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Insulinoma-associated protein 1 (INSM1) – new nuclear marker of neuroendocrine differentiation with high sensitivity and specificity in immunohistochemical diagnostics of neuroendocrine neoplasms – review

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ABSTRACT

Diagnostically difficult cases of neuroendocrine neoplasms require the use of markers of neuroendocrine differentiation. However, even the use of traditional neuroendocrine markers such as synaptophysin, chromogranin, and CD56 yields negative results in 10% to 25% of high-grade neuroendocrine tumors. Insulinoma-associated protein 1 (INSM1) is a novel nuclear marker of neuroendocrine differentiation. In terms of structure, INSM1 is a zinc-finger transcription factor. INSM1 (formerly IA-1) contains five zinc-finger motifs. INSM1 expresses transiently in embryonic neuroendocrine tissues. In adult tissues INSM1 has been identified in multiple tumors of neuroendocrine or neuroepithelial origin. INSM1 is a strong nuclear marker of neuroendocrine differentiation with high sensitivity and specificity. The results of the research analysed in this paper indicate that INSM1 can be very useful in the diagnostics of neuroendocrine neoplasms of the lung, gastrointestinal tract, pancreas, head and neck, uterine cervix, and Merkel cell carcinoma. In order to be included in the review, articles from PubMed (NCBI), Google Scholar, Web of Science and Scopus archive had to fit the following criteria:

- they had to be original articles, case studies and reviews connected with the following key words: neuroendocrine neoplasms, well-differentiated neuroendocrine tumors, poorly-differentiated neuroendocrine carcinomas, INSM1, traditional markers such as chromogranin, synaptophysin and CD56;
- they had to be written in English;
- they had to be published between 1992 and 2020, as the first article about insulinoma-associated protein 1 was written by Goto et al. in 1992.

INTRODUCTION

The term *neuroendocrine system* was introduced in the second half of the 20th century. The neuroendocrine system covers interactions between the nervous system and a variety of endocrine glands such as: the pituitary gland, the thyroid gland, the parathyroid gland, the adrenal gland, the ovaries and testes, the endocrine pancreas, the pineal gland, the gastrointestinal endocrine system, and the respiratory endocrine system. The endocrine/neuroendocrine cells found in these organs and systems synthesize and secrete a number of hormones that have key influence on the metabolism of the body through the interaction of these hormones with target tissues in response to stress and injury. These hormones are also involved in the control of a number of life processes such as growth, development, absorption of nutrients, energy, metabolism, water and electrolyte balance, reproduction, birth, and lactation. The endocrine/neuroendocrine cells appear in the early stages of development and are characterized by a unique pathway of differentiation. According to Lan et al., abnormal differentiation and/or deregulation of these endocrine/ neuroendocrine cells appearing in the pituitary gland, the thyroid gland, the parathyroid gland, the adrenal gland, the ovaries and testes, the endocrine pancreas, the pineal gland, the gastrointestinal endocrine system, and the respiratory endocrine system may lead to the development of neuroendocrine tumors that have a profound effect on the body′s metabolism (Lan, 2009). However, the term neuroendocrine neoplasms (NENs) includes not only tumors developing in the above mentioned organs and systems. Neuroendocrine neoplasms occur throughout the body, in all body organs, including paraganglia and soft tissue (Choi, 2018; Delaloge, 2000; Egashira, 2018; Fujino, 2015; Ramalingam, 2016; Weed, 2003). This prompted the participants of the 2017 WHO conference to accept the term neuroendocrine neoplasms for approval in relation to the classification of the types of tumors mentioned above. According to Rindi et al. the term "neuroendocrine neoplasms"

