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CDX2 expression can predict response to neoadjuvant therapy in gastric carcinoma

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Abstract

Purpose: Caudal-related homeobox transcription factor 2 (CDX2) has recently been proposed as a prognostic factor for gastric carcinoma. However and to the best of our knowledge, no previous report has analyzed CDX2 expression in patients with gastric adenocarcinoma receiving neoadjuvant therapy (NAT). *Patients, Materials and Methods*: This is a retrospective cohort study to analyze the potential role of CDX2 expression to predict response to NAT and prognosis. This study has enrolled 57 patients receiving chemotherapy for locally advanced gastric carcinoma. *Results*: 59.6% of the patients were men; mean age was 64.96 years. Only 8% of the patients showed a complete response to therapy, 10% had grade 1, 28% grade 2, and 54% grade 3 regression, respectively, according to modified Ryan's criteria. On follow-up, 38.6% of the patients showed recurrence of disease (50% distant metastasis) and 28.1% eventually died of it. Twenty-three (40.4%) patients showed intense CDX2 expression. We found a statistically significant association between CDX2 expression and poor regression with NAT, but we found no association with outcome. *Discussion*: Our study indicates that CDX2 expression can predict lack of response to NAT. Our results have not confirmed the association with prognosis shown in previous reports. *Conclusions*: Despite these preliminary results, furthermore studies are necessary to define the potential use of CDX2 in gastric carcinoma.

Keywords: gastric carcinoma, neoadjuvant therapy, regression grade, CDX2 expression.

Introduction

Neoadjuvant therapy (NAT) has recently become a standard therapeutic alternative for some solid tumors [1–4], prior to surgical resection. Response to NAT is an important prognostic factor and can be used to guide adjuvant therapy. Several schemes to grade histopathological response have been proposed, but the *American Joint Committee on Cancer* (AJCC) has recently proposed to use the modified Ryan's system reviewed by the *College of American Pathologists* (CAP) [5]. Response is associated to a better prognosis, but lack of response can delay surgery and even make it impossible due to disease progression.

Caudal-related homeobox transcription factor (CDX2) is a homeobox gene encoding an intestinal transcription factor [6]. CDX2 is aberrantly expressed in many human tumors and several recent reports have associated it to prognosis in colon carcinoma [7]. Some authors have even considered lack of CDX2 expression a potential marker to indicate chemotherapy in stage II colon carcinoma [8].

Several studies have assessed the clinicopathological and prognostic value of CDX2 expression in gastric carcinoma [9–11]. A recent report by Masood *et al.* [12] showed that CDX2 expression indicates good prognosis, as already shown in other tumors [13, 14]. However and to the best of our knowledge, no report has analyzed CDX2 expression in patients receiving NAT for locally advanced gastric carcinoma.

Patients, Materials and Methods

This is a retrospective cohort study performed at a single institution, Hospital Fundación Jiménez Díaz, a large tertiary hospital attending over 400 000 people in Madrid, Spain. From the electronic files of the Department of Surgical Pathology, we have retrieved all the cases of patients undergoing surgery for gastric tubular (former intestinal type in Lauren's classification) carcinoma after chemotherapy, between 2008–2011.

First, we have collected demographic data, like gender or age. We have reviewed the diagnosed endoscopic samples of adenocarcinoma and selected representative areas of the tumor to construct a tissue microarray (TMA) for immunohistochemistry (IHC). One mm cores were identified from the selected areas and re-embedded into a recipient paraffin block with a Manual Tissue Microarrayer (Beecher Instruments, Sun Prairie, WI, USA). All cases were triplicated in the TMA. We obtained 4 μm sections from this TMA and performed CDX2 staining (Dako, FLEX monoclonal mouse anti-human CDX2, clone DAK-CDX2, 1:50 dilution, Catalog No. GA080), with the Dako immunostainer (Dako EnVision). The reaction was highlighted with the Dako EnVision system detection kit. This process is automatized and standardized at the Department of Surgical Pathology, and both negative and positive internal controls are used for every round of staining. For the aim of this study, only nuclear expression was considered positive.

To estimate CDX2 expression, we measured both the percentage of cells with nuclear brown staining (0–100%) in the most intensely stained area of the tumor and also the intensity of the reaction (0–3) (Figures 1–3).

