



Pattern of Expression of CDX2 in Colorectal Cancer and its Role in Prognosis: An Ambispective Observational Study

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Abstract

Introduction Caudal-type homeobox 2 (CDX2), a nuclear protein, is essential for the proliferation and development of intestinal epithelial cells and is frequently down-regulated during tumorigenesis. CDX2 inhibits cell growth as well as stimulates differentiation by activating intestinal specific genes, thus lack of CDX2 favors tumor growth and aggressiveness.

Objectives We aimed to evaluate the pattern of CDX2 expression in all stages of colorectal cancer (CRC) and study its association with baseline characteristics and prognosis.

Materials and Methods Study was conducted as an ambispective observational study, enrolling cases of CRC retrospectively from January 2014 to July 2016 (30 months), and prospectively during next 18-month period till January 2018. We performed CDX2 staining by immunohistochemistry on the available biopsy blocks of CRC patients during the study period. Total 286 patients were registered during the study period, of which only 110 biopsy blocks were available for staining. CDX2 scoring was done by a semiquantitative method on whole tissue section for the intensity and percentage of the cells showing positivity. Correlation of CDX2 expression was done with baseline clinical and histopathologic characteristics, and survival.

Results Of 110 patients, 77 (70%) constituted colon cancer and 33 (30%) were rectal cancer. The median age was 54.2 years, 62 (56.4%) being male and 48 (43.6%) female with male-to-female ratio 1.3:1. In the study cohort, 33 (30%) patients had stage II disease, 30 (27.3%) stage III, and 47 (42.7%) were stage IV. Seventy-three (66.4%) were positive for CDX2 and 37 (33.4%) were negative. Loss of CDX2 expression was significantly associated with advanced stage, rectal site, poor grade of differentiation, and presence of lymphovascular invasion (LVSI). With median follow-up of 16 months, progression-free survival (PFS) at 2 years was 30% for CDX2 negative patients compared

Keywords

- ▶ colorectal cancer
- ▶ CDX2
- ▶ pattern
- ▶ survival
- ▶ prognosis

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with 67% for CDX2 positive ($p = 0.009$), while overall survival (OS) at 2 years was 46% for CDX2 negative versus 77% for positive patients ($p = 0.01$).

Conclusion Loss of CDX2 expression is associated with advanced stage, higher tumor grade, presence of LVSI, and worse PFS and OS and thereby functions as a poor prognostic factor in CRC.

Introduction

Colorectal cancer (CRC) is one of the common cancers diagnosed worldwide and is considered as the second among the leading causes of cancer deaths.¹ The incidence and mortality from CRC is more in developed countries.² Approximately 20% of the patients with CRC presents with distant metastasis at diagnosis.³

Numerous prognostic markers, related to the pathogenesis of CRC have been studied to predict treatment outcome.⁴ Several of these biomarkers are not reproducible and are not robust enough to find place in clinics. A few of these markers have come to clinical practice to help in knowing the prognosis and/or predicting response to particular therapy, either fluorouracil (5FU)/oxaliplatin-based chemotherapy or biological therapy. There is ongoing research to find more novel prognostic or predictive biomarkers or panel of markers at molecular levels to better stratify patients and determine systemic treatment based upon the risk groupings.⁵

Among the novel biomarkers, caudal-type homeobox 2 (CDX2) a nuclear transcription factor, has been explored to predict the survival outcomes in patients with CRC.^{6,7} CDX2 is an intestinal factor, encoded by CDX2 gene which is important for homeostasis and development of the intestinal epithelium.⁸ CDX2 inhibits cell growth as well as stimulates differentiation by activating intestinal specific genes.^{9,10} Loss of CDX2 expression is seen in almost 30% of human CRC and is associated with high tumor grade.¹¹ CDX2 expression can be identified both by gene expressing profile and immunohistochemistry (IHC).¹² A recent study has validated the prognostic and predictive utility of CDX2 in a large cohort of early-stage colon cancer.¹³ Paucity of data on the role of CDX2 expression in advanced colon cancer, in rectal cancer, and in Indian population had urged us to undertake this work.

In our present study, we aimed to characterize the expression of CDX2 by IHC in all stages of colon as well as rectal cancers and to identify its association with various baseline clinical and histopathological parameters. Overall response rates (ORRs) and survival outcomes were evaluated in relation to CDX2 expression within the study cohort.

