Original Article INSM1 is the best marker for the diagnosis of neuroendocrine tumors: comparison with CGA, SYP and CD56

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Received January 25, 2017; Accepted March 22, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Immunohistochemical staining is a powerful diagnostic tool for pathological diagnosis. Neuroendocrine tumors (NETs) consist of heterogeneous groups of neoplasm and range from benign to malignant tumors, a common panel of neuroendocrine (NE) immunohistochemical markers, such as chromogranin A (CGA), synaptophysin (SYP) and CD56 is widely used and often useful for diagnostic distinction from non-NETs. However, it is known that several NETs do not express a common set of NE markers and have metastasized when the tumor is first found. The diagnosis of NETs can be challenging on small biopsy samples. Insulinoma-associated protein 1 (INSM1) is a zinc-finger transcriptional factor expressed in fetal neuroendocrine tissues. INSM1 is a relatively unknown immuno-histochemical marker that is expressed in several NETs exclusively. Here we evaluated INSM1 as a novel NE marker in normal NE tissues and in 102 NETs, then compared INSM1 with conventional NE markers. Stain intensity (O to 3 +) and extent (O to 100%) were scored via H-score (range, O to 300). INSM1 expression was restricted in normal NE tissues and cells, and NETs exclusively. INSM1 was expressed in 100 out of 102 NETs (98%). The distinct nuclear expression of INSM1 enabled us to distinguish NETs from non-NETs clearly. The immunoreactivity for INSM1 was greater than conventional cytoplasmic NE markers as measured by the mean percentage of reactive cells and the mean H-score (INSM/CGA/SYP/CD56, 85%/66%/84%/64%, 211/122/191/117). In addition, INSM1 gene knockdown experiments revealed that INSM1 is a critical modulator of the NE differentiation in several NET cell lines.

Keywords: INSM1, neuroendocrine tumor, immunohistochemical staining

Introduction

Neuroendocrine tumors (NETs) are neoplasms which show neuroendocrine differentiation and originate from neuroendorine or non-neuroendocrine organs or tissues. While NETs are heterogeneous groups and are wide-ranging from benign to malignant tumors, there are several common pathological features and clinical behaviors [1, 2]. Pathological and biological findings of NETs have been thus far identified and leveraged to diagnostic field and management of patients. Although the diagnosis of most cases of histologically typical NETs may not require immunohistological assessment, it is a useful tool in distinguishing between NETs and non-NETs in cases where it may be difficult to discern a definitive diagnosis [1-7].

Poorly differentiated NETs often spread to the lymph nodes or distant sites before final diagnosis is achieved [1, 6-8]. In such cases, NETs can easily be mistaken for a poorly differentiated non-NET as diagnosis is performed on a small biopsy or cytological sample. Numerous studies have demonstrated the utility of several neuroendocrine (NE) biomarkers, such as chromogranin A (CGA), synaptophysin (SYP), and CD56 to give a definitive diagnosis. It has been found, however, that several NETs do not express these NE biomarkers even though their histopathological findings present NE-type features [7]. Thus, there is an urgent need to identify new molecules that can provide pathologists with a highly reliable, sensitive, and specific test.

Insulinoma-associated protein 1 (INSM1) is a zinc-finger transcriptional factor originally isolated from a human insulinoma-glucagonoma subtraction library [9]. INSM1 mRNA is abundantly expressed in fetal NE developmental tissues, however there is also a high occurrence of INSM1 found in NE tumors, such as small cell lung cancer (SCLC), pituitary tumor, medullary thyroid carcinoma, and pheochromocytoma [9-16]. Understanding of INSM1 has been limited and clinical use as a biomarker has not been attempted thus far. We recently reported that INSM1 expresses exclusively in SCLC specimens using immunohistochemistry, and first elucidated that INSM1 regulates the NE differentiation pathway in lung cancer. In addition, we demonstrated the increased sensitivity and specificity compared to other NE biomarkers (CGA, SYP, and CD56) in lung cancer specimens [16].

