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Research

Expression of insulinoma-associated protein 1 (INSM1) in gastric neuroendocrine and non-neuroendocrine neoplasms

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Abstract

Aims Insulinoma-associated protein 1 (INSM1) is a recently added nuclear marker for neuroendocrine differentiation. However, INSM1 expression in gastric neuroendocrine and non-neuroendocrine neoplasms has not been thoroughly investigated.

Methods We examined INSM1 expression in 72 gastric tumors, including 22 gastric neuroendocrine tumors and 50 gastric non-neuroendocrine neoplasms. Synaptophysin and chromogranin immunostaining were also performed for all cases.

Results For gastric neuroendocrine neoplasms, INSM1 immunostaining demonstrated excellent sensitivity (21/22, 95.5%), comparable to synaptophysin (22/22, 100.0%), but had lower specificity (32/50, 64.0%) compared with traditional neuroendocrine markers (synaptophysin (36/50, 72.0%) and chromogranin (42/50, 84.0%)). However, decreased expression of INSM1, measured by H-score, was frequently found among neuroendocrine carcinoma cases. Gastric non-neuroendocrine neoplasms frequently exhibited INSM1 positivity (18/50, 36.0%); however, in most cases (16/18, 88.9%), staining was focal (involving < 10% of tumor cells). Tumor histologic subtype and grade may be associated with INSM1 expression.

Conclusions INSM1 nuclear positivity in gastric neoplasms should be interpreted with caution. INSM1 should not be used as a stand-alone marker for determining neuroendocrine differentiation in gastric tumors. Histologic evaluation with concurrent use of traditional neuroendocrine markers is warranted to accurately demonstrate neuroendocrine differentiation and minimize false positivity and false negativity.

Keywords INSM1 · Neuroendocrine neoplasm · Stomach cancer · Immunohistochemistry

1 Introduction

Neuroendocrine neoplasms (NENs) are a heterogeneous group of tumors that exhibit neuroendocrine differentiation. In the fifth edition of the World Health Organization (WHO) classification of digestive system tumors, NENs in the digestive tract are categorized as neuroendocrine tumors (NETs), neuroendocrine carcinomas (NECs), and mixed neuroendocrine-non-neuroendocrine neoplasms (MiNENs) which contain both adenocarcinoma and NEN components

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[1]. Neuroendocrine markers are used to demonstrate neuroendocrine differentiation. Neuroendocrine biomarkers commonly include synaptophysin, chromogranin, and CD56 [2]. These biomarkers exhibit varying degrees of sensitivity and specificity [3]. Chromogranin expression is typically strong in well-differentiated NENs but can be weak or absent in many NECs [4]. Synaptophysin is a sensitive neuroendocrine marker, but its expression has been reported in some non-neuroendocrine neoplasms [4, 5]. Nonspecific expression of CD56 in non-neuroendocrine neoplasms has been well documented [6].

Insulinoma-associated protein 1 (INSM1), a zinc-finger transcription factor that regulates neuroendocrine differentiation in various tissues and tumor types, is a recently added nuclear marker for neuroendocrine differentiation [7–9]. INSM1 has demonstrated a high degree of sensitivity and specificity in tumors exhibiting neuroendocrine differentiation in many organs, including the thoracic cavity [5], head and neck [10], and digestive tract [11]. However, INSM1 expression has also been reported in non-neuroendocrine tumors, including poorly differentiated lung cancers [12], extraskeletal myxoid chondrosarcoma [13], and other tumors [14]. During routine diagnostic workup, we encountered some stomach adenocarcinoma cases that exhibited aberrant INSM1 expression. This prompted us to investigate the INSM1 expression pattern in gastric neuroendocrine and non-neuroendocrine neoplasms. Although McHugh et al. thoroughly investigated INSM1 expression in the digestive tract, including neuroendocrine and non-neuroendocrine neoplasm [11], their study included only two gastric non-neuroendocrine tumor cases. Therefore, the current research focused on INSM1 expression in gastric neuroendocrine and non-neuroendocrine neoplasms in comparison with traditional neuroendocrine markers.