Material and Methods

Patient Recruitment and Data Collection

The study was conducted as an ambispective observational study. Cases were enrolled into the study both retrospectively (from January 2014 to July 2016; 30 months) and prospectively from July 2016 till January 2018 (18 months). Last case

enrolled was followed up for a minimum of 6 months until the day of study analysis in July 2018. Cases enrolled into this study were identified from the central computerized database in the department of medical oncology at a tertiary cancer care center in South India. All newly diagnosed cases, age ≥ 18 years, any stage (early or advanced), with histopathologically proven diagnosis of CRC were considered for inclusion. Cases were excluded from the study if sufficient tissue material (either a biopsy or postoperative tissue blocks) was not available to perform IHC. Convenience sampling method was used for study enrollment and all consecutive cases meeting the inclusion/exclusion criteria registered during the study period were included into the study. Among 286 patients of CRC registered in the participating department during the study duration of January 2014 to January 2018, 110 cases met the inclusion criteria and were enrolled for analysis. Others ($n = 176$) were excluded due to unavailability of biopsy block or unstained slides.

Baseline clinical, histopathological, treatment, and follow-up details were collected from the medical file records. Baseline biopsies were reviewed for reconfirmation of pathological diagnosis for all eligible patients. Following outcome measures were considered for the study: primary measures: (1) percentage of CDX2 expression in the study cohort, (2) response rate in patients with advanced CRC, and (3) progression-free survival (PFS), and secondary outcome measures: (1) overall survival (OS).

Immunohistochemical Staining and Analysis of CDX2

Whole tissue section from the archived paraffin blocks were used and IHC staining for CDX2 was performed by using monoclonal mouse anti-human CDX2 (clone 88) (DAKO, USA) antibodies. Analysis of these was done by a semiquantitative method using a light microscope with a 40 \times objective at a total magnification of $\times 400$. Area of interest (tumor cell regions avoiding areas containing fibrosis or necrosis) on stained slides was identified and representative 20 high power fields (HPF) were selected for scoring of the cell. The relative percentage of CDX2 positive cells in relation to overall cellularity was calculated for each HPF and the final score for an individual patient was taken as an average of the 20 HPF.

CDX2 Scoring

CDX2 was considered positive only for nuclear staining. Any cytoplasmic positivity was considered artifactual. Positivity of any intensity was considered as positive.⁸ Normal colonic

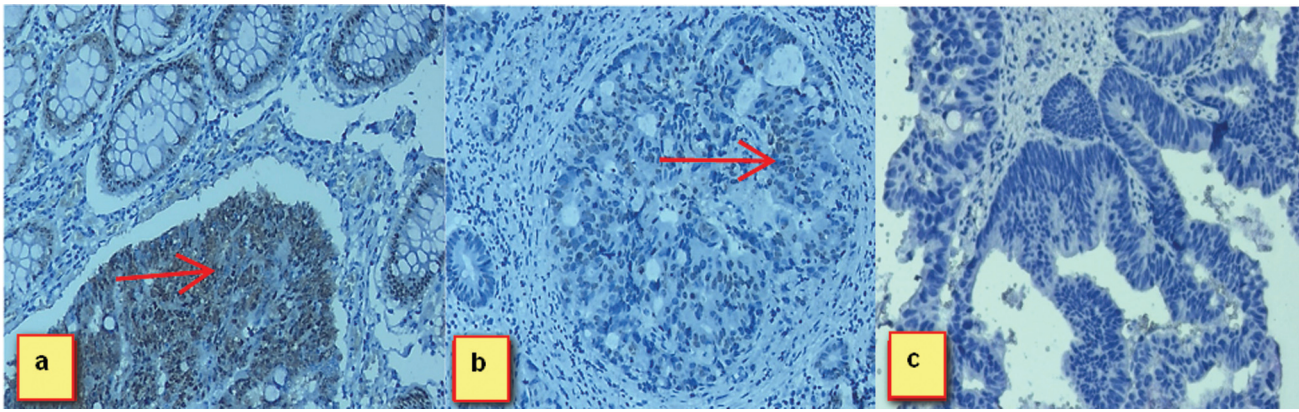


Fig. 1 (A) Immunohistochemistry (IHC) stain, 40 ×. Tumor cells show strong diffuse caudal-type homeobox 2 (CDX2) nuclear positivity along with normal intestinal mucosa. (B) IHC stain, 40 ×. Focal weak nuclear CDX2 expression in tumor cells. (C) IHC stain, 40 ×. Tumor cells are negative for CDX2 expression.

mucosal tissue was used as control. **Fig. 1** demonstrates positive and negative staining of CDX2.

