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Value of SATB2, ISL1, and TTF1 to differentiate rectal from other gastrointestinal and lung well-differentiated neuroendocrine tumors

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ABSTRACT

Background: Management of neuroendocrine tumors (NETs) depends on the primary site, but the location of many well-differentiated (WD) NETs is elusive. Organ-specific markers are required for pathological diagnosis from biopsy. Transcription factors with good organ specificity include TTF1 (thyroid transcription factor 1; lung), CDX2 (caudal type homeobox transcription factor 2; midgut), and ISL1 (ISL LIM homeobox 1) and PAX8 (paired box 8) for the pancreas and rectum. SATB2 (SATB homeobox 2) has shown high sensitivity and specificity in colorectal adenocarcinoma. This study determined the viability of SATB2 and other transcription factors as markers, single or in combination, for WD-NETs of various sites.

Methods: Immunohistochemical staining of 81 WD-NETs from 8 organ sites was performed to identify SATB2, TTF1, CDX2, ISL1, and PAX8. Receiver operating characteristic (ROC) curves were constructed for different combinations of the 5 markers to determine sensitivity and specificity.

Results: Among the WD-NETs, SATB2 was predominantly found in those of the rectum; TTF1 in the lung, larynx, and esophagus; and ISL1 in the duodenum and rectum. PAX8 and CDX2 showed poor organ specificity. ROC profiles showed 50% sensitivity and 96% specificity to lung for TTF1$^+$ ISL1–; and 65% sensitivity and 100% specificity to rectum for SATB2$^+$ ISL1– TTF1–. ISL1$^+$ SATB2– TTF1– showed 83% sensitivity and 85% specificity to the duodenum, and 44% sensitivity and 87% specificity to the pancreas. A literature search showed that there was no significant difference in the expression rates of the five transcription factors (TTF1, CDX2, SATB2, PAX8 and ISL1) between primary and metastatic WD-NETs at the same organ when there was a large sample size.

Conclusion: Among the 5 transcription factors tested, SATB2 may be a viable marker of WE-NETs of the rectum. The combination of SATB2, ISL1, and TTF1 may help estimate the locations of WD-NETs of unknown origin.

1. Introduction

Epidemiological studies from Norway [1], the United States [2], and China [3] have suggested that the incidence of neuroendocrine tumors (NETs) is increasing yearly, especially gastroenteropancreatic NETs. The World Health Organization (WHO) classifies NETs as either well-differentiated neuroendocrine tumors (WD-NETs) or poorly differentiated neuroendocrine carcinomas, mainly depending on histopathological characteristics and proliferative activity [4–6].

The treatment and prognosis of WD-NETs depends on the location of the primary site [7]. At the time of initial diagnosis, nearly 40% of WD-NETs are accompanied by distant metastasis, and in 10% of cases the primary site cannot be determined by exhaustive clinical and imaging methods [7]. As identification of the primary site is so important, pathological diagnosis of biopsy material is often necessary.

In 1963, Williams and Sandler attempted to classify carcinoid tumors (i.e., NETs) as tumors of the foregut, midgut, or hindgut, based on embryological origin [8]. However, NETs from various sites have similar histological characteristics, and hematoxylin and eosin (H&E) staining alone is inadequate to identify the primary site of a metastatic tumor.

Some immunohistochemical studies have focused on transcription factors as organ-specific markers of WD-NETs. Reported transcription factors include TTF1 (thyroid transcription factor 1) for lung [9,10]; CDX2 (caudal type homeobox 2 transcription factor) for midgut [11]; and ISL1 (ISL LIM homeobox 1) and PAX8 (paired box protein Pax-8)

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for the pancreas and rectum [12,13]. However, a single transcription factor often fails to solve the problem of specificity and sensitivity to a particular organ. Therefore, many studies have evaluated combinations of various transcription factors [13,14].

