The Putative Tumor Suppressor Cdx2 Is Overexpressed by Human Colorectal Adenocarcinomas

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Abstract

Purpose: The current paradigm suggests that the homeodomain transcription factor Cdx2, which directs the development and maintenance of the intestinal epithelium, is a tumor suppressor in the colon and rectum. Although a cardinal property of tumor suppressors is their inactivation during carcinogenesis, the expression of Cdx2 in colorectal tumors has not been compared with that in normal mucosa. Here, Cdx2 expression and function was quantified in tumors and matched normal mucosa from patients with colorectal cancer.

Experimental Design: Cdx2 expression was quantified by reverse transcription-PCR, immunoblot analysis, and immunohistochemistry. Transcriptional activity was explored by quantifying expression of an endogenous downstream target of Cdx2, guanylyl cyclase C (GCC), in tissues by quantitative reverse transcription-PCR and expression of exogenous Cdx2-specific luciferase promoter constructs in epithelial cells isolated from tumors and normal mucosa.

Results: Most (>80%) colorectal tumors overexpressed Cdx2 mRNA and protein compared with normal mucosa, with median fold increases of 3.6 and 1.4, respectively (P < 0.002). Concomitantly, immunohistochemistry revealed elevated levels of Cdx2 in nuclei of tumor cells compared with normal epithelial cells. Further, tumors exhibited increased expression of GCC compared with normal mucosa. Moreover, cells isolated from tumors overexpressed a Cdx2-specific luciferase promoter construct compared with normal mucosal cells.

Conclusion: These observations show, for the first time, the structural and functional overexpression of Cdx2 by human colorectal tumors compared with matched normal mucosa. They suggest that loss of Cdx2 expression or transcriptional activity is an infrequent event during tumorigenesis, which does not contribute to molecular mechanisms underlying initiation and progression of most colorectal tumors.

The caudal-related homeobox gene Cdx2 encodes a homeodomain transcription factor required for development and maintenance of the intestinal epithelium (1–3). Cdx2 expression in adults is restricted to intestinal epithelial cells, where it maintains the differentiated phenotype of mature enterocytes by regulating expression of intestine-specific genes, including guanylyl cyclase C (GCC; ref. 4), lactase (5), sucrase-isomaltase (1), and L1-cadherin (6). Also, Cdx2 regulates the expression of ubiquitous genes important in controlling cell dynamics, including adhesion (6), proliferation (7), cell cycle regulation (8), and apoptosis (9). The homeotic function of Cdx2 is underscored by loss of the cognate enterocyte phenotype in intestinal neoplasms arising in the proximal colon of Cdx2+/− mice upon biallelic inactivation of Cdx2 (10, 11). Conversely, ectopic expression of Cdx2 in squamous epithelial cells of the forestomach induces intestinal metaplasia in transgenic mice (12, 13).

Beyond its homeotic role, Cdx2 may be an important tumor suppressor in colorectal carcinogenesis (14, 15). Thus, expression of Cdx2 mRNA and protein is reduced (16, 17) or lost (9, 16, 18–20) in some human colorectal tumors. However, in some analyses, immunohistochemistry revealed Cdx2 protein expression in most human colonic adenocarcinomas examined (21, 22). In animal models, biallelic inactivation of Cdx2 results in the formation of hamartomatous tumors characterized by gastric heteroplasia in the proximal colon (11, 23). Similarly, whereas adenomatous polyposis coli heterozygous (APC+/−) mice develop adenomatous polyposis of the small intestine, APC+/−/Cdx2+/− mice develop polyposis of the colon (24). Moreover, the procarcinogen azoxymethane induced invasive adenocarcinoma of the distal colon in Cdx2+/− mice but not wild-type littermates (10). Further, overexpression of Cdx2 in human colon cancer cells induces a less malignant phenotype, inhibiting proliferation, invasion, and migration
while promoting the expression of genes characteristic of mature enterocytes (2, 9, 17, 25, 26).