2 Materials and methods

2.1 Study population

We retrospectively collected 22 consecutive gastric NETs (9 biopsy and 13 resected cases) that underwent INSM1 immunohistochemistry during diagnostic workup from January 2023 to December 2023 in the Department of Pathology, Kyungpook National University Chilgok Hospital. Additionally, we randomly selected 50 resected gastric non-neuroendocrine tumors during the same period. Clinicopathological data were collected from the hospital's electronic medical records.

2.2 Pathological evaluation

All gastric tumor specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. The paraffin blocks were then cut into 4-μm-thick sections and stained with hematoxylin and eosin. MSK, a specialist in gastrointestinal pathology, reviewed all available slides. Gastric tumors were diagnosed and classified according to the fifth edition of the WHO Classification of Tumors [1]. NETs are defined as a uniform population of cells with round nuclei, finely stippled chromatin, and positive staining for neuroendocrine markers. NECs are sheets or trabeculae of poorly differentiated cells exhibiting small or large cell carcinoma morphology with a high mitotic rate and an elevated Ki-67 proliferation index.

2.3 Immunohistochemistry

FFPE slices were deparaffinized and rehydrated with xylene and alcohol. The sections were then incubated with antibodies against INSM1 (A-8, 1:200; Santa Cruz Biotechnology, Dallas, TX), chromogranin A (mouse monoclonal, clone DAK-A3, 1:500; Dako, Glostrup, Denmark), and synaptophysin (mouse monoclonal, clone DAK-SYNAP, 1:900; Dako). INSM1, synaptophysin, and chromogranin immunostaining were performed for all cases. Representative whole-tissue sections were used for immunostaining the resected cases (63/72, 87.5%). The remainder were whole-tissue sections of biopsies (9/72, 12.5%). The sections were chromogenically visualized using an ultraView Universal DAB Detection Kit (Ventana Medical Systems) or EnVision FLEX/HRP (Agilent Technologies) and subsequently counterstained with hematoxylin. Any nuclear staining of INSM1 was considered positive. Focal staining was defined as staining in less than 10% of tumor cells, whereas diffuse staining was defined as staining in more than 50% of tumor cells [15, 16]. For synaptophysin and chromogranin A, any percentage of granular cytoplasmic staining was considered positive. For INSM1, the intensity (0, 1+, 2+, 3+) and percentage (0% to 100%) of staining were graded. A histoscore (H-score) was calculated by multiplying

the intensity score by the percentage. For mixed adenoneuroendocrine carcinomas (MANEC), neuroendocrine marker expression was evaluated in the neuroendocrine carcinoma component.

2.4 Statistical analysis

H-score distribution according to tumor types were evaluated using Kruskal–Wallis test. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic performance of INSM1. The area under the ROC curve (AUC) was calculated as an overall measure of test accuracy. The sensitivity and specificity at selected cutoff points were determined. *P*-values of < 0.05 were used to indicate a significant difference. All statistical analyses were performed using the R software (version 4.3).

3 Results

3.1 Study cohort

The clinicopathologic details of the study cohort are summarized in Table 1. The median age of the patients was 64 years (range: 28–95 years). Of the total, 50 (69.4%) were male, and 22 (30.6%) were female. The gastric NENs included nine NETs, two large cell neuroendocrine carcinomas, one small cell carcinoma, and 10 mixed adenoneuroendocrine carcinomas. Among the 50 gastric non-neuroendocrine neoplasms, 34 cases were of the intestinal type, and 16 cases were of the diffuse type, according to the Lauren classification.

3.2 INSM1 staining pattern in gastric neuroendocrine neoplasms

Overall, INSM1 was positive in all gastric tumors, showing neuroendocrine differentiation except in one case (Table 2 and Fig. 1). The INSM1 H-score of gastric NENs ranged from 0 to 300. All NETs showed diffuse and intense INSM1 nuclear positivity. However, decreased expression of INSM1 was frequently found among NEC cases. Synaptophysin immunostaining was positive for all cases. Chromogranin staining was positive in 16 out of 22 cases (72.7%).