Despite the understanding of INSM1 in basic research, the clinical utility of INSM1 as a new diagnostic marker and the relevance between INSM1 and the other NE biomarkers in NETs remains largely unclear. To extend previous findings of the significance of INSM1 in NETs, we conducted the following experiments. First, we examined INSM1 protein expression using immunohistochemical (IHC) staining in 102 paraffin-embedded tissue of NETs and 25 non-NETs, and found that the expression of INSM1 was exclusive to NETs. We examined three different commercial monoclonal antibodies against INSM1 and then developed the standard protocol to detect the expression of INSM1 with minimal non-specific background staining. To confirm reproducibility of the IHC method we used, we performed the same protocol in three different pathology laboratories. Second, we conducted a quantitative comparison of IHC staining with INSM1 and the other NE markers (CGA, SYP, and CD56) using the H-score system. INSM1 was superior to the other NE markers in all NETs except pheochromocytomas. Furthermore, to add to our understanding of the roles of INSM1 on the neuroendocrine diiferentiation, we used RNA interference technology on 6 NET cell lines (TT; human thyroid medullary carcinoma, PC-12; rat pheocromocytoma, ECC4; human small cell gastrointestinal carcinoma, RIN-5F; rat islet cell tumor, MtT/SM; rat pituitary tumor, H69; human small cell lung cancer) to elucidate the biological effects of INSM1 in the NE differentiation pathway. The results of western blotting indicated that INSM1 is a critical regulator in the NE differentiation in several cell lines, except rectal NET cell line, ECC4, originating from various organs. From our findings, INSM1 is considered as a critical regulator of the NE differentiation pathway in various types of NETs, and is superior to the other NE markers widely used thus far. This current and novel understanding of INSM1 will advance diagnosis and the management strategy of NETs.

Materials and methods

Tissue samples

Tissue samples of 102 neuroendocrine tumors (NETs) (7 pituitary adenomas, 7 medullary thyroid cancers, 7 merkel cell carcinomas, 7 pheochromocytomas, 11 NETs of pancreas, 31 NETs of gastrointestinal, 5 lung typical carcinoids, 4 large cell neuroendocrine carcinomas, and 19 SCLCs), 25 Non-NETs (5 colon adenocarcinomas, 5 skin carcinomas, 5 hepatocellular carcinomas, 5 lung adenocarcinomas, and 5 lung squamous cell carcinomas), and 25 normal tissues (5 thyroid tissues, 10 intestinal tissues, 5 pancreatic tissues, and 5 lung tissues), resected at Kumamoto University Hospital (Kumamoto, Japan) and National Minami-Kyushu Hospital (Kagoshima, Japan) were obtained from 127 patients for this study. A histopathological diagnosis of the samples was made according to the criteria of the World Health Organization. Additional sections were used for IHC staining. The study followed the guidelines of the Ethics committee of Kumamoto University and National Minami-Kyushu Hospital.

Immunohistochemical staining and evaluation using H-score

INSM1, CGA, SYP and CD56 were detected by IHC on 4-µm-thick sections. The primary antibodies were: CGA (H-300; Santa Cruz, Santa Cruz, CA; 1:100 dilution); SYP (NCL-SYNAP-299; Novocastra laboratories Ltd., Newcastle upon Tyne, UK; 1:50 dilution); CD56 (NCL-CD56-1B6; Novocastra Laboratories Ltd.; 1:50 dilution). Although CGA, SYP, and CD56 were the antibodies widely used in the clinical settings, IHC staining with INSM1 has not been used as a standard diagnostic tool. Before staining of the study material, the protocol for the IHC staining with INSM1 (A-8; Santa Cruz), INSM1 (C-1; Santa Cruz), and INSM1 (ab170876; Abcam, Cambridge, UK) were examined using control

