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Prognostic influence of CDX2 expression in gastric carcinoma after surgery with a curative intent

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ABSTRACT

Introduction and objectives: CDX2 is a specific transcription factor with a significant role in the early differentiation and maintenance of intestinal epithelial cells during gastrointestinal development and also as a tumor suppressor. The aim of this study was to assess the potential role of CDX2 expression as a prognostic predictor.

Material and methods: ninety-two of 206 (44.6%) patients with gastric carcinoma that underwent a curative surgery and had immunohistochemical staining for CDX2 were enrolled into the study; 51.1% were female and the average age was 74.07 years. Overall, 56.5% of tumors were of the intestinal type, 33.7% were diffuse and 9.8% were mixed; 23.9% were T1/T2, 76.1% were T3/T4 and lymph node metastases (N+) were identified in 69.6% of cases; 13% (12) were clinical stage I, 40.2% (37) were stage II and 46.7% (43) were stage III.

Results: a total of 68.5% (63) expressed CDX2. Our study suggests that CDX2 expression (HR = 0.339, $p = 0.024$) represents an independent risk marker together with the Lauren type (HR = 3.471, $p = 0.022$). There was association between a milder clinical stage and CDX2 expression (stage I) ($p = 0.046$). A significant difference was found in overall survival that favored patients with positive CDX2 expression (85.7% vs 65.5%, $p = 0.012$). **Conclusion:** our results confirm that CDX2 expression in gastric carcinoma is associated with improved prognosis, although further studies are needed to draw definitive conclusions.

Key words: CDX2. Gastric carcinoma. Prognosis. Immunohistochemistry.

INTRODUCTION

Stomach carcinoma is the third most common cause of cancer-related mortality worldwide and estimations indicate that over 890,000 people will die from this condition in 2020 (1). Gastric carcinoma is usually diagnosed in advanced stages, when the prognosis is poor (2). Lymph node metastases reduce survival rates, even early in the course of disease (3). Approximately, 90% of gastric malignancies are adenocarcinomas (4). However, the World Health Organization (WHO) recommends the Lauren's histology-based classification system (5), which divides these neoplasms into intestinal (50%), diffuse (33%) and mixed/indeterminate (17%) types. The exact pathogenesis remains unknown. The Correa cascade has been proposed for intestinal-type adenocarcinoma, which involves a series of prior histological changes such as chronic gastritis, atrophy, intestinal metaplasia and dysplasia that ultimately result in adenocarcinoma (6). Furthermore, diffuse carcinoma has a definite molecular basis, the loss of cadherin and a familial phenotype.

The caudal-related homeobox transcription factor 2 (CDX2) is a member of a gene family involved in the proliferation, early differentiation, and maintenance of intestinal phenotypes (7,8). This protein expressed in the intestinal epithelial cells of adult mammals, from the proximal duodenum to the distal rectum (9,10). In fact, some studies suggest a potential role as a tumor suppressor in ovarian, gallbladder, colon and gastric cancer (11-15). An aberrant expression of CDX2 has been reported in

various tumor types. Some studies suggest that the absence of CDX2 expression in colorectal carcinoma is a marker of poor prognosis in stages II and III (16). A meta-analysis by Wang et al. showed that CDX2 expression in gastric carcinoma was associated with an increased survival at five years (17).

The goal of this study was to assess CDX2 expression in 92 patients with gastric carcinoma in a Spanish hospital. The prognostic value and the correlation with histologic type and pathoclinical characteristics were also assessed.

MATERIAL AND METHODS

Two hundred and six patients with gastric adenocarcinoma that underwent surgery with a curative intent from 2002 to 2017 in a sole tertiary site (Hospital Clínico San Carlos, Madrid, Spain) were assessed. Patients with carcinoma at the cardia or gastroesophageal junction and those that had received pre- or post-operative chemotherapy and/or radiotherapy were excluded from the study. The study was reviewed and approved by the hospital ethics committee.

Demographic, clinical and pathologic data were collected from the patient medical records and the pathology department database. The cohort consisted of 92 patients and 51.1% were female with an average age of 74.07 years (range, 32-76 years); 71.7% (66) of patients underwent a subtotal gastrectomy and 28.3% (26) total gastrectomy, 3.3% underwent D1 lymphadenectomy, 21.7% D2 lymphadenectomy and 75% other procedures. All were R0 resections. A total of 1,862 lymph nodes were collected from the surgical specimens, with a median value of 19 (range 2-54). The most common postoperative complications included pancreatic fistula, intraperitoneal abscess, wound infection and anastomotic suture dehiscence.

