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Expression of homeodomain protein CDX2 in colorectal adenoma and adenocarcinoma

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Summary. CDX2 is a homeobox domain-containing transcription factor that is important in the development and differentiation of the intestine. In this study, we examined CDX2 expression in normal and neoplastic human colon using a newly isolated monoclonal antibody. When compared to the intensity observed in adjacent normal mucosal epithelial cells, strong nuclear staining for CDX2 was observed in 10 (100%) of 10 colonic adenomas, 30 (88.2%) of 34 colorectal adenocarcinomas, including 17(94.47%) of 18 well-or moderately differentiated tumors and 13(81.2%) of 16 high-grade tumors. The percentage of CDX2 immunopositive cells was generally lower in carcinomas than in adenomas ($p<0.001$) and lower in moderately or poorly differentiated tumors than in well-differentiated tumors ($p<0.001$). There was an inverse correlation between CDX2 expression and tumor grade, tumor stage and lymph node metastasis (respectively, $p<0.001$; $p<0.05$; $p<0.001$), but this was not associated with age, gender, or tumor location and size. These results indicate that loss of expression of CDX2 protein may play an important role in the tumorigenesis of colorectal cancers. Down-regulation of CDX2 may cause dedifferentiation of gastrointestinal epithelial cells.

Key words: CDX2 protein, Colorectal neoplasm

Introduction

Colorectal carcinogenesis is defined by a complex, multistep process that involves genetic, epigenetic, and environmental factors (Vogelstein and Kinzler, 1993). A candidate gene for colorectal cancer is the caudal-type homeobox gene CDX2 (13q12-13), which is exclusively expressed in adults in intestinal epithelium cells and plays an important role in epithelial cell proliferation, differentiation, and determination of cell fate in different organs along the lower gastrointestinal tract (Drummond et al., 1997; Lorentz et al., 1997).

The human CDX2 genes are the homologues of the caudal-related homeobox gene in the Drosophila. CDX2 proteins have been shown to play a role in the development and differentiation of epithelial cells of the small and large intestine in mammals (Beck et al., 1999; Silberg et al., 2000). The CDX2 gene is expressed in all but the most distal portions of the intestinal tract during development. CDX2 acts as a transcription factor, increasing the expression of several gene products associated with mature intestinal epithelial cells. CDX2 stimulates differentiation by activating transcription of intestine-specific proteins, such as MUC2, sucrase, isomaltase and carbonic anhydrase I (Suh and Traber, 1996; Traber and Silberg, 1996).

Here, expression of CDX2 determined by immunohistochemistry (protein) in primary colorectal tumors was compared to that in normal segments of the colonic epithelium.

Materials and methods

Fourty four colorectal tumor (10 tubular or tubulovillous adenomas and 34 adenocarcinomas) were resected surgically or endoscopically from 42 patients (18 men and 16 women; age 60.5±12.9 years, M±SD) at KSU University Hospital. Representative hematoxylin and eosin stained slides from the tumors were reviewed and one representative block was selected for immunohistochemical staining. Additionally, the grade and histollogic type of colorectal adenocarcinomas were determined according to criteria of the World Health Organization Classification of Tumours. Well and

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moderately differentiated tumors were grouped together as low-grade tumors and compared with high-grade tumors, which included poorly differentiated tumors, mucinous carcinomas, and signet ring cell carcinomas. Tumor stage was classified according to Dukes' criteria (Astler and Coller, 1954). In keeping with embryonic development, the large bowel was divided into left side and right side: (a) the right side included the caecum, ascending and transverse colon (midgut); (b) the left side included the splenic flexure, descending colon, sigmoid, and rectum (hindgut). The clinicopathologic parameters evaluated in each case included the patients' age at diagnosis, sex, location of primary tumor, tumor size, grade, nodal status, numbers of positive and total lymph nodes, overall tumor stage and distant metastasis. Where present, positive lymph nodes with metastatic spread or serosal or mesenteric implants were also evaluated in an identical manner.

