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Can INSM1 and Phox2B be an Alternative to Conventional Neuroendocrine Markers in the Diagnosis of Pancreatic Solid Pseudopapillary Neoplasia and Neuroendocrine Tumors?

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ABSTRACT

Introduction: Solid pseudopapillary neoplasm (SPN) and pancreatic neuroendocrine tumors (PNET) often present diagnostic challenges due to their overlapping clinical and histopathological features. We aimed to investigate the possible role of paired-like homeobox 2B (Phox2B) in this differentiation.

Methods: This study included 12 patients diagnosed with PNET and 7 patients diagnosed with SPN. Clinicopathologic data were collected and immunohistochemical staining was performed for Phox2B, synaptophysin (SYN), chromogranin-A (CHR), and insulinoma-associated protein 1 (INSM1).

Results: Phox2B exhibited limited positivity in PNET (8.3%) and rare focal staining in SPN (considered as negative). Moderate to strong immunoreactivity with SYN and CHR was observed in all PNET cases, while SPN cases showed weak to moderate SYN expression but no staining with CHR. INSM1 showed positive staining in all PNET cases and no staining in SPN cases.

Conclusion: No significant correlation was observed between Phox2B and INSM1 expressions and clinicopathologic parameters. While INSM1 serves as a reliable marker in supporting the diagnosis of neuroendocrine neoplasia in the differential diagnosis of PNET and SPN, Phox2B does not exhibit comparable diagnostic utility.

Keywords: Phox2B, solid pseudopapillary neoplasm, pancreatic neuroendocrine tumors, INSM1, synaptophysin, chromogranin-A

Introduction

Solid pseudopapillary neoplasm (SPN), first described by Frantz in 1959, is a pancreatic tumor characterized by low malignancy potential and favorable prognosis (1). Accounting for 0.9-2.7% of pancreatic neoplasms, this tumor is predominantly observed in young females (2). While pseudocystic areas with pseudopapillary structures are commonly present in SPN, some cases consist entirely of solid areas. Additionally, positive immunoreactivity for neuroendocrine markers such as synaptophysin (SYN) and CD56 necessitates differentiation from well-differentiated neuroendocrine tumors (NETs). Despite findings from electron microscopy and immunohistochemical studies, the cellular origin of SPN, whether epithelial or mesenchymal, remains to be elucidated (3-6). Therefore, the use of specific biomarkers for differentiation will offer valuable diagnostic support.

The *paired-like homeobox 2B (Phox2B*) gene, located on chromosome 4p13, is a crucial transcription factor required for the development of neural crest derivatives. It is expressed in the sympathetic and parasympathetic systems, enteric ganglia, adrenal, and extraadrenal chromaffin cells, as well as glomus cells (7,8). Additionally, the Wnt/β-catenin signaling pathway is involved in the development of neural crest-derived cells (9). Aberrant nuclear expression of β-catenin is observed in SPN and serves as an immunohistochemical marker for differential diagnosis (10,11). Phox2B, a known regulator of the autonomic nervous system and the noradrenergic system, has been utilized as a marker in the diagnosis of neoplasms such as neuroblastoma, ganglioneuroblastoma, and paraganglioma (6,12,13). These findings raise questions regarding Phox2B expression in pancreatic SPN and NETs cases, particularly concerning the potential common pathways or shared origins.

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In this study, we aimed to investigate the potential role of Phox2B in the diagnosis of pancreatic neuroendocrine tumors (PNET) and SPN, as well as its clinicopathological correlations and relationships with other immunohistochemical markers.

Methods

Sample Selection

In our pathology department, our study included 12 cases diagnosed with PNET and 7 cases diagnosed with SPN from 2014 to 2023. One PNET case was obtained through a tru-cut biopsy, while the remaining 18 cases were derived from pancreatic resection specimens, including Whipple procedures and distal pancreatectomies. Patients whose paraffin blocks could not be retrieved from the archive or who had insufficient material in the paraffin blocks were excluded from the study.