SATB2 (special AT-rich sequence-binding protein 2) is a member of a family of matrix attachment region-binding transcription factors. SATB2 participates in craniofacial, neural, osteoblastic, and gastrointestinal differentiation, and is a highly sensitive and specific marker of osteosarcoma [15] and colorectal adenocarcinoma [16]. There has been only one study regarding the presence of SATB2 in intestinal WD-NETs [17].

The present study investigated whether SATB2 and other transcription factors (TTF1, CDX2, ISL1, and PAX8) may be viable markers of WD-NETs in various organ sites, when considered singly or in combination. Also included is a brief summary of the relevant literature.

2. Materials and methods

2.1. Processing specimens and grading tumors

Eighty-one patients with diagnosed primary WD-NETs between 2014 and 2018 were enrolled (70 surgical specimens and 11 biopsy specimens). All specimens were fixed in formalin and paraffin-embedded, and processed as routine surgical pathology specimens. Diagnoses were based on combined clinical information, compatible histology, and evidence of neuroendocrine differentiation. The latter was confirmed by immunohistochemistry showing the positive presence of at least one neuroendocrine marker (chromogranin, synaptophysin, or both). The Ki-67 proliferation index was also performed for all NETs for purposes of grading.

Gastroenteropancreatic-NETs that are graded as 1 or 2 (G1, G2) are within the definition of WD-NETs (The pancreas also contains PanNET G3). In the present study, the specific WHO grade (G1 or G2) was determined according to mitotic count and Ki-67 proliferation index, as recommended by the criteria of the WHO Classification of Tumors of the Digestive System (2010) and WHO Classification of Tumors of Endocrine Organs (2017). Carcinoid and atypical carcinoid of the lung are also classified as WD-NETs. Whether tumors of the lung were carcinoid or atypical carcinoid was determined using the criteria of the WHO Classification of Tumors of the Lung, Pleura, Thymus, and Heart (2015).

All cases were re-diagnosed by two highly experienced pathologists using the latest WHO criteria mentioned above.

2.2. Immunohistochemical staining

Unstained slides (4-μm thick) were prepared from the original paraffin blocks. Immunohistochemical labeling with antibodies to SATB2, TTF1, CDX2, ISL1, and PAX8 were performed using the conventional method, as described previously (Table 1) [18]. For each immunostaining run, positive and negative controls were included. Specifically, the positive controls were the following: normal colonic mucosa for SATB2 and CDX2; normal alveolar epithelium for TTF1; and pancreatic islets for ISL1 and PAX8. The negative controls consisted of incubation with secondary antibody only.

The results were reviewed under a microscope by two pathologists working independently. The tissues were evaluated by both the intensity of the nuclear staining (1+ to 3+), and the percentage of immunoreactivity in the tumor cells (by conventional evaluation methods). Positivity was defined as a staining intensity of at least 1+, in at least 10% of the tumor cells.

Table 1
Antibodies used for immunohistochemical staining.

Antibodies	Species	Manufacturer	Clone	Dilution
TTF1	Mouse	Maxim, Fuzhou, China	8G7G3/1	Prediluted
CDX2	Mouse	Maxim, Fuzhou, China	AMT28	Prediluted
SATB2	Mouse	ZSGB-BIO, Beijing, China	SATBA4B10	Prediluted
PAX8	Rabbit	Maxim, Fuzhou, China	Polyclonal	Prediluted
ISL1	Mouse	www.antibodies-online.com	1H9	1:200

2.3. Statistical analysis

Fisher's exact test was used to assess differences in categorical outcomes between independent groups. The chi-squared test ($\chi 2$) for related proportions was used to assess differences in sensitivity and specificity between 2 methods (individual stains or stain panels) in the same group.

Sensitivity was defined as the percentage of cases, among all cases, for which the considered protein biomarker(s) showed the predefined immunoreactivity at a specific primary site. Specificity for a specific primary site was defined as the percentage of cases for which the considered protein biomarker(s) were not confirmed to show the predefined immunoreactivity for only that primary site. For a specific primary site, the positive predictive value (PPV) of protein biomarker immunoreactivity was the proportion of true cases for which the biomarker(s) tested positive. The negative predictive value (NPV) of protein biomarker immunoreactivity was the proportion of negative cases for which the biomarker(s) tested negative.