CDX2 scoring was done in semiquantitative method for the intensity and percentage of the cells showing positivity. Scoring was done according to the following pattern described by Bayrak et al¹⁴: “less than 5% of tumor cells were given score of 0, 5 to 25% of tumor cells positive for CDX2 considered as 1+, 26 to 50% of tumor cells positive for CDX2 as 2+, 51 to 75% of tumor cells positive for CDX2 were taken 3+, and greater than 75% of tumor cells positive for CDX2 as 4+.” Additionally, positivity for CDX2 was also classified in the following subgroups: focal positivity if less than 50% of tumor cells were positive and diffuse positivity staining in more than 50% of the tumor cells.¹⁴

Statistical Analysis

Descriptive statistics was used for baseline characteristics, disease factors, laboratory, histopathological, treatment details, and CDX2 expression. Correlation of CDX2 expression with baseline clinical and histopathologic characteristics was done using chi-square test/Fisher’s exact test for categorical data. Response rate with respect to CDX2 was calculated using chi-square test. Kaplan–Meier method was used for survival estimation and log-rank test was used for comparison. Median follow-up time for the cohort was calculated using the reverse Kaplan method for potential follow-up. Cox proportional hazards method was used to evaluate significance of CDX2 expression in predicting survival outcomes, while adjusting for other parameters with a significance level below 0.25 at a univariate level. Two-sided *p*-value of less than 0.05 was considered statistically significant. All statistical analysis was done using SPSS version 20.0 and data was censored on June 30, 2018 for survival analysis.

Endpoint definitions: PFS was defined as the time from initial diagnosis to progression at any time, relapse from complete response (CR), initiation of new previously unplanned treatment, or death from any cause. OS was defined as the time from date of registration till date of last follow-up or death from any cause. ORR for advanced stage CRC was defined as partial response or CR to chemotherapy according to response evaluation criteria in solid tumors (RECIST) criteria 1.1.¹⁵

Ethics: The study was approved by the institutional research and ethics committee (vide letter no. JIP/IEC/SC/2016/29/897 dated 12/07/2016). Informed consent was obtained from cases enrolled prospectively while a waiver of consent was granted for the retrospective cases. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013.

Results

A total of 110 cases were included into the study for analysis. Summary of the main results is described below.

Baseline Characteristics

The median age of the study cohort was 51 years (range 21–79 years); 62 (56.4%) were male and 48 (43.6%) were female. Among 110 patients, 77 (70%) had colon cancer; 38 (34.5%) patients had right-sided tumor and 39 (35.5%) had left-sided colon involvement, and 33 (30%) had rectal cancer (**Table 1**). A significant proportion of patients, 42.7% (*n* = 47) had stage IV CRC, while 33 patients (30%) had stage II disease and 30 (27.3%) had stage III disease. Stage II patients had various overlapping high-risk features, mainly inadequate lymph node dissection in 20 (60.6%), and presence of lymphovascular invasion (LVSI) in 7 (21.2%). Serum carcinoembryonic antigen (CEA) levels were available for 78 patients. Median CEA level was 5.5 ng/mL with interquartile range (IQR) of 0.7 to 24.7 ng/mL. Sixty (72.3%) patients had elevated serum CEA levels while 23 (27.7%) had normal serum CEA levels.

Histopathological Details

Of the 110 patients in the analysis, 9 (8.2%) had mucinous histology. Based on grade of differentiation, grade I was the most common, seen in 62 (56.4%) patients, 34 (30.9%) had grade II, and 5 (4.5%) had grade III tumors. Out of 80 patients who underwent definite surgery, 38 (47.5%) had adequate lymph node dissection, 36 (45%) had inadequate lymph node dissection, and no information was available for 6 (7.5%) patients. Among 74 patients out of 80 whose lymph node