Immunohistochemical staining for CDX2

Sections (4mm) from parafin-embedded tissue were prepared for immunohistochemistry and deparaffinized. Endogen peroxidase activity was blocked by incubation for 20 minutes in methanol containing 0.3% H202. The antigen retrieval was performed by microwave heating in 10mM citrate buffer (pH 6.0) for 20 min for CDX2. Sections were then incubated in 1:50 dilution of a monoclonal anti-CDX2 antibody (Novocastra, UK) for 1 h at room temperature. All the tissue was then exposed to biotinylated secondary antibody for 25 min, streptavidin enzyme complex for 25 min, diaminobenzidine as a chromogen for 5 min and hematoxylin counterstain for 5 min. A tumor was recorded positive if greater than 5% of the tumor cells exhibited nuclear staining for CDX2. The positive control was normal colon and it was stained appropriately. Only nuclear staining was considered positive, and it was scored on the following basis: 0 (no detectable staining); 1+ (<25%); 2+ (26-50%); 3+ (51-75%); and 4+ (76-100%). Furthermore, staining in more than 50% of the tumor cells for CDX2 was also recorded as diffuse positive.

Statistics

Statistical analysis of association of variables was done using Kruskal-Wallis analysis of variance followed by post-hoc Mann-Whitney test. p-values<0.05 were accepted as statistically significant.

Results

There were 18 men and 16 women, with ages between 32 and 90 years (median 60 years). Eighteen of 34 adenocarcinomas were low grade (well+moderate differentiated) and 16 were high-grade (poorly differentiated). Ten tumors were classified as stage A, 6 as stage B, 17 as stage C, and 1 as stage D. Sixteen tumors had a maximum dimension <5cm, whereas 18 tumors were ≥ 5cm. Fifteen of 34 patients had tumors localized in the right-sided colon and 19 in the left –sided colon. The expression of CDX2 in the nucleus was present in 30(88.2%) of 34 colorectal cancer. When CDX2 expression in colorectal cancers was examined in low-grade versus high grade tumors, 17 (94.4%) of 18 well or moderately differentiated tumors and 13 (81.2%) of 16 high-grade tumors were positive. Of the high-grade carcinomas, 5 (100%) of 5 of the mucinous and 1 (100%) of 1 of the signet ring cell carcinomas, 7 (70.0%) of 10 of the remaining poorly differentiated carcinomas were positive for CDX2 expression. The proportion of CDX2-negative cases was higher in the poorly differentiated type. Clinically, the loss of CDX2 expression was seen in 4 (23.5%) of 17 tumors corresponding to stage C. Normal colonic mucosa demonstrated moderate nuclear staining. No significant differences in staining intensity were noted along the axis of the crypt, regardless of the original rostral-caudal position of the tissue. Moderate to strong nuclear staining for CDX2 was nearly universal in samples of colonic adenomas (100%; Fig. 1A,B), and was higher in adenomas than in normal epithelium. Whereas in adenomas the staining was generally restricted to nuclei, cytoplasmic positivity was evident in some of the adenocarcinomas. CDX2 was expressed in both nucleus and cytoplasm in five cases. All five tumors with cytoplasmic CDX2 immunoreactivity were well to moderately differentiated. The cytoplasmic immunoreactivity for CDX2 may reflect a non-functional pool of CDX2 protein. The percentage of CDX2 immunopositive cells was generally lower in carcinomas than in adenomas (p<0.001) and lower in

Table1. CDX2 expression by tumor characteristics and patient.

Clinicopathological Parameters	Total n(%)	CDX2 protein positive n(%)	p
Patients			
Male	18	15/18(83.3)	>0.05
Female	16	15/16(93.7)	
Location			
Right	15	12/15(80.0)	>0.05
Left	19	18/19(94.7)	
Tumor size(cm)			
<5	16	16/16(100)	>0.05
≥ 5	18	14/18(77.7)	
Histological grade			
Well	6	6/6(100)	<0.001
Moderate	12	5/6(83.3)	
Poor	16	13/16(81.2)	
Duke's stage			
A	10	10/10(100)	<0.05
B	16	16/16(100)	
C	17	14/17(82.3)	
D	1	1/1(100)	
Primary nodal status			
N0	18	18/18(100)	<0.001
N+	16	12/16(75.0)	

