## Original Article
# SATB2 is a sensitive marker for lower gastrointestinal well-differentiated neuroendocrine tumors

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**Abstract:** Special AT-rich sequence binding protein-2 (SATB2) is selectively expressed in the lower gastrointestinal tract mucosa and has been identified as a sensitive marker for colorectal adenocarcinomas. The goal of this study was to investigate the expression of SATB2 in well-differentiated neuroendocrine tumors to explore its potential as a diagnostic marker for hindgut well-differentiated neuroendocrine tumors. Immunohistochemical staining with a monoclonal antibody to SATB2 was performed on full tissue blocks in 167 well-differentiated neuroendocrine tumors of various origins. The staining was semi-quantitatively scored as 0 (no tumor cell staining), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%) and 4+ (76-100%). Positive SATB2 staining was seen in 17% foregut (14/84, 12/66 primary and 2/18 metastatic), 12% midgut (3/22, 3/18 primary and 0/7 metastatic), and 90% hindgut (52/58, 44/49 primary and 8/9 metastatic) well differentiated neuroendocrine tumors. Most hindgut well-differentiated neuroendocrine tumors (41/58) showed 4+ staining. The specificity of SATB2 for foregut, midgut and hindgut well-differentiated neuroendocrine tumors was 34%, 54% and 84%, respectively. Our results indicate that SATB2 is a sensitive marker for hindgut well-differentiated neuroendocrine tumors though it is not entirely specific. SATB2 should be included in the immunohistochemical panel in working out metastatic well-differentiated neuroendocrine tumor of an unknown origin.

Keywords: SATB2, well-differentiated neuroendocrine tumor, foregut, midgut, hindgut

## Introduction

The incidence of neuroendocrine neoplasms has increased dramatically during the past 30 years [1]. Neuroendocrine neoplasms are divided into well-differentiated neuroendocrine tumors (WDNETs) and poorly differentiated neuroendocrine carcinomas (PDNECs). PDNECs are highly aggressive and treated similarly with platinum-based chemotherapy regardless of their origin, whereas WDNETs exhibit a wide range of biological behavior and are treated differently according to their primary sites [2].

Most WDNETs present with locoregional disease but as many as 20% of them present with distant metastasis [1]. This number is even higher for WDNETs from certain organs such as pancreas and ileojejunum [1]. Even though non-surgical approaches (chemotherapy and/or radiation) are the main treatment for metastatic WDNETs, surgical resection of both primary and metastatic tumors are still beneficial in some of these patients because cytoreduction can reduce acute complication in primary site, minimize endocrine symptoms and decrease the requirement of somatostatin analogs [3, 4]. In addition, chemotherapy and targeted therapy protocols are different for metastatic well differentiated neuroendocrine tumors of different origins [2, 5]. Therefore determination of the primary origins of metastatic WDNETs has some therapeutic implications.

**Table 1.** Immunohistochemical staining result of SATB2 in 84 foregut well differentiated neuroendocrine tumors

| Site of Origin | Primary | Metastatic | Total |
|---|---|---|---|
| Stomach (N = 22) | 2/19 (10%) | 0/3 (0%) | 2/22 (9%) |
| Duodenal (proximal half) (N = 13) | 1/12 (8%) | 0/1 (0%) | 1/13 (8%) |
| Pancreas (N = 27) | 2/13 (15%) | 2/14 (14%) | 4/27 (15%) |
| Gallbladder (N = 1) | 0/1 (0%) | 0/0 | 0/1 (0%) |
| Lung (N = 13) | 3/13 (23%) | 0/0 | 3/13 (23%) |
| Thymus (N = 8) | 4/8 (50%) | 0/0 | 4/8 (50%) |
| Total (N = 84) | 12/66 (18%) | 2/18 (11%) | 14/84 (17%) |