Thus, the prevalent paradigm suggests that Cdx2 is a tumor suppressor whose reduced expression and/or function contributes to initiation and progression of sporadic adenocarcinomas of the colon and rectum (14, 15, 24). This hypothesis presumes that promotion of colorectal carcinogenesis reflects loss of Cdx2 expression and/or function in tumor cells relative to normal intestinal epithelium. However, with the exception of one study (19), expression of Cdx2 in human colorectal tumors has not been compared with matched normal mucosa (15). In the present study, Cdx2 expression and transcriptional activity in colorectal tumors were compared with matched normal mucosa from patients. Unexpectedly, Cdx2 mRNA, protein, and activity were elevated in most colorectal tumors compared with matched normal mucosa. These observations challenge the prevailing paradigm and suggest that loss of Cdx2 expression or function is not a dominant mechanism underlying initiation or promotion of colorectal carcinogenesis.

Materials and Methods

Clinical specimens. Specimens were obtained from the Department of Pathology, Anatomy and Cell Biology of Thomas Jefferson University Hospital (Philadelphia, PA) under an Institutional Review Board–approved protocol (control no. 98.0614). Slides, stained with H&E, were reviewed by a pathologist (I. Palazzo) to confirm that all tumors were adenocarcinoma and all normal mucosal samples were free of disease. Total RNA was extracted from specimens with Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH). Cytoplasmic and nuclear protein fractions were isolated using NE-PER extraction reagents (Pierce, Rockford, IL).

Primary cell isolation. Following resection, tumor and normal mucosal specimens were incubated at 37°C in DMEM/F12 supplemented with 350 units/ml collagenase XI (Sigma, St. Louis, MO). Disaggregated cell suspensions were centrifuged, resuspended in Eagle’s MEM containing 10% fetal bovine serum, insulin (0.1 µg/ml), gentamicin (40 µg/ml), and penicillin/streptomycin (100 units/ml), gentamicin (40 µg/ml), and fungizone (125 µg/ml), and then subjected to 45 cycles at the following conditions: 94°C for 20 seconds and 62°C for 30 seconds. Reactions were incubated at 60°C for 2 minutes, 95°C for 30 seconds, and then subjected to 20 seconds and 62°C for 30 seconds. Reactions were incubated at 70°C for 2 minutes, 95°C for 30 seconds, and then subjected to 20 seconds and 62°C for 30 seconds. Reactions were incubated at 70°C for 2 minutes, 95°C for 30 seconds, and then subjected to 20 seconds and 62°C for 30 seconds. Reactions were incubated at 70°C for 2 minutes, 95°C for 30 seconds, and then subjected to 20 seconds and 62°C for 30 seconds.

Reverse transcription-PCR. RNA harvested from T84 human colon carcinoma cells (American Type Culture Collection, Manassas, VA) was used in a reverse transcription reaction to produce Cdx2, GCC, or β-actin cDNA. The synthesized cDNA was amplified by PCR and subsequently cloned into the Bluescript vector (Stratagene, La Jolla, CA). Purified plasmid DNA was linearized with appropriate restriction enzymes. The conserved 5′ T7 promoter site was used to transcribe target genes using the RibomAX Large Scale RNA Production System-T7 (Promega, Madison, WI). Transcription reactions were treated with DNase to remove template DNA fragments and the cDNA purified on an RNeasy Mini-Column (Qiagen, Foster City, CA). The purity and concentration of cDNA stock solutions was determined by UV spectrophotometry. Stock cRNA solutions underwent serial 10-fold dilutions to generate working standards to establish calibration curves for absolute analyte quantification by quantitative reverse transcription-PCR (RT-PCR). Slopes and intercepts of calibration curves for Cdx2, GCC, and β-actin showed that amplification reactions for these analytes were of comparable high efficiency (Table 1).

Reverse transcription-PCR. RNA (1 µg) isolated from specimens was used in a one-step reverse transcription PCR. The reaction was done using Taqman EZ RT-PCR Core Reagents (Applied Biosystems, Inc., Foster City, CA) and the 7000 Sequence Detection System (Applied Biosystems). A commercially available primer and probe set was used to amplify and detect Cdx2 RNA (Applied Biosystems). The primer and probe sequences used to amplify and detect β-actin and GCC transcripts were as follows (5′-3′): β-actin sense, CCACGACTTGCCCACATCTACC; β-actin antisense, AGGATCTTCATGAG-GTAGTCA-GTACG; probe (FAM)ATGCC-X (TAMRA)-CCCATGCCCACCTGCGGp (X indicates a linker-arm nucleotide and p indicates phosphorylation) and GCC sense, ATTTCACTCATTTCATGACC; GCC antisense, CTGCA-GAACAG-GACATTTTCTC; and probe (6FAM)TACTCGAGGACAATGTCAAGCCCTT (TAMRA). Reactions were incubated at 50°C for 2 minutes, 60°C for 30 minutes, 95°C for 5 minutes, and then subjected to 45 cycles at the following conditions: 94°C for 20 seconds and 62°C for 1 minute.

