Usefulness of CDX2 and TTF-1 in Differentiating Gastrointestinal From Pulmonary Carcinoids

Anjali Saqi, MD, Diane Alexis, Fabrizio Remotti, MD, and Govind Bhagat, MD

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Abstract

Carcinoids of different organs appear morphologically indistinguishable. We studied the differential expression of CDX2 and thyroid transcription factor-1 (TTF-1) in 78 gastrointestinal and pulmonary carcinoids and their metastases (n = 10). CDX2 staining of gastric biopsy specimens with neuroendocrine hyperplasia (n = 11) and various gastritides (n = 10) was also performed. All ileal (6/6 [100%]), 6 (86%) of 7 appendiceal, 3 (75%) of 4 duodenal, 1 (50%) of 2 ampullary, 12 (33%) of 18 rectal, 5 (26%) of 19 pancreatic, and 1 (17%) of 6 gastric carcinoids expressed CDX2 with variable intensity; none of the pulmonary carcinoids stained. Of 15 pulmonary carcinoids, 8 (53%) stained with TTF-1, but none of the gastrointestinal carcinoids did. CDX2 and TTF-1 staining profiles of primary and metastatic carcinoids were similar. CDX2+ gastric endocrine cells had a distribution similar to that of gastrin and enterochromaffin cells but not enterochromaffin-like cells. Our results suggest that CDX2 and TTF-1 have high specificity for gastrointestinal and pulmonary carcinoids, respectively.

Carcinoids arise in diverse sites and are thought to originate from a variety of organ-specific neuroendocrine cells: Kulchitsky cells in the lung; enterochromaffin cells and enterochromaffin-like cells, among others, in the stomach; intraepithelial neuroendocrine cells in the small bowel, colon, and rectum; and subepithelial neuroendocrine cells in the appendix.[1] Although carcinoids of various sites have unique cellular derivations, they are indistinguishable morphologically, resulting in a diagnostic challenge when deciphering the primary site of metastatic neoplasms.

Immunohistochemical antibody panels are used routinely in diagnostic algorithms to ascertain the primary sites of metastatic neoplasms. Antibodies against cytokeratin (CK) 7 and CK20 have been used in an attempt to predict the origin of various carcinomas. Neuroendocrine neoplasms, including carcinoids, also have been reported to differentially express CK7 and CK20. One study reported a high specificity of CK7 for pulmonary carcinoids and CK20 for gastrointestinal carcinoids.[2] However, these findings were not corroborated by a larger survey.[3] A few studies have evaluated other markers,[4] including stains for neuroendocrine granules,[5] to elucidate tissue-specific signatures for carcinoids; although encouraging, the results have not been conclusive. Recent studies using antibodies against relatively organ-specific transcription factors, including CDX2 and thyroid transcription factor-1 (TTF-1), have proved efficacious in diagnosing metastatic adenocarcinomas from unknown primary sites.[6,7]

CDX2 is the human homologue of a *Drosophila* caudal-like gene.[6] Experimental evidence from murine models suggests that CDX2 expression is maintained in the adult small and large intestinal epithelia,[8-10] and its protein product can be detected in epithelial cells from the gastroduodenal junction to

the rectum[8,11] by immunohistochemical staining. CDX2 expression is induced or up-regulated in pathologic states associated with an "intestinal" phenotype, including Barrett esophagus,[12] chronic atrophic gastritis,[13] and adenocarcinomas of gastroesophageal,[11,14] pancreaticobiliary,[6,11] and colorectal[6] origins. Recently, a tumor suppressor function has been suggested for CDX2.[15]

TTF-1, a member of the *Nkx2* family of homeobox genes,[16] is a transcription factor that is expressed in thyroid, lung, and the diencephalon.[17] The immunohistochemical staining profile of TTF-1 has been studied extensively,[17-20] and it is used to confirm or exclude the pulmonary origin of carcinomas.[19] Its expression in pulmonary carcinoids has been reported to range from 45%[20] to 95%.[19] Promiscuous TTF-1 staining in neuroendocrine neoplasms from other organs, including small cell carcinomas[17,18] and large cell neuroendocrine carcinomas,[18] however, has also been reported.

We undertook this study to assess the diagnostic usefulness of CDX2 and TTF-1 immunohistochemical staining in an attempt to distinguish gastrointestinal carcinoids, including pancreatic endocrine neoplasms (PENs), from pulmonary carcinoids. We evaluated the sensitivities and specificities of CDX2 and TTF-1 for carcinoids arising from the foregut, midgut, hindgut, pancreas, and lung. CDX2 and TTF-1 staining of primary tumors and synchronous metastases was also performed in a subset of cases. CDX2 expression was correlated with the hormonal profiles of PENs. The CDX2 staining profiles of gastric neuroendocrine hyperplasia (NEH) and nonneoplastic gastrointestinal tract and pancreatic neuroendocrine cells were also evaluated.

Materials and Methods

Formalin-fixed, paraffin-embedded tissue sections of biopsies and resection specimens of 78 gastrointestinal carcinoids, PENs, and pulmonary carcinoids from 74 patients comprised the study group. The patients included 23 males and 51 females (M/F ratio, 1:2.2) with a median age of 59.5 years (mean, 56.4 years; range, 11-83 years). Fourteen patients had pulmonary carcinoids (mean age, 53.8 years; median, 61 years; range, 13-82 years; M/F ratio, 1:2.5), 40 had gastrointestinal carcinoids (mean age, 56 years; median, 59.5 years; range, 11-83 years; M/F ratio, 1:2.3), 19 had PENs (mean age, 62.4 years; median, 65 years; range, 33-81 years; M/F ratio, 1:1.7), and 1 had a mixed acinar-endocrine carcinoma. Synchronous metastases to lymph nodes (n = 9) and liver (n = 1) were also evaluated.

