International Journal of General Medicine

Dovepress

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ORIGINAL RESEARCH

Caudal-Type Homeobox Transcription Factor 2 is a Favorable Prognostic Indicator in Stage II and III Gastric Cancer Following Curative Surgery

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Background: The study explored the prognostic value of caudal-type homeobox transcription factor 2 (CDX2) in stage II and III gastric cancer.

Methods: This study evaluated the expression level of CDX2 in gastric cancer in a hospital cohort (n=197) using immunohistochemistry. According to a semiquantitative score used to determine CDX2 expression, the cases were divided into a low CDX2 group (116 cases) and a high CDX2 group (81 cases). The RNA-seq expression data from 291 patients with stage II and III gastric cancer from The Cancer Genome Atlas (TCGA) cohort were used to verify the immunohistochemistry results. Based on the median CDX2 expression value, the TCGA patients were divided into a low CDX2 group (145 cases) and a high CDX2 group (146 cases). The relationships among CDX2 expression and clinicopathological features were determined using the Chi-square test. Cox proportional hazards regression models were applied to estimate the independent prognostic factors. The probability of survival was determined using the Kaplan-Meier method and Log rank tests.

Results: Based on the Cox multivariate analysis, CDX2 was the independent prognostic factor in the hospital and TCGA cohorts. In the hospital cohort, CDX2 expression was associated with an improved DFS (hazard ratio [HR] = 0.4076, 95% CI, 0.2675–0.6210, P = 0.0001) and OS (HR = 0.4183, 95% CI, 0.2744–0.6375, P = 0.0002). In the TCGA cohort, CDX2 expression also was associated with an improved DFS (HR = 0.5948, 95% CI, 0.4153–0.8521, P = 0.0054) and OS (HR = 0.5976, 95% CI, 0.4172–0.8561, P = 0.0058). Furthermore, the CDX2 expression level was correlated with an improved DFS (P = 0.0025) and OS (P = 0.0015) using the Kaplan-Meier Plotter database for gastric cancer.

Conclusion: CDX2 is a potential prognostic biomarker for stage II and III gastric cancer. In addition, CDX2 positive cancer patients are more likely to have resectable tumors and exhibit better survival rates.

Keywords: gastric cancer, CDX2, immunoreactivity, chemotherapy, prognostic marker

Introduction

Gastric cancer is the sixth most common malignant tumor and the third cause of cancer-related death worldwide.[1] Global Cancer Statistics 2020 indicates that 1,089,103 new cases are diagnosed, and 768,793 people will die from this disease.[1] Gastric cancer is more common in the Asia Pacific regions, especially in Japan, South Korea, and East Asia.[2] China is a large East Asian country with a high death rate due to gastric cancer.[3] Notably, the incidence and mortality rates for gastric cancer classified by age have steadily decreased.[4] Gastric cancer is often diagnosed at more advanced stages, usually because the initial symptoms are non-specific or overlooked.[5] Based on the stage and tumor location, patients typically are treated with surgery, chemotherapy, and radiotherapy, and the invasiveness of the disease depends on the tumor differentiation.[6] However, patients with stage IV gastric cancer usually are considered inoperable. In the past decade, the survival rate of resectable stage II and III gastric cancer patients has improved to some degree due to the

Received: 1 April 2024
Accepted: 12 September 2024
Published: 27 September 2024

introduction of adjuvant treatment.[7] Nevertheless, the current treatment protocols fail to achieve substantial curative benefits. The lack of convenient and reliable standards to provide accurate prognoses for patients with resectable stage II and III gastric cancer makes it difficult to identify patients who are most likely to benefit from adjuvant or salvage treatment.

Caudal-type homeobox transcription factor 2 (CDX2) is a transcription factor involved in intestinal cell proliferation and differentiation and primarily regulates the intestinal function of progenitor cells.[8–10] CDX2 is expressed in intestinal epithelial cells of adult mammals from the proximal duodenum to the distal rectum.[11,12] The absence of CDX2 expression is related to the prognosis of patients with colorectal carcinoma.[13–15] Yu and colleagues have shown that CDX2 inhibited colon cancer cell proliferation by restricting Wnt/β-catenin signaling.[16] Tomasello et al demonstrated that the expression of CDX2 was a strong positive predictor for stage II and III colorectal cancer patients, and the lack of expression of CDX2 was considered a critical indicator in determining adjuvant chemotherapy.[17] Incremental CDX2 expression was related to lymph node metastasis (LNM) in gastric cancer and, high expression was associated with a more aggressive phenotype in AGS cells.[18] Alternatively, CDX2 silencing significantly inhibited the growth of MGC-803 human cancer cells.[19] Another meta-analysis indicated that CDX2 expression in gastric cancer was associated with an increase in the five-year survival rate.[20] In Xu B's study, circ_0017274 downregulation boosted cisplatin sensitivity by acting on miR-637/CDX2 in cisplatin-resistant gastric cancer cells.[21] Liu H proved that Res inhibits the growth of MKN7 and TMK1 cells by increasing RUNX3 and CDX2 expression levels with the potential involvement of the β-catenin/TCF-4 signaling pathway.[22] Li T's study revealed that the miR-92a-1-5p/FOXD1/NF-κB/CDX2 regulatory axis played critical roles in the generation of the IM phenotype of gastric cells.[23] Finally, CDX2 expression has been linked with gastrointestinal malignancy, but its role in the progression of gastric cancer is unclear.

In this study, CDX2 expression was investigated in a retrospective cohort of stage II and III gastric cancer patients using an immunohistochemical (IHC) approach. Clinicopathologic variables and survival were assessed in these patients. We also analyzed the data from TCGA patients to validate the main findings. Finally, we used the RNA-seq expression data from the TCGA patients to explore potential mechanisms that might be involved.

Materials and Methods

Data Sources

One hundred and ninety-seven patients with stage II and III gastric cancer who underwent surgery from 2013 to 2018 in Daqing Oilfield General Hospital were enrolled. We verified the major findings in two hundred and ninety-one patients with stage II and III gastric cancer who underwent surgery from 2010 to 2014 and were included in The Cancer Genome Atlas (TCGA) database. Participants were considered eligible if they fulfilled the following criteria: (1) presented with stage II and III gastric cancer based on pathological findings; (2) received primary tumor resection; (3) the follow-up information was complete. The exclusion criteria were as follows: (1) a malignant tumor was present at another site, or the tumor was not an adenocarcinoma; (2) the patient exhibited stage I or IV gastric cancer based on pathological findings and critical information was missing; (3) the patient received anti-tumor therapy before surgery.

The clinical and follow-up information was obtained from Daqing Oilfield General Hospital. The tissue samples obtained at this hospital were available for use in the current study. One hundred ninety-seven patients with stage II and III gastric cancer were selected who had CDX2 expression information. These patients provided written informed consent before surgery for the use of the resected samples as well as their clinical and follow-up data. The mRNA sequencing data and TCGA clinical information were downloaded from https://www.cancer.gov/ccg/research/genome-sequencing/tcga on September 14, 2022. Only stage II and III gastric cancer patients were enrolled in this study. Furthermore, 291 patients with stage II and III gastric cancer with CDX2 expression information also were identified. Therefore, 488 patients with II and stage III gastric cancer were included in this study. The Ethical Committee of Daqing Oilfield General Hospital approved this study and the study complied with guidelines established by the Declaration of Helsinki statement of ethical principles for medical research involving human subjects. Based on the publicly available data published in the TCGA database, all patients are required to remove their personal identification without the approval of the ethics committee. The screening assessment allowed the identification of CDX2 as a candidate biomarker

for gastric cancer tissues. We explored the relationship between CDX2 and prognosis among stage II and III gastric cancer patients using subgroup analysis involving a retrospective patient cohort. The flow diagram for the study is presented in Figure 1.