**Table 2.** Immunohistochemical staining result of SATB2 in 25 midgut well differentiated neuroendocrine tumors

| Site of Origin | Primary | Metastatic | Total |
|---|---|---|---|
| Ileum (N = 13) | 1/8 (13%) | 0/5 | 1/13 (8%) |
| Jejunum (N = 3) | 0/2 | 0/1 | 0/3 |
| Appendix (N = 4) | 1/4 (25%) | 0/0 | 1/4 (25%) |
| Cecum and ascending colon (N = 5) | 1/4 | 0/1 | 1/5 (20%) |
| Total (N = 25) | 3/18 (17%) | 0/7 (0%) | 3/25 (12%) |

**Table 3.** Immunohistochemical staining result of SATB2 in 58 hindgut well differentiated neuroendocrine tumors

| Site of Origin | Primary | Metastatic | Total |
|---|---|---|---|
| Sigmoid colon (N = 1) | 1/1 (100%) | 0/0 | 1/1 (100%) |
| Rectum (N = 57) | 43/48 (90%) | 8/9 (89%) | 51/57 (89%) |
| Total (N = 58) | 44/49 (90%) | 8/9 (89%) | 52/58 (90%) |

Determination of the origin of metastatic WDNETs is usually achieved with imaging studies including CT and somatostatic receptor imaging (Octroscan). However, these traditional imaging techniques had a less than 50% successful rate in determining the primary site of metastatic WDNETs of unknown origin [6-8]. Approximately 9-19% of WDNETs still present with unknown origins and such tumors account for approximately 5% of malignancies of unknown origin [2, 9, 10]. Expensive PET/CT tracer may be more sensitive but it is not available in many areas. Therefore, in some instances determination of the origin of metastatic WDNETs still rely on pathology. However, WDNETs from different sites have similar and overlapping morphology, and it is often difficult to determine their origin just based on morphology. In these situations, immunohistochemical markers are often needed to facilitate to determine the primary site of a metastatic WDNET.

Recently, special AT-rich sequence binding protein-2 (SATB2) has been identified as a marker with a highly selective expression pattern in the lower gastrointestinal tract mucosa [11]. About 85-93% colorectal adenocarcinomas were immunohistochemically positive for SATB2 [11, 12]. During our clinical practice, we have observed that SATB2 labels all epithelial cells in lower gastrointestinal mucosa, implicating that SATB2 labels both non-neuroendocrine epithelial cells and neuroendocrine cells. Mucosal neuroendocrine cells give rise to neuroendocrine tumors. In this study, with immunohistochemical staining we investigated SATB2 expression in 167 well-differentiated neuroendocrine tumors from various sites to explore the possible utility of SATB2 as a diagnostic marker for lower gastrointestinal WDNETs especially in the metastatic setting.

## Materials and methods

### Case selection

The study was approved by the Institutional Ethnic Committees (Institutional Review Boards). The surgical pathology files of Peking University Cancer Hospital, the Chinese PLA General Hospital, the Second Affiliated Hospital of Guangzhou Medical College, Hubei Cancer Hospital, and the second Affiliated Hospital of Jilin University were searched for WDNETs/carcinoids. The diagnosis of all WDNETs was confirmed with immunohistochemical staining of chromogranin and synaptophysin. Ki-67 proliferation index was also performed for all gastrointestinal and pancreatic neuroendocrine neoplasms for grading purpose. The grades of gastrointestinal and pancreatic neuroendocrine neoplasms were assigned according to the WHO 2010 criteria (G1: mitotic count < 2 per 10 high power fields (HPFs) and/or ≤ 2% Ki-67 index; G2: mitotic count 2-20 per 10 HPFs and/or 3-20% Ki67 index; G3 mitotic count > 20 per

**Table 4.** Comparison of SATB2 staining in foregut, midgut, and hindgut well differentiated neuroendocrine tumors

| Site of Origin | Percentage of tumors positive for SATB2 staining | P value |
|---|---|---|
| Foregut (N = 84) | 17% (14/84) | P < 0.001 between foregut and hindgut |
| Midgut (N = 25) | 12% (3/25 ) | P < 0.001 between midgut and hindgut |
| Hindgut (N = 58) | 90% (52/58) | P = 0.76 between foregut and midgut |