Neuroendocrine cells in nonneoplastic epithelium adjacent to tumors were evaluated in all cases. In addition, 11 gastric biopsy specimens with NEH related to chronic atrophic autoimmune gastritis (gastric fundus or body, n = 8) and reflux carditis (gastric cardia, n = 1) were studied. Seven antral biopsy specimens (nonspecific reactive features, n = 4; gastrin-cell hyperplasia secondary to proton pump inhibitor therapy, n = 2; and *Helicobacter pylori*–associated chronic active superficial gastritis, n = 1), 2 fundic biopsy specimens (fundic gland polyp, n = 1; nonspecific reactive features, n = 1), and 1 cardiac biopsy specimen (mild reactive features) were also evaluated.

Neuroendocrine Cell Hyperplasia

The different patterns of gastric NEH were categorized according to published criteria[21] after staining with chromogranin.

Immunohistochemical Staining for CDX2

Paraffin sections were deparaffinized and rehydrated. Slides were immersed in Trilogy EDTA (pH 8.0; Cell Marque, Hot Springs, AR) and subjected to heat-induced antigen retrieval by placing the slides in a steamer for 40 minutes. The slides were cooled for 10 minutes, washed with distilled water, and rinsed once with tris(hydroxymethyl)aminomethane-buffered saline containing Tween 20 wash buffer (DAKO, Carpinteria, CA). Staining was performed on an autostainer (Autostainer Plus, DAKO) using a primary mouse monoclonal antibody (clone CDX2-88, dilution 1:300; BioGenex, San Ramon, CA) incubated for 35 minutes at room temperature. Secondary labeling and visualization were performed using the Labeled Streptavidin-Biotin 2 System (DAKO) for 10 minutes at room temperature.

Immunohistochemical Staining for Other Antibodies

Immunohistochemical staining was performed according to standard methods after deparaffinization using an automated immunohistochemical staining machine (Autostainer Plus, DAKO). For a list of primary antibodies, antigen retrieval, and secondary visualization methods used see **Table 1**. The slides were counterstained with hematoxylin before viewing.

Grading of CDX2 Staining

CDX2 staining was scored in a semiquantitative manner. The percentage of stained cells (PSC) was graded from 0 to 4+: 0, no staining; 1+, 1% to 25%; 2+, 26% to 50%; 3+, 51% to 75%; and 4+, 76% to 100%; intensity of staining was graded using a 3-tiered scale: 1+, weak; 2+, moderate; and 3+, strong. Nuclear staining was considered positive; however, the presence of cytoplasmic staining was also noted.

Hormonal profiles of PENs were determined by immunohistochemical staining in all cases. The histologic patterns of all neoplasms were evaluated on H&E-stained slides and classified as solid, trabecular, or glandular when more than 75% of the tumor showed a particular pattern.

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Table 1
Immunohistochemical Stains Used

Antibody/Source	Clone	Dilution	Antigen Retrieval*	Detection System
CDX2/BioGenex (San Ramon, CA)	CDX2-88	1:300	Trilogy (Hot Springs, AR) EDTA (pH 8.0); steamer	LSB2S
TTF-1/DAKO (Carpinteria, CA)	8G7g3/1	1:200	TRS (pH 6.0); steamer	LSB2S
Pancytokeratin/Chemicon (Temecula, CA) and Immunotech (Westbrook, ME)	KL-1; AE1/AE3	1:500	CB; microwave	EM
Synaptophysin/BioGenex	Snp-88	1:40	CB; microwave	EM
Chromogranin/DAKO	DAK-A3	1:100	CB; microwave	EM
Insulin/BioGenex	HB125	1:50	None	EM
Somatostatin/DAKO	1-14 Somatostatin	1:300	None	ER
Gastrin/DAKO	Gastrin-17	1:30	None	ER
Glucagon/BioGenex	Glucagon	1:2	None	ER

CB, citrate buffer (pH = 6); EM, EnVision+ mouse (DAKO); ER, EnVision+ rabbit (DAKO); LSB2S, Labeled Streptavidin-Biotin 2 System (DAKO); TRS, Target Retrieval Solution (DAKO); TTF-1, thyroid transcription factor-1.
* Trilogy EDTA, Cell Marque; steamer, 40 min; microwave, slides placed in microwave at full power for 7 minutes and then decreased to 40% power for 10 minutes.

Statistics

The specificities and sensitivities of CDX2 and TTF-1 were calculated as follows:

Specificity = True Negative/(True Negative + False Positive)

Sensitivity = True Positive/(True Positive + False Negative)

Results

Gastrointestinal and Pulmonary Carcinoids

Carcinoids of the gastrointestinal tract showed regional variations in the PSC and intensity of CDX2 expression (midgut > distal foregut > hindgut > proximal foregut) **Table 2** and **Image 1**. The frequency of CDX2+ carcinoids from different sites is given in Table 2. None of the pulmonary carcinoids stained with CDX2. CDX2 staining was independent of tumor size, architectural pattern, and expression of other neuroendocrine markers. The majority of gastrointestinal carcinoids expressed chromogranin (30/43) and synaptophysin (41/43) (Table 2).

Pancreatic Endocrine Neoplasms

Of 19 PENs, 5 (26%) expressed CDX2, as did a solitary mixed acinar-endocrine carcinoma, but there was marked variability in the staining patterns. The results of immunohistochemical staining for CK, synaptophysin, chromogranin, and various hormones in these tumors are given in **Table 3**. All PENs stained with pan-CK and synaptophysin, but only 17 of 19 cases expressed chromogranin. The mixed acinar-endocrine carcinoma was synaptophysin+ but did not express chromogranin. No correlation was noted between these markers and CDX2. CDX2 expression also was independent of the hormonal profile, functional status, tumor size, and architectural pattern of the neoplasms. One PEN occurred in a patient with multiple endocrine neoplasia type 1 who had hypercalcemia due to a parathyroid adenoma. Two PENs were associated with insulin secretion, and the remaining tumors were nonfunctional.