Table 1 Baseline demographic characteristics

	Features	Patients		Total n = 110
		Colon (77)	Rectum (33)	
1.	Age (median age): 51.47 y (21–79 y)			
	(a) ≤ 30 y	7	3	10 (9.1%)
	(b) 31–60 y	47	20	67 (60.9%)
	(c) > 60 y	23	10	33 (30%)
2.	Gender			
	(a) Male	42	20	62 (56.4%)
	(b) Female	35	13	48 (43.6%)
3.	Performance status			
	(a) 0–1	52	26	78 (70.9%)
	(b) 2	17	7	24 (21.8%)
	(c) 3	8	0	8 (7.3%)
4.	Site of disease			
	(a) Right colon	38	–	38 (34.5%)
	(b) Left colon	39	–	39 (35.5%)
	(c) Rectum	–	33	33 (30%)
5.	Stage of disease			
	(a) Stage II	28	5	33 (30%)
	(b) Stage III	21	9	30 (27.3%)
	(c) Stage IV	28	19	47 (42.7%)
6.	Symptom duration (median): 3 mo (0–24 mo)			
	(a) < 1 mo	13	2	15 (22.7%)
	(b) 1–6 mo	50	25	75 (68.2%)
	(c) > 6 mo	14	6	20 (18.1%)
7.	Symptoms ^a			
	(a) Obstruction and perforation	12	1	13 (11.2%)
	(b) Abdominal pain/distension	57	15	72 (62.1%)
	(c) Vomiting	23	1	24 (20.7%)
	(d) Bleeding PR	15	23	38 (32.8%)
	(e) Altered bowel habit	10	8	18 (15.5%)
	(f) Decreased appetite/weight	3	1	4 (3.4%)

Abbreviation: PR, partial response.

^aOverlapping symptoms may be present.

status was available, 42 (56%) were positive and 32 (44%) had negative lymph node involvement. Positive LVI was seen in 35 patients and 3.7% had unknown LVI status. Molecular studies were available for very few patients. KRAS/NRAS testing was done in 4 patients, 3 were wild-type and one was mutated. Six patients were tested by IHC for mismatch repair (MMR), and all were MMR proficient.

CDX2 Expression

All the 110 patients of CRC were evaluated for CDX2 marker, a nuclear protein, by IHC with anti-CDX2 clone 88 antibodies. With the use of scoring system as described in methodology, 73 (66.4%) patients were positive for CDX2 and 37 (33.6%)

were negative as shown in ► **Table 2**. Median CDX2 positivity was 26.5% (IQR 1–70). In 37 patients who had negative CDX2 expression (< 5% of tumor cells positive), 22 patients had a score of 0, that is, no CDX2 expression at all and 15 patients had CDX2 score of 1 to 4%.

Corelation of CDX2 with Baseline Parameters

The association of CDX2 expression with baseline clinical and histopathological parameters is described in ► **Table 3**. Lack of CDX2 expression was significantly associated with more advanced stage of disease ($p \leq 0.0001$), rectal cancer ($p = 0.04$), higher grade of differentiation ($p = 0.045$), and presence of LVI ($p = 0.028$). Patients with CDX2 positivity

Table 2 CDX2 expression in pathological tissue blocks

CDX2 Expression		Stage II (n = 33)	Stage III (n = 30)	Stage IV (n = 47)	Total, n = 110
CDX2	Positive (\geq 5% of tumor cells +ve)	31	23	19	73 (66.4%)
	Negative (< 5% of tumor cells +ve) ^a	2	7	28	37 (33.4%)
Types of scoring for CDX2 positive patients					
		Stage II	Stage III	Stage IV	n = 73
CDX2 scoring: diffuse/focal	Diffuse	25	14	6	45 (61.6%)
	Focal	6	9	13	28 (38.4%)
CDX2 scoring in percentage	5–25%	3	4	8	15 (20.5%)
	26–50%	3	6	6	15 (20.5%)
	51–75%	15	6	1	22 (30.2%)
	> 75%	10	7	4	21 (28.8%)

Abbreviations: CDX2, caudal-type homeobox 2; IQR, interquartile range.

Note: Median CDX2 positivity: 26.5%, IQR (1–70).

^aIn 37 patients who had negative CDX2 expression (< 5% of tumor cells positive), 22 patients had a score of 0, i.e., no CDX2 expression at all and 15 patients had CDX2 score of 1–4%.

had relatively higher ORR as compared with CDX2 negative patients (57.1% vs. 36.8%, $p = 0.22$) though statistically nonsignificant.

Treatment Particulars

Of 110 patients in the study, intent of treatment at baseline presentation was curative for 73 (66.4%) versus palliative for 37 (33.6%) patients. Definite surgery was done for 68.9% patients, 7.8% had palliative surgery, and 23.3% had no surgical intervention.