tissues (pancreatic islets) and tumor tissues. IHC with INSM1 (A-8; Santa Cruz) was determined as the following standard protocol. Antigen retrieval with 0.01 mol/L citrate buffer (pH 6) for 15 minutes using an autoclave was performed and slides were stained manually overnight at 1:100 dilution. To confirm the standardization of staining protocol, they were tested at three different pathology laboratories (The Department of Pathology and Experimental Medicine, Kumamoto Japan, The Pathology Core of the Centre for Modeling Human Disease, Toronto Centre for Phenogenomics, ON, Canada, and The Pathology Research Program (PRP) Laboratory at the Department of Pathology, Toronto General Hospital, University Health Network, ON, Canada). H.E. and IHC-stained slides were scanned using the 40× magnification on a ScanScope CS slide scanner (Aperio ePathology, Leica Microsystems Inc., ON, Canada). For all markers, both the percentage cells and intensity (0, 1 +, 2 +, 3 +) of immunoreactivity were examined under pathologist supervision. Nuclear immunoreactivity for INSM1, cytoplasms for CGA and CD56, and cell membranes for CD56 were considered positive. Histological scores (H-scores) were obtained by the formula: 1 × (% of 1 + cells) + 2 × (% of 2 + cells) + 3 × (% of 3 + cells). The P-value for the comparison of H-score were determined by Wilcoxon signed-rank test using SPSS version 18 (SPSS Inc., Chicago, IL).

Cell lines

Six NET cell lines (TT; human thyroid medullary carcinoma, PC-12; rat pheocromocytoma, ECC-4; human small cell rectal carcinoma, RIN-5F; rat islet cell tumor, MtT/SM; rat pituitary tumor, H69; human small cell lung cancer) were used for this study. TT and RIN-5F were purchased from ECACC (Salisbury, UK), H69 from ATCC (Manassas, VA), PC-12, ECC4 and MtT/SM from Japan Collection of Research Bioresources Cell Bank (Osaka, Japan).

INSM1 gene silencing experiments

Small interfering RNA (siRNA) targeted at INS-M1 and negative control (scrambled random siRNA) were purchased from Santa Cruz Biotechnology and transfected into cells by using an electroporator (NEPA21 pulse generator; Nepa Gene, Chiba, Japan), as described in the manufacturer's instruction. The cells were harvested at 48 hours after transfection for each experiment.

Western blotting

Whole cell extracts were fractionated by polyacrylamide gels containing 0.1% SDS and transferred to a PVDF membranes (Millipore, Hamburg, Germany) according to the manufacturer's protocol (Bio-Rad). The membrane was blocked with 5% non-fat milk for one hour and incubated with primary antibodies against either ASCL1 (556604; BD Biosciences Pharmingen, San Jose, CA; 1:1000), CD56 (NCL-CD56-1B6; Novocastra Laboratories Ltd.; 1:1000), CGA (H-300; Santa Cruz; 1:1000), SYP (NCL-SYNAP-299; Novocastra Laboratories Ltd.; 1:1000), INSM1 (C-1; Santa Cruz; 1:5000), and B-actin (A-5441, Sigma Aldrich, Ontario, Canada: 1:10.000) overnight at 4°C. The membrane was then washed and incubated with the respective secondary antibodies conjugated with horseradish peroxidase (Amersham Pharmacia Biotech, Buckinghamshire, UK) for 90 minutes. Blots were washed and developed with the electrochemiluminescence system (Santa Cruz Biotechnology, Santa Cruz, CA) according to the manufacturer's protocols.

Results

Selection and optimization of primary antibody against INSM1 using normal NE tissues and cells

To develop reproducible IHC protocol to detect the expression of INSM1, we examined three different commercial antibodies (INSM1 (A-8: Santa Cruz), INSM1 (C-1; Santa Cruz), and INS-M1 (ab170876; Abcam)) using the samples of pancreatic islets. We then developed three useful IHC methods with A-8 antibody, staining manually overnight at 1:100 dilution, staining by using the Ventana Benchmark automated staining system (Ventana Medical System, Tucson, AZ, USA) at 1:200 dilution, or staining by using the Leica Bond Max autostainer (Leica, Bannockburn, IL) at 1:200 dilution, to obtain similar expression patterns. In the present study, to confirm highly-reproducibility of the IHC method we developed, manually stained slides by conducting the same protocol at three different pathology laboratories were evaluated. The expression of INSM1 was restricted in NE tissues and NE cells, such as NE cells in normal bowel tissues and pancreatic islets (Figure 1). The expression pattern of INSM1 was nuclear, and background staining was minimal.