Immunohistochemistry. Sections (5 µm) of paraffin-embedded tissues were mounted on Superfrost Plus-charged slides. Routine deparaffinization from xylene to 95% alcohol was done on a Leica Autostainer (Leica, Inc., Deerfield, IL) and included a 30-minute methanolic peroxide step to block endogenous peroxidase activity. Slides were rehydrated and antigen recovery was done at 70% power in a 1,050 W microwave oven (model EM-F3400, Sanyo, Chatsworth, CA) in EDTA buffer (pH 8.0) for two intervals of 5 minutes each. After a 30-minute cooling period, slides were washed in deionized water and placed in PBS for 5 minutes. Immunostaining was conducted using a DAKO autostainer (model LV-1, DAKO Corporation, Carpinteria, CA). Slides were incubated for 15 minutes each with avidin and blocking solutions (Vector Laboratories, Burlingame, CA), followed by Cdx2 primary antibody (1:1,600; BioGenex, San Ramon, CA) for 60 minutes. After a PBS wash, slides were incubated with DAKO LSAB secondary antibody for 30 minutes followed by DAKO LSAB streptavidin–horseradish peroxidase reagents (DAKO). After another PBS wash, slides were incubated for 5 minutes in 3,3′-diaminobenzidine solution (DAKO), washed in deionized water, counterstained with Harris hematoxylin (SurpIth, Richmond, IL), and dehydrated with xylene before mounting.

Reporter gene analysis. The transcriptional activity of Cdx2 was examined using luciferase reporter constructs, including a pGL-3-Basic luciferase vector (negative control; Promega), a pGL3 construct containing fragment –835 to +117 of the GUCY2C promoter (GCC-Luc), and the identical construct with a TTT to CCC mutation in the Cdx2 consensus site (∆GCC-Luc; ref. 4). Following seeding, primary cells at 30% to 50% confluency were cotransfected with 0.5 µg of the Renilla luciferase control reporter, pRL-TK, driven by a viral thymidine kinase promoter (Promega) with FuGENE 6 Transfection Reagent at 3 µl/µg DNA (Roche Diagnostics, Indianapolis, IN). One day after transfection, cells were harvested and assayed using the protocol and materials in the Dual-Luciferase Reporter Assay System (Promega). Luminescence was measured with a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Luciferase expression was normalized to pRL-TK expression for standardization of transfection efficiency.

Table 1. Amplicon sizes, Y intercepts, and slopes for standard curves for quantitative RT-PCR amplification of marker genes

<table>
<thead>
<tr>
<th>Marker</th>
<th>Amplicon size (bp)</th>
<th>Slope</th>
<th>Intercept</th>
<th>LOD (copies)</th>
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<tr>
<td>Cdx2</td>
<td>50-150*</td>
<td>-3.072±0.139</td>
<td>42.82±0.67</td>
<td>100</td>
</tr>
<tr>
<td>β-Actin</td>
<td>99</td>
<td>-3.515±0.205</td>
<td>42.95±1.07</td>
<td>200</td>
</tr>
<tr>
<td>GCC</td>
<td>84</td>
<td>-3.344±0.108</td>
<td>43.33±0.49</td>
<td>200</td>
</tr>
</tbody>
</table>

Abbreviation: LOD, limit of detection.

*Proprietary information of Applied Biosystems.
Promoter activity was normalized by subtracting the pGL3 control vector ratio from the respective promoter fragment–containing reporter vector ratio. Cdx2-transactivating activity was quantified as the difference between normalized values for GCC-Luc activity and AGCC-Luc activity.