Ethical Statement

This study was approved by the University of Health Sciences Türkiye, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 208, date: 18.08.2023) and was conducted in accordance with the principles of the Helsinki Declaration.

Clinical and Histopathological Evaluation

Information regarding the age, gender, tumor localization, and tumor size of the cases was extracted from the pathology reports in the hospital information system. The histological type/grade of the tumor, as well as lymphovascular and perineural invasion, was re-evaluated. Histological grading for PNET cases was conducted according to the 2019 World Health Organization Criteria for gastrointestinal system tumors (1).

Immunohistochemistry

Following the evaluation of hematoxylin- and eosin-stained slides, appropriate paraffin blocks were subjected to immunohistochemical staining for SYN, chromogranin-A (CHR), insulinoma-associated protein 1 (INSM1), and Phox2B biomarkers. Immunohistochemical reactions were performed on paraffin tissue sections using an automated immunohistochemical stainer (Ventana BenchMark ULTRA, Ventana Medical Systems, Inc., Tucson, AZ), following the manufacturer's protocol. The detection process was facilitated using the Ventana ultraVIEW DAB Detection Kit (Ventana Medical Systems, Inc.). The slides were incubated with the following primary antibodies: Phox2B [EP312](dilution 1:50, Cell Marque, Rocklin, CA, USA), INSM1 [A8] (dilution 1:100, Santa Cruz Biotechnology, Dallas, TX, USA), SYN [SP11] (dilution 1:20, Thermo Fisher Scientific, Waltham, MA, USA), and CHR [LK2H10] (ready-to-use, Ventana Medical Systems, Tucson, AZ, USA).

Evaluation of Immunohistochemistry

The immunohistochemical analysis for INSM1, Phox2B, SYN, and CHR was based on both the percentage of tumor cells exhibiting positive staining and the intensity. The extent of staining was categorized as follows: 0 (<1% stained), 1+ (1-25% stained), 2+ (26-50% stained), 3+ (51-75% stained), and 4+ (>75% stained). Staining intensity was assessed and categorized as follows: no staining (0), weak (1+), moderate (2+), or strong (3+). For INSM1, pancreatic endocrine islets served as positive controls, while a paraganglioma case was utilized as a positive control for Phox2B. Cytoplasmic staining for SYN and CHR, as well as nuclear staining for INSM1 and Phox2B, were deemed positive.

Statistical Analysis

For the statistical analysis of the data in this study, (SPSS, Chicago, IL, USA) version 26.0 was used. Descriptive statistics comprised the mean, standard deviation, median, minimum, and maximum values for continuous variables, while frequency and percentage were calculated for discrete variables. To evaluate the normality of distribution in the initial analysis, both the Kolmogorov-Smirnov and Shapiro-Wilk tests were employed. For non-parametric data between two groups, the Mann-Whitney U test and chi-square test were used. The Pearson correlation test was employed to analyze correlations between binary data. Results were evaluated within a 95% confidence interval, with a p-value of less than 0.05 considered statistically significant.

Results

The clinicopathological findings for the 19 cases, comprising 12 PNET and 7 SPN cases, are summarized in Table 1.

In one PNET case, Phox2B revealed perinuclear dot-like (Golgi zone) cytoplasmic staining (Figure 1 A, B), and, in another PNET case, Phox2B showed nuclear staining of moderate intensity, involving 50% of the tumor cells (Figure 1C). In only one of the SPN cases, sparse nuclear staining was observed in a focal area, comprising less than 1% of the tumor, thus considered negative (Figure 1D). Phox2B expression was not observed in other PNET and SPN cases.

Table 1. The clinicopathological findings of the 19 cases

	Pancreatic neuroendocrine tumor cases, (n=12)	Solid pseudopapillary neoplasm cases, (n=7)	p
Gender			1
Female	8	5	
Male	4	2	
Mean age	52	37	0.057
Tumor location			0.365
Head-uncinate process	7	2	
Body	3	2	
Tail	2	3	
Mean tumor size (cm), range (cm)	4.1 (1.5-8.5)	7.4 (3.5-18)	0.067
Lymphovascular invasion			0.326
Present	3	4	
Absent	9	3	
Perineural invasion			0.129
Present	2	4	
Absent	10	3	
Histologic grade			
Grade 1	2	-	
Grade 2	10	-	

INSM1 staining was detected in all PNET cases, exhibiting moderate to strong intensity, with the extent of expression varying from 30% to 95%. No reaction was detected in any of the SPN cases. In SPN cases, Langerhans islets, entrapped by the tumor in regions adjacent to normal pancreatic tissue, exhibited positive staining for INSM1.