Table 2
Grading of CDX2 Staining in Carcinoids and Pancreatic Endocrine Neoplasms

	No. of Cases			Stained Cells (%)*				Intensity†					
Site	Total	–	+ (%)	1+	2+	3+	4+	1+	2+	3+	No. (%)CS	CHR‡	SYN‡
Stomach	6	5	1 (17)	0	0	0	1	0	1	0	0 (0)	6 (100)	6 (100)
Duodenum	4	1	3 (75)	1	0	1	1	1	1	1	0 (0)	4 (100)	3 (75)
Ampulla	2	1	1 (50)	1	0	0	0	1	0	0	0 (0)	0 (0)	2 (100)
Appendix	7	1	6 (86)	0	0	2	4	0	0	6	6 (86)	7 (100)	7 (100)
Ileum	6	0	6 (100)	0	0	1	5	0	0	6	6 (100)	6 (100)	6 (100)
Rectum	18	12	6 (33)	1	2	2	1	3	2	1	3 (17)	7§ (39)	17 (94)
Pancreas	20‖	14	6 (30)‖	3‖	1	0	2	1	3‖	2	3 (15)	17 (85)	20‖ (100)

CHR, chromogranin; CS, cytoplasmic staining; SYN, synaptophysin.
* The percentage of stained cells was scored according to the following scale: 0, no staining; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; 4+, 76%-100%.
† Intensity of staining was graded according to the following scale: 1+, weak; 2+, moderate; 3+, strong.
‡ Data are given as number (percentage) of positive cases.
§ Seven cases had rare scattered positive cell(s) (<1%) and were considered negative.
‖ Includes 1 mixed endocrine-acinar carcinoma.

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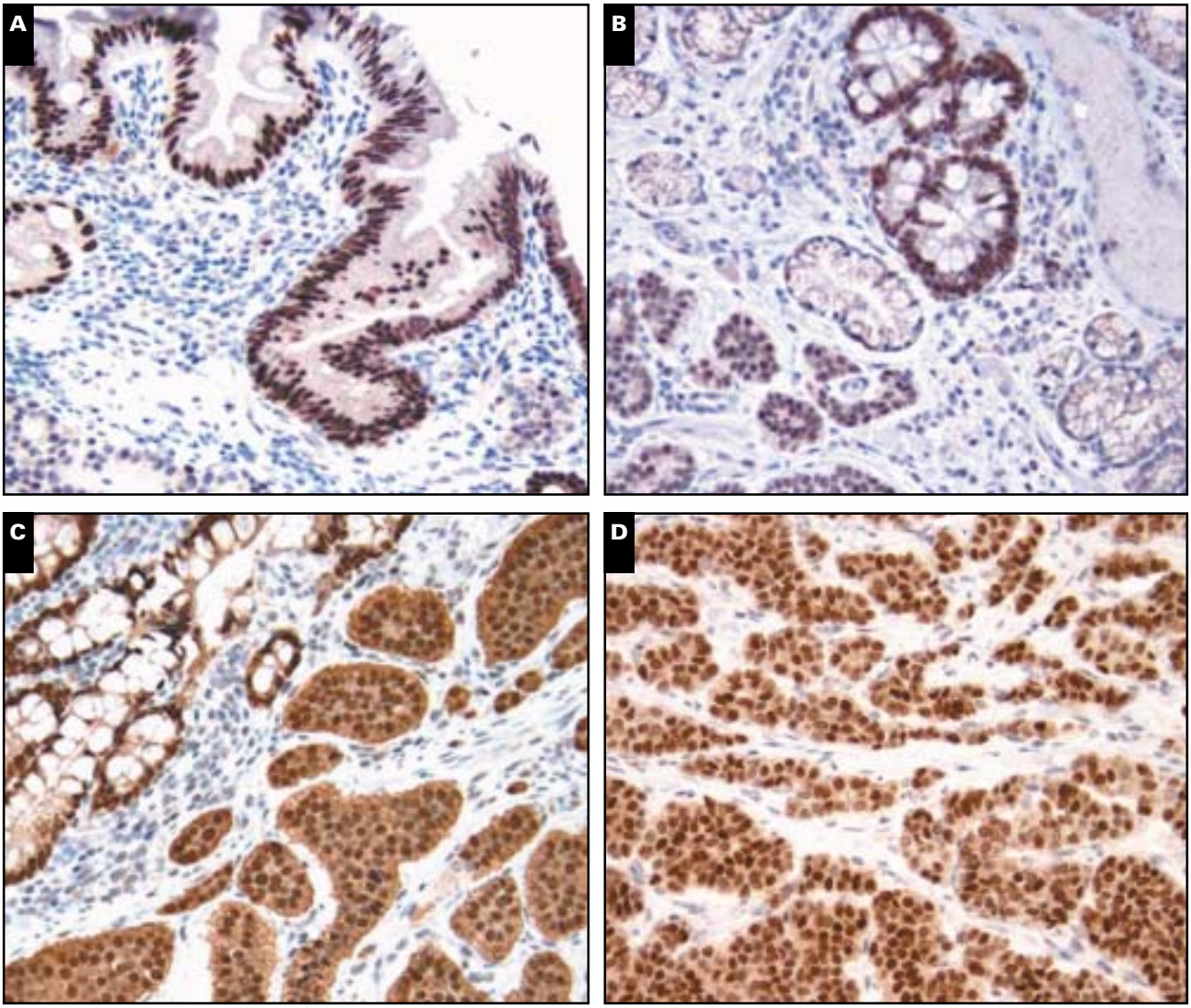

Image 1 CDX2 staining in gastrointestinal carcinoids and pancreatic endocrine neoplasms. **A**, Duodenum. Weak staining (intensity, 1+) of carcinoid and strong CDX2 staining of duodenal epithelium (×200). **B**, Stomach. Moderate staining (intensity, 2+) of carcinoid and strong CDX2 staining in the focus of intestinal metaplasia (×200). **C**, Ileum. Strong CDX2 staining (intensity, 3+) in carcinoid and overlying epithelium. Note intense cytoplasmic staining (×200). **D**, Pancreas. Strong nuclear and cytoplasmic CDX2 staining (intensity, 3+) (×200).