Immunohistochemistry (IHC) Staining and Evaluation

For every enrolled patient in our hospital, 4-μm serial sections were obtained from formalin-fixed, paraffin-embedded tumor tissue blocks and underwent IHC analysis. For CDX2 staining, the primary antibody was purchased from MXB, Fuzhou, China (1:1 dilution, MAB-1056). The CDX2 was localized to the nucleoplasm, and only nuclear CDX2 staining was considered positive. The histological analysis for CDX2 included the density and intensity of stained cells. The density of positively stained cells was assessed as follows: (1) 0 indicated less than 1% of the cells were stained; (2) 1 indicated 1–10% stained cells; (3) 2 indicated 11–50% stained cells; (4) 3 indicated 51–75% stained cells; (5) 4 indicated 76–100% stained cells. The intensity of cellular staining was scored as follows: (1) 0 indicated no tumor cells were stained; (2) 1 for barely perceptible nuclear reactivity in a minority of tumor cells; (3) 2 for moderate nuclear reactivity in a majority of tumor cells; (4) 3 for strong nuclear reactivity in a majority of tumor cells. According to the density and intensity of cells expressing CDX2 expression, the patients were divided into two groups: (1) negative group, 0–1 score; (2) positive group, 2+ score. Representative images of the scores for CDX2 IHC staining are shown in Figure S1.

Follow-Up and Statistical Analysis

Disease-free survival (DFS) was calculated from the primary surgery to the first evidence of localized or remote metastasis that was scored as an event. Overall survival (OS) was calculated from the time of primary surgery to the patient's death due to any reason or the last follow-up assessment. The relationships among CDX2 expression and

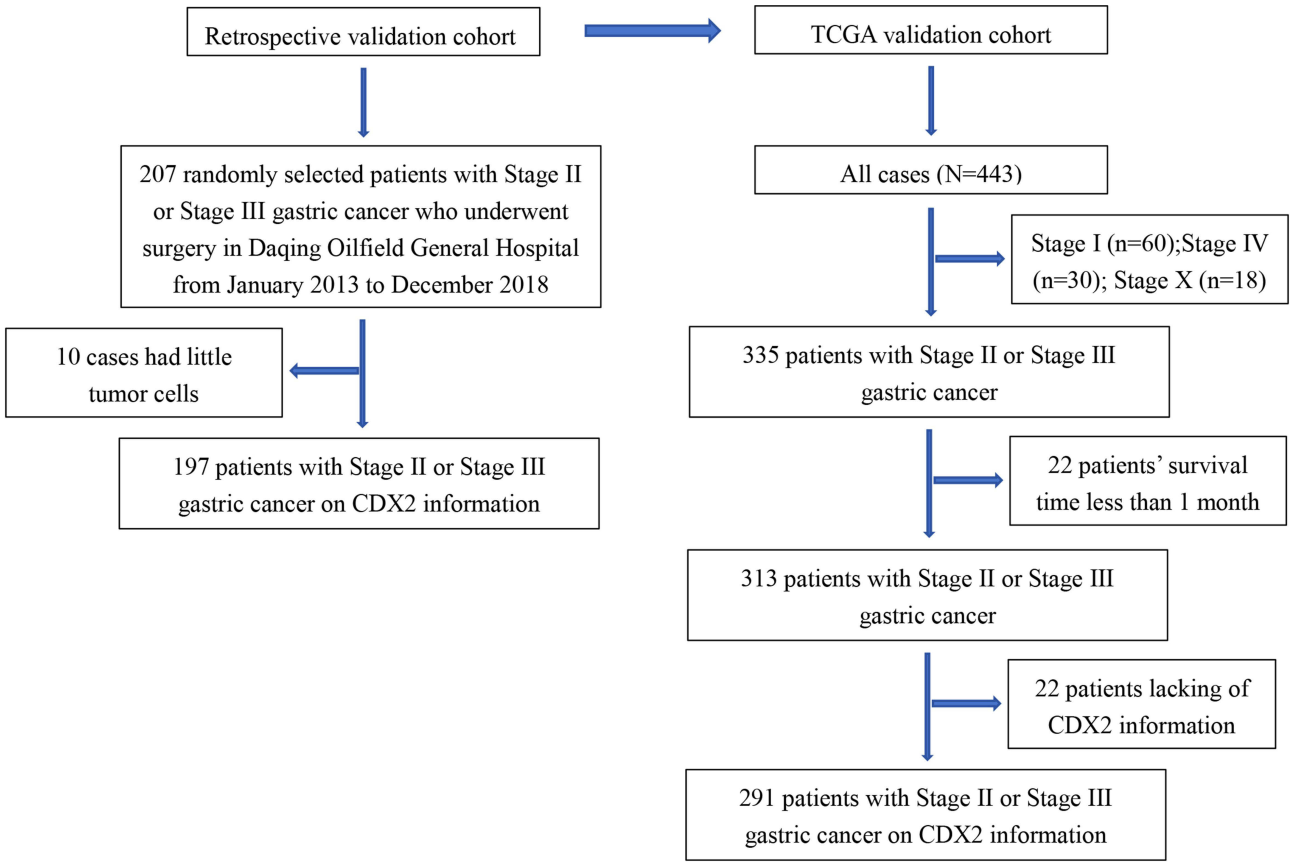

Figure 1 Study flow diagram.

clinical and pathological features were determined using the Chi-square test. Cox proportional hazards regression models were applied to assess the potential independent prognostic factors. The probability of survival was evaluated using the Kaplan-Meier and Log rank tests. Nomogram models were constructed to predict survival time. The statistical analyses were carried out using GraphPad Prism (version 8.0.2, GraphPad Software), and R (version 3.6.0; Vienna, Austria. URL: http://www.R-project.org/). Statistically significant differences were determined by a P-value < 0.05.

Results

Patients

Based on the median value of CDX2 expression, the patients were divided into two groups, a low CDX2 group (145 cases) and a high CDX2 group (146 cases). The baseline clinicopathological characteristics of the TCGA cohorts are summarized in Table 1. There was no significant difference in the clinicopathological characteristics between the low and high CDX2 expression groups (P>0.05). According to the density and intensity of the cellular CDX2 expression, the patients were divided into two groups, a low CDX2 group (116 cases) and a high CDX2 group (81 cases). The baseline clinicopathological characteristics of the hospital data are summarized in Table 2. When compared with the low CDX2 expression group, the high CDX2 expression group was significantly associated with the T stage, N stage, and tumor size (P<0.05).

Table 1 The Baseline Clinicopathological Characteristics of the TCGA Cohort

n	Level	Overall 291	Low CDX2 145	High CDX2 146	p
Age (median [IQR])		66.00 [58.00, 72.00]	65.00 [58.00, 72.00]	68.00 [58.25, 73.75]	0.124
Sex	Male	192 (66.0)	98 (67.6)	94 (64.4)	0.651
	Female	99 (34.0)	47 (32.4)	52 (35.6)	
Histological type	Adenocarcinoma	158 (54.3)	87 (60.0)	71 (48.6)	0.108
	Intestinal Adenocarcinoma	130 (44.7)	56 (38.6)	74 (50.7)	
	Unknown	3 (1.0)	2 (1.4)	1 (0.7)	
T stage	T2	47 (16.2)	18 (12.4)	29 (19.9)	0.221
	T3	157 (54.0)	81 (55.9)	76 (52.1)	
	T4	87 (29.9)	46 (31.7)	41 (28.1)	
N stage	N0	61 (21.0)	28 (19.3)	33 (22.6)	0.951
	N1	90 (30.9)	47 (32.4)	43 (29.5)	
	N2	68 (23.4)	35 (24.1)	33 (22.6)	
	N3	66 (22.7)	32 (22.1)	34 (23.3)	
	NX	6 (2.1)	3 (2.1)	3 (2.1)	
M stage	M0	282 (96.9)	141 (97.2)	141 (96.6)	1.000
	MX	9 (3.1)	4 (2.8)	5 (3.4)	
TNM stage	Stage II	119 (40.9)	54 (37.2)	65 (44.5)	0.253
	Stage III	172 (59.1)	91 (62.8)	81 (55.5)	
Grade	G1	6 (2.1)	1 (0.7)	5 (3.4)	0.105
	G2	90 (30.9)	39 (26.9)	51 (34.9)	
	G3	191 (65.6)	102 (70.3)	89 (61.0)	
	GX	4 (1.4)	3 (2.1)	1 (0.7)	
Anatomic neoplasm subdivision	Gastroesophageal Junction	28 (9.6)	16 (11.0)	12 (8.2)	0.107
	Cardia/Proximal	37 (12.7)	25 (17.2)	12 (8.2)	
	Fundus/Body	101 (34.7)	52 (35.9)	49 (33.6)	
	Antrum/Distal	115 (39.5)	47 (32.4)	68 (46.6)	
	Stomach (NOS)	4 (1.4)	2 (1.4)	2 (1.4)	
	Unknown	6 (2.1)	3 (2.1)	3 (2.1)	