**Table 5.** Sensitivity, specificity, positive predictive value and negative predictive value of SATB2 for 167 well differentiated neuroendocrine tumors

| | Foregut WDNETs | Midgut WDNETs | Hindgut WDNETs |
|---|---|---|---|
| Sensitivity | 17% (14/84) | 12% (3/25) | 90% (52/58) |
| Specificity | 34% (28/83) | 54% (76/142) | 84% (92/109) |
| Positive predictive value | 20% (14/69) | 4% (3/69) | 75% (51/69) |
| Negative predictive value | 29% (28/98) | 78% (76/98) | 94% (92/98) |

*Immunohistochemistry*

One formalin fixed paraffin embedded tissue block from each case was used to generate 4 um unstained slides for immunohistochemical staining with a monoclonal antibody to SATB2 (clone EPNCIR130A, dilution 1:100, OriGene Technologies, Maryland, USA). Appropriate positive and negative controls were included for each batch of immunohistochemical staining. Only nuclear staining was considered positive. The immunohistochemical staining was scored semi-quantitatively as 0 (no tumor cell staining), 1+ (1-25% tumor cells stained), 2+ (26-50%), 3+ (51-75%), and 4+ (76-100%).
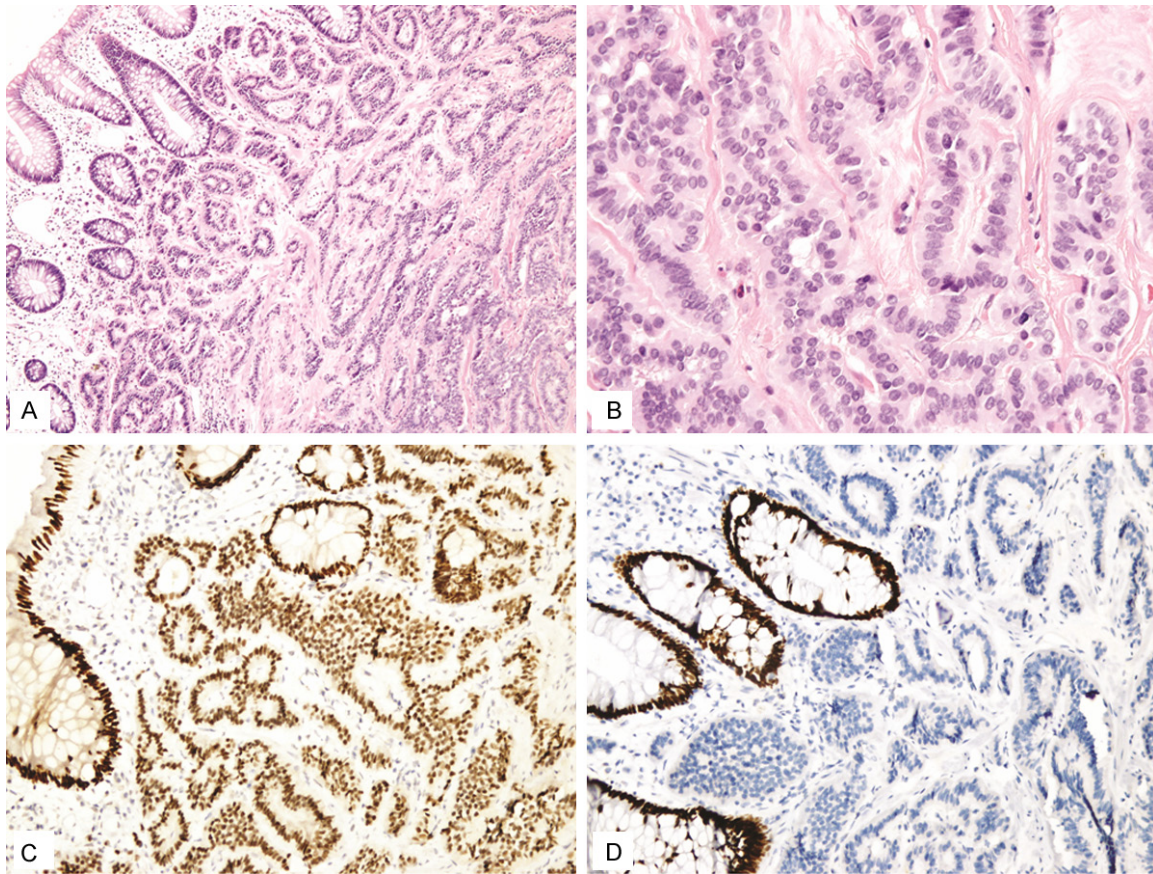
*Statistic analysis*