Nonneoplastic Intestinal, Pancreatic, and Pulmonary Epithelium

The absorptive, goblet, Paneth, and neuroendocrine cells (identified by chromogranin staining) from the duodenum to the rectum stained with CDX2. Virtually all cells along the crypt-villus axis of the small intestine and the crypt lining cells of the colon showed intense staining; only the intraepithelial lymphocytes and exceedingly rare scattered epithelial cells did not stain. Large ducts, intermediate ducts, interacinar ducts, and centroacinar cells of the pancreas were CDX2+, but no staining was identified in the islets of Langerhans. Examination of serial sections revealed that rare chromogranin+ neuroendocrine cells scattered among the ductal epithelial cells seemed to reflect the distribution of CDX2+ duct lining cells. Nonneoplastic pulmonary epithelium did not stain with CDX2.

Gastric Biopsy Specimens With NEH

Gastric fundus, body, antrum, and cardia biopsy specimens showed different patterns of NEH **Table 4**. NEH was highlighted by chromogranin staining in all cases. CDX2+ cells corresponded to areas of gastrin cell hyperplasia in the antrum (2 cases) **Image 2F** and serotoninergic (enterochromaffin) cell hyperplasia in the cardia (1 case) **Image 2B**. No CDX2+ cells

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Table 3
Immunohistochemical Profiles of Pancreatic Endocrine Neoplasms

Case No.	CDX2	PSC*	Intensity†	CK	SYN	CHR	Insulin	SOM	Gastrin	GLUC
1	+	4+	3+	+	+	+	+	–	+F	–
2	–	–	–	+	+	–	–	–	–	+
3	–	–	–	+	+	+	+	+	–	–
4	–	–	–	+	+	+	+	–	–	+
5‡	–	–	–	+	+	+	–	+	–	–
6	+	1+	1+	+	+	+	–	–	–	–
7	–	–	–	+	+	+	+	+	–	–
8	–	–	–	+	+	–	–	–	–	–
9	–	–	–	+	+	+	–	–	–	–
10	–	–	–	+	+	+	–	–	–	+
11	–	–	–	+	+	+	–	–	–	–
12	+	1+	2+	+	+	+	–	–	–	–
13	–	–	–	+	+	+	–	–	+	–
14	+	2+	3+	+	+	+	+	–	–	–
15§	–	–	–	+	+	+	–	–	–	–
16	+	4+	2+	+	+	+	–	+	+	–
17	–	–	–	+	+	+	–	+	–	–
18‖	–	–	–	+	+	+	+	–	–	–
19‖	–	–	–	+	+	+	+	–	–	–
20¶	+	1+	2+	+	+	–	–	–	–	–

CHR, chromogranin; CK, pancytokeratin; F, focal staining in scattered cells; GLUC, glucagon; PSC, percentage of stained cells; SOM, somatostatin; SYN, synaptophysin; +, positive; –, negative.
* The percentage of stained cells was scored according to the following scale: 0, no staining; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; 4+, 76%-100%.
† Intensity of staining was graded according to the following scale: 1+, weak; 2+, moderate; 3+, strong.
‡ Pancreatic endocrine neoplasm in a patient with von Hippel-Lindau disease.
§ Patient with multiple endocrine neoplasia-1.
‖ Patients had functional tumors (insulinomas).
¶ Mixed acinar endocrine carcinoma.

were identified in areas corresponding to enterochromaffin-like hyperplasia of the body or fundus (8 cases) **Image 2D**.

Nonneoplastic Gastric Neuroendocrine Cells

CDX2+ neuroendocrine cells were identified in all biopsy specimens from the antrum, body, and cardia. The neuroendocrine nature of the cells was established by chromogranin staining performed on serial sections and direct visual comparison **Image 3**. Scattered basally oriented cells, beneath the mucous cells of the antrum and cardia, stained with CDX2. A few of these cells had elongated nuclei lying parallel to the basement membrane, whereas others had plump, round to ovoid nuclei communicating with glandular lumens (Image 3). The latter cellular configuration predominated at the gastric glandular junctions. Both architectural types of neuroendocrine cells were also identified in the oxyntic mucosa by chromogranin staining, but CDX2+ neuroendocrine cells (most likely representing enterochromaffin cells) were exceedingly rare in this location. Overall, the distribution and location of the CDX2+ cells correlated with gastrin and enterochromaffin cells (Image 3).

TTF-1 Staining of Carcinoids and PENs

TTF-1 stained 8 (53%) of 15 pulmonary carcinoids, including 2 (67%) of 3 atypical and 6 (50%) of 12 typical carcinoids. The staining intensity varied from case to case. Gastrointestinal carcinoids, gastric NEHs, and PENs did not stain with TTF-1.

Table 4
Gastric Biopsy Specimens With Neuroendocrine Cell Hyperplasia

Case No.	Site	Type of Hyperplasia	Chromogranin	CDX2
1	Body	Linear	+	–
2	Body	Simple	+	–
3	Body	Microglandular	+	–
4	Fundus	Microglandular	+	–
5	Fundus	Microglandular	+	–
6	Fundus	Simple	+	–
7	Fundus	Microglandular	+	–
8	Fundus	Microglandular	+	–
9	Cardia	Linear	+	+
10	Antrum	Microglandular	+	+
11	Antrum	Linear	+	+

+, positive; –, negative.