is the best at "encompassing all tumor classes with predominant neuroendocrine differentiation, including both well and poorly differentiated forms" (Rindi, 2018). Moreover, the authors stated that the "key features defining these neoplasms at any specific anatomic site are, above all, multiple anatomic sources (neural structures, endocrine organs and/or neuroendocrine cells), morphology, and the expression of markers of neuroendocrine differentiation (general and specific)". The expression of neuroendocrine markers may fundamentally differ in different anatomic sites, and at the same time, expression depends on the degree of differentiation. Therefore, different general neuroendocrine markers to define neuroendocrine differentiation are currently applicable in different organs and systems (e.g. only chromogranin and synaptophysin in the gastrointestinal system and pancreas, while chromogranins, synaptophysin, and CD56 in the lung) (Rindi, 2018). According to the latest WHO classification from 2017, neuroendocrine neoplasms include well-differentiated neuroendocrine tumors (NETs) designated carcinoid tumors in some systems, as well as poorly-differentiated neuroendocrine carcinomas (NECs), including two separate morphologic types defined as small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma (Kim, 2016, Lloyd, 2017, Rindi, 2018). The above classification was accepted and adopted by the American Joint Committee on Cancer (8^{th} edition) and the current College of American Pathologists guidelines (Amin, 2017; Burgart, 2020; Shi, 2017; Shi, 2020). Moreover, it was proposed that well-differentiated neuroendocrine tumors be classified in three tiers as G1, G2 or G3 which reflects low-grade, intermediate-grade, and high-grade (Rindi, 2018). Unlike NETs, NECs are always high grade (G3). On the other hand, three grading parameters such as: mitotic count and/or Ki-67 cell labeling index, and/or the presence or absence of necrosis are prognostic (Rindi, 2018). For this reason, the above division of neuroendocrine neoplasms is based on genetic evidence at specific anatomic sites and differences in epidemiology, histology, clinical course and prognosis. NETs, belonging to the family of well-differentiated neoplasms, have potential to metastasize or invade the adjacent tissues depending on tumor site, type, and grade (Klimstra, 2010; Klöppel, 2017). In turn, NECs are characterized by a high degree of malignancy, a very rapid and aggressive course and a poor prognosis. NENs are among a relatively rare group of tumors in the population, ranging from 2.5 to 5 cases per 100,000 people per year (Rodriguez, 2018; Rosenbaum, 2015). However, in recent years, a gradual increase in the incidence of neuroendocrine neoplasms has been observed both in the United States and in other countries (Dasari, 2015; Hallet, 2015; Hauso, 2008). The incidence of NENs has been increasing at all sites, stages and grades (Dasari, 2015), with the main sites of development in the human body affecting the gastrointestinal system and respiratory system (Oronsky, 2017). NENs occur in the population in all age groups, but the highest number of NENs is observed in patients aged 65 years and older (Dasari, 2015).

According to the latest WHO classification from 2017, neuroendocrine neoplasms have epithelial or neuronal/neuroectodermal origin, and share major morphological and protein expression signatures depending on differentiation, despite their different localization in the body (Rindi, 2018). While NENs are characterized by a diverse spectrum of proteins, many of these proteins are identical to proteins present in normal cells, organs or systems with different anatomical localization. Different localization in the body and origin make that NENs a heterogenous group of tumors, yet they share some common features, including presence of neurosecretory granules and typically showing a characteristic histology and immunoprofile (Rindi, 2018). The characteristic markers of general neuroendocrine differentiation occurring in NENs include chromogranin A, chromogranin B, and synaptophysin, as well as site specific markers such as hormones and transcription factors (Inzani, 2017). The following immunohistochemical markers of neuroendocrine differentiation are traditionally used in immunohistochemical diagnostics: synaptophysin, chromogranins and CD56. These immunohistochemical markers are characterized by a relatively low sensitivity and specificity. Research results indicate that synaptophysin shows expression in only 41% to 75% of small cell lung carcinoma (SCLC) and from 58% to 85% of large cell lung carcinoma (LCNEC) cases, chromogranin shows expression in only 23% to 58% of SCLC and 42% to 69% of LCNEC and CD56 showed expression from 72% to 99% of SCLC and 72% to 94% of LCNEC (Hamanaka, 2014; Jiang, 1998;

Kaufmann, 1997). Since none of the above immunohistochemical markers are sufficiently sensitive and specific, they must be used in immunological diagnostics as a group. This creates a situation where diagnostics is overly complicated and expensive. Therefore, the search for a single immunohistochemical marker with high specificity and sensitivity that could be used in the diagnostics of neuroendocrine neoplasms has been going on for many years. Insulinoma-associated protein 1 (INSM1) may fulfill these expectations.

