.159)	0.337		
Sex			1.606 (0.940–2.743)	0.083				
Female	1				1			
Male	1.715 (1.010–2.911)	0.046			1.594 (0.939–2.704)	0.084		
Tumor location								
Rectum	1				1			
Left side	1.034 (0.577–1.853)	0.091			1.065 (0.594–1.908)	0.362		
Right side	1.260 (0.647–2.452)	0.497			1.363 (0.700–2.653)	0.833		
Tumor depth			6.314 (3.108–12.827)	<0.001			4.800 (2.420–9.520)	<0.001
Limited to the serosa (T1–T3)	1				1			
Penetrating the serosa or involving other organs (T4)	6.117 (3.164–11.828)	<0.001			5.641 (2.918–10.904)	<0.001		
Histology								
Well-differentiated	1				1			
Moderately differentiated	1.816 (0.650–5.075)	0.255			1.805 (0.646–5.044)	0.26		
Poorly differentiated	2.861 (0.940–8.709)	0.064			3.007 (0.988–9.149)	0.052		
Regional lymph node metastasis			2.104 (1.208–3.663)	0.009			2.155 (1.243–3.737)	0.006
Negative (N0)	1				1			
Positive (N1–N2)	2.182 (1.279–3.723)	0.004			2.144 (1.258–3.656)	0.005		
Stage			2.436 (1.333–4.129)	0.003			2.313 (1.320–4.053)	0.003
I/II	1				1			
III/IV	2.439 (1.411–4.217)	0.001			2.364 (1.368–4.085)	0.002		
Serum albumin								
≥35 g/L	1				1			
<35 g/L	1.733 (0.950–3.159)	0.073			1.642 (0.901–2.993)	0.105		
Hemoglobin							1.212 (0.708–2.074)	0.484
≥110 g/L	1				1			
<110 g/L	1.647 (0.976–2.781)	0.062			1.743 (1.033–2.943)	0.038		
Serum carcinoembryonic antigen			1.211 (0.698–2.103)	0.496			1.339 (0.778–2.304)	0.292
<10 ng/mL	1				1			
≥10 ng/mL	2.161 (1.295–3.606)	0.003			2.074 (1.243–3.460)	0.005		
CDX2 expression status			5.290 (2.717–10.300)	<0.001			4.732 (2.450–9.142)	<0.001
High expression	1				1			
Low expression	5.903 (3.113–11.196)	<0.001			5.725 (3.020–10.851)	<0.001		

HR, hazard ratio; CI, confidence interval; CDX2, caudal-type homeobox transcription factor 2.

were included in the multivariate analysis (Table 2).

The univariate analysis showed that sex, a high hemoglobin level, a high CEA level, a tumor involving the serosa or beyond, regional lymph node metastasis, a high AJCC stage, and low CDX2 expression were significantly associated with worse DFS and OS ($p < 0.05$). These factors were then used in the multivariate analysis. The depth of tumor invasion, regional lymph node metastasis, AJCC stage, and CDX2 expression status were independent prognostic factors for DFS (HR = 6.314, 95% CI = 3.108–12.827; HR = 2.104, 95% CI = 1.208–3.663; HR = 2.436, 95% CI = 1.333–4.129; HR = 5.290, 95% CI = 2.717–10.300) and OS (HR = 4.800, 95% CI = 2.420–9.520; HR = 2.155, 95% CI = 1.243–3.737; HR = 2.313, 95% CI = 1.320–4.053; HR = 4.732, 95% CI = 2.450–9.142) among patients with CRC.

Survival analysis and prognostic implication of combined CDX2 expression and T stage

In the Kaplan–Meier survival analysis with the log-rank test, the DFS and OS of patients with a high AJCC stage and CEA level ($p = 0.001$ for both DFS and OS) were worse than the DFS and OS of patients with a low AJCC stage and CEA level ($p = 0.002$ for DFS and 0.004 for OS) (Figures 2(a), (b) and 3(a), (b)). Patients in the low CDX2 expression group had a worse outcome than those in the high CDX2 expression group ($p < 0.001$ for DFS and OS) (Figures 2(c) and 3(c)).