Ninety-seven (88.8%) received chemotherapy in the form neoadjuvant chemotherapy/chemoradiotherapy ($n = 17$, 17.5%), adjuvant chemotherapy ($n = 50$, 51.6%), or palliative chemotherapy ($n = 30$, 30.9%). The chemo regimens used were: combination of capecitabine plus oxaliplatin (CapeOX) in 67 (69.1%), combination of 5FU, leucovorin, and oxaliplatin (FOLFOX) in 13 (13.4%), single agent capecitabine in 16 (16.5%), and combination of 5FU, irinotecan, and leucovorin (FOLFIRI) in 1 (1%) patients. Thirty-six (37%) patients required dose modification and there was a delay of chemotherapy between the cycles in 40 (41.2%) patients for various reasons. Among 97 patients, 50 who received neoadjuvant chemotherapy/chemoradiotherapy or palliative chemotherapy were eligible for response assessment. Out of 50 eligible patients, responses were available for 41 (82%) patients. ORR was 19 (38%), 7 (14%) had stable disease and 15 (30%) had progressive disease while in 9 (18%) response was not available, as patients were lost to follow-up. Most common adverse effects with chemotherapy were hand-foot syndrome ($n = 63$, 61.2%), gastrointestinal (nausea; $n = 64$, 62.1%), and hematological (anemia; $n = 45$, 43.7%).

Survival Outcomes

The median follow-up of the study was 16 months. For the entire study cohort median PFS for stage II and III was not reached while for stage IV it was 15.5 months (95% confidence interval [CI] 10.4–20.5 months). Median OS for stage II

and III were also not reached while for stage IV it was 18.7 months (95% CI 15.3–22.1 months). For stage II, III, and IV, PFS at 2 years were 84, 68, and 25% and OS at 2 years 91, 73, and 31%, respectively, as shown in ►Fig. 2. The median PFS and OS were not reached for CDX2 positive tumors compared with CDX2 negative patients who had median PFS of 15.9 months (95% CI 10.3–21.4 months) and median OS of 25.8 months (95% CI 12.1–39.4 months). At 2 years, PFS and OS were 66 and 77% for CDX2 positive and 30 and 46% for CDX2 negative patients, respectively, as shown in ►Fig. 2.

Factors Affecting Survival Outcome

Factors associated with significantly poor PFS in univariate analysis, described in ►Table 4, were advanced stage (stage IV) (hazard ratio [HR] = 6.27, 95% CI: 2.14–18.30), lack of CDX2 expression (HR 2.47, 95% CI: 1.22–5.03), and higher grade of tumor differentiation (HR for grade II and III were 2.08 and 10.24, 95% CI: 0.91–4.74 and 3.46–30.30, respectively). Factors associated with poorer OS were stage IV disease (HR = 8.63, CI: 2.01–37.06), lack of CDX2 expression (HR = 2.43, 95% CI: 1.15–5.12), and higher grade of differentiation (HR for grade II and III were 2.65 and 6.04, 95% CI: 1.06–6.60 and 1.78–20.49, respectively). However, in multivariate analysis only stage and grade were independently associated with both PFS and OS.

Discussion

Risk stratification based upon prognostic markers is applied for treatment and predicting outcomes of CRCs. In early-stage colon cancer, higher grade, presence of obstruction or perforation, higher stage, and MMR deficiency are considered to harbor a poorer prognosis. Recently, CDX2, an intestine-specific nuclear transcription factor, has emerged as a new prognostic biomarker in CRC.^{13,16,17} In this study, we identified the pattern of CDX2 expression in all the stages of CRC and have evaluated the association of CDX2 expression

Table 3 Correlation of CDX2 with baseline parameters and response rate

Features n = 110	Positive CDX2 expression (n = 73)	Lack of CDX2 expression (n = 37)	p
Age			
(a) ≤ 30 y (n = 10)	6 (8.2%)	4 (10.8%)	0.585
(b) 31–60 y (n = 67)	47 (64.4%)	20 (54.1%)	
(c) > 60 y (n = 33)	20 (27.4%)	13 (35.1%)	
Gender (n = 110)			
(a) Male (n = 62)	37 (50.7%)	25 (67.6%)	0.107
(b) Female (n = 48)	36 (49.3%)	12 (32.4%)	
Stage of disease (n = 110)			
(a) Stage II (n = 33)	31 (42.5%)	2 (5.4%)	< 0.0001
(b) Stage III (n = 30)	23 (31.5%)	7 (18.9%)	
(c) Stage IV (n = 47)	19 (26%)	28 (75.7%)	
Site of disease: colon/rectum (n = 110)			
(a) Colon (n = 77)	56 (76.7%)	21 (56.8%)	0.047
(b) Rectum (n = 33)	17 (23.3%)	16 (43.2%)	
Sidedness of primary disease			
(a) Right colon (n = 38)	27 (37%)	11 (29.7%)	0.096
(b) Left colon (n = 39)	29 (39.7%)	10 (27.0%)	
(c) Rectum (n = 33)	17 (23.3%)	16 (43.2%)	
Differentiation (n = 110)			
(a) Grade I (n = 62)	47 (64.4%)	15 (40.5%)	0.045
(b) Grade II (n = 34)	18 (24.7%)	16 (43.2%)	
(c) Grade III (n = 5)	4 (5.5%)	1 (2.7%)	
(d) Mucinous (n = 9)	4 (5.5%)	5 (13.5%)	
LVSI (n = 72): n = 15, n = 57			
(a) Absent (n = 48)	42 (73.7%)	6 (40%)	0.028
(b) Present (n = 24)	15 (26.3%)	9 (60%)	
Response to chemotherapy (NACT/NACTRT or palliative) (n = 40): n = 21, n = 19			
Overall response (complete + partial responses)	12 (57.1%)	7 (36.8%)	0.22
Stable disease + progressive disease	9 (42.8%)	12 (63.2%)	