Fisher exact test was used to compare the SATB2 staining among foregut, midgut and hindgut WDNETs. A P value < 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated with the following formats: sensitivity = number of true positives/(number of true positives + number of false negatives), specificity = number of true negatives/(number of true negatives and number of false positives), PPV = number of true positives/(number of true positives + number of false positives), and NPV = number of true negatives/(number of true negatives and number of false negatives).

**Results**

*SATB2 staining in foregut well differentiated neuroendocrine tumors (N = 84)*

Among the 84 foregut WDNETs, 14 (17%) showed positive SATB2 staining including 12/66 (18%) primary and 2/18 (11%) metastat-

10 HPFs and/or > 20% Ki67 index [13]. G1 and G2 gastrointestinal and pancreatic neuroendocrine neoplasms are classified as WDNETs whereas G3 tumors are PDNECs. In thymus and lung, carcinoid and atypical carcinoid are classified as WDNETs, and small cell carcinoma and large cell neuroendocrine carcinoma are classified as PDNECs.

One hundred sixty-seven (167) WDNETs were collected including 133 primary and 34 metastatic ones (**Tables 1**-**4**). These tumors were assigned to foregut (N = 84), midgut (N = 25) and hindgut (N = 58) origins according to previous criteria [14]. The foregut WDNETs included those from lung (11 carcinoids, 2 atypical carcinoids; all primary), thymus (6 carcinoids, 2 atypical carcinoids; all primary), pancreas (13 G1, 14 G2; 13 primary and 14 metastatic), gallbladder (1 G1, primary), esophagus (N = 0), stomach (14 G1, 8 G2; 19 primary and 3 metastatic) and the proximal half of the duodenum (8 G1, 5 G2; 12 primary and 1 metastatic). WDNETs from the distal half of duodenum (N = 0), jejunum (2 G1, 1 G2; 2 primary and 1 metastatic), ileum (11 G1, 2 G2; 8 primary and 5 metastatic), cecum (1 G2, primary), appendix (4 G1, all primary), ascending colon (3 G1, 1 G2; 3 primary and 1 metastatic), and proximal 1/3 transverse colon (N = 0) are classified as midgut WDNETs. Hindgut WDNETs are those from distal 2/3 transverse colon (N = 0), descending colon (N = 0), sigmoid colon (1 G1, primary) and rectum (45 G1, 12 G2; primary 48, metastatic 9).

**Figure 1.** In this study 90% of rectal well differentiated neuroendocrine tumors (A, B) showed positive staining for SATB2 with most of them showing diffuse staining (C). The normal rectal mucosal epithelium showed diffuse SATB2 staining (C). This tumor was negative for CDX2 (D). Normal rectal mucosal epithelium was positive for CDX2.

ic tumors (**Table 1**). Primary tumors and metastatic ones did not show difference in SATB2 staining (*P* = 0.7238).

Among the 12 (of 66) primary tumors showing positive SATB2 staining, 8 showed staining in no more than 50% tumor cells (1+ in 6/12, 2+ in 2/12) and 4 showed staining in more than 50% tumor cells (3+ in 2/12, 4+ in 2/12). The two metastatic tumors (both from pancreas) showed 2+ and 3+ staining, respectively.