NEW INSIGHT

STRUCTURE AND FUNCTION OF INSULINOMA-ASSOCIATED PROTEIN 1

Insulinoma-associated protein 1 is a zinc-finger transcription factor. At the same time, the protein structure of INSM1 is highly conserved among homologues of different species. INSM1 (formerly IA-1) contains five zinc-finger motifs. Based on the deduced protein sequence, INSM1 can be divided into two major domains. The aminoterminal domain (aa 1-250) contains a high percentage of proline, glycine, and alanine residues. Prolinerich (20-30%) sequences occur in many mammalian transcription factors and serve as protein-protein interacting domains that mediate both transcriptional activation and/or repression (De Caestecker, 2000; Zilfou, 2001). The dibasic amino acids are cleavage recognition sites for processing peptide hormone precursors such as insulin, glucagon, somatostatin and pancreatic polypeptide. An α-amide group is common to many bioactive neuroendocrine peptides. The carboxyl-terminal sequence (aa-251-510) contains five putative Cys2-His2-type zinc-finger motifs. These five zinc-finger motifs are symmetrically spaced at the carboxy terminus. Two tandem repeated zinc-finger motifs from either end are spaced by 45/46 aa from the middle zinc finger (Lan, 2009). The structural features of INSM1 indicates that INSM1 is a zinc-finger DNA-binding protein. INSM1 functions as a transcriptional repressor that simultaneously regulates entry into the cell cycle and controls expression of a neuroendocrine phenotype (Lan, 2009). Moreover, INSM1 is directly responsible for the transcription of synaptophysin and chromogranin (Fujino, 2015). In contrast, INSM1 is regulated by neurogenin 3 (Mellitzer, 2006).

INSM1 shows expression mainly in normal fetal neuroendocrine tissues and tumors of neuroendocrine origin regardless of age. In the fetal period, INSM1 is predominantly expressed in the nervous system in mammals, and plays an important role in early embryonic neurogenesis (Lan, 2009). Moreover, in the fetal period, INSM1 plays an important role in the development of normal neuroendocrine cells in various tissue throughout the body, mainly in the pancreas, digestive system and central nervous system (Gierl, 2006; Goto, 1992; Lan, 1993; Lan, 2009; Xie, 2002). It was found that INSM1 affects both terminal cellular differentiation and cellular proliferation in the pancreas (Gierl, 2006; Osipowich, 2014; Parent, 2008; Zhang, 2012; Zhu, 2002), enteroendocrine cells (Gierl, 2006), the autonomic nervous system (Widner, 2008), the central nervous system (Farkas, 2008; Jacob, 2009), olfactory epithelium (Rosenbaum, 2011), and the pituitary gland (Welcker, 2013). Moreover, INSM1 regulates downstream target genes and exhibits extranuclear activities associated with multiple signaling pathways, including Sonic Hedgehog, PI3K/AKT, MEK/ERK, ADK, p53, Wnt, histone acetylation, LSD1, cyclin D1, Asc1, and N-myc (Chen, 2018; Chen, 2019). However, a disadvantageous phenomenon is that INSM1 expression declines with age (Goto, 1992).