**Immunoblot analysis.** Tissues were homogenized in Laemmli buffer and 30 μg of total lysate proteins were separated on 12% SDS-polyacrylamide gels under reducing conditions and transferred to nitrocellulose (Osmonics, Inc., Minnetonka, MN). After transfer, sheets were subjected to Ponceau S (Sigma) staining, which was consistently identical in all lanes. Following incubation in 5% nonfat dry milk in PBS with 0.5% Tween 20 (Sigma) to block nonspecific protein binding, proteins of interest were identified by incubating with affinity-purified rabbit anti-Cdx2 antibody (1:500; Biosource International, Hopkinton, MA) and rabbit polyclonal anti-α-tubulin antibody (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA) in TBS with 0.1% Tween and 5% nonfat milk (blotting buffer) overnight at 4°C. After several washes, membranes were incubated in horseradish peroxidase–conjugated goat anti-rabbit antibody (1:10,000; Jackson ImmunoResearch Laboratories, West Grove, PA) in blotting buffer at room temperature for 45 minutes. Membranes were washed and protein antibody complexes were visualized by chemiluminescent detection using SuperSignal West Pico Chemiluminescent Substrate (Pierce). Staining intensities on immunoblots were quantified with Kodak ID (v.3.5.4) software (Eastman Kodak, New Haven, CT).

**Fluorescence immunohistochemistry.** Cells in 24-well plates were fixed and permeabilized with −20°C methanol for 20 minutes, washed with PBS, blocked with preimmune goat or rabbit serum at 25°C for 1 hour, and then incubated overnight at 4°C with rabbit anti-Cdx2 or goat anti-α-T-cadherin antibody (1:50; Santa Cruz Biotechnology) in TBS supplemented with 3% bovine serum albumin. Subsequently, cells were washed with TBS and incubated at room temperature for 45 minutes in rhodamine (TRITC)–conjugated goat anti-rabbit or Cyan2−conjugated rabbit anti-goat antibody (1:100; Jackson ImmunoResearch Laboratories) in TBS with 3% bovine serum albumin. Antibody complexes were visualized using fluorescence microscopy (Kodak DC290 ZOOM Digital Camera; Adobe Photoshop 7.0).

**Statistical analysis.** Determinations were done in duplicate or triplicate and results are representative of at least three experiments unless otherwise indicated. Cdx2 and GCC mRNA and protein expression in tumors and matched normal mucosal specimens were compared using the Wilcoxon signed-rank test (27). Correlation of Cdx2 with GCC expression was quantified by the Spearman correlation coefficient, a nonparametric estimate (28). Cdx2 transcriptional activity in tumors and matched normal mucosal specimens were compared using the Student’s t test. In all analyses, P < 0.05 was considered statistically significant.

### Results

**Patient characteristics.** Patients ranged in age from 48 to 94 years (72 ± 12.9 years) and there were no significant differences in the ages of females (53%; range = 48-94 years; 75 ± 11.8 years) and males (47%; range = 51-93 years; 68 ± 13.4 years). All patients had adenocarcinoma of the colon or rectum and their anatomic distribution included the right colon (43%), transverse colon (7%), left colon (7%), sigmoid colon (23%), rectosigmoid colon (10%), and rectum (10%). The distribution of disease stage (29) included stage A (30%), stage B (37%), stage C (20%), and stage D (10%), comparable with distributions observed previously (30). Moreover, the distribution of tumor grade included well-differentiated (10%), moderately well differentiated (3%), moderately differentiated (70%), moderately poorly differentiated (13%), and poorly differentiated (3%; Table 2).

**Cdx2 mRNA is overexpressed by colorectal tumors compared with matched normal mucosa.** External calibration standards (Fig. 1A and B) were used in quantitative RT-PCR to quantify Cdx2 and β-actin mRNA expression in tissues (Table 2). For clinical specimens, mRNA levels were not routinely normalized to β-actin, reflecting the heterogeneity of contribution of epithelial and stromal cells to different specimens; the variability of expression of housekeeping genes in different cells, tissues, and patients; and the general acceptance of normalization to amount of total RNA analyzed (31). However, specimens yielding <10^3 copies of β-actin mRNA per microgram of total RNA were deemed insufficient and omitted from further analysis.