(*Continued*)

Table 1 (Continued).

n	Level	Overall 291	Low CDX2 145	High CDX2 146	p
Family history of stomach cancer	No	226 (77.7)	110 (75.9)	116 (79.5)	0.495
	Yes	16 (5.5)	7 (4.8)	9 (6.2)	
	Unknown	49 (16.8)	28 (19.3)	21 (14.4)	
Race	White	188 (64.6)	92 (63.4)	96 (65.8)	0.681
	Asian	68 (23.4)	34 (23.4)	34 (23.3)	
	Others	8 (2.7)	3 (2.1)	5 (3.4)	
	Unknown	27 (9.3)	16 (11.0)	11 (7.5)	
Chemotherapy	No	137 (47.1)	64 (44.1)	73 (50.0)	0.377
	Yes	154 (52.9)	81 (55.9)	73 (50.0)	
Radiation therapy	No	115 (39.5)	57 (39.3)	58 (39.7)	0.670
	Yes	41 (14.1)	18 (12.4)	23 (15.8)	
	Unknown	135 (46.4)	70 (48.3)	65 (44.5)	
Radical resection	R0	244 (83.8)	118 (81.4)	126 (86.3)	0.372
	R1	16 (5.5)	7 (4.8)	9 (6.2)	
	R2	3 (1.0)	2 (1.4)	1 (0.7)	
	RX	28 (9.6)	18 (12.4)	10 (6.8)	

Abbreviation: IQR, interquartile range.

Table 2 The Baseline Clinicopathological Characteristics of the Hospital Cohort

n	Level	Overall 197	Low CDX2 116	High CDX2 81	p
Sex	Male	131 (66.5)	81 (69.8)	50 (61.7)	0.302
	Female	66 (33.5)	35 (30.2)	31 (38.3)	
Age	≤60	94 (47.7)	55 (47.4)	39 (48.1)	1.000
	>60	103 (52.3)	61 (52.6)	42 (51.9)	
BMI	≤22.0	96 (48.7)	55 (47.4)	41 (50.6)	0.766
	>22.0	101 (51.3)	61 (52.6)	40 (49.4)	
TNM stage	II	20 (10.2)	10 (8.6)	10 (12.3)	0.540
	III	177 (89.8)	106 (91.4)	71 (87.7)	
T stage	T2	1 (0.5)	1 (0.9)	0 (0.0)	0.015
	T3	64 (32.5)	28 (24.1)	36 (44.4)	
	T4	130 (66.0)	85 (73.3)	45 (55.6)	
	Tx	2 (1.0)	2 (1.7)	0 (0.0)	
N stage	N0	23 (11.7)	13 (11.2)	10 (12.3)	0.044
	N1	25 (12.7)	13 (11.2)	12 (14.8)	
	N2	68 (34.5)	49 (42.2)	19 (23.5)	
	N3	79 (40.1)	39 (33.6)	40 (49.4)	
	Nx	2 (1.0)	2 (1.7)	0 (0.0)	
Radical resection	R0	160 (81.2)	99 (85.3)	61 (75.3)	0.116
	R1	23 (11.7)	9 (7.8)	14 (17.3)	
	R2	14 (7.1)	8 (6.9)	6 (7.4)	
Type of surgery	Distal gastrectomy	157 (79.7)	91 (78.4)	66 (81.5)	0.499
	Proximal gastrectomy	9 (4.6)	7 (6.0)	2 (2.5)	
	Total gastrectomy	31 (15.7)	18 (15.5)	13 (16.0)	
Primary tumor site	Upper 1/3	17 (8.6)	8 (6.9)	9 (11.1)	0.722
	Middle 1/3	20 (10.2)	11 (9.5)	9 (11.1)	
	Low 1/3	133 (67.5)	81 (69.8)	52 (64.2)	
	Whole	27 (13.7)	16 (13.8)	11 (13.6)	

(*Continued*)

Table 2 (Continued).

n	Level	Overall 197	Low CDX2 116	High CDX2 81	p
Borrmann type	Borrmann 0	2 (1.0)	1 (0.9)	1 (1.2)	0.149
	Borrmann I	7 (3.6)	2 (1.7)	5 (6.2)	
	Borrmann II	37 (18.8)	22 (19.0)	15 (18.5)	
	Borrmann III	130 (66.0)	74 (63.8)	56 (69.1)	
	Borrmann IV	16 (8.1)	12 (10.3)	4 (4.9)	
	Borrmann V	5 (2.5)	5 (4.3)	0 (0.0)	
Tumor size	≤20mm	31 (15.7)	18 (15.5)	13 (16.0)	0.013
	>20 and <50mm	92 (46.7)	45 (38.8)	47 (58.0)	
	≥50mm	74 (37.6)	53 (45.7)	21 (25.9)	
Differentiation	Poorly differentiated	96 (48.7)	58 (50.0)	38 (46.9)	0.892
	Moderately differentiated	99 (50.3)	57 (49.1)	42 (51.9)	
	Well differentiated	2 (1.0)	1 (0.9)	1 (1.2)	
Pathology	Adenocarcinoma	70 (35.5)	34 (29.3)	36 (44.4)	0.223
	Mucinous carcinoma	8 (4.1)	5 (4.3)	3 (3.7)	
	Signet ring cell carcinoma	8 (4.1)	6 (5.2)	2 (2.5)	
	Mixed carcinoma	110 (55.8)	70 (60.3)	40 (49.4)	
	Others	1 (0.5)	1 (0.9)	0 (0.0)	
Lauren type	Intestinal	87 (44.2)	44 (37.9)	43 (53.1)	0.107
	Diffuse	51 (25.9)	33 (28.4)	18 (22.2)	
	Mixed	59 (29.9)	39 (33.6)	20 (24.7)	
Postoperative chemotherapy	No	91 (46.2)	56 (48.3)	35 (43.2)	0.578
	Yes	106 (53.8)	60 (51.7)	46 (56.8)	
Total lymph nodes (TLN)	≤25	98 (49.7)	67 (57.8)	31 (38.3)	0.011
	>25	99 (50.3)	49 (42.2)	50 (61.7)	
Positive lymph nodes (PLN)	≤5	96 (48.7)	58 (50.0)	38 (46.9)	0.778
	>5	101 (51.3)	58 (50.0)	43 (53.1)	
CK	Negative	24 (12.2)	20 (17.2)	4 (4.9)	0.017
	Positive	173 (87.8)	96 (82.8)	77 (95.1)	
D2-40	Negative	125 (63.5)	110 (94.8)	15 (18.5)	<0.001
	Positive	72 (36.5)	6 (5.2)	66 (81.5)	
HER2	Negative	184 (93.4)	108 (93.1)	76 (93.8)	1.000
	Positive	13 (6.6)	8 (6.9)	5 (6.2)	
S100	Negative	31 (15.7)	23 (19.8)	8 (9.9)	0.091
	Positive	166 (84.3)	93 (80.2)	73 (90.1)	

Expression Levels of CDX2 and Common Hematological Markers

Utilizing the hospital data, we explored the associations between the patients' CDX2 status and common hematological markers. The CDX2 status was significantly associated with PALB and FIB ($P<0.05$; Table 3).