Metastases

Nine carcinoids (ileum, 5; rectum, 1; and lung, 3) and 1 mixed acinar-endocrine carcinoma had synchronous metastases to the lymph nodes or liver. None of the PENs were associated with metastases. The architectural patterns of the primary and metastatic neoplasms were similar, and the CDX2 staining patterns of all intestinal carcinoids and their metastases were virtually identical **Image 4**. A minor difference in staining intensity was detected between the mixed acinar-endocrine neoplasm and its metastasis (primary tumor intensity, 2+; metastasis intensity, 1+) and in the staining profile of

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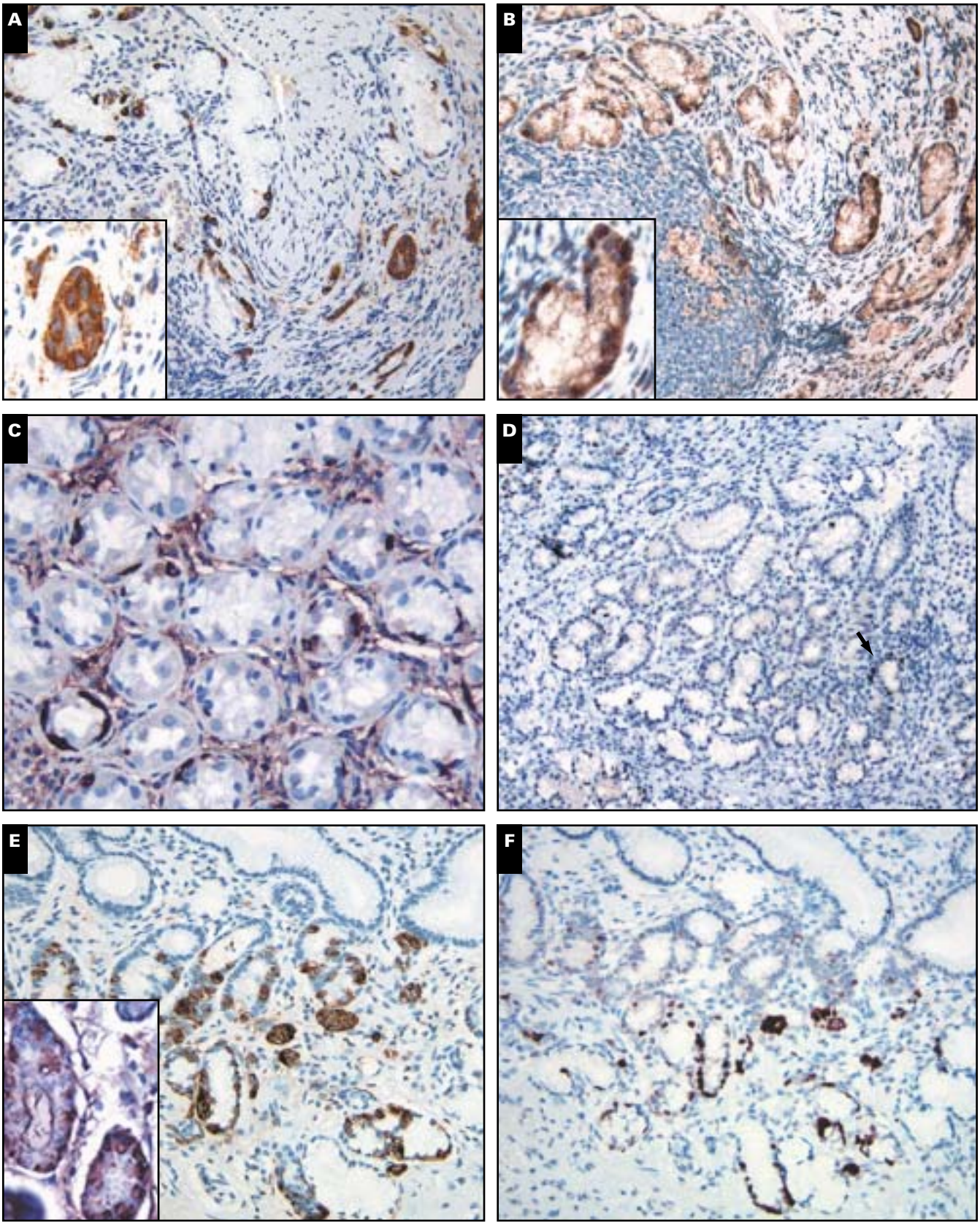

Image 2 Gastric neuroendocrine cell hyperplasia. Chromogranin demonstrates linear hyperplasia in the cardia (**A**; inset, higher magnification of linear hyperplasia, ×400) and microglandular hyperplasia in the body (**C**) and antrum (**E**; inset, higher magnification of microglandular hyperplasia, ×400). CDX2 stains hyperplastic neuroendocrine cells in the cardia (**B**; inset, higher magnification of CDX2 staining in a linear pattern, ×400) and antrum (**F**); only occasional CDX2+ neuroendocrine cells are seen in the body (arrow) (**D**) (**A-F**, ×100).