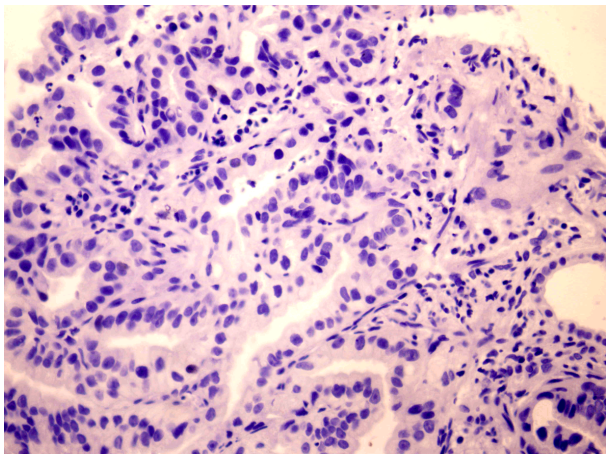

Figure 1 – ***Lack of CDX2 expression (IHC staining for CDX2, ×400).***

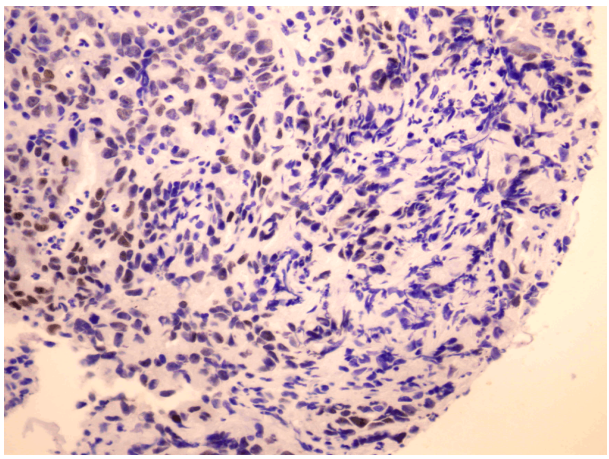

Figure 2 – ***CDX2 weak nuclear expression (Hematoxylin–Eosin staining for CDX2, ×400).***

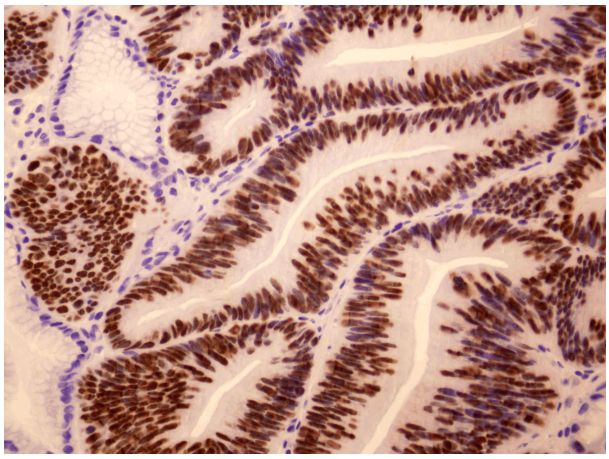

Figure 3 – ***CDX2 high expression (IHC staining for CDX2, ×400).***

We multiplied both values to obtain a *z*-score ranging from 0–300. To define the cut-off values to separate the cases into negative and positive ones we performed a receiver-operator curve analysis to define the best value predicting regression. This cut-off value was established in 40, with a sensitivity of 30% and specificity of 60%.

In the post-NAT gastrectomy specimen, we have measured the regression grade according to Becker's and modified Ryan's criteria, which is the main outcome measure in our study [15, 16].

Prognosis has been analyzed with the disease-free survival (defined as the time elapsed between surgery with curative intent and reappearance of disease, either locally or distant in months) and the overall survival (defined as the time elapsed between surgery with curative intent and death due to disease in months).

All the pathological data have been independently reviewed by two pathologists (AC and MJFA) blinded to the outcome of the patients and cases with discordance have been conjointly reviewed to reach consensus or else reviewed by a third pathologist (JP).

All data were saved in an Excel file and subsequently analyzed with Statistical Package for the Social Sciences (SPSS) 20.0 for Windows. First, we described our results with absolute numbers (and percentages) for qualitative variables and mean (standard deviation) for quantitative ones. Times were represented with the median value. Association between CDX2 expression and the different variables was analyzed with χ^2 (*chi*)-squared test or Student's *t*-test, as indicated. Non-parametric Mann–Whitney *U*-test was used for time variables. Significance was established in a p-value <0.05, as usual.