CDX2 Expression Effects on Patient Survival

In the TCGA cohort, CDX2 expression was associated with an improved DFS (HR = 0.5948, 95% CI, 0.4153–0.8521, $P = 0.0054$) and OS (HR = 0.5976, 95% CI, 0.4172–0.8561, $P = 0.0058$) (Figure S2). In the hospital cohort, CDX2 expression also was associated with an improved DFS (HR = 0.4076, 95% CI, 0.2675–0.6210, $P = 0.0001$) and OS (HR = 0.4183, 95% CI, 0.2744–0.6375, $P = 0.0002$) (Figure 2). We investigated the associations between CDX2 expression and the prognosis of gastric adenocarcinoma obtained from the Kaplan-Meier Plotter database for gastric cancer. The results indicated that CDX2 expression was associated with an improved DFS ($P = 0.0025$) and OS ($P = 0.0015$) (Figure S3).

Table 3 Expression Levels of CDX2 and Common Hematological Markers

n	Level	Overall 197	Low CDX2 116	High CDX2 81	p
Total protein (TP)	≤67.00	98 (49.7)	51 (44.0)	47 (58.0)	0.072
	>67.00	99 (50.3)	65 (56.0)	34 (42.0)	
Albumin (ALB)	≤40.00	89 (45.2)	48 (41.4)	41 (50.6)	0.256
	>40.00	108 (54.8)	68 (58.6)	40 (49.4)	
Prealbumin (PALB)	≤230.0	98 (49.7)	45 (38.8)	53 (65.4)	<0.001
	>230.0	99 (50.3)	71 (61.2)	28 (34.6)	
Neutrophils (N)	≤3.70	99 (50.3)	59 (50.9)	40 (49.4)	0.953
	>3.70	98 (49.7)	57 (49.1)	41 (50.6)	
Lymphocyte (L)	≤1.88	97 (49.2)	60 (51.7)	37 (45.7)	0.490
	>1.88	100 (50.8)	56 (48.3)	44 (54.3)	
Monocyte (M)	≤0.46	97 (49.2)	60 (51.7)	37 (45.7)	0.490
	>0.46	100 (50.8)	56 (48.3)	44 (54.3)	
Fibrinogen (FIB)	≤3.07	99 (50.3)	67 (57.8)	32 (39.5)	0.017
	>3.07	98 (49.7)	49 (42.2)	49 (60.5)	
CEA	≤2.09	96 (48.7)	59 (50.9)	37 (45.7)	0.568
	>2.09	101 (51.3)	57 (49.1)	44 (54.3)	
CA125	≤10.59	99 (50.3)	55 (47.4)	44 (54.3)	0.418
	>10.59	98 (49.7)	61 (52.6)	37 (45.7)	
CA724	≤2.47	99 (50.3)	54 (46.6)	45 (55.6)	0.272
	>2.47	98 (49.7)	62 (53.4)	36 (44.4)	

To estimate whether CDX2 expression was an independent prognostic factor in the hospital cohort, we analyzed the prognostic relevance of CDX2 using Cox multivariate proportional hazards models adjusted for several risk factors, including sex, age, BMI, TNM stage, radical resection, type of surgery, tumor size, differentiation, Laurén classification type, postoperative chemotherapy, TP, ALB, PALB, N, L, M, FIB, CEA, CA125, CA724, TLN, PLN, CDX2, CK, D2-40, HER2, and S100. The results revealed that CDX2, D2-40, TNM stage, Laurén classification type, PALB, postoperative chemotherapy, and TLN were the independent prognostic factors for DFS. Furthermore, CDX2, D2-40, TNM stage, Laurén classification type, PALB, postoperative chemotherapy, TLN, tumor size, and type of surgery were the independent prognostic factors for OS. Although neoplasm staging was a robust prognostic factor, CDX2 expression also was an independent prognostic factor (Table 4).

We analyzed the prognostic relevance of CDX2 in the TCGA cohort using Cox multivariate proportional hazards models that utilized the following risk factors: sex, age, race, family history of stomach cancer, anatomic neoplasm subdivision, histological type, residual tumor, grade, T stage, N stage, TNM stage, CDX2, chemotherapy, and radiation therapy. The results indicated that age, residual tumor, TNM stage, CDX2, and chemotherapy were the potential independent prognostic factors for DFS and OS (Table S1).

CDX2 Expression and Benefits from Adjuvant Chemotherapy

Based on the Cox multivariate proportional hazards models, chemotherapy was the independent prognostic factor in the hospital and TCGA cohorts. Subsequently, we examined the relationship between the CDX2 status and clinical outcomes among patients who did or did not accept adjuvant chemotherapy. In our hospital, 106 patients received chemotherapy after their surgery. Of these patients, CDX2 expression was significantly correlated with PLN, tumor size, CK, and D2-40 ($P<0.05$; Table S2). According to the Kaplan-Meier assay, the CDX2 expression was associated with an improved DFS ($P = 0.0335$) and OS ($P = 0.0231$) (Figure S4). Similarly, 154 patients received chemotherapy in the TCGA cohort. Of these patients, CDX2 expression was significantly associated with the histological type ($P<0.05$; Table S3). We also analyzed the prognostic relevance of CDX2 in the TCGA cohort for the patients who received chemotherapy. According to the Kaplan-Meier assay, CDX2 expression was associated with an improved DFS ($P = 0.0006$) and OS ($P = 0.0004$; Figure S5).