What is interesting, however, is that when it comes to tumors, the presence of INSM1 can be found in a number of tumors with neuroendocrine differentiation, such as pheochromocytoma (Sandgren, 2010), medullary thyroid carcinoma, pituitary adenoma (Goto, 1992), hypothalamic hamartoma (Parent, 2008), retinoblastoma, small cell lung carcinoma (Amelung, 2010; Goto, 1992; Lan, 1993; Taniwaki, 2006), and medulloblastoma (Breslin, 2002; De Smaele, 2008; Gilbertson, 2004; Pomeroy, 2002). INSM1 was found not only in human tumors but also in mice and rats (Farkas, 2008; Jacob, 2009; Kawaguchi, 2008; Xie, 2002). Initially, it was thought that INSM1 did not appear in the normal tissue of adults (Breslin, 2003; Duggan, 2008; Gierl, 2006; Goto, 1992; Welcker, 2013; Widner, 2008; Zhu, 2002). However, further research has shown INSM1 expression in normal adult cells such as neuroendocrine cells present in the gastrointestinal tract, pancreatic tract, bronchopulmonary system, adrenal medullary tissues, and in occasional individual cells in nonneoplastic prostate glands

(Ames, 2018; Rosenbaum, 2015; Yoshida, 2018).

INSM1 is encoded by the insulinoma associated-1 (IA-1) gene of cDNA. This gene was first identified by Goto et al. in 1992 in human pancreatic insulinoma tissues and murine insulinoma cell lines, which influenced its name (insulinoma associated protein 1) (Goto, 1992). However, the localization of the *INSM1* gene at the start arm of chromosome 20 was revealed by Lan et al. two years later (Lan, 1994). Research conducted on human lung cancer cell lines has shown that *INSM1* gene expression occurs in small cell lung carcinoma and carcinoid tumors, while expression of this gene does not occur in non-small cell lung carcinoma (Lan, 1993). Subsequent studies have shown that the expression of *INSM1* gene is not limited to small cell lung carcinoma but also occurs elsewhere of the body, including neuroendocrine tumors of the gastrointestinal tract, cervical cancer, prostate cancer, pheochromocytoma, medullary thyroid carcinoma, insulinoma, or pituitary tumors (De Smaele, 2008; Gilbertson, 2004; Goto, 1992; Parent, 2008; Pomeroy, 2002; Sandgren, 2010; Xin, 2018).

INSM1 AS IMMUNOHISTOCHEMICAL AND MOLECULAR MARKER

INSM1 shows high expression in tumors of neuroendocrine origin, with INSM1 expression significantly increased in neoplastic tissue compared to non-neoplastic tissue (Doxtader, 2018; Lan, 2009; Nakra, 2019; Rodriguez, 2018; Rosenbaum, 2015). Moreover, research conducted by many authors has confirmed that INSM1 is a strong nuclear, immunohistochemical marker of neuroendocrine differentiation in neoplastic human tissues (González, 2019; Rosenbaum, 2015; Roy, 2019; Staaf, 2020; Viswanathan, 2019). For this reason, INSM1, the only available nuclear neuroendocrine marker, is increasingly used in immunohistochemistry diagnostics (Ames, 2018; Kuji, 2017; Rooper, 2018; Rosenbaum, 2015; Xin, 2018). INSM1 in immunohistochemical staining gives a positive nuclear reaction, in contrast to synaptophysin and chromogranin, which show a granular cytoplasmic reaction. In turn, CD56 is both cytoplasmic or membrane positive.

REVIEW AND DISCUSSION

NEUROENDOCRINE NEOPLASMS IN THE LUNG

In one of the first large studies involving 111 primary thoracic neuroendocrine neoplasms (small cell carcinoma, large cell carcinoma, atypical carcinoid, typical carcinoid and mediastinal paraganglioma) and 156 non-neuroendocrine tumors (adenocarcinoma, and squamous cell carcinoma), the authors assessed immunohistochemistry sensitivity and specificity of INSM1 in surgical specimens and compared its performance to traditional neuroendocrine markers (synaptophysin, chromogranin and CD56) (Rooper, 2017). For this purpose, they used material from the surgical pathology archives from 1997-2017, but did not include thoracic mixed tumors in the study. In the presented study, the sensitivity of INSM1 for small cell lung carcinomas and large cell neuroendocrine carcinomas was significantly higher (94%, 91.3%) than the sensitivity of the panel of the three traditional neuroendocrine markers (74.4%, 78.3%). In addition, the authors found positive staining for INSM1 in all the atypical carcinoids, typical carcinoids and mediastinal paragangliomas. The sensitivity of INSM1 across all grades of thoracic neuroendocrine tumors was 96.4%, and significantly exceeded the sensitivity of the panel of traditional neuroendocrine markers (87.4%). However, in non-neuroendocrine tumors staining positive for INSM1, they observed only 3.3% of adenocarcinomas and 4.2% of squamous cell carcinomas.

