https://doi.org/10.15218/zjms.2019.014

Immunohistochemical expression of CDX2 gene in colorectal carcinoma

Received: 14/12/2017 Accepted: 18/2/2018

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Abstract

Background and objective: Colorectal cancer is a multi-factorial disease process, with etiology encompassing genetic factors, environmental exposures, and inflammatory conditions of the digestive tract. This study aimed to investigate the immunohistochemical expression of CDX2 as well as clinical and histopathological parameters of patients with colorectal cancer in Erbil, Kurdistan.

Methods: A retrospective study including about 100 colorectal cancer cases in Erbil city, Kurdistan was conducted from January 2015 to January 2017. Tumor type, site, size, histological grade, lymph node status, and pathological stage with CDX2 expression were investigated.

Results: CDX2 was expressed in 86 out of 100 (86%) patients. It was more expressed (focal or diffuse staining) in nonmucinous carcinoma more than signet ring and mucinous carcinoma. Loss of CDX2 expression in colorectal cancer is associated with a high tumor grade and stage. Significant associations between CDX2 expression with both tumor type and tumor grade were observed. Although CDX2 expression was found to be reduced in proximal location (right colon) and higher stage, however, no significant associations between CDX2 expressions in colorectal cancer were detected with tumor site, nodal status, and tumor stage.

Conclusion: Reduced and loss CDX2 expression in colorectal cancer associated with high tumor grades as well as with the mucinous and signet ring cell carcinomas may reflect aggressive tumor behavior probably because of the CDX2 tumor-inhibitory properties. However, the full extent of CDX2's antitumor effects has yet to be elucidated.

Keywords: Colorectal cancer; CDX2 expression; Immunohistochemistry.

Introduction

Colorectal cancer, the most common type of gastrointestinal cancer, is the third most commonly diagnosed cancer in both men and women.[1] In Iraq, It is the 7th most frequent cancer, while in Kurdistan, it forms the 4th most prevalent cancer.[2] This cancer affects nearly 1 million patients worldwide every year, with an average survival rate of less than 50% which forms the third major cause of cancer-related deaths.[3] Risk factors for developing colorectal cancer include prolonged inflammation in the bowel, low physical activity, obesity, high alcohol consumption as well as high intake of red meat.[4] Development of colorectal cancer is a multistep process, wherein specific genes are more to be mutated or over expressed than others.[5] CDX2 gene maps to chromosome 13q12.3 which has three exons and encodes a 313 amino acids protein.[6] It is expressed during endoderm development and in adult intestine.[7] The role of CDX2 started to be established when it was observed in the undifferentiated intestinal cell line (IEC-6) which arrests proliferation and initializes an epithelial polarity program.[8] Cell proliferation and differentiation were thus tightly controlled in the normal intestinal epithelium. Various genes and transcription factors may be involved in this process;

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one of these is CDX2 which is a nuclear transcription factor that is essential for regulating genes related to epithelial functions,[9] and controlling the balance between differentiation and proliferation of IECs.[8] The *caudal*-related CDX2 gene encodes an intestinal-specific transcription factor that has been suggested to be crucial for the development of the intestinal epithelium.[10] CDX2 was required for columnar morphogenesis and cell differentiation in the normal intestinal epithelium.[11] CDX2 had also been suggested to be a tumor suppressor in the colon.[12] Furthermore, loss of CDX2 expression promotes tumor progression in genetically, and chemically induced colorectal cancer.[13] This study aimed to investigate the immunohistochemical expression of CDX2 as well as clinical and histopathological parameters of patients with colorectal cancer in Erbil, Kurdistan.