Interestingly, we found that the combination of the T stage with the CDX2 expression status had a better prognostic prediction value. In the Kaplan–Meier survival analysis, patients with tumors penetrating the serosa (T4) and low CDX2

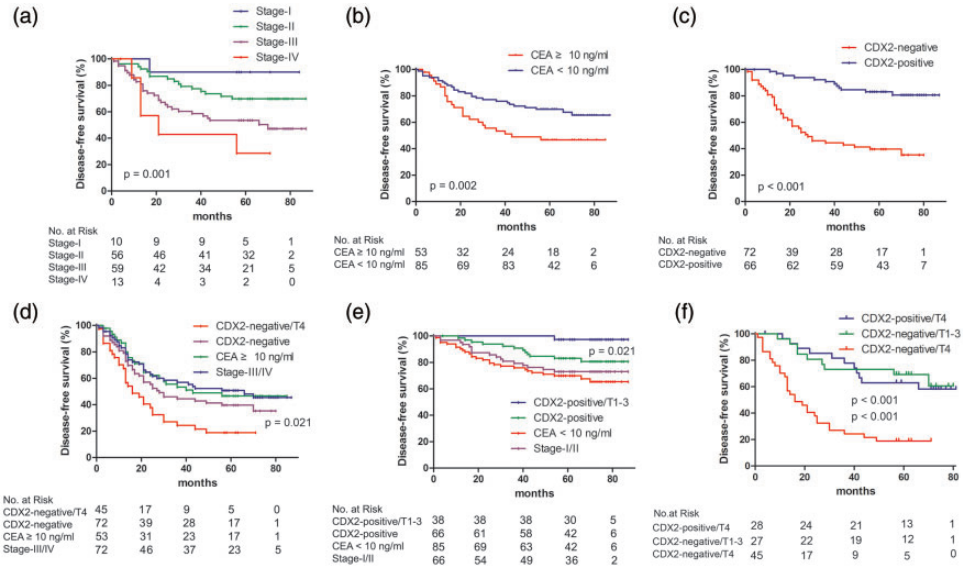

Figure 2. Kaplan–Meier survival curves depicting outcomes of disease-free survival (DFS) according to the TNM stage, preoperative CEA level, CDX2 expression status, and CDX2 expression combined with T stage. (a) TNM stage with DFS. (b) Preoperative CEA level with DFS. (c) CDX2 expression status with DFS. (d–f) CDX2 expression combined with the T stage was more effective in prognostic prediction of DFS than were the other markers. CDX2, caudal-type homeobox transcription factor 2; CEA, carcinoembryonic antigen.

expression had worse outcomes with respect to DFS and OS ($p < 0.05$ for both) compared with the other groups. In addition, these patients had significantly worse DFS ($p = 0.021$) and OS ($p = 0.047$) than those with only low CDX2 expression (Figures 2(d) and 3(d)). In contrast, patients with high CDX2 expression and tumors confined to the serosa (T1–T3) had the best prognosis among all subgroups ($p < 0.05$). The survival curve showed that these patients had significantly better DFS and OS than those with only high CDX2 expression ($p = 0.021$, $p = 0.022$) (Figures 2(e) and 3(e)). In addition, we analyzed survival in patients with low CDX2 expression with T4 stage CRC, low CDX2 expression with T1–T3 stage CRC, and high CDX2 expression with T4 stage CRC. As shown in Figures 2(f) and 3(f), patients with low CDX2 expression with T4 stage CRC had the worst prognosis with respect to both DFS and OS than the other two groups ($p < 0.001$, $p < 0.001$) (Figures 2(f) and 3(f)).

A Cox proportional hazards regression analysis was performed to further evaluate the prognostic prediction value of the combination of CDX2 expression and the T stage. Because the integrated indictor was derived from the CDX2 expression status and T stage, we alternately removed CDX2 expression status and T stage to reduce the bias of the derivative indicators when they were analyzed together; we then evaluated the prognostic value of the integrated indictor separately. In the univariate analysis, low CDX2 expression combined with stage T4 CRC showed a significant association with worse DFS ($p < 0.001$) and OS ($p < 0.001$). Furthermore, the multivariate analysis showed that the combination of low CDX2 expression and stage T4 CRC was an independent prognostic factor