Expression of Cdx2 mRNA was compared in 30 paired colorectal tumors and histologically normal adjacent mucosas (Fig. 1C and D; Table 2). Quantitative RT-PCR revealed substantial interindividual variation in expression of Cdx2 mRNA in normal mucosa (1.2 × 10^6 ± 1.9 × 10^6 copies/μg total RNA; mean ± SD) and adenocarcinomas (7.7 × 10^6 ± 1.9 × 10^7 copies/μg total RNA; mean ± SD), with a range of ~2 orders of magnitude. However, Cdx2 mRNA was significantly overexpressed in 25 of 30 adenocarcinomas (~83%) compared with matched normal adjacent mucosas (P < 0.002; Fig. 1C and D). Indeed, the median difference in expression of Cdx2 mRNA between tumors and matched normal adjacent mucosas, 8.3 × 10^7, with a range of ~5.0 × 10^7 to 7.9 × 10^7 copies, reflected a 3.6-fold median increase in Cdx2 mRNA expression in tumors compared with matched normal mucosa. Although most tumors were moderately differentiated and were from patients with stage B or C disease, Cdx2 mRNA copy number did not obviously correlate with tumor grade or stage (Table 2). Of significance, four of five tumor specimens that expressed less Cdx2 mRNA compared with matched normal adjacent mucosas were of an early stage (stage A and B; Table 2) in contradistinction to suggestions that Cdx2 expression decreases during tumor progression (16, 17).

**Cdx2 protein is overexpressed by colorectal tumors compared with matched normal mucosa.** Immunoblot analysis revealed elevated Cdx2 protein in tumors that overexpress Cdx2 mRNA compared with matched normal adjacent mucosas (Fig. 2A). In every tumor examined in which Cdx2 mRNA was overexpressed (n = 10), Cdx2 protein also was elevated compared with matched normal mucosa. The mean increase in Cdx2 protein in tumors was ~150% compared with matched normal mucosa (P < 0.002; Fig. 2B). Further, overexpression of Cdx2 mRNA and protein detected by immunoblot analysis was associated with an increase in Cdx2 detected by immunohistochemistry in tumors compared with matched normal mucosas (n = 5; Fig. 2C). Cdx2 expressed by tumors and normal mucosa was appropriately localized in the nucleus (Fig. 2C).

**Cdx2 transcriptional activity is elevated in colorectal cancer compared with matched normal mucosa.** Cdx2 transcriptional activity, which regulates expression of proteins that define the differentiated enterocyte phenotype (1–4, 10, 32), can be examined in intestinal cells by quantifying the expression of genes that are downstream targets (17, 25). GCC is an epithelial cell receptor in the intestine that regulates fluid and electrolyte homeostasis (33) and proliferation along the crypt-villus axis (34–36) whose tissue-specific expression is defined by Cdx2 (4). Thus, Cdx2 transcriptional activity in colorectal tumors and
matched normal mucosa (n = 26) was compared by quantifying the expression of GCC using quantitative RT-PCR. Calibration curves of in vitro transcribed cRNA encoding GCC were used in quantitative RT-PCR to quantify GCC mRNA expression levels (Fig. 3A; Table 2). GCC mRNA levels were significantly elevated in tumors exhibiting overexpression of Cdx2 mRNA compared with matched normal mucosa (P < 0.01; Fig. 3B and C). The median difference in expression of GCC mRNA in tumor compared with matched normal mucosa, 6.0/10^4 with a range of 9.2/10^4 to 1.0/10^6 copies, reflected a median 2.4-fold increase in GCC mRNA expression in tumors compared with matched normal mucosa. The precursor-product relationship between Cdx2 and GCC is highlighted by the close correlation of the quantities of their cognate transcripts in individual tissues (r = 0.53; P < 0.01; Fig. 3D).

The foregoing is consistent with a mechanism in which increased expression of GCC mRNA reflects overexpression of Cdx2 in tumors compared with matched normal mucosa. However, expression of GCC mRNA reflects the integrated activities of multiple transcriptional regulatory proteins at the promoter and differences in expression could reflect the activities of transcriptional factors other than Cdx2 (4, 37). Thus, Cdx2 transcriptional activity was directly quantified in isolated tumor cells and matched normal enterocytes by reporter gene analysis. In these studies, Cdx2-specific transactivation was quantified as the difference between luciferase activities generated by a construct driven by the wild-type GCC promoter (~835 to +117) and the identical construct with a TTT-to-CCC mutation in the Cdx2 consensus site (4). Following surgical resection, colorectal tumors and matched normal adjacent mucosa were disaggregated and established in culture. To confirm identity, isolated cells were stained with antibody against Cdx2 and LI-cadherin, nuclear and membrane proteins exclusively expressed by intestinal epithelial cells (Fig. 4A). Cdx2-specific transcriptional activity was 2-fold greater in isolated tumor cells compared with matched normal adjacent mucosal cells (P < 0.05; Fig. 4B).