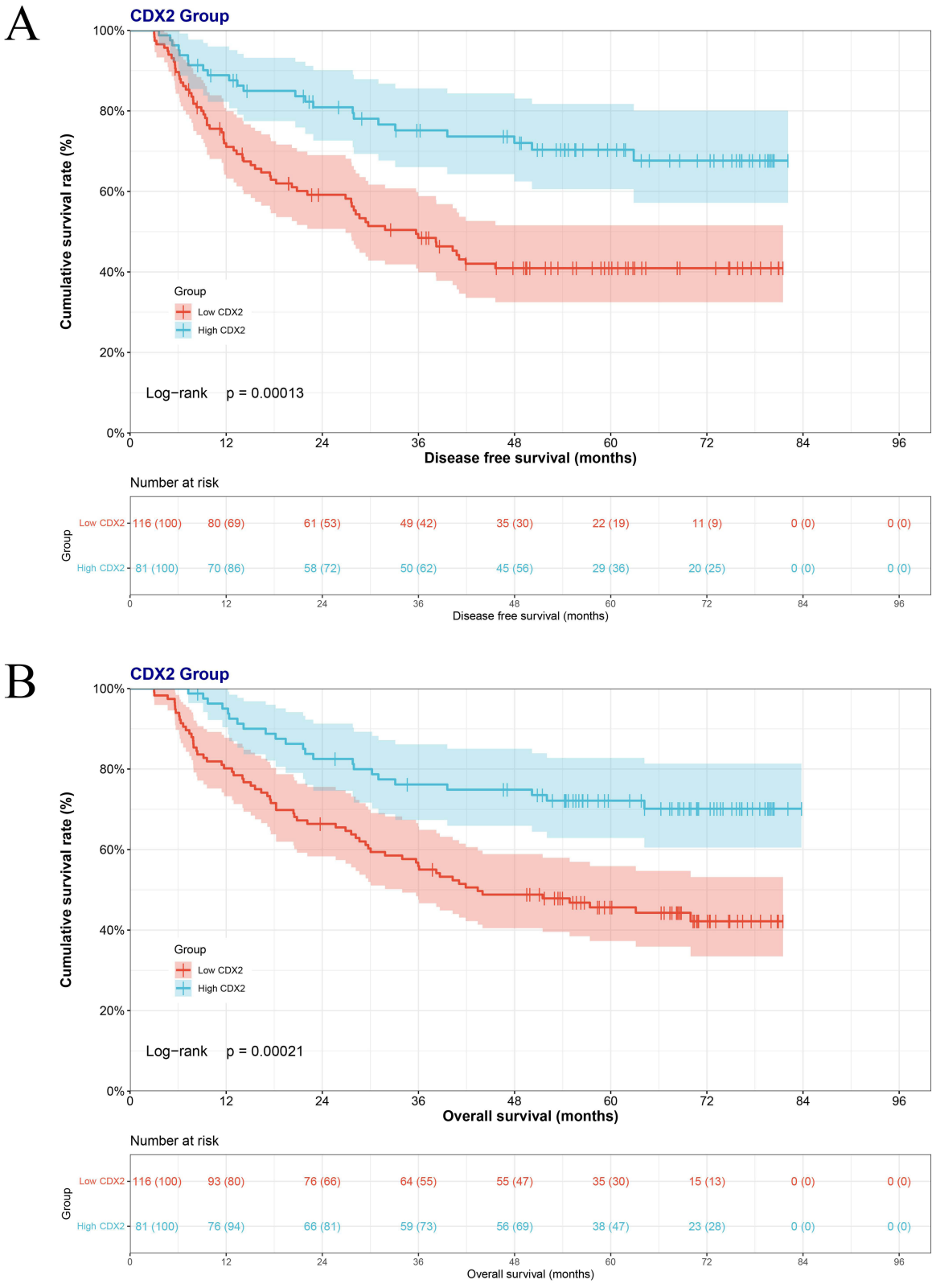

Figure 2 Relationship between CDX2 status and patient disease-free survival (**A**) and overall survival (**B**) in the hospital cohort.

Table 4 The Univariate and Multivariate COX Regression Analyses for the Hospital Cohort

Characteristics		DFS						OS					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Sex	Male	1(Ref.)						1(Ref.)					
	Female	1.189	0.769–1.838	0.437				1.171	0.757–1.811	0.478			
Age	≤60	1(Ref.)						1(Ref.)			1(Ref.)		
	>60	1.462	0.950–2.249	0.084				1.645	1.069–2.531	0.024	1.249	0.782–1.995	0.352
BMI	≤22.0	1(Ref.)						1(Ref.)					
	>22.0	1.109	0.728–1.690	0.629				1.119	0.735–1.706	0.600			
TNM stage	II	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	III	1.933	1.237–3.021	0.004	1.666	1.061–2.617	0.027	1.914	1.194–3.069	0.007	1.958	1.239–3.095	0.004
Radical resection	R0	1(Ref.)		0.160				1(Ref.)		0.121			
	R1	1.808	0.786–4.156	0.163				2.002	0.858–4.673	0.109			
	R2	2.412	0.827–7.038	0.107				2.474	0.814–7.513	0.110			
Type of surgery	Distal gastrectomy	1(Ref.)		0.125				1(Ref.)		0.020	1(Ref.)		0.015
	Proximal gastrectomy	0.552	0.160–1.899	0.346				0.763	0.221–2.628	0.668	0.575	0.140–2.367	0.444
	Total gastrectomy	1.831	0.949–3.531	0.071				2.568	1.320–4.997	0.005	2.620	1.336–5.139	0.005
Tumor size	≤20mm	1(Ref.)		0.003	1(Ref.)		0.001	1(Ref.)		0.004	1(Ref.)		0.001
	>20 and <50mm	4.055	1.476–11.139	0.007	4.725	1.808–12.350	0.002	2.683	0.984–7.318	0.054	3.511	1.352–9.117	0.010
	≥50mm	6.325	2.150–18.607	0.001	6.210	2.357–16.364	0.000	5.102	1.794–14.506	0.002	5.870	2.252–15.303	0.000
Differentiation	Poorly differentiated	1(Ref.)		0.611				1(Ref.)		0.330			
	Moderately differentiated	1.090	0.589–2.019	0.783				1.435	0.766–2.688	0.260			
	Well differentiated	3.191	0.323–31.530	0.321				4.516	0.439–46.491	0.205			
Lauren type	Intestinal	1(Ref.)		0.035	1(Ref.)		0.006	1(Ref.)		0.035	1(Ref.)		0.013
	Diffuse	1.917	1.001–3.672	0.050	1.865	1.045–3.328	0.035	1.812	1.019–3.221	0.043	2.043	1.134–3.681	0.017
	Mixed	2.335	1.162–4.694	0.017	2.272	1.467–5.050	0.001	2.157	1.181–3.938	0.012	2.434	1.313–4.510	0.005
Postoperative chemotherapy	No	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	Yes	1.687	1.093–2.602	0.018	2.085	1.308–3.324	0.002	1.768	1.145–2.729	0.010	2.171	1.364–3.456	0.001
Total protein (TP)	≤67.00	1(Ref.)						1(Ref.)					
	>67.00	1.181	0.776–1.799	0.438				1.184	0.777–1.803	0.432			
Albumin (ALB)	≤40.00	1(Ref.)						1(Ref.)					
	>40.00	0.943	0.619–1.438	0.787				0.947	0.621–1.444	0.801			
Prealbumin (PALB)	≤230.0	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	>230.0	0.494	0.320–0.765	0.002	0.528	0.317–0.880	0.014	0.492	0.318–0.761	0.001	0.487	0.284–0.836	0.009
Neutrophils (N)	≤3.70	1(Ref.)						1(Ref.)					
	>3.70	0.989	0.649–1.507	0.959				0.941	0.618–1.434	0.778			

(*Continued*)

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Table 4 (Continued).

Characteristics		DFS						OS					
		Univariate			Multivariate			Univariate			Multivariate		
		HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Lymphocyte (L)	≤1.88	1(Ref.)						1(Ref.)					
	>1.88	0.746	0.489–1.139	0.175				0.782	0.512–1.193	0.253			
Monocyte (M)	≤0.46	1(Ref.)						1(Ref.)					
	>0.46	0.751	0.492–1.146	0.184				0.780	0.512–1.191	0.250			
Fibrinogen (FIB)	≤3.07	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	>3.07	1.774	1.155–2.725	0.009	1.514	0.949–2.414	0.082	1.819	1.184–2.793	0.006	1.581	0.992–2.520	0.054
CEA	≤2.09	1(Ref.)						1(Ref.)					
	>2.09	0.897	0.589–1.367	0.614				0.923	0.606–1.406	0.708			
CA125	≤10.59	1(Ref.)						1(Ref.)					
	>10.59	0.937	0.615–1.427	0.762				0.977	0.641–1.487	0.913			
CA724	≤2.47	1(Ref.)						1(Ref.)					
	>2.47	1.170	0.768–1.782	0.465				1.172	0.769–1.786	0.459			
Total lymph nodes (TLN)	≤25	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	>25	0.598	0.391–0.914	0.018	0.595	0.370–0.957	0.032	0.558	0.365–0.854	0.007	0.524	0.323–0.850	0.009
Positive lymph nodes (PLN)	≤5	1(Ref.)						1(Ref.)					
	>5	1.237	0.812–1.885	0.321				1.165	0.765–1.774	0.477			
CDX2	Low expression	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	High expression	0.405	0.251–0.653	0.000	0.351	0.212–0.581	0.000	0.417	0.259–0.672	0.000	0.331	0.200–0.547	0.000
Cytokeratin (CK)	Negative	1(Ref.)						1(Ref.)					
	Positive	0.977	0.519–1.839	0.942				1.024	0.544–1.927	0.941			
D2-40	Negative	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	Positive	9.931	3.723–26.492	0.000	15.984	6.543–39.050	0.000	8.701	3.449–21.951	0.000	13.931	5.816–33.367	0.000
HER2	Negative	1(Ref.)						1(Ref.)					
	Positive	0.703	0.285–1.736	0.445				0.787	0.319–1.942	0.604			
S100	Negative	1(Ref.)			1(Ref.)			1(Ref.)			1(Ref.)		
	Positive	2.174	1.050–4.502	0.037	1.596	0.729–3.492	0.242	2.152	1.040–4.455	0.039	1.652	0.762–3.582	0.204

Nomograms Verified the Predictive Accuracy

Nomogram models were established using multivariate analysis to predict the DFS and OS of patients for one-, three-, and five-year survival rates. The corresponding scores were calculated based on CDX2, D2-40, TNM stage, Laurén classification type, PALB, postoperative chemotherapy, and TLN. They were further summarized and projected to the total subscale to predict the probability of different DFS results in individual gastric cancer patients (Figure 3A). The corresponding scores were calculated based on CDX2, D2-40, TNM stage, Laurén classification type, PALB, postoperative chemotherapy, TLN, tumor size, and type of surgery. The results were further summarized and projected to the total subscale to predict the probability of different OS in individual gastric cancer patients (Figure 3B). The calibration curve results demonstrated that the prediction line correlated with the reference line for the postoperative one-, three-, and five-year survival rates (Figure 4). The results of the decision curve analysis indicated that the one-, three-, and five-year survival rates that were determined using the nomograms exhibited superior ability to predict the clinical application compared to CDX2 (Figure 5).