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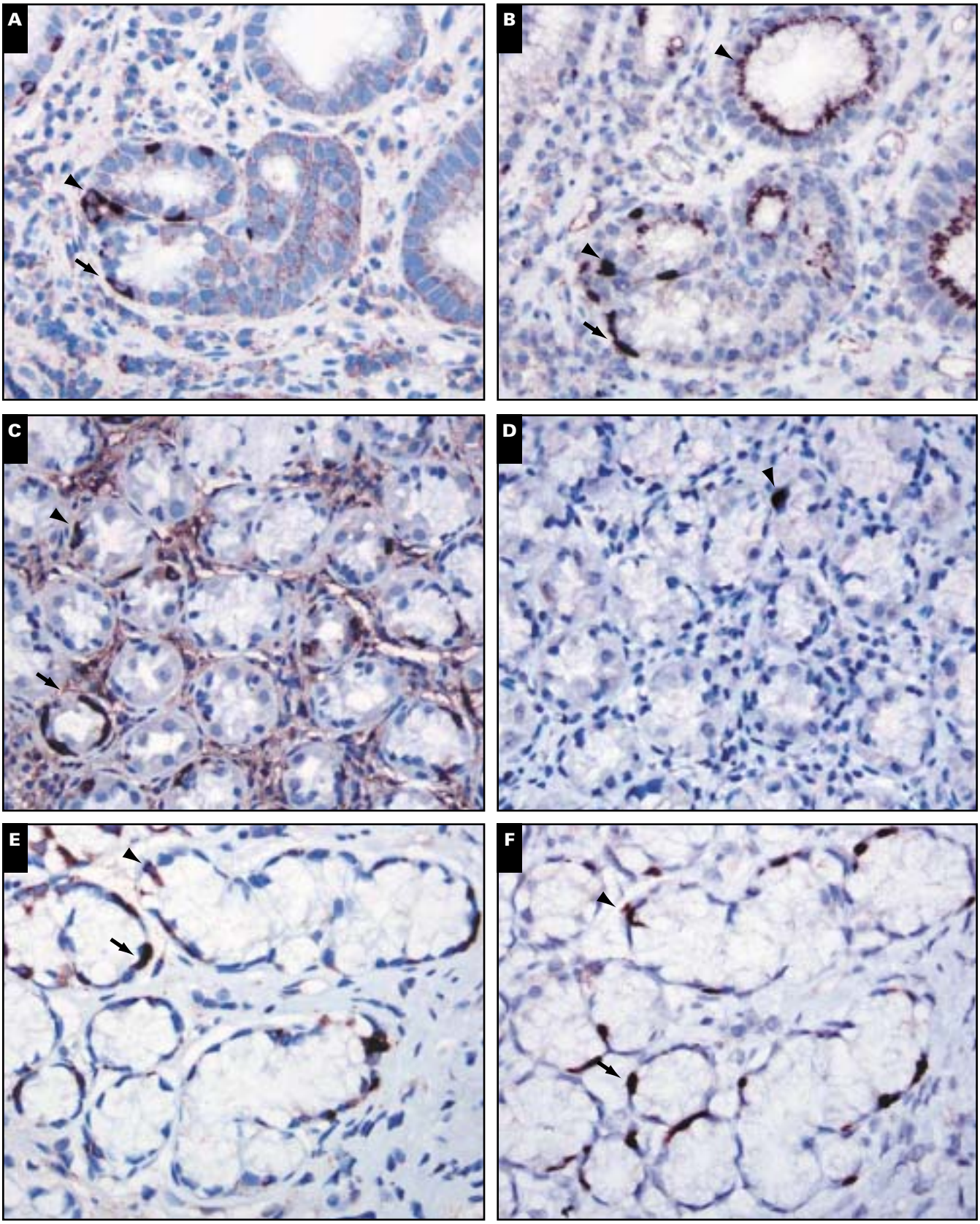

Image 3 Normal gastric mucosa. Chromogranin demonstrates flat (arrows) and cuboidal (arrowheads) neuroendocrine cells in the cardia (**A**), fundus (**C**) and antrum (**E**). CDX2 also highlights flat (arrows) and cuboidal (lower arrowhead) neuroendocrine cells in similar locations on serial sections in the cardia (**B**) and antrum (**F**). Supranuclear, granular cytoplasmic staining also is seen in 1 cardiac gland (upper arrowhead) (**B**). Only rare CDX2+ neuroendocrine cells (arrowhead) were seen in the fundus (**D**) (**A-F**, ×400).

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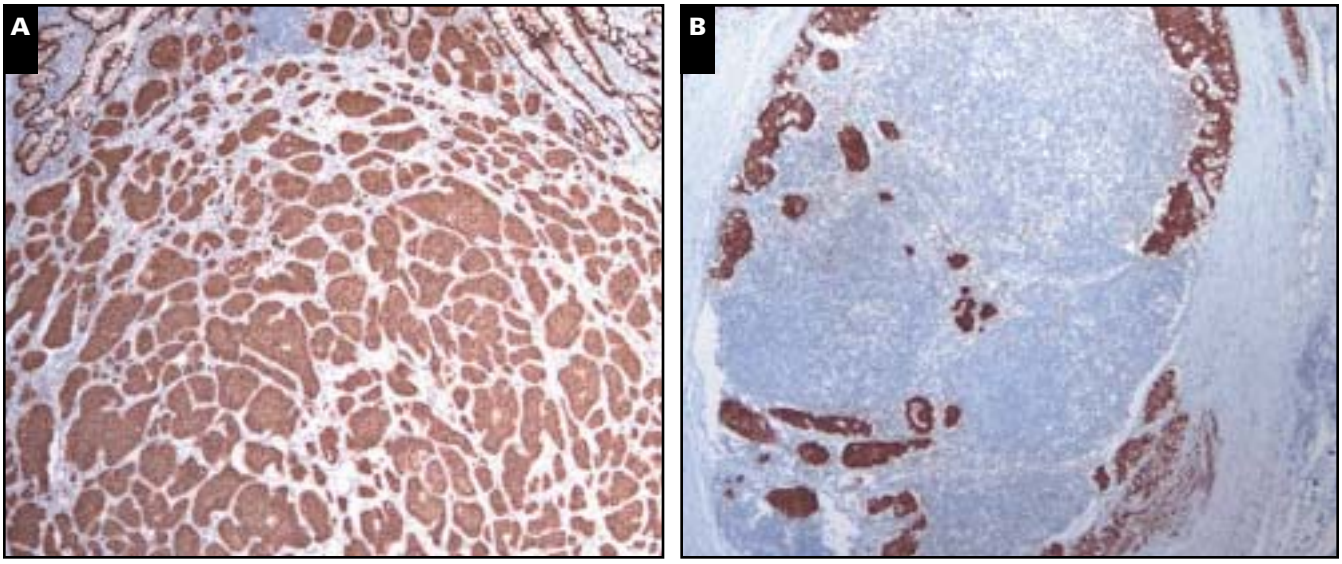

Image 4 An ileal carcinoid (**A**) and its lymph node metastasis (**B**) with similar percentages of stained cells, intensity, and histologic appearance (**A**, CDX2, ×100; **B**,CDX2, ×100).

an atypical pulmonary carcinoid and its metastasis (primary tumor PSC, 1+ and intensity, 1+; metastasis PSC, 3+ and intensity, 2+).