Immunoreactivity with SYN was observed in all PNET and SPN cases. In PNET cases, SYN showed moderate to strong intensity and immunoreactivity, with staining extent ranging from 50% to 100%. In SPN cases, however, the expression, was weak to moderate, with staining extent ranging from 10% to 100%. No staining for CHR was observed in any of the SPN cases. In contrast, PNET cases exhibited moderate to strong intensity and expression, with staining extent ranging from 60% to 100%.

Immunohistochemical expressions of SYN, CHR, INSM1 and Phox2B, representative of most SPN cases in our study, are shown in Figure 2.

No statistically significant correlation was observed between Phox2B staining patterns in the PNET, SPN groups, and age, gender, tumor size, tumor localization, histological grade (in PNET), lymphovascular invasion, or perineural invasion.

Discussion

In recent years, with the advancement of disease-specific follow-up and treatment protocols, histopathological diagnosis has become critically important. Diagnostic procedures are increasingly being carried out using minimally invasive methods, which places pressure on pathologists to make definitive pathological diagnoses with limited material. PNET and SPN occasionally exhibit overlapping features in macroscopic, histopathological, and immunohistochemical aspects. This challenge becomes more pronounced in cytology and tru-cut biopsies. While certain immunohistochemical markers, such as CHR, E-cadherin, and β-catenin, have been reported to be useful in differential diagnosis, their roles are not well-defined (2). PNET, which originates from neuroendocrine cells derived from endodermal stem cells, expresses conventional neuroendocrine markers such as SYN, CHR, and CD56 (3). In contrast, SPN, with an unclear cellular origin, remains a subject of ongoing research (4). The absence of staining with most epithelial markers (e.g., pancytokeratin, e-cadherin) in SPN may indicate a deviation from an epithelial origin, while positivity with markers such as vimentin may suggest a mesenchymal origin (2,5). Furthermore, the frequent expression of SYN in SPN raises questions about its potential association with neuroendocrine or neural crest-derived tumors.

Phox2B is currently utilized in the diagnosis of Hirschsprung's disease and tumors of neural crest origin, such as neuroblastoma, ganglioneuroma, and paraganglioma (6). However, in NETs, the results have been inconsistent and somewhat confusing, with only a limited number of studies available. To our knowledge, no research has yet been conducted on Phox2B in SPN. Given the limited research on Phox2B, the uncertain cellular origin of SPN, and the necessity to differentiate it from PNET, these factors prompted our study.

In reviewing the literature, Phox2B positivity in paragangliomas has been reported to range from 25% to 100% (6-8). In the same studies, Lee et al. (7) and Nonaka et al. (8) did not detect Phox2B expression in any cases