Receiver operating characteristic (ROC) curves were generated that plotted sensitivity opposed to 1 – specificity. The performance of stain panels was compared using the area under the ROC curve. All analyses were performed using SPSS software (version 18.0; SPSS, IL, USA). A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Pathological characteristics of the cases

Among the 81 patients, there were 41 (51%) men and 40 (49%) women. Their ages ranged from 20 to 78 years old, with a mean of 50 years and median of 49 years. The most common primary sites of WD-NETs in this study cohort were rectum (31, 38%), pancreas (18, 22%), lung (14, 17%), stomach (7, 9%), and duodenum (6, 7%). Also included in the study were 3 cases from the larynx, and one case each from the esophagus and appendix (Table 2). According to the WHO criteria for histologic grading, 52 (64%) and 29 (36%) tumors were G1 and G2, respectively (Table 2). In the rectum, pancreas, and lung, 83.9%, 50.0% and 35.7% of the tumors were G1, a significant difference ($\chi 2 = 11.628$, $P = 0.003$).

3.2. Individual markers

SATB2 positivity was mainly observed in rectal WD-NETs (25/31, 81%; Fig. 1A), with rare positivity seen in those of the lung (1/14, 7%;

Table 2
Immunoreactivity of TTF1, CDX2, SATB2, PAX8 and ISL1 in primary WD-NETs[a].

	Cases	G1/G2, *n*/*n*	TTF1	CDX2	SATB2	PAX8	ISL1
Lung	14 (17)	5/9	7 (50)	1 (7)	1 (7)	0	0
Larynx	3 (4)	0/3	3 (100)	0	0	1 (33)	1 (33)
Esophagus	1 (1)	0/1	1 (100)	0	0	0	0
Stomach	7 (9)	5/2	0	0	0	0	0
Duodenum	6 (7)	6/0	0	3 (50)	0	2 (33)	5 (83)
Pancreas	18 (22)	9/9	0	5 (28)	0	3 (17)	8 (44)
Appendix	1 (1)	1/0	0	1 (100)	0	0	0
Rectum	31 (38)	26/5	0	10 (32)	25 (81)	2 (7)	23 (74)

[a] Reported as *n* (%), unless indicated otherwise.