The study has been reviewed and approved by the Ethical Committee at Hospital Fundación Jiménez Díaz. Patients have given written consent for study enrolment and all data have been anonymized in accordance to Spanish regulations regarding personal data protection.

Results

In this series, we have enrolled 57 patients fulfilling inclusion criteria. 59.6% of the patients were men; mean age was 64.96 years. Most patients received chemotherapy based on the ECX protocol (namely, Epirubicin, Cisplatin and Capecitabine) and only 10% received ECF (changing Capecitabine for 5-Fluoruracil), following standard management recommendations in our Hospital. Most patients were clinical and image stages T3 N+ (85%), with 10% T2 N+ and 5% T4 N+. All patients were operated after NAT. Table 1 summarizes the main results of our study, according to CDX2 expression. Only 8% of the patients showed a complete response to therapy, 10% had grade 1, 28% grade 2 and 54% grade 3 regression, according to modified Ryan's criteria. In other words, 82% of tumors showed minor response to neoadjuvant treatment (Ryan's regression grades 2 or 3) and 18% of them showed major response (complete response or grade 1 regression). On follow-up, 38.6% of the patients showed recurrence of disease (50% distant metastasis) and 28.1% eventually died of it. Twenty-three (40.4%) patients showed intense CDX2 expression.

We have found a statistically significant association between gender and CDX2 expression. In our series, 70.5% of the patients without CDX2 expression were males. As for regression, CDX2 expression was significantly associated to the lack of response to NAT, for only 5.5% of the patients with high CDX2 expression showed major response as opposed to 25% of the patients with low CDX2 expression (p=0.04).

Table 1 – ***Summary of the results according to CDX2 expression***

Feature	CDX2 negative (34 patients; 59.6%)	CDX2 positive (23 patients; 40.4%)	*P*-value for the association
Gender	Males: 70.5% Females: 29.5%	Males: 43.4% Females: 57.6%	0.03
ypT	T1–T2: 26.5% T3–T4: 73.5%	T1–T2: 10% T3–T4: 90%	0.18
ypN	N0: 44.1% N+: 55.9%	N0: 30.4% N+: 69.6%	0.28
Ryan's regression grade[a]	Minor: 75% Major: 25%	Minor: 94.5% Major: 5.5%	0.04
Age [years] (mean and SD)	66.06 (7.2)	63.15 (12.05)	0.2
Differentiation grade[b]	Low grade: 66.7% High grade: 33.3%	Low grade: 58.3% High grade: 41.7%	0.5
Disease-free survival (median [months]; range)	23.5 (5–34)	22 (9–40)	0.6
Overall survival (median [months]; range)	24 (7–43)	25.5 (11–56)	0.7
Recurrence	No: 58.8% Yes: 41.2%	No: 65.2% Yes: 34.8%	0.7
Death due to disease	No: 73.5% Yes: 36.5%	No: 69.5% Yes: 30.5%	0.6

[a]Minor: Ryan's regression grades 2 and 3; Major: Complete response and Ryan's regression grade 1. [b]Differentiation grade: Low grade (well to moderately differentiated, ≥50% gland formation); High grade (poorly differentiated, <50% gland formation). CDX2: Caudal-related homeobox transcription factor 2; SD: Standard deviation.

Our study has not been able to show any association with differentiation or N or T stage, as shown by other authors.

Discussion

Surgery remains as the best therapeutic option for tumor management, but in recent times, targeted therapies and neoadjuvant therapy have broadened the therapeutic alternatives trying to improve patients' outcome. However, it is a well-known fact that some patients do not respond to NAT and in this moment, there is an increasing interest in defining factors that can predict response to NAT. This is a well-explored field in many tumors [17–19], but it has not been widely analyzed in gastric carcinoma [20, 21].

CDX2 is a homeobox gene encoding an intestinal transcription factor. It has been shown to be aberrantly expressed in many human tumors, including gastric carcinoma. Recent reports have established that lack of CDX2 expression in colorectal carcinoma is a feature of bad prognosis and some authors have proposed to use this factor to decide chemotherapy in stage II disease in an attempt to improve outcome [8].