Methods

One hundred formalin-fixed, paraffin-embedded specimens of colorectal cancer were randomly selected from histopathology departments of the Rizgary Teaching Hospital and private histopathology labs during the period, between January 2015 and January 2017. The study was approved by the Kurdistan Board of Medical Specialties (KBMS). The single exclusion criterion was a CDX2 expression in secondary metastatic colorectal adenocarcinoma. Demographic data (age at diagnosis and sex) and the topography of the tumor (location, histological type of tumor, nodal status) were obtained from the patient's request forms. All blocks were examined, and the best-represented tumor blocks (no necrosis, no much mesenchymal tissue) were selected. New sections were made and stained with Hematoxylin and Eosin H&E for reevaluation. Tumors were divided according to the anatomical location into right, left, and rectal. Histological grading was based on reviewing the H&E stained slides and labeled as the G1 for well-moderately differentiated, G2 for poorly differentiated cases, and histological tumor type as mucinous and nonmucinous. The staging was performed according to the American Joint Committee on Cancer (AJCC) and the Union international center le cancer (UICC), by grouping the various TNM components, as T1-T2 and T3-T4. CDX2 immunoscore measurement was assessed by two investigators; only nuclear staining was considered positive. Cytoplasmic positivity was infrequently encountered and was considered as an artifact. Tumors were graded according to the percentage of positive cells and were scored quantitatively according to the 3-tiered system as following.[14] 1= negative (<5%), 2= focal positive (< 50%), 3= diffuse positive (≥50%). In general, cases showing 2 and three staining patterns also had strong, intense staining, so the intensity was not used in the determination of the final reactivity score. Normal colonic mucosal tissue was used as a CDX2-positive control. For negative controls, the primary antibody was omitted in each run.[15] A Mouse Monoclonal Anti-Human CDX2 Ready-to-Use (Dako Autostainer/ Autostainer Plus) was used. Labeled polymer and enhanced polymer system (Dako EnVision ^{TM} Flex) method according to Dako recommendation was used to stain tissue by the Anti-CDX2 antibody.

Statistical analysis

All patients' data were analyzed using computerized statistical software, the statistical package for the social sciences (version 20). Descriptive statistics were presented as mean± standard deviation and percentages. Multiple contingency tables conducted and appropriate statistical tests were performed. Chi-square was used for categorical variables. Fisher's exact test was performed when the expected count of more than 20% of the cells of the table was less than 5. In all statistical analysis, the level of significance (*P* value) was set at ≤0.05.

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Results

A total of 100 patients with colorectal cancer (median age: 43 years, range: 19 - 85 years) were included in this study. The male to female ratio was 0.75:1 (43 males and 57 females). Clinicopathological variables are presented in Table 1. Sixty-eight patients had left colon and sigmoid cancer, whereas 32 patients had right colon cancer. Only 13 patients were diagnosed with mucinous adenocarcinoma, while the remaining 87 had non-mucinous adenocarcinoma. A total of 47 cases were at stage T1, T2 colorectal cancer while 53 patients showed stage T3, T4 colorectal cancer, and 53 patients had positive lymph nodes at the time of diagnosis.

Table 1: Descriptive data of cases.

Variables	Categories	No.	%
Age groups	< 50 years	35	35
	≥ 50 years	65	65
Gender	Male	43	43
	Female	57	57
Site of cancer	Right colon	32	32
	Left colon and sigmoid	68	68
Tumor type	Non-mucinous	87	87
	Mucinous & signet ring cell carcinomas	13	13
Tumor grade	Well - moderate	89	89
	Poor	11	11
Nodal status	Negative	47	47
	Positive	53	53
Stage of cancer	T1, T2	47	47
	T3, T4	53	53
Total		100	100

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There was a significant association (P = 0.04) between tumor type with a CDX2 expression level. Eighty-nine cases were well-moderate differentiated, and 11 were poorly differentiated tumors. A significant association between tumor grade (well-moderate versus poor) (P = 0.006) was found. No significant association between nodal status with an expression of CDX2 was demonstrated (Table 2).

Table 2: Association of CDX2 expression with patient's age, gender and tumor measures.

