SATB.2 is a Sensitive Marker for well Differentiated Neuroendocrine Tumors

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ABSTRACT

Introduction: Neuroendocrine tumors (NET) constitute a heterogeneous group of rare neoplasms, with a sustained increase in incidence in recent years. They originate in neuroendocrine cells, the most common are those of the gastrointestinal system and most are sporadic, although they can be part of hereditary syndromes. Neuroendocrine neoplasms are divided into well-differentiated neuroendocrine tumors (WDNET) and poorly differentiated neuroendocrine carcinomas (PDNEC). **Material and methods:** We retrospectively evaluated 78 WDNET of the anterior, middle and posterior intestine, in a period of 20 years (01/01/2000 to 07/31/2020), with the SATB2 antibody, in which it is considered a diagnostic marker for WDNET of the hindgut tract. **Results:** Of the 40 WDNET of the anterior intestine, 2 (5%) tumors showed reactivity with SATB 2. Positive staining with SATB2 was observed in 1/17 (5.88%) of WDNET of the midgut. Among the 21 WDNET of the hindgut, we observed SATB2 staining in 19 (90.57%) of the tumors. **Conclusion:** Our results indicate that SATB2 is a sensitive marker for hindgut WDNET, although it is not specific.

KEYWORDS

Stab2 protein, Carcinoid tumors, Gastroenteropancreatic neuroendocrine tumors, Neuroendocrine tumor, Colorectal neuroendocrine tumors

SATB.2 ES UN SENSIBLE MARCADOR PARA LOS TUMORES NEUROENDOCRINOS BIEN DIFERENCIADOS DEL TRACTO GASTROINTESTINAL BAJO

RESUMEN

Introducción: Los tumores neuroendocrinos (TNE) constituyen un grupo heterogéneo de neoplasias poco frecuentes, con un aumento sostenido de la incidencia en los últimos años. Se originan en las células neuroendocrinas, los más frecuentes son los del sistema gastrointestinal y la mayoría son esporádicos, aunque pueden ser parte de síndromes hereditarios. Las neoplasias neuroendocrinas se dividen en tumores neuroendocrinos bien diferenciados (TNEBD) y carcinomas neuroendocrinos poco diferenciados (CNEPD). **Material y métodos**: Evaluamos en forma retrospectiva 78 TNEBD de intestino anterior, medio y posterior, en un periodo de 20 años (01/01/2000 al 31/07/2020), con el anticuerpo SATB2, un factor de transcripción nuclear que es considerado marcador diagnóstico para TNEBD del tracto del intestino posterior. **Resultados:** De los 40

TNEBD del intestino anterior, 2 (5%) tumores mostraron reactividad con SATB 2.Se **observó** tinción positiva con SATB2 en 1/17 (5,88%) de TNEBD del intestino medio. Entre los 21 TNEBD del intestino posterior observamos tinción con SATB2 en 19 (90,57%) de los tumores. **Conclusión:** Nuestros resultados indican que SATB2 es un marcador sensible para TNEBD de intestino posterior, aunque no es específico.

PALABRAS CLAVE

Stab2 protein, Carcinoid tumors, Gastroenteropancreatic neuroendocrine tumors, Neuroendocrine tumor, Colorectal neuroendocrine tumors

INTRODUCTION

Gastrointestinal neuroendocrine neoplasms are defined as epithelial tumors with predominantly neuroendocrine differentiation and constitute a heterogeneous group of pathologies. They are originated from neuroendocrine cells, which in the gastrointestinal tract will be distributed mainly in the mucosa and submucosa. These neuroendocrine cells, in the embryonic stage, are distributed throughout the body through endocrine glands, diffuse endocrine system, neural crests and islets, therefore, they can be located in any organ of the body[1].

Neuroendocrine neoplasms are divided into well-differentiated neuroendocrine tumors (WDNET) and poorly differentiated neuroendocrine carcinomas (PDNEC). PDNECs are highly aggressive and are treated similarly with platinum-based chemotherapy regardless of their origin, while WDNETs exhibit a wide range of biological behavior and are treated differently based on their primary sites [2].

Most WDNET tumors present locoregional disease, but up to 20% of them present distant metastasis. This number is even higher for the WDNETs of certain organs such as the pancreas, ileum, and jejunum[3]. Although nonsurgical approaches (chemotherapy and / or radiation) are the main treatment for metastatic WDNET, surgical resection of primary or metastatic tumors is still beneficial in some of these patients, because cytoreduction can reduce the acute complication at the site. primary, minimize endocrine symptoms and decrease the requirement for somatostatin analogues.[4] Furthermore, chemotherapy and targeted therapy protocols are different for well-differentiated metastatic neuroendocrine tumors of different origins [2] [5].