Microscopic characteristics were assessed and TNM pathologic staging was performed (AJCC, 8th edition). Overall, 56.5% (52) of tumors were of the intestinal type, 33.7% (31) of the diffuse type and 9.8% (9) of the mixed type. A total of 54.3% (50) of carcinomas were high-grade and signet ring cells were identified in 40.2% (37/92) of cases. With regard to the pT stage, 23.9% (22) were T1/T2 and 76.1% were T3/T4. Lymph node metastases (N+) were identified in 69.6% (64) of patients. Thirteen percent (12) were clinical stage I, 40.2% (37) clinical stage II and 46.7% (43) clinical stage III.

Cancer sections were reviewed and tissue blocks were selected from surgical specimens that were diagnosed as adenocarcinoma. Two tumor-representative areas were marked in order to build a tissue microarray (TMA). TMAs were made using a manual arrayer (Beecher Instruments, Silver Spring, MD, USA), which collects cores with a 1 mm diameter from all selected areas, in order to place them in the recipient block. Then 4- μ m sections were collected from the TMA and an Autostainer (Dako) device was used for CDX2 immunohistochemical staining (Dako, FLEX monoclonal mouse anti-human CDX2, clone DAK-CDX2, 1:50 dilution, Catalog No. GA080). Both the positivity range intensity (1-3) and the percentage of stained cells were assessed, which resulted in a score of 0-300. Only nuclear staining was considered as positive, according to established test interpretation criteria. For the subsequent analysis, a ROC curve-based cut-off was established and cases with moderate intensity in at least 5% of cells were considered as positive (scores > 10).

Patients with GC that underwent a resection with a curative intent had follow-up visits every three months for the first two years, every six months for the next three years and every year thereafter. During each visit, the clinical history was taken and a physical examination, abdominal CT and complete blood testing were performed. Gastroscopy was included every six months for the first two years and every year thereafter. Disease-free survival (DFS), defined as the time elapsed from curative-intent surgery to local or distant recurrence in months and overall survival (OS), defined as the time elapsed from surgery to disease-related death in months, were used as measures of prognosis.

All data were digitally recorded in an Excel database and subsequently analyzed using the SPSS 20.0 for Windows statistical package. Quantitative variables were described as the mean (standard deviation) or median (range) values and qualitative variables were expressed as percentages. The χ^2 test, with all the relevant adjustments, was used to assess the association between CDX2 expression and quantitative variables. The Student's t-test was used to compare differences in the mean variables between the groups. Cumulative survival was estimated using the Kaplan-Meier method and differences between the survival curves were analyzed using the log-rank test. The influence of each variable on survival was analyzed using the Cox's proportional

hazards model. Differences were considered as statistically significant at $p < 0.05$.

RESULTS

Of 206 cases with gastric adenocarcinoma, 44.6% (92 patients) met the study criteria. Of these, CDX2 expression was found in 68.5% of subjects (63) (Figs. 1 and 2). Table 1 summarizes the results of the association between CDX2 expression and clinicopathological parameters in patients with gastric cancer. CDX2 expression was significantly associated with clinical stage and there was a higher expression in earlier stages (stage I) ($p = 0.046$). CDX2 expression was not associated with the assessed clinicopathological parameters such as gender, age, Lauren's histological classification, differentiation degree and lymph node metastasis.

Overall survival based on the follow-up of 92 patients was 79.3%, with a median survival of 34 months (range, 0-188). The Kaplan-Meier analysis showed that patients with positive CDX2 expression had a significantly longer overall survival (OS) compared to patients without CDX2 expression (85.7% vs 65.5%, $p = 0.012$) (Fig. 3). However, disease-free survival or clinical stage (I, II, III) were not associated with CDX2 expression. The Cox multivariate survival analysis showed that CDX2 expression (HR, 0.339; $p = 0.024$) and the Lauren's tubular and diffuse types (HR, 3.471; $p = 0.022$) had an independent prognostic value.

DISCUSSION

Gastric carcinoma is a highly heterogeneous disease and the prognosis cannot be predicted with only the clinicopathological characteristics. Therefore, the identification of useful biologic markers to categorize patients according to prognosis may guide treatment decision making.

CDX2 is a specific transcription factor that plays a relevant role in the early differentiation and maintenance of intestinal epithelial cells during gastrointestinal development (7). Furthermore, studies indicate that CDX2 expression is involved in the onset and development of intestinal metaplasia (18,19). However, its role in the gastric carcinogenesis process remains controversial.