*SATB2 staining in midgut well differentiated neuroendocrine tumors (N = 25)*

Positive SATB2 staining was seen in 3/25 (12%) midgut WDNETs including 3/18 (17%) primary and 0/7 (0%) metastatic ones (**Table 2**). The percentage of tumor cells showing SATB2 staining was no more than 50% in all 3 cases (1+ in 1, 2+ in 2).

*SATB2 staining in hindgut well differentiated neuroendocrine tumors (N = 58)*

Among the 58 hindgut WDNETs, positive SATB2 staining was seen in 52 (89%) tumors including 44/49 (90%) primary (**Figure 1**) and 8/9 (89%) metastatic tumors (**Figure 2**; **Table 3**). The staining pattern distribution in 44 SATB2-positive primary tumors was 1+ in 4 (4/44, 9%), 2+ in 4 (4/44, 9%), 3+ in 2 (2/44, 5%), 4+ in 34 (34/44, 77%). Among the 8 (of 9) SATB2-positive metastatic hindgut WDNETs, 7 (88%) showed 4+ staining and 1 (12%) showed 3+ staining. Overall 41 of 58 (71%) hindgut WDNETs showed 4+ staining.

*Sensitivity and specificity of SATB2 for well-differentiated neuroendocrine tumors from foregut, midgut and hindgut*

In this study, the sensitivity of SATB2 for labeling foregut, midgut and hindgut WDNETs was
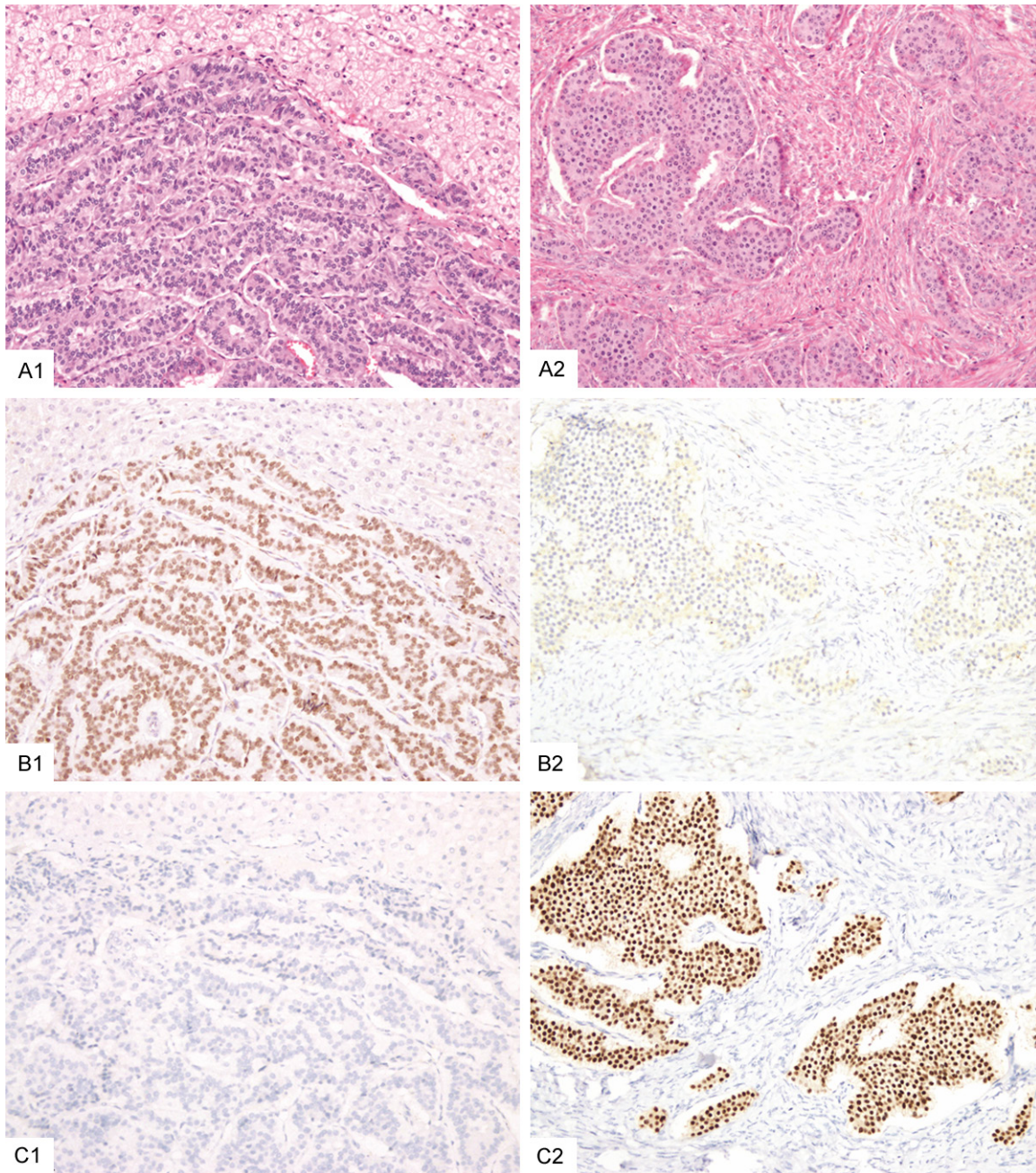
**Figure 2.** This rectal well differentiated neuroendocrine tumor that metastasized to the liver (A1) was positive for SATB2 (B1) but negative for CDX2 (C1). In contrast, an ileal well differentiated neuroendocrine tumor that metastasized to one ovary (A2) showed negative staining for SATB2 (B2) but was positive for CDX2 (C2).