3.3 INSM1 staining pattern in gastric non-neuroendocrine neoplasms

INSM1 positivity was frequently found in gastric non-neuroendocrine neoplasms (18/50, 36.0%), with higher frequencies in intestinal-type adenocarcinoma (16/34, 47.1%) than in diffuse-type adenocarcinoma (2/16, 12.5%) according to the Lauren classification (Table 2 and Fig. 2). All poorly differentiated intestinal-type adenocarcinoma cases showed INSM1 positivity (5/5, 100%) (Supplementary Table 1). The INSM1 H-score of gastric non-neuroendocrine neoplasms

Table 1 Characteristics of the patient cohort

Variables	N (total = 72)	(%)
Sex		
Male	50	69.4
Female	22	30.6
Age (median)	64 (28–95)	
Type of sampling		
Resection	63	87.5
Biopsy	9	12.5
Histologic type		
Neuroendocrine tumor	9	12.5
Mixed adenoneuroendocrine carcinoma	10	13.9
Large cell neuroendocrine carcinoma	2	2.8
Small cell neuroendocrine carcinoma	1	1.4
Intestinal type adenocarcinoma	16	22.2
Diffuse type adenocarcinoma	34	47.2

Table 2 INSM1, synaptophysin, and chromogranin expression in gastric neuroendocrine and non-neuroendocrine neoplasms

Tumor type	Positive/total (%)		
	ISNM1	Synaptophysin	Chromogranin
Neuroendocrine tumor	9/9 (100%)	9/9 (100%)	9/9 (100%)
Neuroendocrine carcinoma (including MANEC)	12/13 (92.3%)	13/13 (100.0%)	7/13 (53.8%)
Intestinal type adenocarcinoma	16/34 (47.1%)	14/34 (41.2%)	8/34 (23.5%)
Diffuse type adenocarcinoma	2/16 (12.5%)	0/16 (0.0%)	0/16 (0.0%)