Abbreviations: CDX2, caudal-type homeobox 2; LVSI, lymphovascular invasion; NACT, neoadjuvant chemotherapy; NACTRT, neoadjuvant chemoradiotherapy.

with baseline clinical and histopathological features, response to chemotherapy, and survival outcomes. Our study has shown that lack of CDX2 expression is associated with advanced stage, higher grade, presence of LVSI, and poorer PFS and OS.

CDX2, a nuclear protein expressed by the intestinal epithelium cells throughout the gut, is essential for the proliferation and growth of epithelial cells and is frequently downregulated during tumorigenesis.^{18–20} Malignant progression of colorectal adenocarcinomas is characterized by the expression of oncoprotein β -catenin^{21,22} during tumor cell invasion and metastasis. CDX2 reduces the cellular proliferation and inhibits the β -catenin/T cell transcription factor transcriptional activity in colon cancer.^{23,24} Lack of CDX2 is associated with

loss of differentiation of gastrointestinal epithelium leading to unregulated proliferation.^{25–28} Though the precise mechanisms for loss of CDX2 expression are not well characterized, low expression by IHC/gene expression has been associated with poorer prognosis, especially in early-stage CRC.¹³ Since IHC is fast, reliable, and economical, we evaluated the pattern of expression of CDX2 by IHC in all stages of CRC and its association with prognosis.

There is variation in loss of CDX2 expression reported in different studies ranging from 4 to 30%, depending on the patient population, proportion of early and advanced stage colon cancers, proportion of rectal cancers, method for studying CDX2 (gene expression profile or IHC), tissue heterogeneity depending on use of tissue microarray or