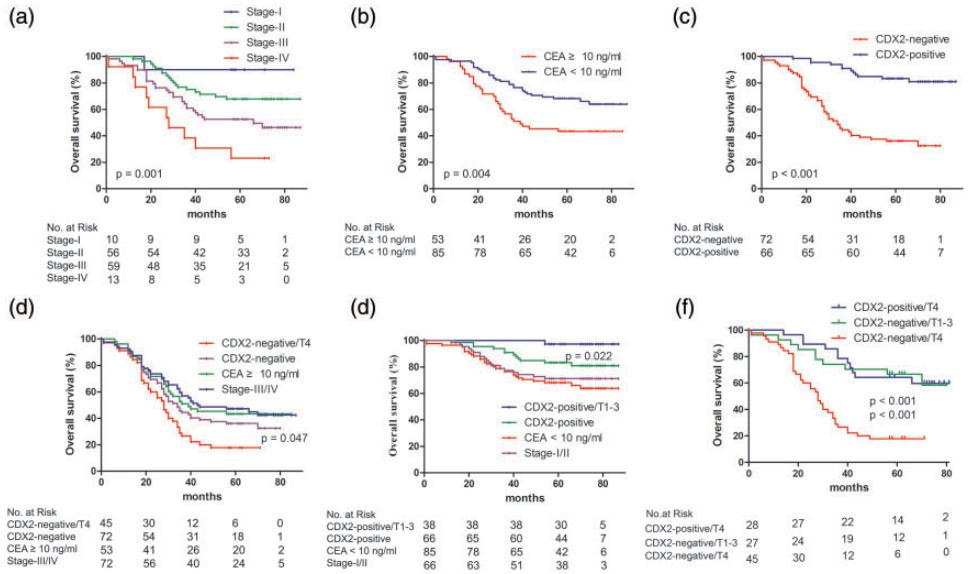

Figure 3. Kaplan–Meier survival curves depicting outcomes of overall survival (OS) according to the TNM stage, preoperative CEA level, CDX2 expression status, and CDX2 expression combined with T stage. (a) TNM stage with OS. (b) Preoperative CEA level with OS. (c) CDX2 expression status with OS. (d–f) CDX2 expression combined with the T stage was more effective in prognostic prediction of OS than were the other markers. CDX2, caudal-type homeobox transcription factor 2; CEA, carcinoembryonic antigen.

for poor DFS (HR = 7.020, 95% CI = 3.922–12.564) and OS (HR = 5.176, 95% CI = 3.237–10.091) (Table 3).

Relationship between low CDX2 expression and tumor features in CRC

In the present study, 72 (52.2%) patients were confirmed to have low CDX2 expression. To investigate the potential correlation between CDX2 expression and tumor features, we further analyzed the clinical pathological features among these 72 patients. Only 9 (12.5%) patients were diagnosed with well-differentiated tumors, and 45 (62.5%) had tumor penetration of the serosa. These data further indicated that loss of CDX2 could cause a poor prognosis in patients with CRC. Detailed data are shown in Table 4.

Discussion

Establishment of an accurate prognostic prediction system or markers is crucial in classifying high- and low-risk patients and developing appropriate treatment and follow-up strategies for CRC. In the present study, we investigated the influence of multiple factors on the prognosis of patients with CRC, including CDX2 expression, TNM stage, and CEA level.

Our data demonstrated that low CDX2 expression was a poor prognostic factor for CRC, which is consistent with the findings of previous studies.[17,19] In the Cox proportional hazards model, we found that CDX2 expression was closely associated with the outcome of CRC, while the differentiation of the tumor was not significantly associated with either DFS or OS. This further implicates CDX2 as a novel prognostic marker independent of differentiation. In addition, the combination of CDX2 expression and the T stage showed a better prognostic prediction value. Specifically, patients with high CDX2 expression and a tumor confined within the serosa (T1–T3) showed longer OS or DFS than patients in the other groups. Meanwhile, patients with low CDX2 expression and a tumor penetrating the serosa (T4) had the worst prognosis. Therefore, a combination of CDX2 expression and a stage T4 tumor might provide a reference for choosing treatment modalities. For example, to improve survival, the treatment should be more proactive and the follow-up more frequent in patients with low CDX2 expression and a T4 tumor. Determining the depth of tumor invasion via imaging and CDX2 expression via pathological analysis during the preoperative period may be helpful in predicting the prognosis and developing treatment strategies for patients with CRC.