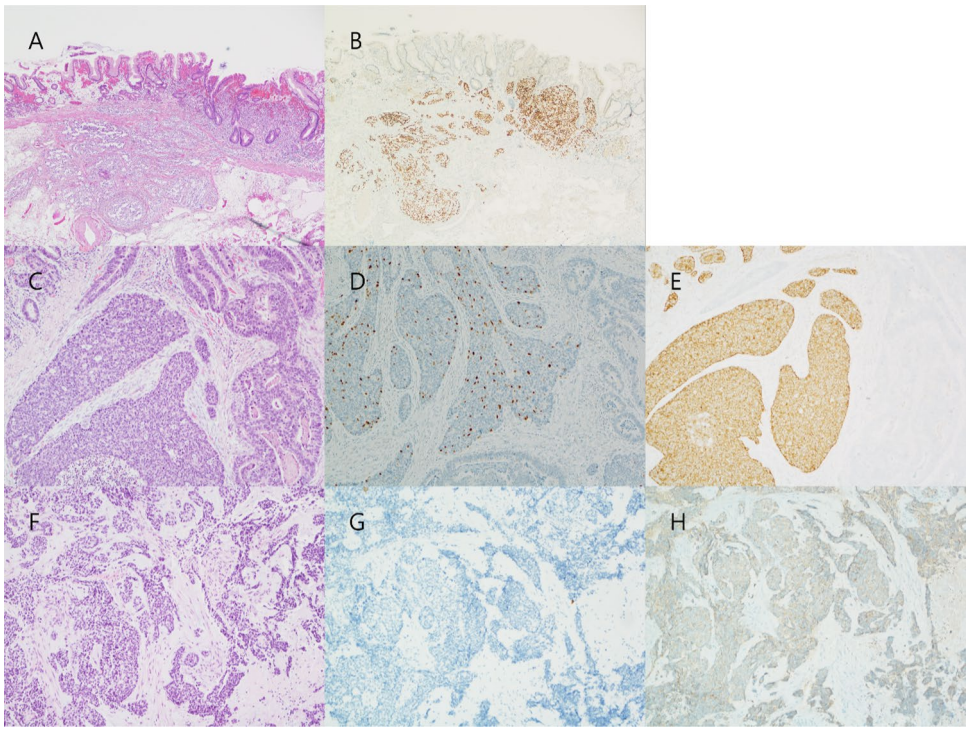

Fig. 1 INSM1 expression in gastric neuroendocrine neoplasms. **A** H&E image of well-differentiated neuroendocrine tumor and (**B** INSM1 immunostaining. Almost all tumor cells demonstrate high-intensity nuclear positivity of INSM1. **C** H&E image of mixed adenoneuroendocrine carcinoma composed of conventional tubular adenocarcinoma area and large cell neuroendocrine carcinoma area. **D** Only a few neuroendocrine carcinoma tumor cells were positive for INSM1 with strong intensity. In contrast, synaptophysin immunostaining (**E**) demonstrated diffuse positive staining (**F**) H&E image of small cell carcinoma showing scant cytoplasm without visible nucleoli. **G** None of the tumor cells were positive for INSM1 with strong intensity, whereas (**H**) synaptophysin immunostaining shows diffuse positivity. Original magnifications: (**A**) and (**B**): × 100, (**C**–**H**): × 100

ranged from 0 to 30. Among INSM1-positive gastric non-neuroendocrine carcinomas, the majority (16/18, 88.9%) showed a focal staining pattern, and none showed diffuse staining. Gastric non-neuroendocrine neoplasms with solid or cribriform growth patterns that may mimic neuroendocrine neoplasms were identified in 19 cases (19/50, 38.0%). The H-score distribution was not significantly different between gastric cancers with a cribriform pattern and those without ($P = 0.376$). Among the 18 cases with aberrant INSM1 positivity, concurrent synaptophysin and chromogranin expression were observed in 11 and eight cases, respectively.

3.4 Sensitivity and specificity of INSM1 in gastric neoplasms

INSM1 demonstrated excellent sensitivity (21/22, 95.5%), comparable to synaptophysin (22/22, 100.0%) and superior to chromogranin (16/22, 72.7%). However, the specificity of INSM1 was somewhat lower (32/50, 64.0%) than that of traditional neuroendocrine markers (synaptophysin (36/50 (72.0%) and 42/50 (84.0%)) (Table 3). In addition to the original cutoff point (defined as any nuclear positivity of INSM1), we further plotted ROC curve and analyzed the sensitivity