Molecular Characteristics of the Different CDX2 Expression Subgroups

According to RNA-sequencing data for the stage II and III gastric cancer patients from the TCGA cohort, there were 19 upregulated genes and 17 downregulated genes in the CDX2-positive subgroup compared with the CDX2-negative subgroup ($P<0.05$, Table S4 and Figure 6A). The heatmap demonstrates the expression fold changes for the 19 upregulated and 17 downregulated genes (Figure 6B and C). Then GSEA was used to assess the gene sets enriched in the different CDX2 expression subgroups (Figure 6D). The results indicated that graft-versus-host disease, mismatch repair, pentose and glucuronate interconversions, as well as the ascorbate and aldarate metabolism pathways were associated with the CDX2 expression subgroups.

Discussion

CDX2 is becoming a promising biomarker in gastric and colorectal cancer.[24,25] To determine the prognostic value of CDX2 expression in gastric adenocarcinoma, we retrospectively conducted CDX2 immunohistochemical staining in tissue samples from patients in our hospital and mRNA sequencing data from the TCGA database. We found that gastric cancer patients with high CDX2 expression experienced improved disease-free survival as well as overall survival. The main findings of this study were verified using an independent validation cohort (n=197) and external public datasets (n=291).

Considering the histological evaluation, the gastric cancer tissue sections were clearly stained, and the monoclonal CDX2 antibodies used in our study did not exhibit non-specific immunoreactivity or excessive background reactivity. Based on the density and intensity of cellular CDX2 expression in this study, 116 cases were included in the negative group with scores of 0–1, and 81 cases were in the positive group with scores of 2+. In the hospital cohort, the relationships between CDX2 expression and clinicopathological characteristics revealed that compared with the low CDX2 expression group, the high CDX2 expression group was related to the T stage, N stage, and tumor size. In the study by Camilo V, CDX2 expression was significantly related to a lower TNM stage ($P = 0.002$) and a lower T stage ($P = 0.007$).[26] Thus, the results reported by Camilo V were consistent with the results obtained in this study. On the other hand, no relationship was observed between CDX2 expression and TNM stage in the TCGA cohort. We also analyzed the relationships between CDX2 expression and common hematological markers; the results indicated that the CDX2 status was significantly related to PALB and FIB ($P<0.05$). According to the Oñate-Ocaña LF study, CDX2 was related to the albumin/monocyte ratio (AMR) (OR 1.06, 95% CI 1.01–1.1), and was independently associated with OS.[27]

Our most interesting outcome was that the prognostic ability of CDX2 expression was enhanced when either the protein-based methods or RNA sequencing were used. The results indicated that CDX2 expression was associated with improved DFS and OS based on the hospital data, the TCGA cohort, and the Kaplan-Meier Plotter database. Dalerba et al found that CDX2 expression was verified in a subgroup of patients with low-risk stage II colon cancer who benefited from adjuvant chemotherapy.[28] Mukohyama et al reported that low CDX2 expression was associated with a decrease in overall survival (37.67 months vs 25.32 months, $P=0.03$), and tended to be related to a decrease in progression-free

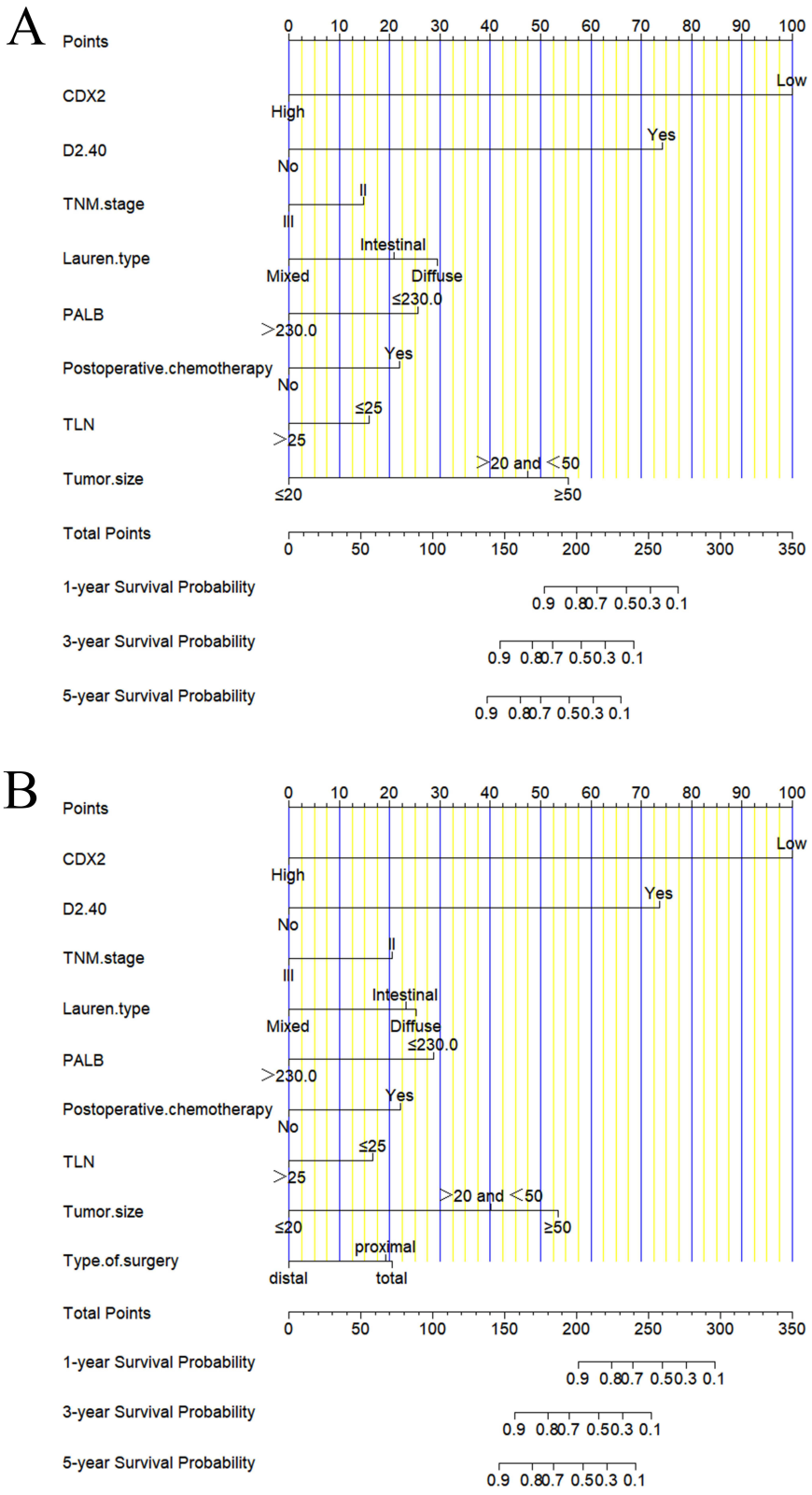

Figure 3 Nomograms for disease-free survival (**A**) and overall survival (**B**).
Abbreviations: CDX2, caudal-type homeobox transcription factor 2; TNM, Tumor node metastasis; PALB, prealbumin; TLN, total lymph node.