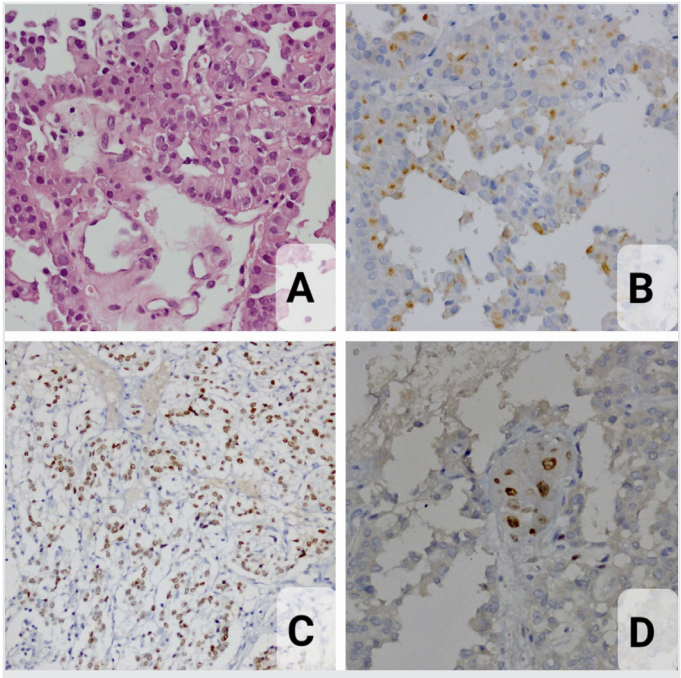

Figure 1. Phox2B staining patterns in cases of pancreatic neuroendocrine tumors and solid pseudopapillary neoplasm. [(A) Hematoxylin-eosin, x400]. The tumor shows perinuclear dot-like (Golgi zone) cytoplasmic staining for Phox2B [(B): IHC, x200]. Another PNET case showed nuclear staining with moderate intensity, accounting for 50% of cells for Phox2B [(C): IHC, x200]. One SPN case shows focal nuclear staining less than 1% of the tumor area, considered negative for Phox2B [(D) IHC, x400]
Phox2B: Paired-like homeobox 2B, IHC: Immunohistochemistry, PNET: Pancreatic neuroendocrine tumors, SPN: Solid pseudopapillary neoplasm

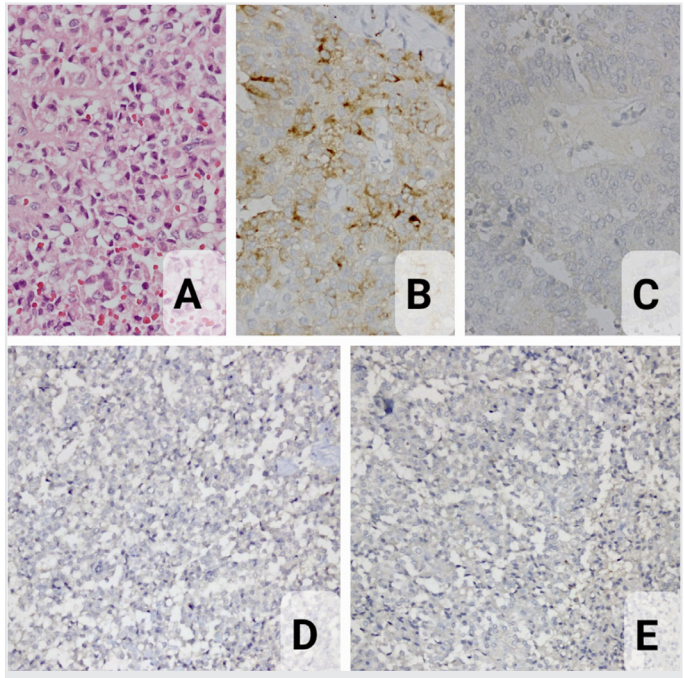

Figure 2. Solid pseudopapillary neoplasm [(A): Hematoxylin-eosin, x400]. Tumor shows patchy and moderate staining positivity for synaptophysin [(B): IHC, x400]. Chromogranin-A [(C): IHC, x400], INSM1 [(D): IHC, x200] and Phox2B [(E): IHC, x200] are negative
IHC: Immunohistochemistry, INSM1: Insulinoma-associated protein 1, Phox2B: Paired-like homeobox 2B