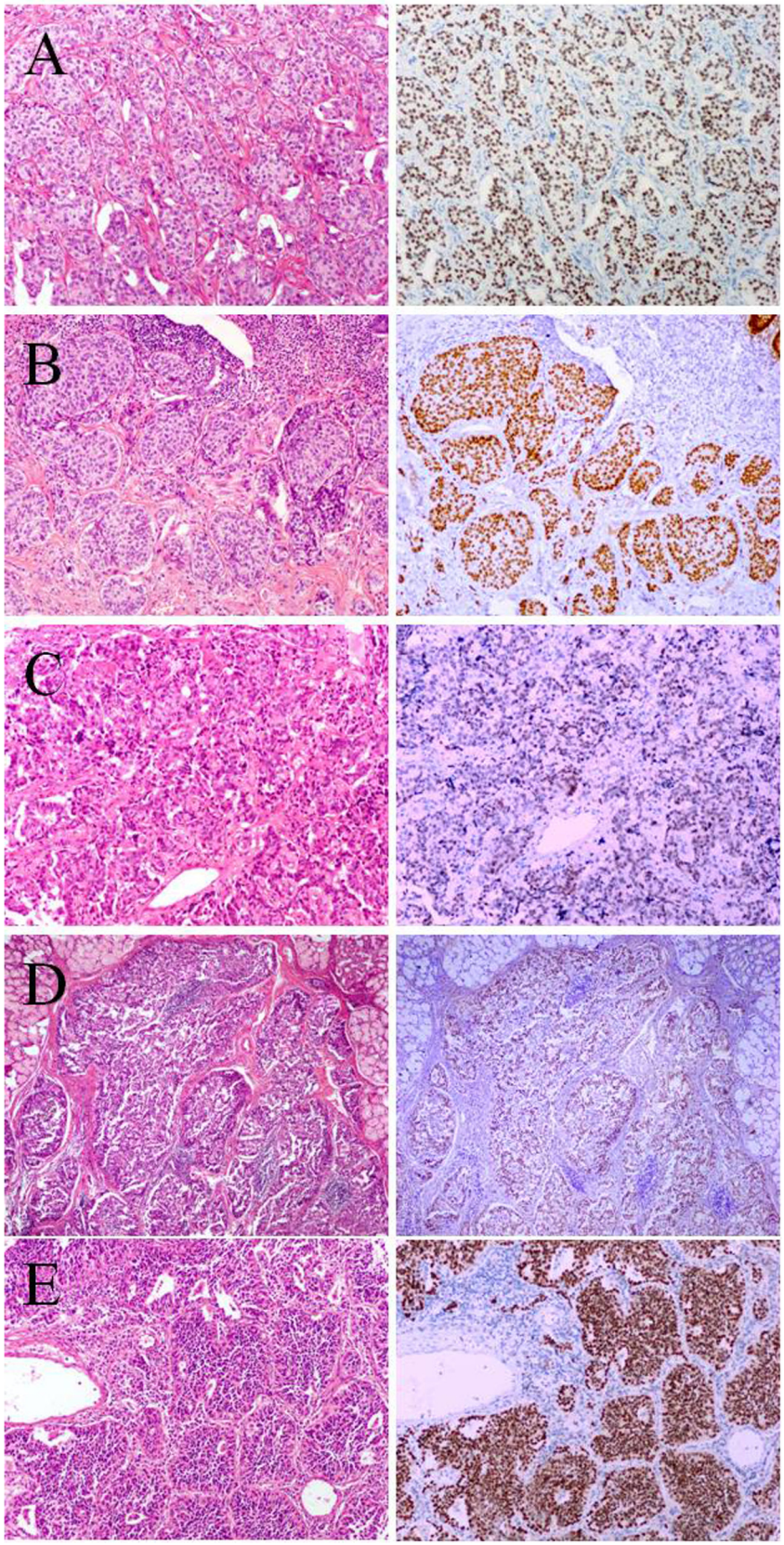

Fig. 1. Representative H&E stain and marker positivity in WD-NETs of specific organs. (A) Rectum, H&E, and SATB2. (B) Appendix, H&E, and CDX2. (C) Pancreas, H &E, and PAX8. (D) Duodenum, H&E, and ISL1. (E) Lung, H&E, and TTF1.

Table 2). No SATB2 labeling was observed in other digestive organs, including pancreas, duodenum, stomach, larynx, esophagus, and appendix. Among the SATB2$^+$ tumors, 22 and 4 were G1 and G2, respectively. In SATB2– tumors, 30 and 25 were G1 and G2. SATB2 positivity was significantly more likely in G1 relative to G2 WD-NETs ($\chi2 = 6.945$, $P = 0.008$).

Positive CDX2 labeling was seen in most WD-NETs that originated from the digestive organs, specifically in 28% and 32% of the pancreas and rectum, respectively, and in 50% and 100% of the duodenum and appendix (Fig. 1B; Table 2). However, while no CDX2 positivity was observed in WD-NETs of the larynx, stomach, or esophagus. Outside the digestive system, 7% of lung WD-NETs was positive for CDX2. The presence of CDX2 was not significantly associated with grade; the percentages of G2 tumors in the CDX2$^+$ (30.0%) and CDX2– (37.7%) subgroups did not differ significantly ($\chi2 = 0.389$, $P = 0.533$).