Some studies suggest that CDX2 may be a tumor suppressor in many carcinomas (11-15) and loss of expression was associated with a poor prognosis in colorectal carcinoma (16). Xie et al. and Wei et al. showed that CDX2 overexpression inhibited the progression of gastric cancer, both *in vitro* and *in vivo* (11,20) and several studies have associated this marker with prognosis in gastric carcinoma. Camilo et al. reviewed a series of 201 patients with gastric carcinoma and confirmed that tumors with CDX2 expression and no SOX2 expression had an improved survival (21). Masood et al. assessed CDX2 expression in 101 patients with gastric cancer and found that positive cases were more likely to have resectable tumors and an improved survival (22). Thirteen studies were reviewed that included a total of 1,513 patients in a meta-analysis by Wang et al. CDX2 expression was found to be significantly associated with a milder clinical stage, good histological differentiation, lower vascular invasion rate and improved survival (17).

We found a significant difference in survival rates for patients with a positive CDX2 expression, which was not identified for disease-free survival. This finding is consistent with the literature. Multivariate analysis revealed that CDX2 represents an independent risk indicator together with the Lauren's type. Furthermore, an association was found between a milder clinical stage and CDX2 expression. However, our findings did not validate any association with the male gender, differentiation degree, vascular invasion, or lower lymph node metastasis rate, which has been previously reported. In contrast, the proportion of low-grade (well/moderately differentiated) adenocarcinomas with CDX2 overexpression in our cohort was lower compared to high-grade (poorly differentiated) tumors. The proportions were 45.5% and 54.5% for low and high grade, respectively, in contrast to the report by Wang et al. This finding may be due to the retrospective study design with a small population (n = 92). Furthermore, there was a wide variation among the lymph nodes collected from surgical specimens (2-54) that may have partly contributed to our study findings (23,24). Furthermore, most of the clinical III-stage patients (n = 43) received surgery before 2009, when some reports were available on the benefits of adjuvant chemotherapy. However, the evidence is inadequate to support its systematic use in the protocol of our center (25,26). In fact, the treatment is contraindicated in some

patients older than 80 years with a poor baseline status, heart disease and postoperative periods longer than two months that delayed treatment onset.

The primary limitations of the study include its retrospective nature and small patient population, which limits the generalization of the available data. Furthermore, the scarce availability of the lymph node dissection surgical technique that was used also limited our assessment of its contribution to the study findings.

CONCLUSION

Our results have confirmed that the presence of CDX2 expression in gastric carcinoma is a marker of improved prognosis. Therefore, it may play a role in risk stratification for patients with gastric carcinoma and therapy decision making after surgery. However, further studies are needed in order to draw definite conclusions.

ETHICAL COMMITMENTS

All the study procedures performed in human subjects complied with the ethics guidelines established by institutional and/or national research committee, as well as with the Declaration of Helsinki of 1964 and subsequent amendments or other comparable ethics guidelines.

No formal consent was required for this retrospective study.

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Accepted Article

Table 1. Association between CDX2 expression and multiple variables in gastric carcinoma

Accepted Article

<i>Characteristics</i>	<i>CDX2 negative</i>	<i>CDX2 positive</i>	<i>Total</i>	<i>p</i>
Age				
Mean, SD	73.45 (10.742)	74.35 (10.760)		0.710
Median	76 (32-87)	77 (43-91)		
Gender				
Male	14 (31.1%)	31 (68.9%)	45	1.00
Female	15 (31.9%)	32 (68.1%)	47	
Location				
Fundus/body	14 (35%)	26 (65%)	40	0.687
Antrum/pylorus	15 (28.8%)	37 (71.2%)	52	
Lauren classification				
Intestinal	15 (28.8%)	37 (71.2%)	52	
Diffuse	11 (35.5%)	20 (64.5%)	31	0.874
Mixed	3 (33.3%)	6 (66.7%)	9	
Grade*				
Low (well/moderately differentiated)	12 (28.6%)	30 (71.4%)	42	0.739
High (poorly differentiated)	17 (34%)	33 (66%)	50	
Signet ring cells				
No	15 (27.3%)	40 (72.7%)	55	0.401
Yes	14 (37.8%)	23 (62.2%)	37	
Vascular invasion				
Absent	13 (26.5%)	36 (73.5%)	49	0.382
Present	16 (37.2%)	27 (62.8%)	43	
pT stage				
T1/T2	4 (18.2%)	18 (81.8%)	22	0.188
T3/T4	25 (35.7%)	45 (64.3%)	70	
N stage				
N0	11 (39.3%)	17 (60.7%)	28	0.414
N+	18 (28.1%)	46 (71.9%)	64	
Clinical TNM stage				
I	2 (16.7%)	10 (83.3%)	12	
II	17 (45.9%)	20 (54.1%)	37	0.046