In another large study, researchers examined a large series of whole-tissue sections of primary lung neoplasms (345), including 152 neuroendocrine tumors (64 small cell lung carcinomas, 24 large cell neuroendocrine carcinomas, 48 typical carcinoid tumors, 16 atypical carcinoid tumors), and 163 non-neuroendocrine tumors (130 adenocarcino-mas, 33 squamous cell carcinomas) for sensitivity and specificity of INSM1 (Mukhopadhyay, 2019). The analyzed material also included mixed tumors. In this study, the sensitivity of INSM1 for neuroendocrine neoplasms *as a group* (95%) was similar to synaptophysin and CD56 (98%, 97%), but higher than chromogranin (84%). In contrast, the specificity of INSM1 and chromogranin (97%, 98%) was higher than the specificity of synaptophysin and CD56 (90%, 87%). The sensitivity of INSM1 in small cell carcinoma was similar to the sensitivity of synaptophysin and CD56 (98%, 100% and

95%), but was higher than the sensitivity of chromogranin (83%). For large cell neuroendocrine carcinomas, similar sensitivity for CD56 and synaptophysin (92%, 88%) was observed, while the sensitivity of INSM1 and chromogranin was unquestionably less (75%, 46%). Except for one case of atypical carcinoid tumor, all carcinoid tumors were positive for INSM1, chromogranin, synaptophysin and CD56.

A third study looked at surgically resected 54 primary lung neuroendocrine tumors (including 24 small cell lung carcinomas, 23 large cell lung carcinomas, 5 typical carcinoid tumors and 2 atypical carcinoid tumors) as well as 623 non-small cell lung carcinomas (Staaf, 2020). There were also mixed tumors in the material studied. Here, the authors determined the diagnostic value of INSM1 in comparison to the previously used traditional neuroendocrine markers (CD56, synaptophysin and chromogranin A). They observed positive INSM1 staining in 39 cases of 54 pulmonary neuroendocrine tumors (72%) and in 6 cases of 623 non-small cell lung carcinomas (1%). On the other hand, a positive CD56 staining for primary lung neuroendocrine tumors and non-small cell lung carcinomas were 47 of 54 (87%) and 14 of 626 (2%), for synaptophysin 46 of 54 (85%) and 49 of 630 (8%), and for chromogranin A 30 of 54 (56%) and 6 of 629 (1%).

Other authors tested for whether INSM1 could be used in cytology (Cellient) cell blocks and whether these results correlated with surgical pathology specimens (Doxtader, 2018). The aim was to compare the sensitivity and specificity of INSM1 with the sensitivity and specificity of synaptophysin, chromogranin and CD56. The study was conducted on seventy-four primary lung neoplasms, including 52 primary lung neuroendocrine neoplasms (41 small cell lung carcinomas, 1 large cell neuroendocrine carcinoma, 10 carcinoid tumors) and 22 non-neuroendocrine primary lung tumors (11 adenocarcinomas, 9 squamous cell carcinomas, 1 mesothelioma, and 1 poorly differentiated non-small cell lung carcinoma). In 20 cases, INSM1 staining was performed simultaneously on paired surgical pathology specimens (biopsy or resection). The specimens tested positive for INSM1 in all 20 paired surgical pathology cases. However, in cytology cell blocks, positive INSM1 results were found in 48 of 52 cases of primary lung neuroendocrine neoplasms (92%), including 38 of 41 small cell lung carcinomas (93%), in one case large cell neuroendocrine carcinoma (100%) and in 9 cases out of 10 carcinoid tumors (90%). The specificity of INSM1 for primary pulmonary neuroendocrine neoplasms *as a group* was identical to the specificity of chromogranin (100%), but was higher than the specificity of synaptophysin (95%) and CD56 (95%).