Figure 1. Selection and optimization of primary antibody against INSM1 using normal NE tissues and cells. Immunohistochemical staining for insulinoma-associated protein 1 (INSM1) in serial sections revealed that INSM1 was expressed exclusively in intestinal neuroendocrine (NE) cells and an islet of pancreas consistent with the expression pattern of traditional NE markers (chromogranin A and synaptophysin).

Tumor type	Ab	Positive cases	0%	1-5%	6-20%	21-50%	>50%	Positive cells (%)	H score
Pituitary Adenoma	INSM1	7/7	0	0	0	0	7	96	262
	CGA	7/7	0	0	0	0	7	89	221
	SYP	7/7	0	0	0	0	7	97	253
	CD56	6/7	1	0	0	2	4	63	82
Medullary Thyroid Carcinoma	INSM1	7/7	0	0	0	0	7	96	257
	CGA	6/7	1	0	0	2	4	70	119
	SYP	7/7	0	0	0	0	7	96	260
	CD56	6/7	1	0	0	0	6	77	171
Merkel Cell Carcinoma	INSM1	7/7	0	0	0	0	7	91	223
	CGA	7/7	0	0	1	1	5	46	53
	SYP	5/7	2	0	0	0	5	67	128
	CD56	5/7	2	0	0	0	5	64	118
Pheochromocytoma	INSM1	5/7	2	0	1	4	0	26	31
	CGA	5/7	2	0	0	0	5	67	175
	SYP	7/7	0	0	0	0	7	93	239
	CD56	7/7	0	0	0	2	5	80	147
NET (Pancreas)	INSM1	13/13	0	0	0	0	13	92	253
	CGA	10/13	3	1	2	0	7	49	88
	SYP	13/13	0	0	0	2	11	92	232
	CD56	10/13	3	2	2	1	5	47	67
NET (Gastrointestinal)	INSM1	31/31	0	0	0	2	29	90	235
	CGA	26/31	5	1	1	7	17	57	96
	SYP	27/31	4	0	0	4	23	77	142
	CD56	25/31	6	6	0	1	18	51	72
Lung Carcinoid	INSM1	5/5	0	0	0	0	5	82	158
5	CGA	5/5	0	0	0	0	5	78	56
	SYP	5/5	0	0	0	1	4	80.6	149
	CD56	5/5	2	0	0	0	3	54	63
LCNEC	INSM1	4/4	0	0	0	0	4	96	235
	CGA	2/4	2	0	0	0	2	57	72
	SYP	4/4	0	0	0	0	4	93	193
	CD56	4/4	0	0	0	0	4	79	162
SCLC	INSM1	19/19	0	0	0	1	18	94	246
	CGA	14/19	5	2	0	0	12	58	87
	SYP	11/19	8	0	0	0	11	54	84
	CD56	13/19	6	1	4	0	8	39	38
Small Cell Carcinoma (Uterus)	INSM1	2/2	0	0	0	0	2	89	218
	CGA	2/2	0	0	0	0	2	91	252
	SYP	2/2	0	0	0	0	2	94	228
	CD56	2/2	0	0	0	0	2	88	258
Total	INSM1	-/ - 100/102 (98%)	2	0	1	7	- 92	85	211
	CGA	84/102 (82.3%)	18	4	4	10	66	66	122
	SYP	88/102 (86.2%)	14	0	0	-0	81	84	191
	CD56	81/102 (79.4%)	21	9	6	6	60	64	117

Table 1. Results of immunohistochemical staining of human NETs surgically resected samples

Abbreviations: Ab, Antibody; CGA, chromogranin A; INSM1, insulinoma-associated protein 1; LCNEC, large Cell Neuroendocrine Carcinoma of the lung; SCLC, small-cell lung carcinoma; SYP, synaptophysin. 85%/66%/84%/64%, 211/122/191/117.