17%, 12% and 90%, respectively ($P < 0.001$ between foregut and hindgut, $P < 0.001$ between midgut and hindgut, $P = 0.76$ between foregut and midgut) (**Table 4**). The specificity of SATB2 for foregut, midgut and hindgut WDNETs was 34%, 54% and 84%, respectively (**Table 5**). The positive predictive value of SATB2 for foregut, midgut and hindgut WDNETs was 20%, 4%, and 75%, respectively (**Table 5**). The negative

predictive value of SATB2 for foregut, midgut and hindgut WDNETs was 29%, 78%, and 94%, respectively (**Table 5**).

**Discussion**

SATB2 is a DNA-binding protein that interacts with transcription factors to regulate craniofacial development, cortical neuron differentia-

tion, immunoglobulin mu gene expression, skeletal development and osteoblastic differentiation [15-18]. In a previous study [11], Magnusson et al first showed that SATB2 was a sensitive marker (85% sensitivity) for colorectal adenocarcinomas, which was later confirmed by another study (93% sensitivity) [12]. In addition, SATB2 was also a sensitive osteoblastic marker [19, 20].

In this study, we investigated the expression of SATB2 in a large series of 167 WDNETs. We found that SATB2 staining was seen in 17% foregut, 12% midgut and 90% hindgut WDNETs. Among those 52 (of 58) SATB2-positive hindgut WDNETs, most of them (44/52 or 85%) showed staining in more than 50% tumor cells and 80% (41/52) labeled more than 90% tumor cells (4 + staining). Our study indicates that among WDNETs, SATB2 is preferentially expressed in hindgut WDNETs and is therefore a sensitive marker for hindgut WDNETs. The high sensitivity of SATB2 for hindgut WDNETs holds not only for primary tumors (44/49 or 90%) but also for metastatic ones (8/9 or 89%).