In another study, the authors compared the diagnostic utility of INSM1, CD56, synaptophysin and chromogranin in the largest cohort (143) of pulmonary cytology cell blocks (11 typical carcinoid tumors, 11 atypical carcinoid tumors, 9 small cell lung carcinomas, 8 large cell neuroendocrine carcinomas, 9 squamous cell carcinomas and 95 adenocarcinomas) and the largest available material (563) of surgical specimens including 17 typical carcinoid tumors, 14 atypical carcinoid tumors, 8 small cell lung carcinomas, 10 large cell neuroendocrine carcinomas, 58 squamous cell carcinomas, 415 adenocarcinomas, 17 large cell carcinomas and 24 other tumor types (Viswanathan, 2019). These authors obtained sensitivity and specificity for INSM1 of 92.3% and 100% in the cytology cell blocks, while the sensitivity and specificity for INSM1 in the surgical specimens was lower (89.8%, 98.1%). The sensitivity and specificity for CD56 were 97.4% and 93.3% in the cytology cell blocks and 93.9% and 93.6% in the surgical specimens. The sensitivity and specificity for synaptophysin and chromogranin were significantly lower in both the cytology cell blocks and the surgical specimens.

In the next study, the authors performed manual immunohistochemistry on small biopsies of INSM1 and immunocytochemistry on direct smears of INSM1 on archival material from 60 patients diagnosed with small cell lung carcinoma in order to check the suitability of each of these methods in the diagnostics of this tumor (Nakra, 2019). Of these 60 patients, 37 were tested for INSM1 immunohistochemistry on small biopsies and 36 were tested for INSM1 immunocytochemistry on direct smears. The sensitivity for INSM1 immunohistochemistry (small biopsies) was 97% (36 of 37 cases), while the sensitivity for INSM1 immunocytochemistry (direct smears) was lower, only 91% (30 of 33 cases). Moreover, INSM1 reactions were performed on 10 cases of non-small cell lung carcinoma on spare direct smears and on small biopsies, obtaining 100% specificity (all cases were negative for INSM1).

In yet another study, the authors performed immunohistochemistry staining for INSM1 on cytology samples from 32 patients with neuroendocrine tumors of the lung (8) and tumors with neuroendocrine differentiation of lung origin (22 lymph node, 1 chest wall mass, 1 thyroid) (Rodriguez, 2018). All the neuroendocrine tumors used in the study were small cell carcinomas. The material taken was from multiple aspirations. INSM1 was positively identified in 31 of 32 cases (97%). In the control group of non-neuroendocrine tumors all 13 cases were negative for INSM1. The sensitivity of CD56 in small cell carcinoma was 96%.

NEUROENDOCRINE NEOPLASMS IN THE GASTROINTESTINAL TRACT AND PANCREATICOBILIARY TRACT

In a retrospective study covering the archive material from 2013-2015, the authors examined 30 patients with primary gastroenteropancreatic neuroendocrine neoplasms and their metastatic diseases in the liver in terms of INSM1 sensitivity assessment and compared it with the sensitivity of chromogranin-A and synaptophysin (Gonzalez, 2019). Moreover, they assessed the changes in the expression of these markers in the material from primary and metastatic diseases. Most of the cases studied were small intestine and neoplasms were present in ileum, duodenum, Meckel′s diverticulum, pancreas, stomach, rectum and caecum. All studied cases of primary gastroenteropan-creatic neuroendocrine neoplasms were reactive for INSM1 and synaptophysin (100%), while the sensitivity of chromogranin-A was weaker (97%). In the material from metastatic neoplasms, sensitivity of INSM1 was weaker (94%) than the sensitivity of synaptophysin (100%) and chromogranin-A (97%). The specificity of INSM1 (96%) was comparable to the specificity of chromogranin-A (97%), and higher than that of synaptophysin (54%).