In the stomach, CDX2 expression has been linked to intestinal metaplasia, a well-known risk factor for tumor development [22]. Shin *et al.* [22] showed that CDX2 mRNA expression was increased in patients with *Helicobacter pylori* infection and that response to triple therapy led to a reduction of CDX2 expression in these cases, associated to the disappearance of intestinal metaplasia in gastric biopsies. Camilo *et al.* reviewed a series of 201 patients with gastric carcinoma and showed that those tumors with CDX2 expression and lack of SOX2 had a better outcome [11]. Similar results have been obtained in a recent report by Masood *et al.* with 100 patients [12]. In their series, 30.7% of the patients showed CDX2 expression and had a significantly better prognosis compared to negative cases. These results are in accordance with those reported in colon and small intestine adenocarcinoma [13, 14].

Few reports have tried to predict response in gastric tumors and to the best of our knowledge none of them have based on CDX2 expression in the diagnostic of endoscopic biopsies. Despite the small number of cases (57 patients), our results are not in accordance with the reported good prognostic influence indicated by other authors in gastric carcinoma. In fact, our results indicate that CDX2 expression is a harbinger of a poor response to therapy. Besides, our study has not been able to confirm the association to less lymph node involvement or better differentiation, as shown by other authors [13]. We do not have a definite explanation for these apparently contradictory findings, but our patients had received NAT and the outcome should necessarily been influenced by it. It might be tumors with CDX2 expression are less aggressive, as already shown by some authors and therefore respond less than high-grade ones to therapy. Another intriguing fact is that men show in our series a significantly lower rate of CDX2 expression in contrast to the results shown by Wang *et al.* in their meta-analysis [9]. This is a fact for which we have no clear explanation.

Our study has several limitations. The most important ones are its retrospective nature and the small number of cases (57 patients). The high number of males (almost 60% in our series) could have influenced results due to the lower frequency of CDX2 expression in men, although this situation reflects the gender ratio in gastric carcinoma.

Conclusions

Several studies have shown that CDX2 expression in malignant tumors and specifically in gastric cancer is associated with better prognosis. In our series of patients treated with neoadjuvant therapy, we have not been able to demonstrate an association between CDX2 expression and tumor stage or differentiation. On the contrary, we have found a relationship between CDX2 expression and lack of response to neoadjuvant therapy. We feel furthermore studies are necessary before any conclusion can be used regarding potential significance of CDX2 study to predict response to NAT.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Salem ME, Hartley M, Unger K, Marshall JL. Neoadjuvant combined-modality therapy for locally advanced rectal cancer and its future direction. Oncology (Williston Park), 2016, 30(6):546–562.

[2] Russell MC. Comparison of neoadjuvant *versus* a surgery first approach for gastric and esophagogastric cancer. J Surg Oncol, 2016, 114(3):296–303.

[3] Metha RS. Dose-dense and/or metronomic schedules of specific chemotherapies consolidate the chemosensitivity of triple-negative breast cancer: a step toward reversing triple-negative paradox. J Clin Oncol, 2008, 26(19):3286–3288; author reply 3288.

[4] Shubert CR, Bergquist JR, Groeschl RT, Habermann EB, Wilson PM, Truty MJ, Smoot RL, Kendrick ML, Nagorney DM, Farnell MB. Overall survival is increased among stage III pancreatic adenocarcinoma patients receiving neoadjuvant chemotherapy compared to surgery first and adjuvant chemotherapy: an intention to treat analysis of the National Cancer Database. Surgery, 2016, 160(4):1080–1096.

[5] Ryan JE, Warrier SK, Lynch AC, Ramsay RG, Phillips WA, Heriot AG. Predicting pathological complete response to neo-adjuvant chemoradiotherapy in locally advanced rectal cancer: a systematic review. Colorectal Dis, 2016, 18(3):234–246.

[6] Saad RS, Ghorab Z, Khalifa MA, Xu M. CDX2 as a marker for intestinal differentiation: its utility and limitations. World J Gastrointest Surg, 2011, 3(11):159–166.

[7] Gross I, Duluc I, Benameur T, Calon A, Martin E, Brabletz T, Kedinger M, Domon-Dell C, Freund JN. The intestine-specific homeobox gene Cdx2 decreases mobility and antagonizes dissemination of colon cancer cells. Oncogene, 2008, 27(1): 107–115.

[8] Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, Wilcox-Fogel N, Forgó E, Rajendran PS, Miranda SP, Hisamori S, Hutchison J, Kalisky T, Qian D, Wolmark N, Fisher GA, van de Rijn M, Clarke MF. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med, 2016, 374(3):211–222.