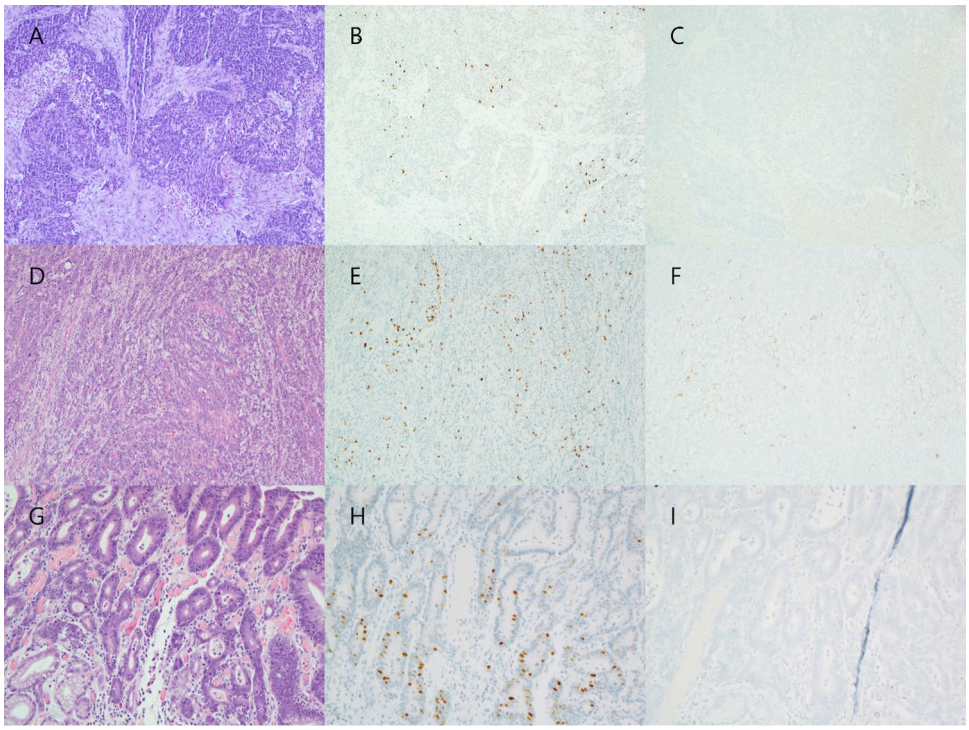

Fig. 2 INSM1 expression in gastric non-neuroendocrine neoplasms. **A**–**C** H&E image of tubular adenocarcinoma, poorly differentiated (**A**) and INSM1 (**B**) and synaptophysin (**C**) immunostaining. While INSM1 positivity was found in some tumor cells, the tumor cells were negative for synaptophysin staining. **D**–**F** H&E image of tubular adenocarcinoma, poorly differentiated (**D**) and INSM1 (**E**) and synaptophysin (**F**) immunostaining. In this case, tumor cells show focal staining for INSM1 and synaptophysin. **G**–**I** H&E image of tubular adenocarcinoma, well-differentiated (**G**) and INSM1 (**H**) and synaptophysin (**I**) immunostaining. While aberrant INSM1 immunostaining was frequently found in poorly differentiated adenocarcinoma, this well-differentiated.adenocarcinoma shows INSM1 expression. Original magnifications: (**A**–**F**): × 100, (**G**–**I**): × 200

and specificity of INSM1 immunostaining at other cutoff points (10, 30, and 100) (by H-score) (Fig. 3). Overall, the AUC was 0.945. The sensitivity and specificity of INSM1 were 91.0% and 80.0% (cutoff point = 10), 77.3% and 96.0% (cutoff point = 30), and 63.6% and 100.0% (cutoff point = 100), respectively. The sensitivity and specificity of diffuse and strong INSM1 immunostaining (more than 50% of tumor cells showing 3 + nuclear INSM1 expression) were 54.5% and 100.0%.

4 Discussion

This study evaluated INSM1 expression in both gastric neuroendocrine and non-neuroendocrine neoplasms. INSM1, a newly added neuroendocrine marker, is a sensitive and specific marker for demonstrating neuroendocrine differentiation in malignant tumors [5]. Some authors have even suggested that INSM1 can be a stand-alone neuroendocrine marker

Table 3 Sensitivity and specificity of INSM1, synaptophysin, and chromogranin in gastric neuroendocrine neoplasms

Variables	Positive/total (%)		
	INSM1	Synaptophysin	Chromogranin
Sensitivity	21/22 (95.5)	22/22 (100.0)	16/22 (72.7)
Specificity	32/50 (64.0)	36/50 (72.0)	42/50 (84.0)
Positive predictive value	21/39 (53.8)	22/36 (61.1)	16/24 (66.7)
Negative predictive value	32/33 (97.0)	36/36 (100.0)	42/48 (87.5)

Sensitivity = true positive/true positives + false negatives; specificity = true negatives/true negatives + false positives; positive predictive value = true positives/true positives + false positives; negative predictive value = true negatives/true negatives + false negatives