Discussion

A cardinal property defining tumor suppressors is their inactivation in cancer cells compared with normal cells, resulting in disequilibrium in molecular mechanisms regulating proliferation and survival (38). The current paradigm suggests that Cdx2 is a tumor suppressor (14, 15, 24) whose loss of function, reflecting loss of expression (16), mutation

Table 2. Patient demographics, tumor characteristics, and Cdx2 mRNA copy number

<table>
<thead>
<tr>
<th>Sex</th>
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<td></td>
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(39), posttranslational modification (40), or regulation (17), contributes to mechanisms underlying colorectal carcinogenesis. In stark contrast, the current study shows that Cdx2 is overexpressed in most sporadic human colorectal tumors compared with matched normal mucosa. Overexpression of mRNA and protein is associated with elevated Cdx2 transcriptional activity and overexpression of GCC, an endogenous downstream gene target of Cdx2 (4). These observations show that Cdx2 is structurally and functionally overexpressed in most sporadic human colorectal tumors. They are in close agreement with immunohistochemical studies in which >80% of human colorectal tumors retained expression of Cdx2 (17, 21, 22, 41). Taken together, these observations show that loss of Cdx2 function is not a principle mechanism underlying the initiation or promotion of most sporadic human colorectal tumors.

The present study does not eliminate a role for Cdx2 as a tumor suppressor in colorectal carcinogenesis. Indeed, <20% of sporadic colorectal tumors exhibit reduced or absent Cdx2 expression (17, 21, 22, 41). Also, Cdx2 expression seems to be lost in large cell minimally differentiated colon carcinomas (16). Additionally, ~10% of sporadic colorectal tumors exhibit loss of heterozygosity with respect to Cdx2 (42, 43). Moreover,
Cdx2 transcription is silenced by a dominant repression mechanism in ~10% of sporadic colorectal tumors (17). These observations are consistent with a role for Cdx2 as a tumor suppressor in a subset of colorectal tumors. However, it remains unclear whether loss of Cdx2 expression in those tumors is intrinsic to mechanisms underlying carcinogenesis or reflects the effects of genetic instability associated with neoplastic transformation.

Although Cdx2 is not a classic tumor suppressor in most colorectal neoplasms, it may play a role in tumor progression in a restricted spatial and temporal window. Cells that metastasize from the invasive front of primary colorectal tumors undergo transient dedifferentiation, only to reestablish the differentiated phenotype at the metastatic site (44–46). Although Cdx2 is expressed in most colorectal cancer cells, it is transiently lost in cells at the invasive edge of the tumor (25). Transient loss of Cdx2 is initiated by cell interaction with collagen type I, mediated by cell surface β-integrin and nuclear β-catenin signaling, and is presumed to be the mechanism mediating the epithelial-mesenchymal transition characterizing metastasis (25). Establishment of tumor metastases at distant sites results in the reexpression of Cdx2 associated with recovery of the differentiated phenotype (25). In the context of the present study, these observations suggest that rather than a tumor suppressor gene, Cdx2 may be an important metastasis suppressor gene (47) with a role restricted spatially to the invasive front in tumors accessible to collagen type I and temporally to the perimetastasis period (25).

Although Cdx2 is overexpressed by most colorectal tumors, the precise mechanisms underlying its overexpression remain undefined. Overexpression may reflect the genetic instability characterizing carcinogenesis wherein Cdx2 expressed by tumors is a bystander in the process of neoplastic transformation. Thus, 35% of sporadic colorectal tumors exhibit polyplody of chromosome 13q12.13-q12.2 where the gene encoding Cdx2 resides (48). Also, most colorectal tumors constitutively overexpress nuclear factor-kB (49), a potent transactivator of Cdx2 expression (50). Moreover, the Cdx2 promoter has Cdx2 binding sites that mediate transactivation and increases in expression reflecting genetic instability will be amplified by autoregulation (51).

Alternatively, overexpression of Cdx2 might reflect compensatory mechanisms directed at reestablishing the equilibrium in proliferation, differentiation, and survival characteristics of epithelial cells that is disrupted during carcinogenesis. Biallelic inactivation of Cdx2 in epithelial cells results in the formation of hyperplastic colonic polyps in Cdx2-/- mice (11, 23, 24). Conversely, conditional expression of Cdx2 in colon carcinoma cells in vitro suppressed their proliferation (9, 17). Further, conditional expression of Cdx2 in HT-29 colon carcinoma cells induced the expression of the mitogen-activated protein kinase MOK, which mediates growth arrest (52). Ectopic expression of Cdx2 in the foregut of transgenic mice induces the development of the differentiated intestinal cell phenotype (13). Similarly, overexpression of Cdx2 in human colon cancer cells induces the expression of genes characteristic of differentiated enterocytes and goblet cells (2, 25, 26, 52). Moreover, conditional expression of Cdx2 increased the sensitivity of human colon carcinoma cells to apoptosis (9).