Therefore, determining the primary origins of metastatic WDNET has some therapeutic implications. Determination of the origin of metastatic WDNET is generally accomplished with imaging studies such as computed tomography and somatostatic receptor imaging (Octroscan). However, these imaging techniques saw a less than 50% success rate in determining the primary site of WDNET[6][7][2]. Approximately 9 to 19% of WDNETs are still present with unknown origins[8].

For this reason, in some cases, the determination of origin of metastatic WDNET still depends on the pathology. However, WDNETs from different sites have similar and overlapping morphology, and their origin is often difficult to determine based on morphology alone. In these situations they often need to be marked by immunohistochemistry , to facilitate the determination of the primary site of a metastatic WDNET[2] [9].

Recently, the special AT-rich sequence binding protein 2 (SATB2) has been identified as a marker with a highly selective expression pattern of the mucosa of the lower gastrointestinal tract. Approximately 85 to 93% of colorectal adenocarcinomas were immunohistochemically positive for SATB2[9][10].

In view of the importance of a correctly directed diagnosis, in this study we will investigate by immunohistochemistry the expression of SATB2 in well-differentiated neuroendocrine tumors from various sites (lung, esophagus, stomach, duodenum, pancreas, jejunum, ileum, cecal appendix, colon, rectum and upper part of the anal canal), and thus demonstrate whether it is a potential sensitive and specific marker of well-differentiated neuroendocrine tumors of the colon, rectum and upper part of the anal canal (hindgut), especially in the metastatic environment .

MATERIALS AND METHODS

A quantitative clinical investigation was realized. In the selection of cases, well-differentiated neuroendocrine tumors diagnosed from January 2000 to July 2020 were searched in the pathology files of Dr. Antonio Arra's laboratory. The diagnosis of all WDNET was confirmed with immunohistochemical staining with chromogranin and synaptophysin. The cell proliferation index evaluated with the Ki67 antibody was also performed for all neuroendocrine neoplasms. Grades for gastrointestinal and pancreatic neuroendocrine neoplasms were assigned according to the 2010 WHO criteria:

- **Grade 1** - Mitotic count less than 2 per 10 high power fields (HPF) and / or less than 2% Ki67 index;
- **Grade 2** - Mitotic count of 2 to 20 per10 high power fields (HPF) and / or a Ki67 index of 3 to 20%;
- **Grade 3** - Mitotic count greater than 20 per 10 high power fields (HPF) and / or more than 20% of the Ki67 index.

Grade 1 and grade 2 gastrointestinal and pancreatic neuroendocrine neoplasms are classified as WDNET, while grade 3 tumors are PDNEC. In lung, thymus, carcinoids and atypical carcinoids are classified as WDNET. Small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma are classified as PDNEC[2].
In immunohistochemistry, a 10% formalin-fixed paraffin-embedded tissue block was used from each case to generate unstained 4-millimicron slides for immunohistochemistry with a monoclonal antibody against SATB2 with a 1: 100 cell mark dilution.
Appropriate positive and negative controls were included for each lot of immunohistochemical staining. Only nuclear staining was considered positive.

RESULTS

Table 1 - Result of immunohistochemical staining of SATB2 in 40 well-differentiated neuroendocrine tumors of the foregut.

SITE OF ORIGIN	PRIMARY	TOTAL
Stomach (N = 10)	1/10 (10%)	1/10 (10%)
Duodenum (N = 5)	0/5 (0%)	0/5 (0%)
Pancreas (N = 15)	1/15 (6,66%)	1/15 (6,66%)
Gallbladder (N = 1)	0/1 (0%)	0/1 (0%)
Thymus (N = 2)	0/2 (0%)	0/2 (0%)
Lung (N = 7)	0/7 (0%)	0/7 (0%)
TOTAL (N=40)	2/40 (5%)	2/40 (5%)

Table 2 - Result of immunohistochemical staining of SATB2 in 17 well differentiated neuroendocrine tumors of the midgut.

SITE OF ORIGIN	PRIMARY	TOTAL
Ileum (N = 4)	0/4 (0%)	0/4 (0%)
Jejunum (N = 1)	0/1 (0%)	0/1 (0%)
Appendix (N = 8)	1/8 (12,5%)	1/8 (12,5%)
Ascending colon (N = 4)	0/4 (0%)	0/4 (0%)
TOTAL (N=17)	1/17 (5,88%)	1/17 (5,88%)

Table 3 - Result of immunohistochemical staining of SATB2 in 21 well-differentiated neuroendocrine tumors of the hindgut.