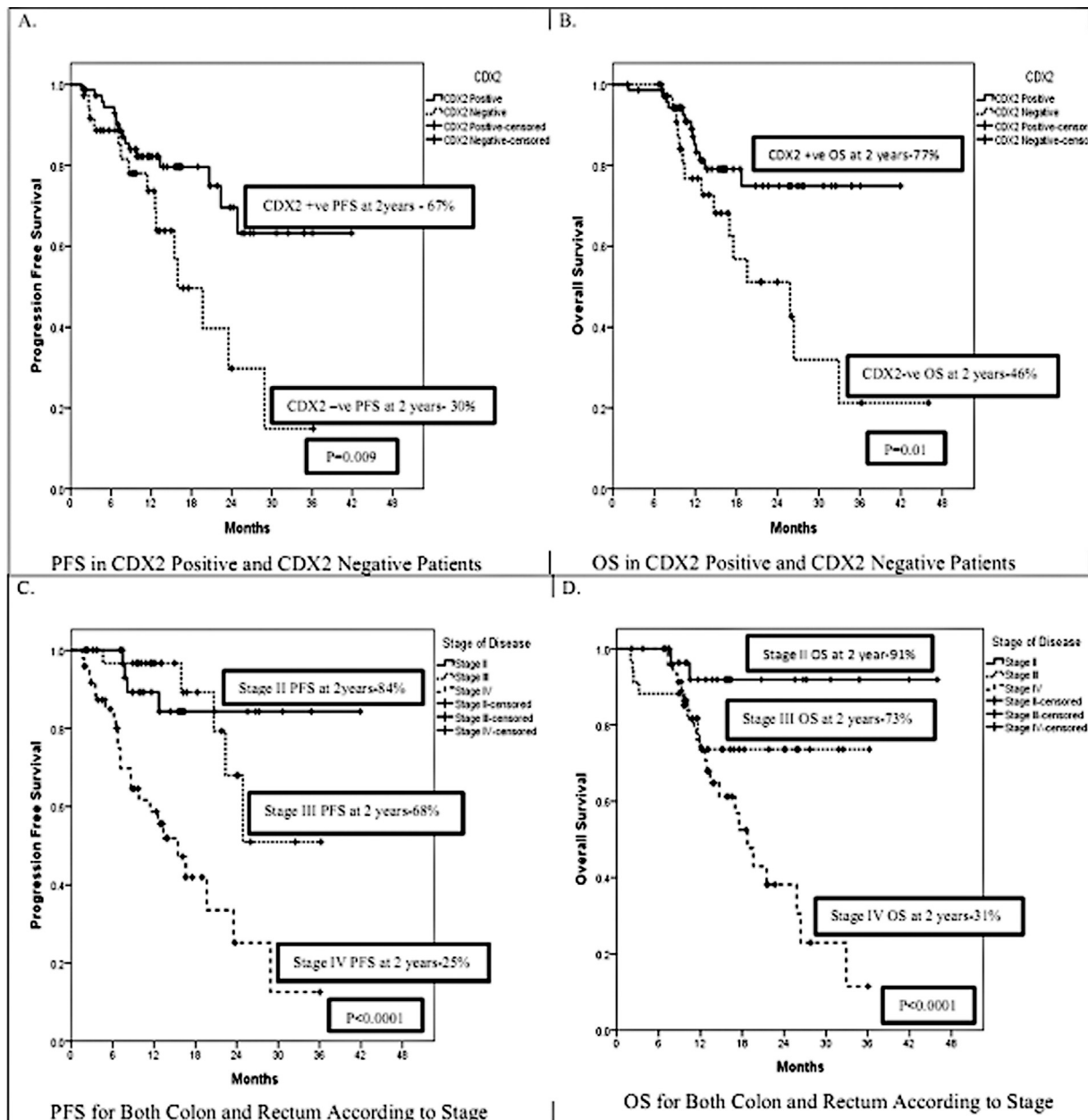


Fig. 2 (A) Progression-free survival for caudal-type homeobox 2 (CDX2) positive and negative patients. (B) Overall survival for CDX2 positive and negative patients. (C) Progression-free survival for stage II, III, and IV colorectal cancer. (D) Overall survival for stage II, III and IV colorectal cancer.

whole tissue section for IHC, and cutoff chosen for CDX2 positivity.^{13,16,29} In our study, we have found a higher rate of loss of CDX2 expression (33.6%) overall, possibly because of inclusion of patients with rectal cancers, a significant proportion of patients with stage IV disease, and use of whole tissue section for IHC. Our results are comparable to the study by Baba et al who reported loss of CDX2 in 29% of patient population of all stages of CRC.¹⁶ Notably, CDX2 expression varies with the stage of CRC showing significantly low or loss of expression in advanced stage of the disease.¹⁶ In our study around 2% of stage II CRC lacked CDX2 as compared with 6 and 25% of stage III and IV cases. In contrast to the study by Bae et al where female gender was associated with loss of CDX2 expression,⁷ we did not find any significant association with age or gender, possibly due to population

heterogeneity. Disease-related parameters like higher stage of disease, rectal site, higher grade of differentiation, and presence of LVSI were significantly associated with lack of CDX2 expression in our as well as in other studies.^{7,13,16,30–32} In our study, in patients receiving neoadjuvant or palliative chemotherapy, loss of CDX2 expression was not associated with response rate possibly due to small assessable number ($n=40$) in this subgroup. Association of CDX2 expression with response rate to chemotherapy in the neoadjuvant or palliative setting has not been evaluated in any other study.