CDX2: caudal-related homeobox transcription factor 2; SD: standard deviation; p: association value. *Differentiation grade: low grade (well to moderately differentiated, gland formation $\geq 50\%$); high grade (poorly differentiated, gland formation $< 50\%$).

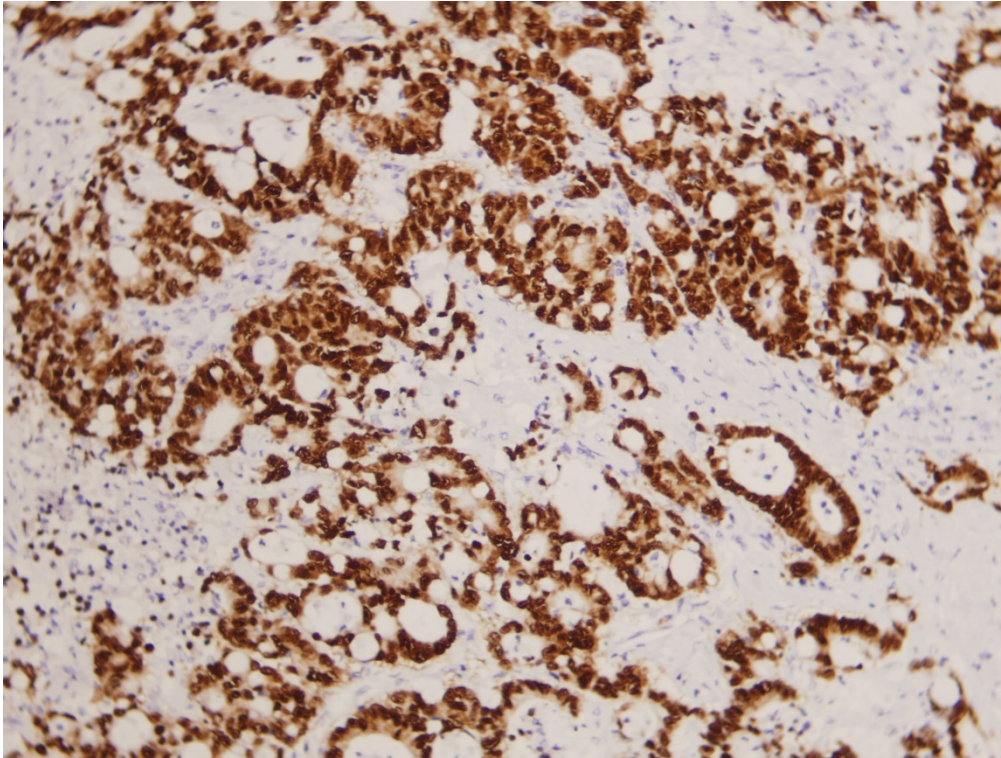


Fig. 1. Intense CDX2 expression (IHC staining for CDX2, $\times 200$).