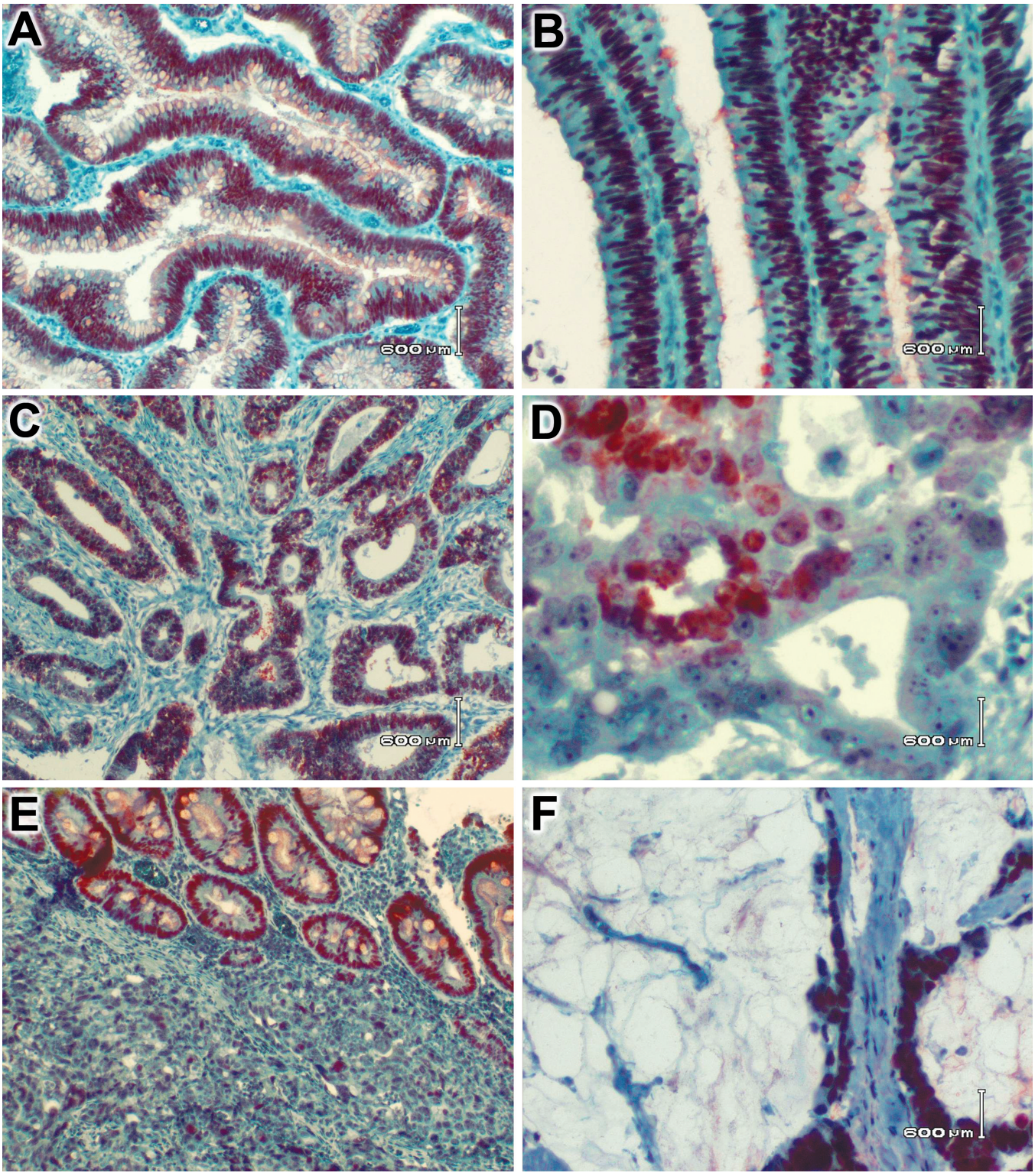

Fig. 1. CDX2 immunostaining in primary colorectal tumors (whole tissue sections). Tubular **(A)** or villous adenoma **(B)** with strong nuclear staining. **C.** Well-differentiated colonic adenocarcinoma with positive staining. **D.** The view of staining in moderately differentiated colonic adenocarcinoma. **E.** The staining was focal and weaker in some of the poorly differentiated colonic adenocarcinoma. **F.** Mucinous adenocarcinoma with positive staining.

moderately or poorly differentiated tumors than in well-differentiated tumors (26±9% versus 9±6%, 74±2% respectively; $p<0.001$ in each case). CDX2 expression has been found to decrease with tumor grade in human cancers. The expression of nuclear CDX2 was found to correlate with histologic grade, depth of wall invasion and metastasis of lymph node. No significant association was observed between nuclear CDX2 expression and age, gender, maximum tumor diameter or location. (Table1). Figure 1 (C through F) shows the CDX2 staining patterns among representative sections of histologic subtypes of colorectal cancers.