Characteristics		Number (No.) and % of CDX2 expression						Total	*P* value
		Negative		Focal		Diffuse			
		No.	%	No.	%	No.	%		
Age	More than 50 years	10	(15.4)	28	(43.1)	27	(41.5)	65	0.84
	Less than 50 years	4	(11.4)	15	(42.9)	16	(45.7)	35	
Gender	Male	7	(15.9)	19	(43.2)	18	(40.9)	44	0.86
	Female	7	(12.5)	24	(42.9)	25	(44.6)	56	
Tumor type	Non-mucinous	10	(11.5)	36	(41.4)	41	(47.1)	87	0.04
	Mucinous	4	(30.8)	7	(53.8)	2	(15.4)	13	
Tumor grade	Well- moderate	9	(10.1)	40	(44.9)	40	(44.9)	89	0.006
	Poor	5	(45.4)	3	(27.3)	3	(27.3)	11	
Tumor stage	Stage T1, T2	4	(8.5)	19	(40.4)	24	(51.1)	47	0.18
	Stage T3, T4	10	(8.9)	24	(45.3)	19	(35.8)	53	
Nodal status	Negative	4	(8.5)	20	(42.6)	23	(48.9)	47	0.26
	Positive	10	(18.9)	23	(43.3)	20	(37.7)	53	
Tumor site	Right colon	6	(18.8)	13	(40.6)	13	(40.6)	32	0.64
	Left colon	8	(11.8)	30	(44.1)	30	(44.1)	68	

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Although CDX2 expression was found to be reduced in proximal location (right colon), there was no significant relation between negative, focal or diffuse CDX2 expression with tumor sites. Expressions of CDX2 determined by nuclear immunoreactivity are shown in Figure 1.

Discussion

In this study, CDX2 was expressed in 86 (86%) cases of colorectal adenocarcinomas. This is similar to what has been seen by Kaimaktchiev et al. study (85.7%).[16] Nuclear staining for CDX2 in 10 (100%) of 10 colonic adenomas and 30 (88.2%) of 34 colorectal adenocarcinomas,[17]whereas another study found reduced CDX2 expression in 4% of differentiated adenocarcinomas.[18] Loss of CDX2 has been detected in 29% of cases while expressed in 71%.[19] Choi et al. in their study on 123 colorectal adenocarcinoma cases, reported a loss of CDX2 immunostaining in 23.6% while expressed in 76.4% of cases.[20] On the other hand, it was stated that CDX2 expression was found in 97% of colorectal adenocarcinomas.[15] Loss and reduction of CDX2 expression in colorectal cancer were seen to be associated with high tumor grade and high stage tumors, although the association with tumor stage was not significant. This may be explained by the variability in some cases and to the heterogeneity of CDX2 expression by tumor cells. Previous reports support our