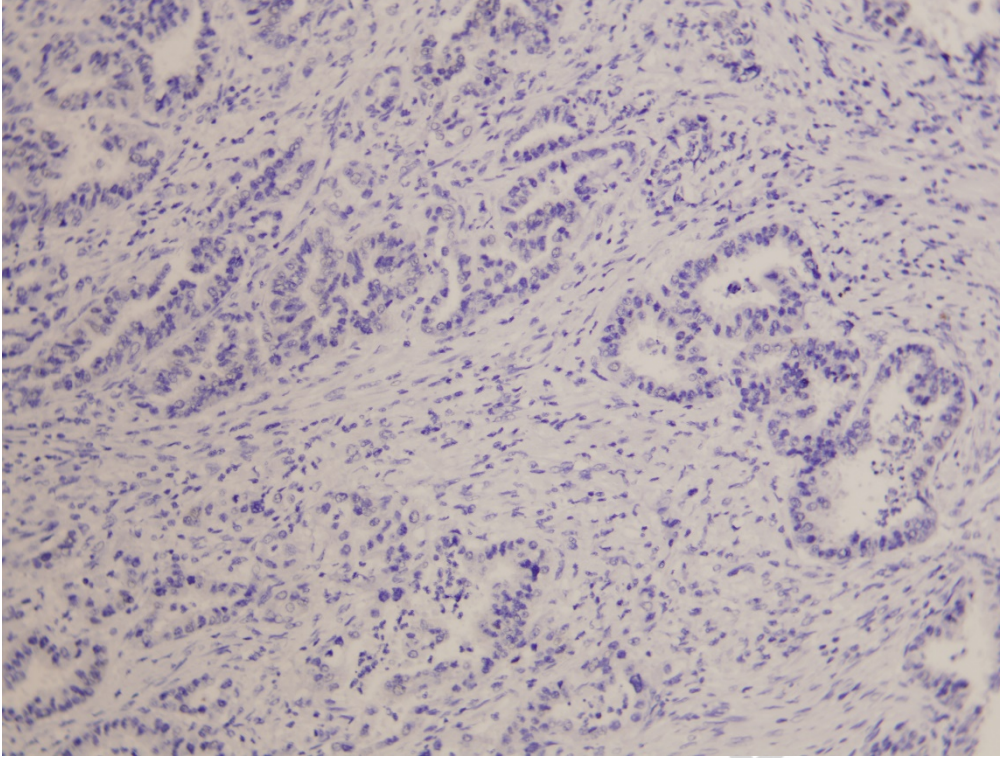


Fig. 2. No CDX2 expression (IHC staining for CDX2, $\times 200$).

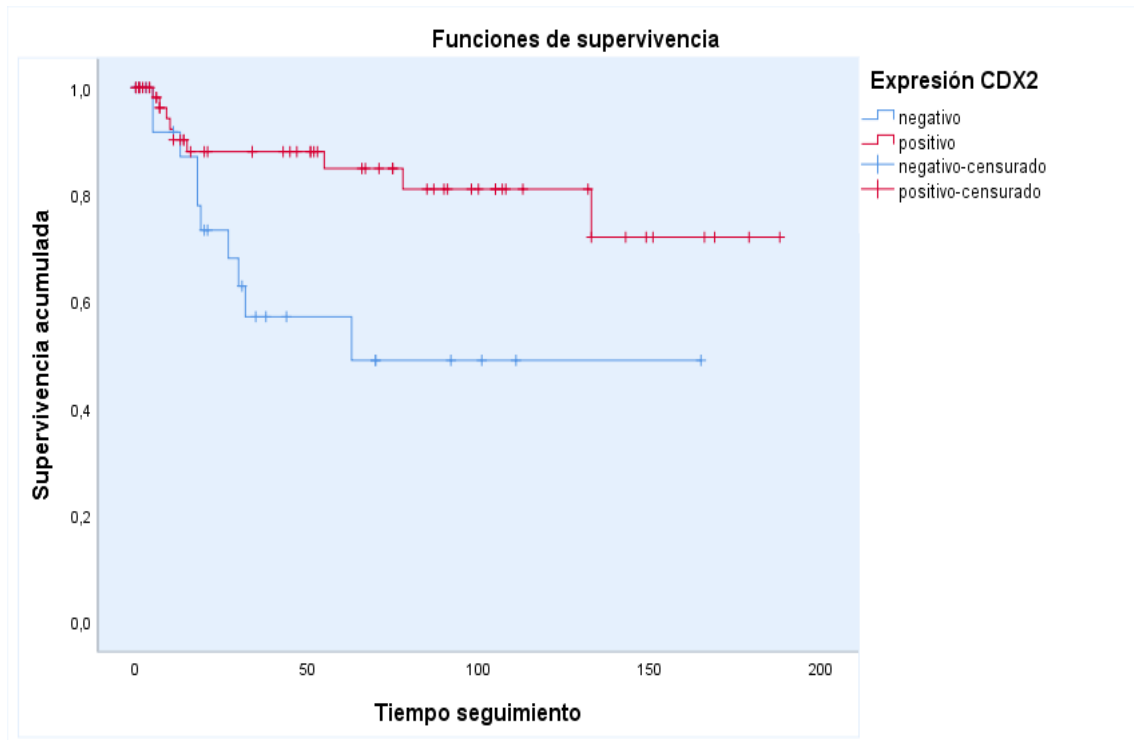


Fig. 3. Survival analysis of patients with gastric carcinoma with group comparisons according to CDX2 expression.