[9] Wang XT, Wei WY, Kong FB, Lian C, Luo W, Xiao Q, Xie YB. Prognostic significance of Cdx2 immunohistochemical expression in gastric cancer: a meta-analysis of published literatures. J Exp Clin Cancer Res, 2012, 31(1):98.

[10] Okayama H, Kumamoto K, Saitou K, Hayase S, Kofunato Y, Sato Y, Miyamoto K, Nakamura I, Ohki S, Sekikawa K, Takenoshita S. CD44v6, MMP-7 and nuclear Cdx2 are significant biomarkers for prediction of lymph node metastasis in gastric cancer. Oncol Rep, 2009, 22(4):745–755.

[11] Camilo V, Barros R, Celestino R. Castro P, Vieira J, Teixeira MR, Carneiro F, Pinto-de-Sousa J, David L, Almeida R. Immuno-histochemical molecular phenotypes of gastric cancer based on SOX2 and CDX2 predict patient outcome. BMC Cancer, 2014, 14:753.

[12] Masood MA, Loya A, Yusuf MA. CDX2 as a prognostic marker in gastric cancer. Acta Gastroenterol Belg, 2016, 79(2):197–200.

[13] Jun SY, Eom DW, Park H, Bae YK, Jang KT, Yu E, Hong SM. Prognostic significance of CDX2 and mucin expression in small intestinal adenocarcinoma. Mod Pathol, 2014, 27(10): 1364–1374.

[14] Baba Y, Nosho K, Shima K, Freed E, Irahara N, Philips J, Meyerhardt JA, Hornick JL, Shivdasani RA, Fuchs CS, Ogino S. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clin Cancer Res, 2009, 15(14):4665–4673.

[15] Becker K, Mueller JD, Schulmacher C, Ott K, Fink U, Busch R, Böttcher K, Siewert JR, Höfler H. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. Cancer, 2003, 98(7):1521–1530.

[16] Ryan R, Gibbons D, Hyland JM, Treanor D, White A, Mulcahy HE, O'Donoghue DP, Moriarty M, Fennelly D, Sheahan K. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. Histopathology, 2005, 47(2):141–146.

[17] Toxopeus EL, Nieboer D, Shapiro J, Biermann K, van der Gaast A, van Rij CM, Steyerberg EW, van Lanschot JJ, Wijnhoven BP. Nomogram for predicting pathologically complete response after neoadjuvant chemoradiotherapy for oesophageal cancer. Radiother Oncol, 2015, 115(3):392–398.

[18] Sun W, Li G, Wan J, Zhu J, Shen W, Zhang Z. Circulating tumor cells: a promising marker of predicting tumor response in rectal cancer patients receiving neoadjuvant chemo-radiation therapy. Oncotarget, 2016, 7(43):69507–69517.

[19] Tromberg BJ, Zhang Z, Leproux A, O'Sullivan TD, Cerussi AE, Carpenter PM, Mehta RS, Roblyer D, Yang W, Paulsen KD, Poque BW, Jiang S, Kaufman PA, Yodh AG, Chung SH, Schnall M, Snyder BS, Hylton N, Boas DA, Carp SA, Isakoff SJ, Mankoff D; ACRIN 6691 Investigators. Predicting responses to neoadjuvant chemotherapy in breast cancer: ACRIN 6691 Trial of Diffuse Optical Spectroscopic Imaging (DOSI). Cancer Res, 2016, 76(20):5933–5944.

[20] Tang L, Li ZY, Li ZW, Zhang XP, Li YL, Li XT, Wang ZL, Ji JF, Sun YS. Evaluating the response of gastric carcinomas to neoadjuvant chemotherapy using iodine concentration on spectral CT: a comparison with pathological regression. Clin Radiol, 2015, 70(11):1198–1204.

[21] Sun Z, Zhang N. Clinical evaluation of CEA, CA19-9, CA72-4 and CA125 in gastric cancer patients with neoadjuvant chemo-therapy. World J Surg Oncol, 2014, 12:397.

[22] Shin CM, Kim N, Chang H, Kim JS, Lee DH, Jung HC. Follow-up study on CDX1 and CDX2 mRNA expression in noncancerous gastric mucosae after *Helicobacter pylori* eradication. Dig Dis Sci, 2016, 61(4):1051–1059.

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