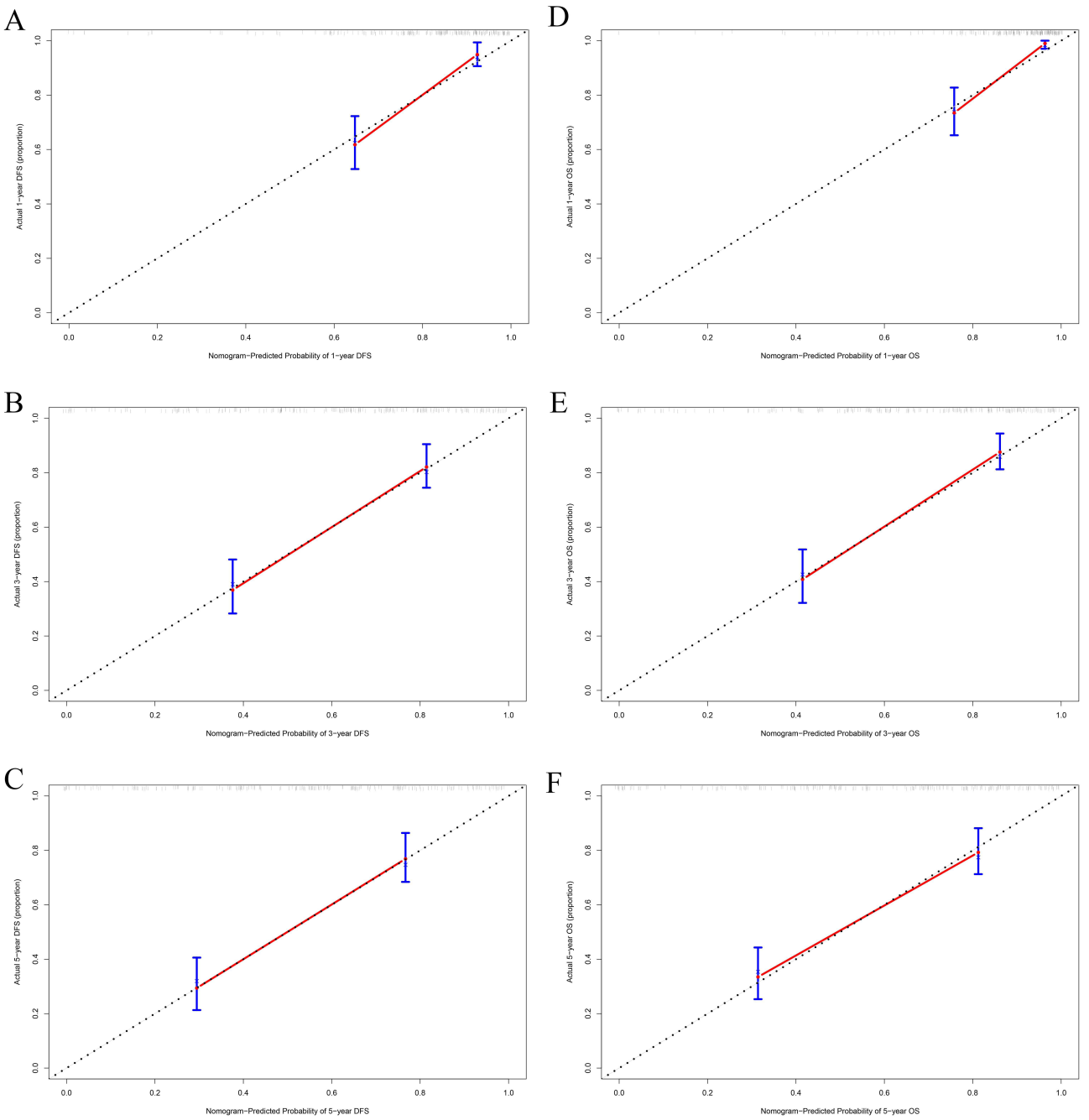

Figure 4 Calibration curve for one-, three-, and five-year disease-free survival (**A**–**C**) and one-, three-, and five-year overall survival (**D**–**F**).

survival (17.4 months vs 12.9 months, $P = 0.37$).[29] They concluded that CDX2 expression might be a prognostic indicator for unresectable metastatic colorectal cancer.[29] Another study indicated that osteopontin was a valuable prognostic biomarker for gastric cancer, and might have a particular advantage in predicting the survival of patients with advanced gastric cancer when combined with CDX2 expression.[30] Based on the multivariate COX analysis, CDX2 was an independent prognostic factor for DFS and OS in the hospital and TCGA cohorts. Previous studies have shown that CDX2-negative tumors were typically correlated to several adverse prognostic variables, including TNM stage, differentiation, and vascular invasion.[31–33] We also constructed a CDX2-based nomogram model to verify the predictive accuracy of CDX2 using calibration curve and decision curve analyses. The model displayed better predictive clinical

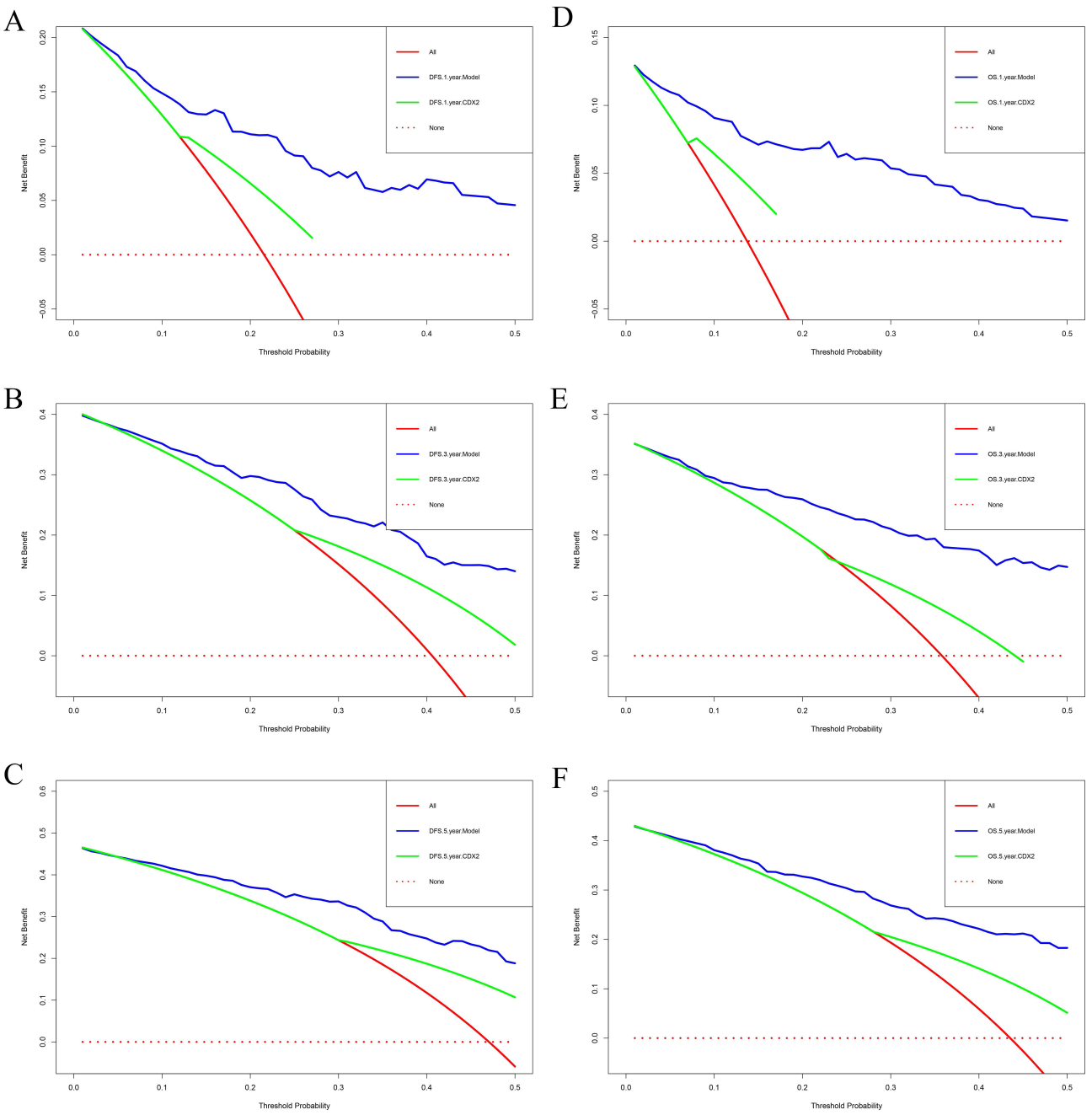

Figure 5 Decision curve analysis for one-, three-, and five-year disease-free survival (**A**–**C**) and one-, three-, and five-year overall survival (**D**–**F**).

application than using only CDX2 expression. The nomogram model included the TNM stage, chemotherapy, Laurén classification type, and type of surgery.