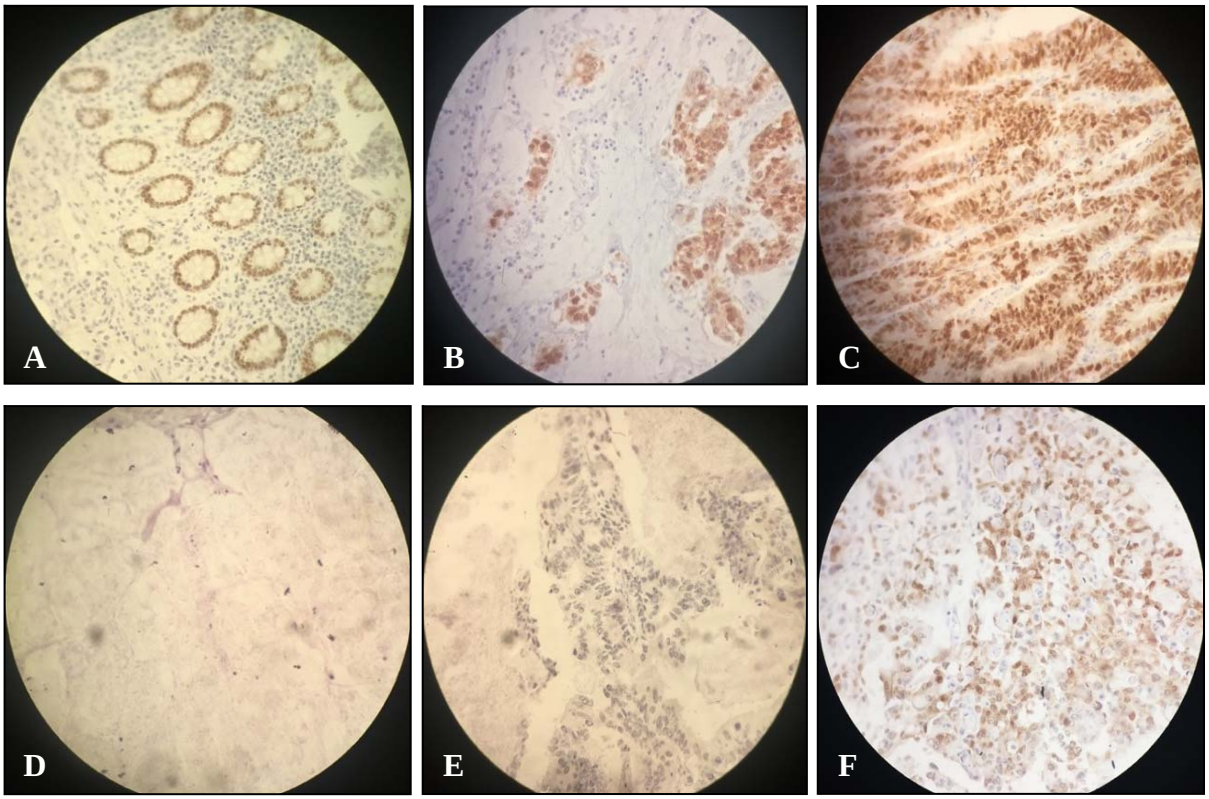

Figure1: Expression of CDX2 determined by immunoreactivity in representative sections of colorectal carcinoma. (A) Positive control of CDX2 expression in nuclei of normal intestinal epithelial cells. (B) Tumor with Focal (<50%) expression of nuclear CDX2. (C) Tumor with Diffuse (≥50%) expression of nuclear CDX2. (D) Colorectal cancer mucinous adenocarcinoma with negative expression of CDX2. (E) Poorly differentiated colorectal adenocarcinoma with negative expression of CDX2. (F) Colorectal cancer signet ring adenocarcinoma with focal expression of CDX2. (microscopy images magnification: 400x).

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findings considering the loss of CDX2 expression in colorectal cancer associated with high tumor grade or advanced tumor stage.[16-18] The staining of CDX2 was more (focal or diffuse staining) in nonmucinous carcinoma than signet ring and mucinous carcinomas. This may indicate that there is a loss of CDX2 expression in mucinous and signet ring carcinomas. Some studies reported CDX2 loss association with advanced TNM stage, higher tumor grades, mucinous and signet ring cell histology.[19] As well, it is in agreement with others who found a negative association between CDX2 expression and tumor stage.[16,20-22] In contrast, another study showed no association among CDX2 expression with the anatomical location, tumor stage (T), or nodal status.[14] However, Olsen et al. had noticed that CDX2 expression was probably correlated to tumor grade, stage, and right-sided tumors.[23] Another study reported that not all histological subtypes of high-grade tumors exhibit loss of CDX2 expression.[24] Also, the predilection for female gender and right-sided tumor location has also been observed in other studies.[19,25] Expression of CDX2 between matched lymph nodes and primary colorectal cancers was also noticed. These results appear to indicate that a further evolution leading to loss of CDX2 after lymph node spread is unlikely.[25] The full extent of CDX2's antitumor effects has yet to be elucidated.[26] Moreover, CDX2 plays an important role in the processes of intestinal cell proliferation, differentiation, adhesion, and apoptosis in addition to its contribution as a tumor suppressor gene.[27-31] Indeed, its expression is often reduced in colorectal cancer and cell differentiation is poor in tumors that lose CDX2[27-29,32] Such concepts indicate that loss of expression of CDX2 may play an important role in the tumorigenesis of colorectal cancers and may categorize patients at high risk.[20]

Conclusion

In conclusion, reduction and loss of CDX2 expression in colorectal cancer were associated with a high tumor grade, advanced tumor stage, mucinous and signet ring cell carcinomas. This indicates that loss of expression of CDX2 may play an important role in the tumor progression of colorectal cancers. It is recommended that CDX2 should always be used as a part of a broader immunohistochemical panel.

Competing interests

The authors declare that they have no competing interests.

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