PAX8 labeling was positive in WD-NETs of the following organs (Table 2): larynx, 1 of 3 (33%); duodenum, 2 of 6 (33%); pancreas, 3 of 18 (17%; Fig. 1C); and rectum, 2 of 31 (7%). No labeling was seen in pulmonary (0/14), gastric (0/7), esophageal (0/1), or appendiceal (0/1) WD-NETs. The PAX8$^+$ (37.5%) and PAX8– (35.6%) WD-NETs were equally likely to be G2 tumors ($\chi2 = 0$, $P = 1$, continuity correction).

WD-NETs of the following organs tested positive for ISL1 labeling (Table 2): duodenum, 5/6 (83%; Fig. 1D); pancreas, 8/18 (44%); rectum, 23/31 (74%); and larynx, 1/3 (33%). ISL1 labeling was negative in WD-NETs of other sites (lung, stomach, appendix, and esophagus). G2 WD-NETs were significantly more likely to be ISL1– (50.0%) than ISL1$^+$ (18.9%; $\chi2 = 8.448$, $P = 0.004$).

TTF1 labeling was positive in WD-NETs of the following organs (Table 2): lung, 7/14 (50%; Fig. 1E); larynx, 3/3 (100%); and esophagus, 1/1 (100%) WD-NETs. All other WD-NETs were negative for TTF1. G2 WD-NETs were significantly more likely to be TTF1$^+$ (9/11, 81.8%) than TTF1– (20/70, 28.6%; $\chi2 = 8.448$, $P = 0.004$)

Among the 5 markers considered individually, the highest sensitivity and specificity results were shown in WD-NETs of the following sites: in lung, TTF1$^+$ (50% and 94%, respectively); rectum, SATB2$^+$ (80.6%, 98%); pancreas, SATB2– (100%, 41.3%); and duodenum, ISL1$^+$ (83.3%, 57.3%).

3.3. Two- and three-marker profiles

The sensitivities and specificities of various combinations of markers in WD-NETs were assessed (Table 3). The TTF1$^+$ISL1– profile was seen predominantly in known WD-NETs of the lung (50% sensitivity, 96% specificity). SATB2$^+$ISL1– TTF1– was observed only in the WD-NETs of the rectum (64.5% sensitivity, 100% specificity). ISL1$^+$SATB2–TTF1– was seen predominantly in WD-NETs of the duodenum (83% sensitivity, 85% specificity), and pancreas (44% sensitivity, 87% specificity).

3.4. Results of literature retrieval

Our literature search results showed that there was no significant difference in the expression rates of the five transcription factors (TTF1, CDX2, SATB2, PAX8 and ISL1) between primary and metastatic WD-NETs when there was a large sample size (see Tables 4 and 5 for details).

Table 3
Combined immunohistochemical stains for predicting site of origin in primary WD-NETs[a].

	Pattern of immunoreactivity	Sensitivity, specificity	PPV, NPV
Lung	TTF1$^+$, ISL1–	50, 96	70, 90
Rectum	SATB2$^+$, TTF1–, ISL1–	65, 100	100, 82
Duodenum	ISL1$^+$, TTF1–, SATB2–	83, 85	31, 98
Pancreas	ISL1$^+$, TTF1–, SATB2–	44, 87	50, 85

PPV, positive predictive value; NPV, negative predictive value.
[a] Sensitivity, specificity, PPV, and NPV reported as %.

4. Discussion

This study determined the viability of 5 transcription factors (SATB2, TTF1, CDX2, ISL1, and PAX8) as organ-specific markers of primary WD-NETs, utilizing data from a single center collected from 2014 to 2018. The presence of these factors in WD-NETs from 8 organ sites (rectum, pancreas, lung, stomach, and duodenum, larynx, esophagus, and appendix) was determined through immunohistochemical staining, and ROC curves were constructed to determine sensitivity and specificity. For individual markers, the highest sensitivity and specificity results were shown by TTF1$^+$ (WD-NETs of the lung), SATB2$^+$ (rectum), SATB2– (pancreas), and ISL1$^+$ (duodenum). Combinations of markers of potential interest included TTF1$^+$ISL1– (lung), SATB2$^+$ISL1– TTF1– (rectum), and ISL1$^+$SATB2–TTF1– (duodenum, pancreas).