SITE OF ORIGIN	PRIMARY	TOTAL
Sigmoid colon (N = 2)	2/2 (100%)	2/2 (100%)
Recto (N = 19)	17/19 (89,47%)	17/19 (89,47%)
TOTAL (N=21)	19/21 (90,47%)	19/21 (90,47%)

Table 4 - Comparison of SATB2 staining in well-differentiated neuroendocrine tumors of the anterior, middle and posterior intestine.

SITE OF ORIGIN	PERCENTAGEOFPOSITIVETUMORSFOR SATB2
Foregut (N = 40)	5% (2/40)
Middle intestine (N = 17)	5,88% (1/17)
Posteriorintestine(N=21)	90,47% (19/21)

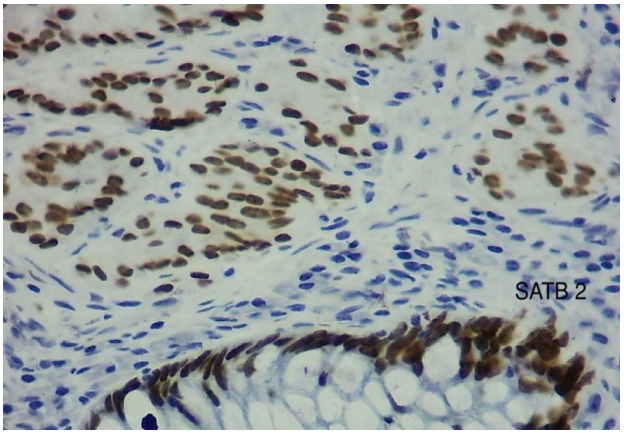

FIGURE 1: STAB2 400X WDNET FROM SIGMOID COLON

Among the 40 WDNET of the anterior intestine, 2 (5%) showed positive staining with STAB2. Positive staining with STAB2 was observed in 1/17 (5.88%) of WDNET of the middle intestine. Among the 21-posterior intestine WDNET, STAB2 staining was observed in 19 (90.47 %) of the tumors.