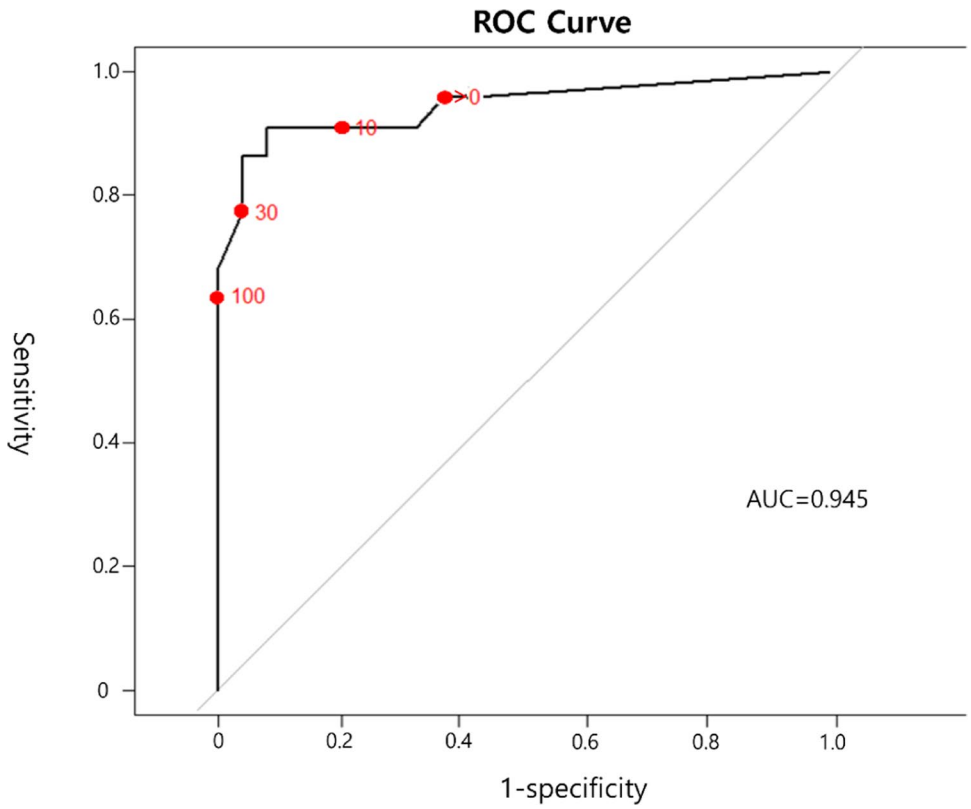

Fig. 3 Receiver operating characteristic (ROC) curve of INSM1 immunostaining by H-score. Cutoff points > 0, 10, 30, and 100 are shown

[5, 17]. Most of the gastric NENs included in this study expressed nuclear INSM1 positivity, demonstrating the high sensitivity of INSM1 for identifying neuroendocrine differentiation, consistent with previous studies [18, 19]. However, the expression level of INSM1 tended to decrease in NECs compared to NETs (Fig. 4). Decreased INSM1 expression in NECs has also been reported in lung NECs [4, 12]. Currently, the reason for decreased INSM1 expression in neuroendocrine carcinoma remains unclear. Recent studies have shown that neuroendocrine carcinomas comprise heterogeneous subgroups. Therefore, some subgroups may be less dependent on the INSM1 transcription factor for neuroendocrine differentiation, as evidenced by YAP1-high and POU2F3-high small cell lung cancer subtypes [20]. Since many gastric NECs with decreased INSM1 expression demonstrated diffuse synaptophysin staining, as shown in Fig. 1, the concurrent use of additional neuroendocrine markers alongside INSM1 may be helpful for correctly identifying tumors with neuroendocrine differentiation, particularly in small biopsy samples, to reduce the risk of false-negative results.