The epidemiological characteristics of NETs vary by country, and by regions in the same country. In Norway between 1993 to 2010, a review of 16,075 cases concluded that the most common primary sites were the lung (48.1%) and the gastroenteropancreatic system (18.0%), and the most common sites of gastroenteropancreatic-NETs were the small intestine (32.5%), appendix (23.4%), pancreas (15.7%), colon (10.6%), rectum (9.2%), stomach (7.1%), and esophagus (1.3%) [1]. In the United States from 1950 to 2007, an epidemiological survey of 51,849 gastroenteropancreatic NETs determined that the most common sites were the small intestine (18.3%), specifically the ileum (8.6%) and duodenum (3.4%), and the rectum (15.1%), colon (10.1%), pancreas (6.9%), and stomach (5.2%) [2]. Fan et al. [3] summarized the epidemiological data from all 7 regions of China during the years 2001 through 2010, and among 2010 gastroenteropancreatic NETs the most common primary sites were the pancreas (31.5%), rectum (29.6%), stomach (27.0%), small intestine (5.6%), and colon (3.0%). In the present study, the main primary sites of WD-NETs were the rectum (38%), pancreas (22%), lung (17%), stomach (9%), and duodenum (7%).

CDX2 is a transcription factor that participates in the development of the small and large intestines. One study showed that 90% of colonic adenocarcinomas tested positive for CDX2, but CDX2 was in other gastrointestinal adenocarcinomas [19]· Other studies have found CDX2 in NETs, mainly of the midgut [9–11,13,20–23] (Tables 4, 5), specifically the appendix (76–100% and 75–100% of primary and metastatic, respectively); small intestine (52–100%, 61–100%); and colon (75–93%, 50–100%; with the exception of Schmitt et al. [22], primary 0/2, 0%, but metastatic 1/2, 50%).

In hindgut NETs, CDX2 was found mainly in primary rectal (0–52%), but not metastatic rectal NETs [9–11,13,20–23]. CDX2 positivity was also seen in foregut NETs, specifically the stomach (0–50% and 0–100% of primary and metastatic, respectively), duodenum (0–83%, 0–100%), pancreas (0–30%, 0–38%), and ampulla (50% of primary) [9–11,13,20–23]. Only one study showed CDX2 in lung NETs (primary 4/20, 20%, but metastatic 0/3, 0%) [22]. CDX2 positivity was also encountered in midgut and hindgut WD-NETs, but not in foregut WD-NETs such as the stomach, larynx, and esophagus, with the rare exception in lung (1/14, 7%).

We retrieved only one document (Zhongwu et al. [17]) that concerned the presence of SATB2 in WD-NETs. They showed rates of SATB2$^+$ in foregut WD-NETs of 17% (14/84), specifically: primary stomach (2/19, 10%); duodenum (1/12, 8%); pancreas (2/13, 15%); lung (3/13, 23%); pancreas (2/14, 14%, metastatic only); and 3 other sites were negative (Tables 4, 5). In midgut WD-NETs, rates of SATB2$^+$ were 12% (3/25), including: the appendix (primary 1/4, 25%, metastatic 0/0, 0%); small intestine (primary 1/10, 10%, metastatic 0/6, 0%); and cecum and ascending colon (primary 1/4, 25%, metastatic 0/1, 0%). The highest rate of SATB2$^+$ (90%, 52/58) was observed in

Table 4
Immunoreactivity of markers TTF1, CDX2, SATB2, PAX8 and ISL1 in primary WD-NETs in the literature by organ[a].