INSM1 is the best IHC marker in various NETs compared to conventional NE markers

As shown in Table 1 and Figure 2, to determine the sensitivity and specificity of IHC for INSM1, we examined that 102 various types of NETs and compared with conventional NE markers, CGA, SYP, and CD56 using H-score. INSM1 was positive in 100 out of 102 NETs (98%), and was not detected in non-NE normal tissues as well as 25 non-NETs. INSM1 had consistently strong and diffuse reactivity in all NETs except pheochromocytomas, the mean reactivity of INSM1/ CGA/SYP/CD56 in NETs was 85%/66%/84%/ 64% (Table 1 and Figure 2). The mean H-score was superior or more than equivalent in the majority of NETs (INSM1/CGA/SYP/CD56, 211/ 122/191/117, P<0.0001) (Figure 3). In cases with low level of expression of traditional cytoplasmic NE markers, INSM1 was expressed diffusely. Furthermore, nuclear expression pattern of INSM1 was much more distinct and clear when compared to conventional cytoplasmic NE markers. Two cases of SCLCs, two cases of rectal NET and one case of pancreatic NET, which did not have any positive staining for the conventional NE markers, showed distinct expression of INSM1. Although in vast majority NETs had 80-100% of tumor cells strongly labeling for INSM1, the mean H-score of conventional NE markers were superior to INSM1 (H-score: CGA/SYP/CD56 vs INSM1; 175, 239, 147 vs 31) in pheocromocytomas.

Effect of INSM1 in NE differentiation pathway of NET cell lines

To investigate the biological significance of INSM1 in NET cell lines, we conducted INSM1 knockdown experiments using siRNA in TT, PC-12, ECC4, RIN-5F, MtT/SM, and H69 cells. Knockdown of INSM1 expression in TT, PC-12, RIN-5F, MtT/SM, and H69 resulted in significant reduction of the expression of CGA and SYP (**Figure 4A**). The reduction of those expressions was not observed in ECC4.

In addition, we examined the impact of the suppression of INSM1 on the expression of ASCL1, which is reported as critical for the proper development of neuronal and NE populations in various organs. Suppression of INSM1 resulted in the significant reduction of ASCL1 expression in TT, H69, and RIN-5F, while a slight decrease of ASCL1 expression was observed in ECC4 (Figure 4B). On the other hand, increased ASCL1 expression was observed in PC-12 and MtT/SM. Neurogenin-3, a master regulator of pancreatic NE development, was increased by the suppression of INSM1 expression (Figure 4C). As a result it is found that the suppression of INSM1 modulates the expression of master genes in several cell lineages, suggesting that INSM1 has an important effect on NE differentiation pathways in various types of NETs.

Discussion

The present study extends previous understandings of INSM1 in NETs. NETs are a heterogeneous group of neoplasms regarding morphological features and clinical characteristics. In some cases, patients with NETs present with metastatic disease at diagnosis, therefore the diagnosis is often performed on a small biopsy or cytological sample [6-8]. However, these small samples present a challenge in the differential diagnosis due to scant tumor cells, crush artifacts, and/or necrosis. Immunohistochemical NE markers (CGA, SYP, and CD56) are used as important complementary tools for the diagnosis of NETs, however in several NETs, all of them are not positive and the diagnosis could still be challenge [1-6].