An intriguing possibility not considered previously is that overexpression of Cdx2 in tumors reflects its role in mediating neoplastic transformation. Ectopic expression of Cdx2 in hematopoietic progenitor cells induces acute myeloid leukemia in transgenic mice (53). Similarly, although ectopic expression of Cdx2 in the foregut induces intestinal metaplasia in mice (12, 13), these lesions ultimately progress to frank

![Fig. 3. A, in vitro transcribed cRNA was used to generate a quantitative RT-PCR standard curve to quantify GCC mRNA expression. B and C, GCC mRNA copy numbers from human colorectal tumors that overexpressed Cdx2 RNA were quantified by RT-PCR (n = 25; *P < 0.01). Box and whiskers plots in (C) are similar to those in Fig. 1. D, correlation of Cdx2 and GCC mRNA expression in human colorectal tumors (n = 25; r = 0.53; P < 0.01).](image)
mucosal cells using the promoter of GCC with wild-type (GCC-Luc) and mutated cultures of tumor and normal adjacent mucosal cells confirms the presence of

Traber PG, Silberg DG. Intestine-specific gene

References

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adenocarcinoma (54). Although the precise role of Cdx2 in neoplastic transformation in bone marrow (53) and foregut (54) remains unclear, carcinomas arising from its ectopic expression exhibit elevated proliferating cell nuclear antigen as well as genetic instability reflected by mutations in APC and p53 (54). Conversely, hamartomas deficient in Cdx2 arising in the proximal colon of Cdx2−/− mice fail to incorporate bromodeoxyuridine, a marker of proliferation, in contrast with normal adjacent mucosa expressing Cdx2, which suggests a proproliferative function for this transcription factor (55). Moreover, conditional expression of Cdx2 in IEC-6 undifferentiated intestinal epithelial cells increased their proliferation and migration (2, 7). Further, Cdx2 induces the expression of E2F3 (56), a member of the E2F family of transcription factors that regulate G1-S phase transition and DNA replication (57). Although these observations do not establish Cdx2 as an oncogene, they underscore the potential for this transcription factor to contribute to the cancer phenotype. In the context of overexpression in tumors compared with normal mucosa, they suggest a need to define the role of Cdx2 in gastrointestinal tumorigenesis.

In conclusion, Cdx2 is overexpressed in most colorectal tumors compared with matched normal mucosa. Overexpression of Cdx2 mRNA was associated with increased Cdx2 protein expression localized in the nuclei of tumor cells. Similarly, increased protein expression was associated with elevated Cdx2-dependent transcriptional activity. Moreover, increased transactivation was associated with overexpression of GCC, an endogenous downstream target of Cdx2. The structural and functional overexpression of Cdx2 in tumors, compared with normal mucosa, suggests that, in contrast to the prevailing paradigm, Cdx2 does not serve as a tumor suppressor in the development of most sporadic colorectal tumors. Rather, in the context of earlier observations of its role in promoting the neoplastic phenotype in some cells and tissues, the present observations suggest the intriguing possibility that Cdx2 could serve as an oncogene in the gastrointestinal tract. The ability to regulate proneoplastic and antineoplastic pathways highlights the importance of further studies to define the complex roles for Cdx2 in molecular mechanisms underlying human colorectal carcinogenesis.

Fig. 4. A, immunofluorescent detection of Cdx2 and LI-cadherin protein in primary cultures of tumor and normal adjacent mucosal cells confirms the presence of intestinal epithelial cells. B, reporter analysis of primary tumor and normal adjacent mucosal cells using the promoter of GCC with wild-type (GCC-Luc) and mutated (TTT-to-GCC) Cdx2 binding sequences (ΔGCC-Luc). GCC-Luc and ΔGCC-Luc reporter activities were normalized to a TK-driven Renilla reporter. Values reflect the arithmetic difference between activities obtained with GCC-Luc and ΔGCC-Luc (n = 4; *, P < 0.05).


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