	Foregut				Midgut			Hindgut
	Lung	Stomach	Duodenum	Pancreas	Jejuno-ileum	Appendix	Colon	Rectum
TTF1	49 (72/147)	2 (1/62)	3 (1/29)	2 (4/253)	1 (1/123)	0 (0/59)	15 (3/20)	1 (1/79)
CDX2	3 (4/157)	13 (9/70)	66 (19/29)	17 (43/253)	83 (107/129)	95 (56/59)	80 (16/20)	26 (22/86)
SATB2	23 (3/13)	10 (2/19)	8 (1/12)	15 (2/13)	10 (1/10)	25 (1/4)	0	90 (43/48)
PAX8	9 (8/92)	13 (7/54)	74 (23/31)	65 (134/206)	0 (0/102)	14 (5/36)	7 (1/14)	48 (32/67)
ISL1	11 (9/82)	32 (10/31)	75 (15/20)	75 (120/161)	3 (2/70)	27 (7/26)	31 (5/16)	79 (30/38)

[a] Reported as % (*n*/total *n*).

Table 5
Immunoreactivity of TTF1, CDX2, SATB2, PAX8, and ISL1 in metastatic WD-NETs in the literature[a].

	Foregut				Midgut			Hindgut
	Lung	Stomach	Duodenum	Pancreas	Jejuno-ileum	Appendix	Colon	Rectum
TTF1	50 (9/18)	0 (0/4)	0 (0/3)	1 (1/85)	0 (0/90)	0 (0/10)	0 (0/5)	0 (0/16)
CDX2	0 (0/18)	25 (1/4)	67 (2/3)	7 (6/85)	84 (75/89)	90 (9/10)	80 (4/5)	0 (0/16)
SATB2	0	0 (0/3)	0 (0/1)	14 (2/14)	0 (0/6)	0	0 (0/1)	89 (8/9)
PAX8	0 (0/10)	0	0 (0/3)	58 (49/85)	0 (0/66)	0 (0/4)	0 (0/1)	10 (1/10)
ISL1	6 (1/16)	0 (0/3)	67 (2/3)	70 (59/84)	4 (3/83)	13 (1/8)	0 (0/3)	94 (15/16)

[a] Reported as % (*n*/total *n*).

hindgut WD-NETs, including primary (44/49, 90%) and metastatic (8/9, 89%). The specificities for SATB2 in the foregut, midgut, and hindgut were 34%, 54% and 84%, respectively, and the sensitivities were 17%, 12%, and 90%. Results of the present study showed SATB2 to have a relatively narrow WD-NET organ-specific profile, being mainly found in the hindgut (25/31, 81%), and not in the midgut or foregut, except rarely in the lung (1/14, 7%). The rates of SATB2 positivity in the hindgut reported in the present study and that of Zhongwu et al. [17] were approximately the same (81% and 89%, respectively), but were inconsistent for the foregut and midgut. This may be due to a limited number of cases.

PAX8 is crucial for development of the kidney, thyroid gland, and Müllerian system. Therefore, in pathology laboratories PAX8 is often used as a specific marker for epithelial tumors from these organs [24]. The diagnostic utility of PAX8 in NETs from various organs has been investigated [13,22,24,25] (Tables 4, 5), including the duodenum, pancreas, stomach, appendix, rectum, colon, lung, and others. Liau et al. [25] determined that cross-reaction of the PAX8 polyclonal antibody to other members of the PAX family, such as PAX2, PAX5, and PAX6, may compromise the diagnostic utility of PAX8 in NETs at various sites. The current experiment also used the PAX8 polyclonal antibody, and found that PAX8 was present in almost all organs of the foregut and hindgut, but not in the lung. The above research suggests that the results for PAX8 for differential diagnosis of NETs should be interpreted with appropriate caution by pathologists.

ISL1 is widely expressed in subsets of neurons of the adrenal medulla and dorsal root ganglion, inner nuclear and ganglion cell layers of the retina, and the pineal gland and other regions of the brain (UniProt entry P61371). ISL1 immunoreactivity was also found positive in all endocrine cells of pancreatic islets [22]. Some studies showed ISL1 in the majority of primary (75%) and metastatic (70%) NETs of the pancreas [13,14,22,23] (Tables 4, 5). Subsequent studies found high concentrations of ISL1 in the NETs of the duodenum (75% and 67% in primary and metastatic, respectively) [22,23], and rectum (79% and 94%) [13,14,22,23]. ISL1 was also expressed in the primary and metastatic NETs of other sites, as follows: stomach (32% and 0%, respectively) [13,14,22,23]; appendix (27%, 13%) [22,23]; colon (31%, 0%) [13,22,23]; jejuno-ileum (3%, 4%) [13,22,23]; and even lung (11%, 6%) [13,14,22,23]. In the present study, ISL1 was found in primary WD-NETs of the rectum (23%), pancreas (8%) and duodenum (5%), and larynx (33%), but not lung (0/14). These findings suggest that pathologists should carefully interpret the results when using ISL1 antibodies to judge the origin of metastatic NETs.