Discussion

CDX2 represents one of the latest transcription factors, which include myo-D1(Dias et al., 1990; Wang et al., 1995) and myogenin (Wang et al., 1995) as markers of skeletal muscle differentiation, thyroid transcription factor-1 as a marker of lung and thyroid (Bejarano et al.,1996) and microphthalmia transcription factor as a marker of melanocytes (Busam et al., 2001). As a transcription factor, CDX2 has a key role in regulating the proliferation and differentiation of intestinal cells and maintaining intestinal phenotypes. Thus, CDX2 is thought to be a marker for intestinal epithelium. In the present study, we analyzed expression patterns of CDX2 protein in primary colorectal carcinomas. Nuclear staining for CDX2 was present in 30 (88.2%) of 34 colorectal cancer. Based on differentiation grade, CDX2 was expressed in 100% (6/6), 91.6% (11/12) and 81.2% (13/16) of well, moderately, and high-grade differentiated colorectal cancers, respectively. Of the high-grade carcinomas, 5 of 5 of the mucinous and 1 of 1 of the signet ring cell carcinomas were positive for CDX2 expression. In the remaining poorly differentiated carcinomas, 7 of 10 were positive for CDX2 expression. The current study of high-grade colorectal carcinomas revealed that not all histologic subtypes of high-grade tumors exhibit loss of CDX2 expression. Nuclear CDX2 expression was very strong in the tumor cells of well-differentiated cancers, but significantly decreased in poorly differentiated carcinomas. Among colorectal adenocarcinomas, the relationship between tumor grade and CDX2 staining has been controversial. Werling et al. (2003) noted that 74 of 75 cases of colonic adenocarcinomas were CDX2-positive, independent of tumor grade. The study reported that there did not seem to be any correlation between tumor grade and the percentage of cells staining, in spite of some high-grade adenocarcinomas that showed fewer immunoreactive cells. Our data support the contention that high-grade colorectal adenocarcinomas demonstrate a lower frequency of CDX2 staining than lower-grade tumors. There was a statistically significant inverse correlation between tumor grade and CDX2 staining ($p<0.001$). This confirms a trend previously observed in much smaller studies by Hinoi et al. (2003). The loss of CDX2 in high-grade carcinomas may reflect a deregulation of the mechanisms that control CDX2 expression. While it initially appeared that high-grade colorectal carcinomas showed decreased CDX2 staining when compared to low-grade tumors, further analysis of staining among histologic subtypes of high-grade tumors revealed that the decrease in tumor staining observed in these tumors is likely a reflection of tumor histology and not tumor grade alone (Ee et al., 1995; Hinoi et al., 2001). The number of cases analyzed in this study is not sufficient to draw any definitive conclusion. Reports from other investigators will be necessary for a prognosis and frequency of its occurrence. In this study, decreased CDX2 staining correlated with increased tumor stage ($p<0.05$). Our data are consistent with previous reports, in which expression of CDX2 showed significant correlation with tumor grade and stage of colorectal carcinomas (Ee et al., 1995; Hinoi et al., 2003; Kaimaktchiev et al., 2004). There was also a negative correlation between nuclear CDX2 expression and lymph node metastasis ($p<0.001$). These findings are entirely consistent with those of Werling et al. (2003) and Barbareschi et al. (2003), and support the use of this marker in the evaluation of metastatic adenocarcinoma of unknown primary. A loss of CDX2 at the invasive front may lead directly to transient tumor cell dedifferentiation, which triggers dissemination of tumor cells through blood and lymphatic vessels (Brabletz et al., 2004). In the current study, reduced CDX2 expression was statistically closely associated with differentiation grade of tumor cells, lymph node metastasis and higher clinical stage. Reduced CDX2 expression was not associated with tumor location. Right-sided tumors showed lower mean CDX2 expression than left-sided tumors (respectively 0.23±0.18; 0.30±0.28), but this result was not statisticaly significant ($p>0.05$). Hinoi et al. (2001) reported reduced CDX2 expression in colorectal carcinomas which were predominantly right sided.

Furthermore, the adenomas studied also showed strong nuclear staining for CDX2. In this study, both tubular and villous adenoma were shown the expression CDX2 proteins at roughly uniform levels. The percentage of CDX2 immunopositive cells was generally lower in carcinomas than in adenomas ($p<0.001$). This finding may indicate the possibility of an early event in colorectal carcinogenesis. In conclusion, the premalignant adenoma derived cell lines we have studied provide a model for elucidating the role of CDX2 in the control of colonic epithelial growth and differentiation. These results indicate that increased CDX2 expression may be related to growth and malignant transformation of adenomas, and may play an important role in the progression of colorectal adenomatous change.

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