Cytoplasmic Staining of CDX2

Cytoplasmic staining, in addition to nuclear staining, was observed in some cases. The majority of ileal and appendiceal carcinoids and a minority of rectal carcinoids and PENs showed intense homogeneous cytoplasmic staining (Images 1C and 1D, Table 2). No cytoplasmic staining was present in gastric, duodenal, or ampullary carcinoids.

Sensitivity and Specificity of CDX2 and TTF-1

CDX2 was 100% specific for gastrointestinal tract carcinoids and PENs. However, the sensitivity of CDX2 within the gastrointestinal tract was variable, ranging from 100% (ileum) to 17% (stomach) (Table 2). TTF-1 was 100% specific for pulmonary carcinoids, but the sensitivity was only 53%.

Discussion

Carcinoids are well-differentiated, usually indolent, neuroendocrine neoplasms. They are encountered incidentally, often in conjunction with chronic atrophic gastritis or acute appendicitis or during routine colonoscopy.[1] Depending on their location, they may produce symptoms due to local obstructive effects, the sequelae of metastases, or the manifestations of carcinoid syndrome. The site, size, functional status, and histologic characteristics of these tumors are important predictors of their biologic behavior.[1]

Carcinoids are relatively uncommon, accounting for 2% of all gastrointestinal tract malignant neoplasms[22] and 1% to 2% of all lung tumors.[23] Females appear to have a higher incidence of gastrointestinal carcinoids[24,25] and pulmonary carcinoids,[26] a finding also observed in our series.

Numerous studies have used a variety of markers in an attempt to define organ-specific profiles for carcinoids and PENs. Differences in the hormonal profiles of gastrointestinal and pulmonary carcinoids have been described.[27] Advances in immunohistochemical staining techniques have led to the use of novel markers to help distinguish carcinoids from different organs. Differences in CK7 and CK20 expression[2,3] and various neuroendocrine markers have been reported for foregut, midgut, and hindgut carcinoids.[5]

Cdx2, a member of the ParaHox cluster of homeobox genes, is responsible for endodermal intestinal fate specification and seems critical for intestinal development.[28] CDX2 (protein product of the *Cdx2* gene[29] located on chromosome 13q12-13[30]) is thought to be responsible for the maintenance of a differentiated epithelial phenotype. In adults, high levels of gene expression persist in the proximal colon, gradually falling off proximally and distally.[10] Studies of adenocarcinomas arising in various anatomic sites have documented CDX2 expression in tumors with intestinal[6,11,13,14,31] and, rarely, non-intestinal phenotypes.[11,14]

TTF-1 is a 38-kd homeodomain containing DNA-binding protein[32] essential for lung development.[33-36] Immunohistochemical staining for TTF-1 is used routinely in determining a pulmonary origin of metastatic adenocarcinomas.[37] Although a few studies have comprehensively evaluated TTF-1 expression in neuroendocrine carcinomas[18,19] and pulmonary carcinoids,[20]

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only limited data are available for CDX2 expression in well-differentiated neuroendocrine neoplasms.[11,14]

Our results suggest that there is a wide, albeit region-specific, variation in the frequency (sensitivity) of CDX2 expression and the PSC and intensity of CDX2 staining in intestinal carcinoids. The sensitivity is highest for ileal (100%) and appendiceal (86%) carcinoids and decreases both proximally and distally (Image 1). This gradient is similar to that described for chromogranin expression in intestinal carcinoids[5] and mimics the expression of CDX2 messenger RNA transcripts in normal intestine as observed by in situ hybridization.[10]

Werling et al[11] described CDX2 expression in 42% of gastrointestinal carcinoids, but because the carcinoids were not subclassified by site, it is not possible to compare our results with theirs. Moskaluk et al[14] subdivided gastrointestinal carcinoids and observed a higher proportion of CDX2+ carcinoids arising from the midgut (8/9 [89%]) relative to the hindgut (4/9 [44%]), similar to our results (midgut > distal foregut > hindgut) (Table 2). We further categorized the intestinal carcinoids into distinct anatomic locations (ampulla, duodenum, ileum, and appendix) and were able to show differences in CDX2 expression, even among carcinoids that have common embryological derivations.

In nonneoplastic intestinal tissue, contrary to experimental murine data that demonstrated a gradient of CDX2 expression along the crypt-villus axis,[8] we observed an equal intensity of CDX2 staining in virtually all epithelial cells of the small intestinal villi and crypts, as well as the colonic crypts. Similar observations in colonic crypts were reported by Qualtrough et al.[38] Silberg et al[8] reported a decreasing gradient of CDX2 from the small intestine to the distal colon in mice as assessed by immunohistochemical staining. However, we could not detect any difference in the intensity of CDX2 staining between different segments of the small or large intestine.

Absorptive cells, goblet cells, Paneth cells, and the majority of neuroendocrine cells expressed CDX2, and only intraepithelial lymphocytes and exceedingly rare cells (possibly subtypes of neuroendocrine cells) did not stain. Owing to intense epithelial staining and crowding of cells in the crypt bases, it is unclear whether CDX2 expression is restricted to particular architectural types of neuroendocrine cells or neuroendocrine cells that secrete specific hormones.

Numerous genes, including another ParaHox family member (*Pdx1*),[39,40] have been implicated in the development and differentiation of intestinal neuroendocrine cells. CDX2 has an important role in the development and maintenance of intestinal secretory and absorptive epithelial cells.[28] A role for CDX2 in the development and function of neuroendocrine cells, however, has not been explored and deserves further study.