TTF1 is essential for transcriptional activation during embryogenesis in the thyroid, diencephalon, and lung. Immunohistochemical staining of TTF1 is routinely applied to determine a pulmonary origin of metastatic adenocarcinoma [26] and NETs [9,10,13,20–23]. Studies concerning WD-NETs showed that TTF1 was mainly found in the lung (49% and 50% of primary and metastatic, respectively), while much lower rates of TTF1 positivity were reported in the intestinal tract, such as colon (15%, 0%), duodenum (3%, 0%), and other sites [9,10,13,20–23] (Tables 4, 5). The present study indicated that TTF1 was mainly expressed in WD-NETs of the lung (50%), larynx (100%), and esophagus (100%), but not in that of the rectum, pancreas, duodenum, stomach, or appendix.

Special note should be paid to the high rate of TTF1$^+$ in WD-NETs of the larynx (3/3) and esophagus (1/1) observed in the present study. Although the number of cases was small and more evidence is required, pathologists still need to pay attention to interpreting results concerning TTF1. Altogether, the evidence suggests that TTF1 positivity is relatively specific, but not a highly sensitive marker of pulmonary WD-NETs, and TTF1 has some diagnostic value in determining the site of origin of WD-NETs.

It is difficult for a single marker to achieve high specificity and sensitivity to a specific organ. Therefore, in the present study statistical analyses were applied to determine better the specificity and sensitivity of combinations of markers. Acceptable sensitivity and specificity was demonstrated for TTF1$^+$/ ISL1– in WD-NETs of the lung, SATB2$^+$/ TTF1–/ ISL1– in that of rectum, ISL1$^+$/ TTF1–/SATB2– in that of the duodenum and pancreas, and also the latter in duodenum and pancreas combined. Of note, Yang et al. [14] found that a 3-marker panel (TTF1/CDX2/ISL1) showed sensitivities of 81%, 89%, and 63% and specificities of 100%, 94%, and 100%, respectively, for differentiating metastatic WD-NETs in 3 major primary sites: pancreas/rectum, small intestine, and lung. Therefore, in our daily work, we recommend that multiple immunohistochemical markers should be used to assist in identifying the primary site of metastatic NETs.

Regrettably, our cases did not include metastatic WD-NETs. The metastatic WD-NETs mostly were of biopsy samples and were not included in this study because the number of samples was too small. However, the results of the literature retrieval showed that there was no difference in the expressions of 5 transcription factors in metastatic

WD-NETs of different origins (when the number of samples was large). This result is not surprising, as WD-NETs remain WD-NETs when they transfer to other sites.

5. Conclusions

In conclusion, in this study the positivity rates were investigated of 5 markers (TTF1, CDX2, SATB2, PAX8 and ISL1) in WD-NETs taken from various organs. The rate of SATB2 was particularly high in WD-NETs of the rectum. In addition, the combination of SATB2/ISL1/TTF1 can be applied to estimate the location of WD-NETs of unknown origin.

Conflicts of interest

The authors declare that they have no competing interests.

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Zhao LH and Chen C designed the study, selected the cases, and wrote the manuscript. Mao CY and Fu P performed and evaluated the immunostaining. Xiao H conducted the statistical analyses. Xiao HL and Zhao LH reevaluated the immunostaining and re-diagnosed all the cases. Wang G contributed helpfully to the discussion and critically revised the manuscript.

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