Interestingly, the CDX2 expression level also was closely related to chemotherapy. In this study, chemotherapy was the independent prognostic factor in the hospital and TCGA cohorts. Moreover, for patients who received chemotherapy, CDX2 expression also was associated with improved DFS and OS. In the Gao X study, patients with CDX2 expression deficiency experienced worse clinical outcomes without systemic therapy.[34] However, many patients benefited considerably from postoperative adjuvant chemotherapy.[34] Moreover, patients with positive CDX2 expression exhibited much lower sensitivity to systemic chemotherapy and did not benefit from adjuvant chemotherapy.[34]

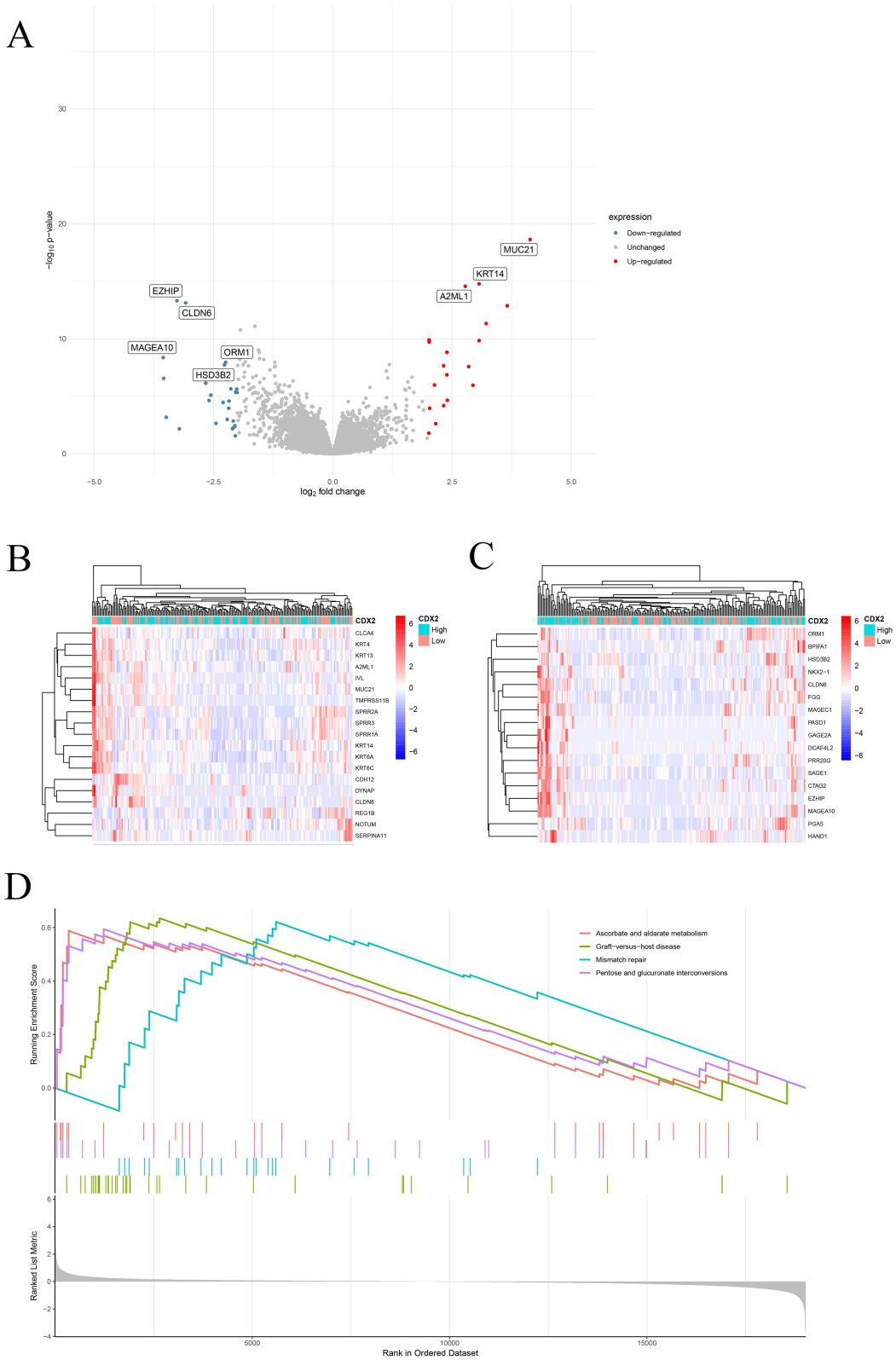

Figure 6 Molecular characteristics of different CDX2 expression subgroups. (**A**) Volcano plot of differentially expressed mRNAs in the TCGA cohort; (**B** and **C**) Heat map showing expression fold changes of the significant up- and downregulated genes; (**D**) Gene sets enriched in different CDX2 expression subgroups ($P < 0.05$, FDR < 0.25).

A study of colorectal cancer reported that the absence of CDX2 expression was a poor prognostic biomarker, but was associated with benefits achieved with stage III adjuvant chemotherapy.[35] Another study demonstrated that the expression of CDX2 and MDR1 might be effective in predicting anticancer drug resistance.[36] CDX2 and MDR1 expression also might be predictive of the potential effectiveness of novel chemotherapy regimens in ovarian mucinous adenocarcinoma.[36] CDX2 has been proposed as a potential prognostic factor for gastric cancer, and the CDX2 expression can predict a lack of response to neoadjuvant therapy.[37]

The tumor microenvironment seriously affects patient response to tumor treatment and their overall survival.[38,39] Currently, several studies have reported that low tumor purity was related to a malignant phenotype and poor prognosis.[40–42] Our results showed that 19 genes were upregulated and 17 genes downregulated in the two patient groups. Furthermore, the graft-versus-host disease, mismatch repair, pentose and glucuronate interconversions, and the ascorbate and aldarate metabolism pathways by GSEA were associated with the CDX2 expression subgroups.

Several limitations of the current study should be considered. First, the CDX2 immunohistochemistry data analyzed in our study were obtained from only one medical center and the sample size was limited. Therefore, more patients should be enrolled from multiple centers to offset this limitation. Second, the nomogram models need additional validation due to the limited inclusion of variables. Finally, CDX2 expression was statistically significant as a prognostic factor, but postoperative treatment efficacy might be a confounding factor for patient survival.

Conclusions

In conclusion, we demonstrated that CDX2 could be a prognostic biomarker for stage II and III gastric cancer. Compared with CDX2 negative gastric cancer patients, CDX2 positive cancer patients appeared more likely to have resectable tumors and better survival rates. The expression level of CDX2 also was related to benefits derived from chemotherapy. Our findings would be strengthened by additional validation through future randomized adjuvant trials.

Data Sharing Statement

Data are provided within the manuscript or supplementary information files.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Daqing Oilfield General Hospital (Approved No.20240125). The patients from Daqing Oilfield General Hospital provided written informed consent before surgery for the use of the resected samples as well as their clinical and follow-up data.

Author Contributions

All authors contributed substantially to part or all of this study, including the conception, study design, execution, data acquisition, analysis, and interpretation. The authors also took part in drafting, revising, or critically reviewing the manuscript and gave final approval of the version to be published. The authors have agreed on the journal to which the article has been submitted and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest.

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