The key findings of this study is that INSM1 appears greatly superior to other conventional NE markers (CGA, SYP, and CD56) as an immunohistochemical biomarker. We demonstrated that INSM1 expresses strongly in various NETs; the reactivity and intensity for INSM1 were consistently high and strong. Notably, 5 cases (2 SCLCs, 2 NETs of rectums, 1 NET of pancreas) which were negative for all the other NE markers showed the expression of INSM1, suggesting a higher utility of INSM1. As we previously reported, INSM1 could be one of the factors which contribute to initiate NE differentiation in lung cancer cell lines. Therefore, we speculate that we can detect a very early phase of NE differentiation using the INSM1 marker. Furthermore, INSM1 is a nuclear protein, which is less prone to potential non-specific reactivity and difficulties in evaluation as could happen with cytoplasmic and cell membrane NE markers such as CGA. SYP. and CD56. In addition, we confirmed high reproducibility of IHC staining using INSM1 by examining in three different pathology laboratories. Although the present comparative study was achieved using manu-







Figure 2. Examples of INSM1 versus traditional neuroendocrine (NE) markers in various neuroendocrine tumors (NETs). Hematoxylin and eosin (H.E.) staining, and IHC staining for INSM1, CGA, SYP, and CD56 were examined in 102 cases of various NETs. Representative images of pituitary adenomas (A), medullary thyroid carcinomas (B), Merkel cell carcinomas (C), pheochromocytomas (D), NETs of stomach (E), NETs of Rectum (F), NETs of pancreas (G), and small cell lung carcinomas (SCLC) (H) were shown. INSM1 expressions are strongly and diffusely positive in NET cell nuclei, except pheochromocytomas. INSM1 expression does not always co-exist with traditional NE markers (CGA, SYP, and CD56).

ally stained slides with a monoclonal antibody against INSM1 (A-8; Santa Cruz), we have established two other protocols using automated IHC instruments (the Ventana Benchmark automated staining system and the Leica Bond Max autostainer) to obtain the similar expression patterns. The highly- sensitivity, highlyspecificity, and reproducibility of INSM1 IHC staining will result in the improvement of accuracy in diagnosing NETs. Additional validation studies are needed in order to examine the utility of IHC with INSM1 in NETs. We experienced that several NETs, such as pheocromocytoma, did not show INSM1 expression as strongly as the other NE markers, but 5 of 7 cases were positive for INSM1, thus we need further analysis using various types of NETs to extend the understanding of the significance of INSM1 in NETs.

The second key finding is that INSM1 has a role of regulator in the neuroendocrine pathway of



Figure 3. Comparison of INSM1 with CGA, SYP, and CD56 in various neuroendocrine tumors (NETs). Histological score (H-score) of INSM1, chromogranin A (CGA), synaptophysin (SYP), and CD56 were evaluated on 102 neuroendocrine tumors, including pituitary adenomas, medullary thyroid carcinomas, merkel cell carcinomas, pheochromocytomas, neuroendocrine tumors (NETs) of pancreas, gastrointestinal NETs, lung carcinoids, large cell neuroendocrine tumors of the lungs (LCNEC), small cell lung carcinomas (SCLCs), small cell carcinomas of the uterus. H scores were obtained by the formula: $1 \times (\% \text{ of } 1 + \text{cells}) + 2 \times (\% \text{ of } 2 + \text{cells}) + 3 \times (\% \text{ of } 3 + \text{cells})$. INSM1 is superior to conventional neuroendocrine tumors for the diagnosis of neuroendocrine tumors. The *P*-value for the comparison of H-score were determined by Wilcoxon signed-rank test using SPSS version 18 (SPSS Inc., Chicago, IL) *P<0.0001.

several cell lines of NETs. In the present study, all NET cell lines had the expression of INSM1, and INSM1 knockdown study suggested that INSM1 could work as a NE differentiation-promoting regulator in NETs, with the exception that a rectal NET cell line did not decrease NE markers after INSM1 gene knockdown. NE differentiation is regulated by overlapping sets of



various transcription factors; for example, Math1 in enterocyte, neurogenin 3 in pancreatic islet cells, Pit-1 in pituitary cells, Math1 in skin neuroendocrine cells, and Hand2 in adrenal medulla cells, and the sets and their regulatory systems seem to be diverse in each neuroendocrine tissues and cells [7, 17-22]. We previously reported that the INSM1-ASCL1 axis is crucial in determination of neuroendocrine differentiation in the lung NE cells and neoplasms [16]. In the present study, we confirmed that this regulatory system for NE differentiation did not always consistently work in determining NE fate in the other organs, while knockdown of INSM1 expression modulated the expression of NE molecules, ASCL1, and neurogenin-3 in all the cell lines examined.