Previous studies have identified several immunohistochemical markers for determining the origins of metastatic WDNETs. These markers included thyroid transcription factor 1 (TTF1), caudal type homeobox 2 (CDX2), neuroendocrine secretory protein-55 (NESP-55), (PDX1), paired box 8 (PAX8), and Islet1 [2, 21-48]. TTF1 is a relatively specific marker for pulmonary carcinoid/atypical carcinoids (the overall sensitivity is 32%) [2, 21, 22, 24, 25, 31, 32, 35]. CDX2 is considered a sensitive marker for midgut WDNETs (86-92% sensitivity) but some foregut (3% to 33%) and hindgut (29-43%) WDNETs also show immunoreactivity [2, 23-32, 35, 36]. NESP55 is relatively specific for pancreatic WDNETs but with low sensitivity (50% sensitivity) [2, 31-34]. PDX1 mainly labeled gastroduodenopancreatic WDNETs (46-60% sensitivity) but it also labeled a variable percentage of lung (3/39 or 8%), appendiceal (7/17 or 41%) and rectal (2/14 or 14%) WDNETs [2, 31, 35-39]. PAX8 and Islet1 mainly labeled foregut (61-68% and 80-89% duodenopancreatic WDNETs, respectively) and hindgut WDNETs (56% and 88% rectal WDNETs, respectively) [2, 39-48]. When compared to these previously reported markers, SATB2 showed similar sensitivity to Islet1 for rectal WDNETs (89% versus 88%, *P* =

1.0) and was more sensitive than PAX8 for these tumors (89% versus 56%, P < 0.01). The specificity of SATB2, Islet1 and PAX8 for rectal WDNETs was 84%, 55%, and 63%, respectively (*P* < 0.01 between SATB2 versus Islet1, *P* < 0.01 between SATB2 and PAX8) (specificity for Islet1 and PAX8 was calculated based on the data from literature as summarized in **Table 6**). Therefore even though SATB2 and Islet1 show similar sensitivity for rectal WDNETs, SATB2 is more specific than Islet1 for these tumors. SATB2 is both more sensitive and specific than PAX8 for rectal WDNETs. Another marker for rectal WDNETs that is not usually used but reported in the early literature is prostatic acid phosphatase (PASP) [49-53]. The reported positive rate of PSAP in large series of rectal WDNETs (carcinoids) was ranging from 67-82% [49-53], not as sensitive as SATB2. Although SATB2 is not specific for hindgut WDNETs, it has a high negative predictive value (94%) i.e. a WDNET negative for SATB2 is unlikely from the hindgut region.

The proportions of WDNETs arising foregut, midgut and hindgut origins are highly variable among different racial groups. In the USA SEER data, in Caucasians foregut WDNETs were most common (45%) followed by midgut (25%) and hindgut ones (12-16%) whereas in Asians/Pacific islanders, the most common WDNETs were from hindgut (approximately 41-45%, more than 90% hindgut WDNETs were rectal) followed by foregut (34%) and midgut (7%) [1]. In east Asian countries such as China, Korea and Japan, hindgut WDNETs (vast majority are rectal) account for 50-70% of gastrointestinal WDNETs and only 10% of gastrointestinal WDNETs are of midgut origin [54, 59]. The distant metastasis rate of rectal WDNETs is related to the tumor size, ranging from 5.5% in less than 1.0 cm tumors to 4-30% in 1.0-1.9 cm tumors to 70-80% in tumors 2.0 cm or larger [54-59]. Given the large number of population in East Asian countries, the number of patients with metastatic rectal WDNETs is not small [54-59]. Even in USA, in a recent study 13% (11/85) rectal WDNETs were metastatic at presentation and additional 5% (4/85) had metastasis on follow-up [60]. Therefore even though rectal WDNETs have a lower metastasis rate than that of midgut and pancreatic WDNETs, the number of metastatic rectal WDNETs is actually not that small. Most metastasis occurred in the liver but