of NET. These studies included 15 and 67 cases of PNET, respectively. Manethova et al. (14) detected Phox2B staining in 10 of 91 NET cases but did not report any staining in the PNET cases included in their study. Of the ten cases with Phox2B expression, five were of lung origin, four were appendiceal, and one was of small intestine origin (14). On the other hand, Miyauchi et al. (12) reported immunoreactivity for Phox2B in 4 of 123 NET cases, but did not specify the origins of these NETs. In our study, moderate nuclear staining was observed in 50% of the tumor cells in one PNET case (1 out of 12 cases, or 8.3%), while perinuclear (dot-like) cytoplasmic staining was detected in another PNET case. While conflicting results regarding Phox2B expression in PNET cases have been reported in the literature, it is evident that Phox2B immunoreactivity is absent in most cases. Additionally, the antibodies, dilution ratios, and immunohistochemical methods employed in these studies vary. In our study, weak nuclear expression of Phox2B was observed in a small fraction of tumor cells (less than 1%), which was considered negative, in one case of SPN. Neither cytoplasmic nor nuclear staining was observed in other SPN cases. Due to the focal staining and the limited number of cases, interpretation remains challenging. However, it is evident that further studies with larger sample sizes are necessary to elucidate the role of Phox2B in the diagnosis of PNET and SPN.

INSM1, encoded by the *INSM1* gene, is a zinc-finger transcription factor expressed by cells undergoing terminal neuroendocrine differentiation. Originally isolated from pancreatic insulinoma and glucagonoma samples, INSM1 has been detected in various NETs, including pheochromocytoma, medullary thyroid carcinoma, pituitary tumors, small-cell lung carcinoma, and merkel cell carcinoma (15,16). INSM1 also plays a role in cell growth (17). In our study, positive staining with INSM1 was observed in all PNET cases, whereas no nuclear staining was detected in any SPN cases. Most studies in the literature have also reported positive INSM1 staining in all PNET cases (18-20). Additionally, Kim et al. (20) reported that INSM1 expression varies according to the grade of PNETs, with a decrease in INSM1 expression observed in G3 PNET cases compared to G1 and G2 PNETs. Consistent with our findings, Tanigawa et al. (18) did not detect nuclear expression of INSM1 in 5 cases of SPN; and Guo et al. (19) also reported no nuclear expression of INSM1 in 22 SPN cases. However, Tanigawa et al. (18) determined weak cytoplasmic staining in 4 of 5 cases. Additionally, Kim et al. (20) reported focal weak nuclear INSM1 staining in 5 of 14 SPN cases, while McHugh et al. (21) detected focal but strong nuclear staining in 1 of 19 cases. Although these findings may appear contradictory, the differences could be attributed to variations in antibody clones, fixation procedures, and immunohistochemical methods used. We believe that INSM1 is a strong supportive factor in the differential diagnosis of PNET and SPN, towards neuroendocrine neoplasia.

In our study, both PNET and SPN cases exhibited positive staining with SYN; however, the extent and intensity of staining varied between the two types of tumors. CHR staining was absent in all SPN cases, while positive staining was observed in all PNET cases. In the literature, SYN positivity has been reported in nearly 100% of PNET cases, whereas CHR positivity ranges between 76% and 100% (18,20,21). Similar to our study, Tanigawa et al. (18) and Kim et al. (20) reported the absence of CHR staining in SPN cases. On the other hand, McHugh et al. (21) reported CHR positivity in 3 of 15 SPN cases.

Study Limitations

Although our study's retrospective nature and limited case count are notable drawbacks, it is, to our knowledge, the first investigation specifically focusing on Phox2B in SPN cases.

Conclusion

Our study found that Phox2B did not prove useful in differentiating between PNET and SPN. On the other hand, INSM1 exhibited more specific reactions in PNET cases compared to other neuroendocrine markers such as SYN, and CHR. The complete absence of INSM1 staining in all SPN cases proved highly valuable for differential diagnosis. Particularly, for the diagnosis of SPN, we believe that further investigation with larger study cohorts is needed to elucidate the role of Phox2B.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Türkiye, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 208, date: 18.08.2023).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions: Surgical and Medical Practices - M.C., E.Y., M.M.S.; Concept - M.C., Ö.G., C.L.; Design - M.C., Ö.G., C.L.; Data Collection or Processing - M.C., Ö.G., S.C., M.M.S.; Analysis or Interpretation - M.C., Ö.G., E.Y., C.L.; Literature Search - M.C., Ö.G., S.C., Writing - M.C., Ö.G., S.C., C.L.

Conflict of Interest: No conflict of interest was declared by the authors.

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