As CDX2 has an important role in impeding proliferation and promoting cellular differentiation,³³ loss of CDX2 expression is associated with poor outcomes. CDX2 loss contributes to aggressive disease behavior,³⁴ thereby increasing the likelihood of advanced stage.³⁵ In our study,

Table 4 Univariate analysis for progression-free survival and overall survival of study cohort

Features	Progression-free survival		Overall survival	
	HR (95% CI)	p	HR (95% CI)	p
Age (n = 110)				
(a) < 30 y (n = 10)	1		1	
(b) 31–60 y (n = 67)	0.54 (0.18–1.61)	0.27	0.49 (0.17–1.49)	0.21
(c) > 60 y (n = 33)	0.64 (0.19–2.10)	0.47	0.65 (0.20–2.13)	0.48
Gender (n = 110)				
(a) Male (n = 62)	1		1	
(b) Female (n = 48)	1.02 (0.50–2.05)	0.93	1.58 (0.75–3.33)	0.22
Stage of disease (n = 110)				
(a) Stage II (n = 33)	1		1	
(b) Stage III (n = 30)	1.36 (0.36–5.11)	0.64	2.68 (0.51–13.93)	0.23
(c) Stage IV (n = 47)	6.27 (2.14–18.30)	0.001	8.63 (2.01–37.06)	0.004
Site of disease (n = 110)				
(a) Colon (n = 77)	1		1	
(b) Rectum (n = 33)	1.26 (0.60–2.64)	0.54	1.13 (0.52–2.46)	0.74
Serum CEA levels (n = 78)				
(a) Normal (n = 22)	1		1	
(b) Elevated (n = 56)	1.89 (0.63–5.61)	0.25	3.85 (0.89–16.67)	0.07
CDX2 (n = 110)				
(a) Positive (n = 73)	1		1	
(b) Negative (n = 37)	2.47 (1.22–5.03)	0.01	2.43 (1.15–5.12)	0.01
Differentiation (n = 110)				
(a) Grade I (n = 62)	1		1	
(b) Grade II (n = 34)	2.08 (0.91–4.74)	0.07	2.65 (1.06–6.60)	0.03
(c) Grade III (n = 5)	10.24 (3.46–30.30)	< 0.0001	6.04 (1.78–20.49)	0.004
(d) Mucinous (n = 9)	3.31 (0.69–9.24)	0.16	4.92 (1.56–15.51)	0.006
LVSI (n = 72)				
(a) Absent (n = 48)	1		1	
(b) Present (n = 24)	1.23 (0.37–4.12)	0.72	2.14 (0.61–7.43)	0.22

Abbreviations: CDX2, caudal-type homeobox 2; CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; LVSI, lymphovascular invasion.

loss of CDX2 in CRC patients was associated with both poor PFS and OS in univariate analysis, but no significance was seen in multivariate analysis possibly due to small sample size. Independent predictors of poor survival in multivariate analysis in our patient cohort were the widely established factors of advanced stage and higher grade. Independent prognostic effect of loss of CDX2 expression on survival outcomes has diverse results across studies. Results similar to our study of the significance of loss of CDX2 with poor survival outcomes in univariate but not in multivariate analysis in CRC was observed by Baba et al and Lugli et al,^{16,36} though other studies have shown that loss of CDX2 expression was independently associated with poor prognosis mostly in early-stage CRC.^{7,13,37}

The limitations of our study were inclusion of a heterogeneous patient population, small sample size due to unavail-

ability of many tissue blocks, some missing baseline information in the retrospective cohort, and short follow-up. Also, absence of MMR status for majority of cases was another main limitation. Use of whole tissue sections instead of tissue microarray, though labor-intensive, avoids tumor heterogeneity and false negative rates and this was one of the strengths of our study.

Conclusion

In conclusion, we found that loss of CDX2 expression is associated with markers of poor prognosis such as advanced disease stage, higher tumor grade, and presence of LVSI in CRC. Loss of CDX2 could possibly drive the evolution to poor biologic characteristics in the tumor. Loss of CDX2 was associated with worse PFS and OS in univariate analysis

and our results attests its role as a poor prognostic factor in CRC. However, independent prognostic role of CDX2 in survival outcomes of CRC needs to be confirmed in larger multicenter data set with relatively longer follow-up.

Authors' Contributions

Study concept and design: S.K., J.S., B.D., N.G.R. Provision of patient and study material: J.S., N.G.R., B.D., S.K., P.G., N.K.M., I.C. Performance and interpretation of IHC (immunohistochemistry): J.S., N.G.R., I.C. Data collection and analysis: J.S., N.G.R., S.K., B.D., K.K.M., I.C. Manuscript writing: J.S., S.K., B.D., P.G., N.G.R., N.K.M. Final approval of manuscript: all authors.

Declaration

Manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and all author believes that the manuscript represents honest work.

Justification for authors more than 6: this study was an interdepartmental work, initiated by department of medical oncology in collaboration with departments of pathology and surgery. Authorship has been attributed to all the researchers who have made substantial contributions to the work as per the ICMJE authorship criteria.

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Conflict of Interest

All authors declare no conflict of interest.

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