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internal control.

The first identification of INSM1 was conducted by Goto et al. in 1993 using a human insulinoma-glucagonoma subtraction library [9]. INSM1 is a zinc-finger transcription factor, and is expressed exclusively in embryonic developing NE tissues, has been reported that INSM1 has a critical role in the development of several NE tissues [9-14]. The uniqueness of this molecule is that the expression of INSM1 is exclusive in developing NE tissues in an embryonic phase, the expression of INSM1 is confined in normal NE cells after birth, but a marked INSM1 expression occurs in NETs, such as SCLCs, pituitary tumors, and medullary thyroid carcinomas [9-15]. Lan, M et al. reported that 30 of 31 SCLC cell lines and four NSCLC cell lines with NE phenotype expressed INSM1 mRNA and a high concordance with CGA and L-dopa decarboxylase using the Northern blotting method in 1993 [23]. Several microarray gene expression studies also reported that INSM1 is a prominent diagnostic marker for SCLC [24, 25]. However, the validation of utility in clinical practice as a diagnostic marker has remained dormant until recently because of the lack of a proper antibody. Recently, we reported that INSM1 is a superior immunohistochemical diagnostic marker for SCLC compare to NE markers (CGA, SYP, and CD56) used in the clinical pathological diagnosis field [16]. Also recently, Rosenbaum et al. examined 129 neuroendocrine neoplasm specimens and found that INSM1 was positive in 88.3% of cases using IHC staining (15). Although these two studies presented the highly-specificity of INSM1 compared with the other NE markers, the main contribution of the current study is that we evaluated the utility of IHC staining with INSM1 compared to CGA, SYP. and CD56 using the H-score method to establish the objective evidence for clinical use. Although H-scores vary on each type of NET, INSM1 is a superior immunohistochemical NE marker to conventional NE markers. Our results show that a combination INSM1 and SYP could increase the diagnostic yield for NETs.

We recently reported that INSM1 is involved in cell proliferation and apoptosis in lung cancer. Knockdown of INSM1 in SCLC cell lines suppressed cell proliferation and increased the apoptosis pathway [16]. Therefore, we speculate that INSM1 has an impact on malignancy, and are conducting a prospective study to clarify whether INSM1 has relevance in regards to the prognosis of lung cancer patients. In the present study, 3 cases of grade 3 gastrointestinal NETs and 2 cases of the pancreas were examined. Grade3 NET is classified as malignant or poorly differentiated, and had a propensity for lower INSM1 expression compared to grade 1 and grade 2 NETs in the present study. Our hypothesis stated that high expression levels of INSM1 should have poor prognostic outcomes for patients; however, for grade 3 NETs it was found that INSM1 was expressed at low levels. This may be due to the fact that this was a small pilot study in which our analysis included only a small number of cases. There are a lot of genes known as either tumor suppressors or oncogenes. More samples of NETs are needed in order to continue with additional experiments which will provide a better understanding of the significance of patient prognosis using INSM1 marker.

In conclusion, we found that INSM1 is a superior immunohistochemical biomarker when

compared to the traditional NE markers (CGA, SYP, and CD56). In addition, we showed that INSM1 is involved in NE differentiation in medullary thyroid carcinoma, pheochromocytoma, intestinal NE carcinoma, islet cell tumor, pituitary tumor, and SCLC cell lines. Further study of INSM1 in NETs may provide the significance of INSM1 as a NE diagnostic marker and the biological meaningfulness. The present study hopefully improves the diagnostic yield of NETs and promotes our understandings of INSM1.

Acknowledgements

This study was partially supported by a Grantin-Aid for Scientific Research (C; No. 23220010) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Disclosure of conflict of interest

None.

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