CDX2 is an intestine-specific tumor suppressor that may inhibit progression and metastasis in CRC.[20–22] Loss of CDX2 expression is related to a poor outcome, suggesting an invasive phenotype, advanced TNM stage, or poor differentiation.[15,20,23–25] The carcinogenesis of CRC could be associated with chromosomal instability, genotype mutations (e.g., *APC*, *KRAS*, *BRAF*), a high level of microsatellite instability (MSI), and a CpG island methylator phenotype (CIMP), among others.[26–28] Previous research has shown that low CDX2 expression is an independent and highly specific predictor of a poor prognosis in patients with MSI-high CRC.[23,25] Moreover, loss of CDX2 expression may be a significant contributing factor to the development of a CIMP-high phenotype in CRC, which could compromise the patient's prognosis.[29] However, in their multivariate analysis, Baba et al.[30] found that loss of CDX2 expression was significantly associated with CIMP but not with MSI. Thus, according to the close relationship between CIMP and CDX2 expression, we speculate that CpG island hypermethylation could play a potential role in silencing CDX2

Table 3. Prognostic value of CDX2 expression combined with tumor depth for disease-free survival and overall survival in patients with colorectal cancer.

Variable	Disease-free survival				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Sex, male vs. female	1.715 (1.010–2.911)	0.046	1.532 (0.896–2.621)	0.119	1.594 (0.939–2.704)	0.084		
Hemoglobin, ≥110 vs. <110 g/L	1.647 (0.976–2.781)	0.062			1.743 (1.033–2.943)	0.038	1.471 (0.862–2.510)	0.157
CEA, ≥10 vs. <10 ng/mL	2.161 (1.295–3.606)	0.003	1.361 (0.784–2.362)	0.274	2.074 (1.243–3.460)	0.005	1.453 (0.845–2.497)	0.177
Regional lymph node metastasis, positive vs. negative	2.182 (1.279–3.723)	0.004	2.016 (1.159–3.506)	0.013	2.144 (1.258–3.656)	0.005	2.034 (1.176–3.517)	0.011
Stage, I/II vs. III/IV	2.439 (1.411–4.217)	0.001	2.262 (1.288–3.972)	0.005	2.364 (1.368–4.085)	0.002	2.209 (1.264–3.859)	0.005
CDX2/T-stage, $CDX2^{low}$/T4 vs. others	7.946 (4.581–13.783)	<0.001	7.020 (3.922–12.564)	<0.001	6.881 (3.984–11.885)	<0.001	5.716 (3.237–10.091)	<0.001

HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; CDX2, caudal-type homeobox transcription factor 2.

expression. Further evidence is needed to determine other roles of CDX2 expression in CRC carcinogenesis.

Low CDX2 expression has also been associated with genotype mutations. One study showed that 23 of 24 (96%) *BRAF*-mutated cancer samples demonstrated loss of CDX2 expression, indicating that loss of CDX2 expression is highly specific for *BRAF* mutation. However, *KRAS* mutation was not significantly associated with CDX2 expression in this study.[31] Lack of CDX2 expression is considered to be a potential marker of the serrated neoplasia pathway in CRC because of its association with high MIS, high CIMP, and BRAF mutations.[32] As such, CDX2 might be an indicator of cancer molecular biology.

The AJCC TNM staging system is widely accepted as an important prognostic predictor with which to estimate survival of patients with CRC. This system puts more emphasis on the N and M status in advanced cases. In the present study, we found that patients with stage T4 CRC had a significantly worse prognosis than those with earlier stages. Furthermore, stage T4 CRC was an independent factor for shorter OS.

Our study confirms the challenges posed by T4 carcinomas to the operating surgeon. In this study, all patients with CRC diagnosed with stage T4 tumors showed worse survival than patients with stage T1 to T3 tumors. Notably, 35 (47.9%) of these patients developed recurrence or metastasis, while only 6 (9.2%) of those with stage T1 to T3 had recurrence or metastasis. Liver or lung metastasis was the most common type of metastasis (62.9%) in the patients with stage T4 CRC. Given that almost 50% of the patients with stage T4 CRC were more likely to develop a worse prognosis may indicate that primary tumors penetrating the serosa or involving other organs have a higher risk of recurrence and metastasis.

Table 4. Relationship between low CDX2 expression and tumor features in colorectal cancer.