CDX2 expression was seen in 5 (26%) of 19 PENs and in 1 mixed acinar-endocrine carcinoma. Our results are similar to those reported by Moskaluk et al,[14] who found CDX2 expression in 4 (29%) of 14 islet cell tumors. Lack of CDX2 expression in the majority of PENs argues against a critical role in tumoral growth regulation for this transcription factor. In nonneoplastic pancreatic tissue, CDX2 stained virtually all epithelial cells lining ducts of different calibers but not islet cells, as reported previously.[14]

CDX2 does not seem to have an important role in the development of the pancreas because mice heterozygous for CDX2 do not show pancreatic defects.[41] This could relate to the possible functional redundancy of other transcription factors.[42] However, it remains possible that low levels of CDX2 might be sufficient for appropriate development of the pancreas, and levels of the CDX2 transcript might have been below the threshold of detection (by in situ hybridization or immunohistochemical staining) in published studies. A role for CDX2 in endocrine cell function is supported by studies that have shown that CDX2, interacting with other transcription factors, regulates glucagon synthesis in islet cells.[43,44]

A single (1/6) gastric carcinoid expressed CDX2 in our series, in contrast with 0 of 3 gastric carcinoids studied by Moskaluk et al.[14] We studied only type 1 carcinoids arising in the background of chronic atrophic (autoimmune) gastritis because these are encountered more commonly. A larger study including all types of gastric carcinoids, including sporadic carcinoids, is warranted to determine the spectrum of CDX2 expression in gastric neuroendocrine neoplasms. CDX2 expression in occasional gastric carcinoids could represent aberrant up-regulation of this transcription factor, as reported for other homeobox transcription factors.[45] However, absence of CDX2 in the majority of gastric carcinoids suggests that other undetermined factors are responsible for the neoplastic transformation of gastric neuroendocrine cells.

In nonneoplastic gastric tissue, CDX2+ cells had the same distribution as quiescent or hyperplastic antral gastrin-producing cells, as well as antral and cardiac enterochromaffin cells (Image 3). Normal or hyperplastic enterochromaffin-like cells did not stain (Images 2 and 3). These observations have not been reported previously. Because gastric neuroendocrine cells, especially the antropyloric neuroendocrine cells, represent a minority of gastric epithelial cells early in development,[46] CDX2 expression in these cells could have been overlooked in murine studies.[41] Distinct trophic factors and signaling cascades have been determined for various gastric neuroendocrine cells,[47] and our observations suggest possible roles for CDX2 in the development or the function of specific gastric neuroendocrine cell subtypes. A delineation of all the different CDX2+ neuroendocrine cells would require a comprehensive double immunohistochemical staining analysis using CDX2 and different hormonal products.

No CDX2 staining was identified in the pulmonary carcinoids. Our results are similar to those of Barbareschi et al[6] but differ from those of Moskaluk et al,[14] who reported 1+ (<25% cells) staining in 2 of 7 pulmonary carcinoids. No role for CDX2 has been demonstrated in normal lung development. Dysregulated expression of CDX2 might occur in a subset of

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pulmonary carcinoids because this phenomenon has been observed in lung adenocarcinomas.[48]

In our series, TTF-1 expression was seen in 53% of pulmonary carcinoids (8/15), with atypical carcinoids expressing TTF-1 more often than typical carcinoids, similar to results described in the literature.[20] None of the gastrointestinal carcinoids or PENs expressed TTF-1, making TTF-1 staining 100% specific for pulmonary carcinoids. Rare TTF-1+ gastrointestinal carcinoids (exact organs not specified) were reported in 2 studies,[2,18] but this observation has not been substantiated in gastrointestinal carcinoids[17,19] or PENs.[17]

In metastatic carcinoids, the presence or absence of CDX2 and TTF-1 staining was identical to that in the primary tumors with only minor discrepancies in 2 cases. This observation argues against a tumor suppressor role for TTF-1 and CDX2 in well-differentiated pulmonary and gastrointestinal endocrine neoplasms. The differences in staining could be attributed to variable tissue fixation or sampling.

Cytoplasmic CDX2 staining (in addition to nuclear staining) was detected in numerous carcinoids, especially from the ileum and appendix (Table 2). The significance of this observation is unclear. This phenomenon was also noted by others[11,12,49-51] but dismissed as a possible artifact by Werling et al.[11] We also observed cytoplasmic granular staining in the absence of nuclear staining in a few carcinoids, as has been described in nonintestinalized gastric epithelium of patients with *H pylori* gastritis[49] and in submucosal glands and inflamed squamous epithelium adjacent to intestinal metaplasia at the squamocolumnar junction of Barrett esophagus.[12] Although cytoplasmic staining might reflect leaching of the nuclear protein into the cytoplasm due to antigen retrieval, excess production and/or cytoplasmic retention of this transcription factor is also a possibility. Ishikawa et al[51] detected CDX2 messenger RNA by reverse transcriptase–polymerase chain reaction in neoplasms that demonstrated cytoplasmic CDX2 staining. Studies have revealed novel cytoplasmic functions for transcription factors,[52] including p53[53]; however, this possibility has not been explored for CDX2.

CDX2 and TTF-1 have high specificities for gastrointestinal and pulmonary carcinoids, respectively; overall sensitivities of TTF-1 (53%) and CDX2 (46% [29/63; Table 2]) are low, however. Because CDX2 and TTF-1 expression are maintained in metastases, these markers can be incorporated into antibody panels to distinguish between pulmonary and gastrointestinal carcinoids. Our study was limited to well-differentiated neuroendocrine neoplasms of the gastrointestinal tract and lung because these are the most common primary sites of metastatic carcinoids. A larger survey encompassing small cell and large cell neuroendocrine carcinomas and carcinoids arising in other organs is necessary to evaluate the true organ specificity of CDX2. Finally, we noted CDX2 expression in subsets of gastric neuroendocrine cells and NEH. Further studies are warranted to identify additional CDX2-expressing gastrointestinal neuroendocrine cells and define a role for CDX2 in the development and/or function of these cells.

From the Department of Pathology, Columbia University Medical Center, New York, NY.

Address reprint requests to Dr Bhagat: Dept of Pathology, Columbia University Medical Center, 630 W 168th St, VC14-215, New York, NY 10032.

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