**Table 6.** Comparison of SATB2 to other well differentiated neuroendocrine tumor markers in the literature

| Markers | Foregut Origin | | | | Midgut origin | | | | Hindgut Origin | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lung | Stomach | Pancreas | Duodenum | Jejunoilecum | Appendix | Cecum | Colon | Rectum | |
| TTF1 | 32% (189/588) | | | | 0.03% (4/928) | | | | | [2, 21-22, 24-25, 31-32, 35] |
| CDX2 | 3% (8/233) | 15% (12/80) | 16% (81/499) | 33% (17/52) | 90% (306/339) | 92% (72/78) | 86% (6/7) | 43% (10/23) | 29% (32/112) | [2, 23-32, 35-36] |
| NESP55 | 10% (2/20) | 0% (0/9) | 50% (49/99) | 0% (0/5) | 0% (0/57) | 0 (0/11) | NA | NA | 7% (1/16) | [2, 31-34] |
| PDX1 | 8% (3/39) | 60% (3/5) | 56% (124/220) | 46% (18/39) | 0% (0/49) | 41% (7/17) | 0% (0/3) | 0% (0/2) | 14% (2/14) | [2, 35-39] |
| PAX8 | 6% (7/117) | 14% (5/37) | 68% (145/213) | 61% (11/18) | 0% (0/152) | 17% (5/30) | NA | 0% (0/1) | 56% (28/50) | [2, 42, 44-48] |
| Islet1 | 11% (11/101) | 0% (0/22) | 80% (256/322) | 89% (25/28) | 1% (2/137) | 15% (7/46) | 0% (0/1) | 23% (3/13) | 88% (22/25) | [2, 39-43] |
| SATB2 | 23% (3/13) | 9% (2/22) | 15% (4/27) | 8% (1/13) | 6% (1/16) | 25% (1/4) | 0% (0/1) | 40% (2/5: ascending 1/4, sigmoid 1/1) | 89% (51/57) | This study |

rectal WDNETs can metastasize to lung and pancreas, creating significant difficulty in differentiating them from primary pulmonary and pancreatic WDNETs [54-60]. In such situations, use of immunohistochemical markers is critical to make such distinction. Sensitive hindgut WDNET markers such as SATB2 are particularly useful in this aspect. Such diagnostic utility is even more appreciated in eastern Asian countries given the large patient population and high proportion of rectal WDNETs.

It should be pointed out that even though SATB2 is a sensitive marker for hindgut WDNETs, it is not specific. As a matter of fact, except TTF1 (TTF1 is relatively specific for pulmonary WDNETs and thyroidal medullary carcinoma), none of the currently used WDNET markers is specific for WDNET from any particular site. Therefore a panel of immunohistochemical markers is often needed in determining the primary site of a metastatic WDNET. Bellizzi [2] recommended an initial panel to include TTF1, CDX2 and PAX8 (or Islet1). We would recommend to add SATB2 to this panel to increase sensitivity and specificity for working out metastatic WDNETs of unknown origin.

Obviously SATB2 should be always used in conjunction with tumor morphology. SATB2 was highly expressed in colorectal adenocarcinomas and occasionally in some other types of carcinomas [11, 12]. Therefore SATB2 is not useful to distinguish WDNET from adenocarcinoma in the hindgut region. Their distinction relies on other parameters (morphology, mitotic figures/Ki67 index, and neuroendocrine markers chromogranin and synaptophysin). In addition, we have found that SATB2 is also expressed in approximately one third of PDNECs of various organs (data not published). Therefore SATB2 is not useful to distinguish WDNETs from PDNECs.

One limitation of our study is that the number of metastatic WDNETs is relatively small (foregut 18, midgut 7 and hindgut 9). Further studies are needed to include more WDNETs from various sites particularly metastatic ones to test whether the high sensitivity of SATB2 for metastatic hindgut WDNETs can be maintained.

In summary, we investigated SATB2 expression in a large series of WDNETs. Our results indicate that SATB2 is a sensitive marker for hindgut WDNETs though it is not specific. SATB2 should be included in the immunohistochemical panel for working out metastatic WDNETs from an unknown origin.

## Disclosure of conflict of interest

None.

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