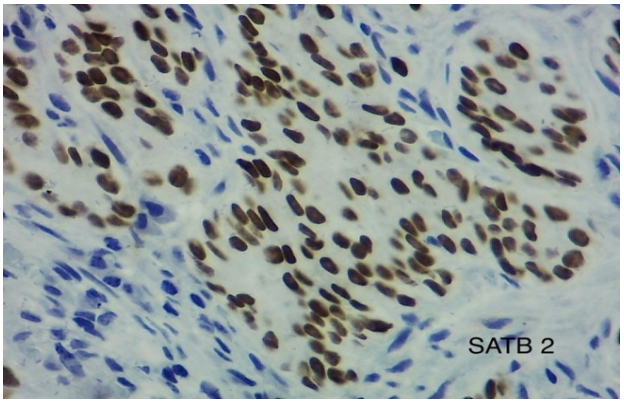

FIGURE 2: STAB 2 400X WDNET FROM SIGMOID COLON

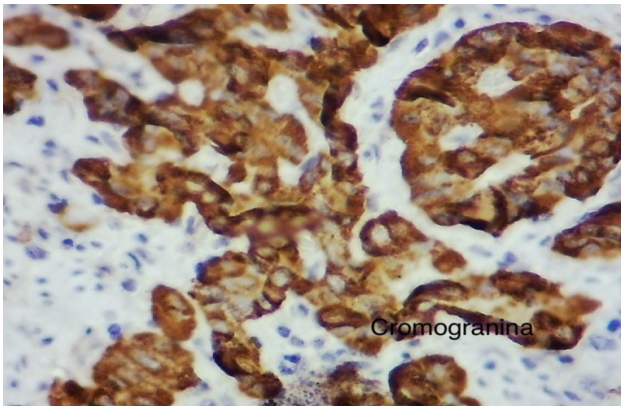

FIGURE 3: CROMOGRANINA 400X WDNET FROM SIGMOID COLON

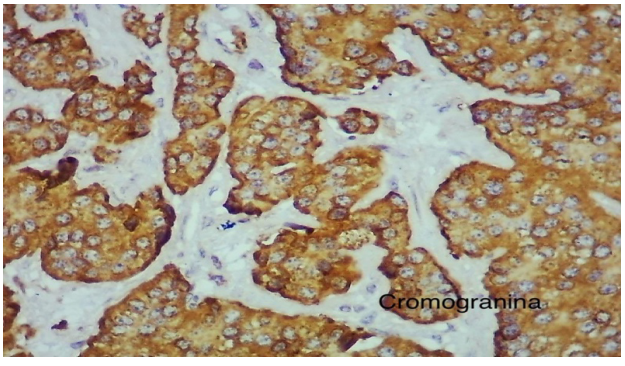

FIGURE 4: STAB2 400X WDNET FROM CECAL APPENDIX

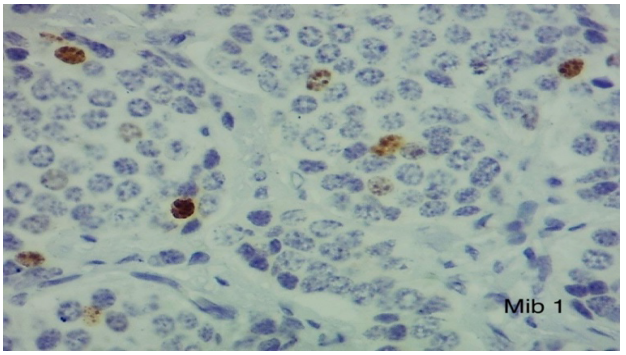

FIGURE 5: CROMOGRANINA 400X WDNET FROM CECAL APPENDIX

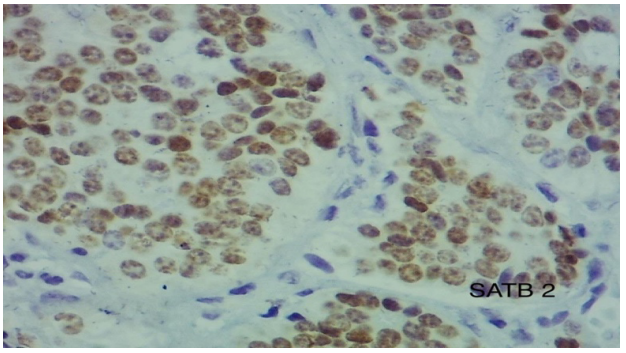

FIGURE 6: MIB-1 (KI67) 400X WDNET

DISCUSSION

In this study, the sensitivity of STAB2 to diagnose WDNET of the anterior, middle, and posterior intestine was 5%, 5.88%, and 90.47%, respectively (Table 4).

STAB2 is a DNA-binding protein that interacts with transcription factors to regulate craniofacial development, cortical neuron differentiation, MU immunoglobulin gene expression, skeletal development, and osteoblastic differentiation[11]in the form of deletions and balanced translocations, points to the existence of a locus on 2q32-q33, for which haploinsufficiency results in isolated cleft palate (CPO[12].

In a previous study Magnusson and Cols. showed first that STAB2 was a sensitive marker (85% sensitivity) for colorectal adenocarcinoma, which was later confirmed by another study (93% sensitivity) [10]a nuclear matrix-associated transcription factor and epigenetic regulator, was identified as a tissue type-specific protein when screening protein expression patterns in human normal and cancer tissues

using an antibody-based proteomics approach. In this respect, the SATB2 protein shows a selective pattern of expression and, within cells of epithelial lineages, SATB2 expression is restricted to glandular cells lining the lower gastrointestinal tract. The expression of SATB2 protein is primarily preserved in cancer cells of colorectal origin, indicating that SATB2 could function as a clinically useful diagnostic marker to distinguish colorectal cancer (CRC[9].
In addition STAB2 was also a sensitive osteoblastic marker [13][14].
In that study, we investigated the expression of STAB2 in a series of 78 WDNET. We have found that STAB2 staining is observed in 5% of the anterior intestine, 5.88% in the middle intestine, and 90.47% of WDNET of the posterior intestine.
Our study indicates that among WDNET, STAB2 was preferentially expressed in WDNET of the posterior intestine. It is important to note, although STAB2 is a sensitive marker for posterior intestine WDNET, it is not specific, so obviously STAB2 should always be used in conjunction with tumor morphology. STAB2 was highly expressed in colorectal adenocarcinomas and occasionally in some other types of carcinomas.
Therefore, STAB2 is not useful to distinguish WDNET from adenocarcinoma in the posterior intestine region, its distinction is based on other parameters (morphology, mitotic figures / Ki67 index, and chromogranin and synaptophysin type neuroendocrine markers).
In summary, we investigated the expression of STAB2 in a series of WDNET and our results indicate that STAB2 is a sensitive marker for posterior intestine WDNET, although it is not specific. STAB2 must be included in the immunohistochemical panel to work with metastatic WDNET of unknown origin.

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