Clinical pathological features	Low CDX2 expression (n = 72)
Tumor site	
Rectum	31 (43.0)
Left side	22 (30.6)
Right side	19 (26.4)
Histology	
Well-differentiated	9 (12.5)
Moderately differentiated	47 (65.3)
Poorly differentiated	16 (22.2)
T stage	
T1	0 (0.0)
T2	6 (8.3)
T3	21 (29.2)
T4	45 (62.5)
N stage	
N0	31 (43.1)
N1	25 (34.7)
N2	16 (22.2)
Cancer stage	
I	3 (4.2)
II	26 (36.1)
III	33 (45.8)
IV	10 (13.9)
CEA	
$<$10 ng/mL	37 (51.4)
$\geq$10 ng/mL	35 (48.6)

Data are expressed as n (%). CDX2, caudal-type homeobox transcription factor 2; CEA, carcinoembryonic antigen.

Several researchers have recently focused on the prognostic value of the T stage.[33–35] A retrospective nationwide study of 889 patients with colon cancer revealed a 5-year cancer-specific survival rate of 50% and 30% for patients with stage II and III disease, respectively, among those with stage T4 cancer.[36] These findings indicate that stage T4 might be the best histopathological indicator of a poor prognosis in stage II disease and a major poor prognostic predictor in stage III disease.[36] Rottoli et al.[37] also demonstrated that T4N0 cancer had a worse oncologic outcome than other stage II tumors and had a similar outcome to advanced stage III cancer. A study that evaluated several microscopic features of stage T4 cancer showed that tumors perforating the visceral peritoneum and directly invading other organs or structures through malignant invasion instead of inflammatory adhesion are associated with poor survival.[38] These findings further support the clinical value of the tumor stage. We consider that the depth of tumor invasion might reflect the invasiveness of the phenotype.

Prognostic evaluation in patients with CRC remains challenging because of the heterogeneity of the disease. The combination of CDX2 expression and the tumor stage was the most effective prognostic marker in our study. In our study cohort, 30 of the 45 patients with low CDX2 expression and a low tumor stage developed malignant recurrence and metastasis, which led to a poor outcome. One possible explanation is that abnormal changes in molecular biology including high MSI, high CIMP, and point mutations could decrease CDX2 expression, thus adversely influencing the patient's prognosis. Second, tumors penetrating the serosa or involving other organs could be an independent contributing factor of malignant recurrence and metastasis, which further compromise the patient's quality of life and eventually lead to worse outcomes. Thus, we hypothesized that these two poor prognostic factors could play a synergistic effect in worsening survival. This hypothesis supports the promising value of these factors in predicting prognosis.

From the results of this study, we can stipulate that CDX2 expression is associated with the depth of tumor invasion and that low CDX2 expression accelerates tumor invasion, thus increasing the risk of tumors penetrating the serosa or involving other organs. Determining the exact mechanism by which CDX2 is correlated with

the depth of tumor invasion may be helpful in improving the accuracy of prognostic prediction and identification of new therapeutic targets.

CEA is another widely applied tumor marker for CRC, particularly in tumor surveillance.[39] It has high sensitivity in predicting recurrence, but not in predicting OS. This might partly explain why it did not have clinical value in our study. Other factors such as the hemoglobin level might have played a role in the short-term outcome of the surgery, but it showed limited value in long-term survival.

Our study had some limitations. First, some selection bias may have been present because all patients included in the study were diagnosed and managed only by colorectal surgeons in our department. Second, because of the retrospective nature and long duration of this study, loss to follow-up and incomplete clinical data were inevitable. Third, our study was limited by its single-center design and insufficient sample size, which might limit the generalizability of the results. Multicenter studies with large sample sizes should be performed to further confirm our findings.

Conclusions

The T stage combined with the CDX2 expression status had better prognostic prediction value in terms of OS. Patients with T4 stage CRC with low CDX2 expression have the highest risk of poor survival. The combination of CDX2 expression and the T stage could be a novel effective prognostic indicator for patients with CRC. If the prognosis is accurately predicted, treatment and follow-up strategies could be adjusted accordingly to prolong survival.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Peng Du and Yingwei Chen conceived the study. All authors collected the data. Weimin Xu, Wenjun Ding, Tingyu Wu, and Yuegui Guo completed the follow-up. Weimin Xu analyzed the data. Weimin Xu and Yilian Zhu wrote the manuscript. Yingwei Chen and Long Cui revised the manuscript. Weimin Xu and Yilian Zhu contributed equally to this work. All authors reviewed the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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