This study reports that aberrant INSM1 positivity can frequently be frequently present in gastric non-neuroendocrine neoplasms, occurring in approximately one-third of cases, although the majority exhibited focal staining. While several previous studies have reported INSM1 expression in non-neuroendocrine tumors [11, 14], the frequency of INSM1 positivity in this study was higher than previously documented. The use of whole-section slides instead of tissue

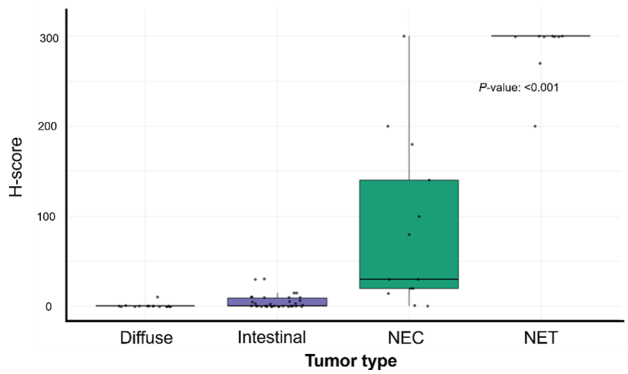

Fig. 4 Boxplot showing INSM1 expression level (H-score) according to gastric tumor. *NEC* neuroendocrine carcinoma, *NET* neuroendocrine tumor

microarrays may have contributed to the higher frequencies of INSM1 positivity in gastric non-neuroendocrine neoplasms in this study. The clinical implications of limited INSM1 positivity in gastric non-neuroendocrine neoplasms are unclear and require further investigation. Given that gastric non-neuroendocrine neoplasms with INSM1 positivity co-expressed traditional neuroendocrine markers in approximately half of the cases, this low-level INSM1 expression likely represents focal neuroendocrine differentiation rather than antibody cross-reactivity. Intestinal-type adenocarcinoma showed higher levels of INSM1 expression than diffuse-type adenocarcinoma (Fig. 4). All poorly differentiated intestinal-type adenocarcinomas showed INSM1 positivity, although the sample size was small. Thus, tumor histologic subtype and grade may be associated with INSM1 expression.

This study has some limitations. Although we used whole-section slides of gastric cancer to accurately assess the expression pattern of INSM1 immunostaining, the number of gastric cancer cases in this study was limited. Thus, subsequent studies with a larger number of gastric cancer cases will be needed to provide stronger justification and validation of our findings. We did not perform additional immunostaining that could further enhance sensitivity and specificity in determining neuroendocrine differentiation, such as ASCL1, NEUROD1, YAP1, SOX2, or POU2F3 [20–23]. Currently, there is no standard cut-off for INSM1 positivity. We thus considered any nuclear positivity of INSM1 as positive INSM1 immunostaining; this may partially explain the high percentage of INSM1 expression in non-neuroendocrine tumor specimens included in this study. As shown in the ROC curve, adopting a higher INSM1 cutoff criterion may increase the specificity of INSM1 immunostaining while decreasing sensitivity.

Overall, INSM1 showed excellent sensitivity (95.5%), similar to synaptophysin (100.0%). However, INMS1 expression was decreased in gastric NECs. The specificity of INSM1 was somewhat lower (32/50, 64.0%) than traditional neuroendocrine markers and frequently showed aberrant positivity in gastric non-neuroendocrine carcinomas. Therefore, INSM1 immunostaining in gastric tumors should be interpreted cautiously, particularly in small biopsy specimens. Neuroendocrine differentiation in gastric tumors should be determined through histologic evaluation and the combined use of neuroendocrine markers.

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Author contributions MSK conceived and designed the manuscript. SJK, JSS, YJS drafted the manuscript. SJK, JSS, YJS analyzed previous articles on INSM1 and neuroendocrine tumors. MSK reviewed and revised the manuscript carefully. All authors have read and approved the final manuscript.

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Data availability All data generated or analysed during this study are included in this published article and its supplementary information file.

Declarations

Ethics approval and consent to participate The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Kyungpook National University Chilgok Hospital Institutional Review Board (No. KNUCH IRB 2023-09-014). The requirement for written informed consent from the patients was waived because of the retrospective nature of the study.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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