In turn, other authors compared the sensitivity and specificity of INSM1 with other neuroendocrine markers (synaptophysin, chromogranin and CD56) and the performance of the antibody according to site and differentiation of the tumor (Rodriguez, 2018). This study was performed using 134 specimens, including 91 neuroendocrine tumors with neuroendocrine features (taken from pancreas, liver, gastric and perigastric mass, abdomen, parotid gland, and other organs such as: lymph node, lung, soft tissue, vertebra, buttock, soft tissue of vagina, and pelvic wall). In this material, INSM1 showed a sensitivity of 99% and a specificity of 97%, while CD56 had a sensitivity only slightly lower (95.5%), but the specificity was very low (69.2%). In contrast, chromogranin had the weakest sensitivity (82.5%), while synaptophysin had the weakest specificity (66.7%). In contrast, among 10 cases diagnosed as non-neoplastic lesions, only two cases (pancreatic neuroendocrine islet cells and benign adrenal cells) were positive for INSM1.

In another study, the authors assessed the sensitivity and specificity of INSM1 in material covering 110 cases of primary neuroendocrine neoplasms of the gastrointestinal tract, appendix, and pancreas (McHugh, 2020). At the same time, they performed a sensitivity and specificity check of synaptophysin, chromogranin, CD56 and Ki67. INSM1 was positive in 89 of 110 (80.9%) primary gastrointestinal, appendiceal and pancreatic neuroendocrine neoplasms, while synaptophysin was positive 99.1%, chromogranin 88%, CD56 95.3%. In contrast, the specificity of INSM1 (95.7%) was higher than that of synaptophysin (86.0%), chromogranin (87.3%), and CD56 (86.0%).

Other authors studied INSM1 in conjunction with chromogranin, synaptophysin, and CD56 in 36 appendiceal adenocarcinoma ex-goblet carcinoid (21 primaries, 15 metastases) (Yang, 2019). In primary adenocarcinoma ex-goblet carcinoid, they obtained positive results for INSM1 62%, for chromogranin 86%, for synaptophysin 86% and for CD56 47%. In contrast, metastatic adenocarcinoma exgoblet carcinoid showed staining for INSM1 53%, for chromogranin 73%, for synaptophysin 80% and for CD56 21%.

NEUROENDOCRINE NEOPLASMS OF PANCREAS

In a retrospective study, the authors examined the usefulness of INSM1 for identifying pancreatic neuroendocrine tumors in 26 cell blocks and 29 surgical resections (Kim, 2020). Additionally, they performed INSM1 staining in other primary pancreatic tumors such as solid pseudopapillary neoplasms (14 cases), 11 acinar cell carcinomas and 21 pancreatic ductal adenocarcinomas. They obtained in all 55 cases of pancreatic neuroendocrine tumors a positive

nuclear test for INSM1, both in cell blocks and surgical resections (100% sensitivity), while sensitivity of synaptophysin was 97%, chromogranin 92%, and CD56 85%.

In turn, other authors assessed the expression of INSM1 in 14 cytology specimens obtained from endoscopic ultrasound-guided fine needle aspiration cytology during diagnostics of pancreatic neuroendocrine tumors (Takase, 2018). These authors used cytological specimens from 15 cases diagnosed as pancreatic ductal adenocarcinoma as a control group. In all 14 pancreatic neuroendocrine tumor cases, INSM1 showed expression in the tumor cells (100% sensitivity). In the control group, these authors observed INSM1-expressing cells within the adenocarcinoma cell cluster, but found no expression of INSM1 in the pancreatic duct cells or acinar cells.

The presented study was aimed at detected INSM1, chromogranin, synaptophysin and neural cell adhesion molecule immunohistochemically, in 25 cases of pure pancreatic neuroendocrine tumors and 2 mixed adenoneuroendocrine carcinomas (Tanigawa, 2018). As a control group, they used 5 cases of solid-pseudopapillary neoplasm, 7 cases of acinar cell carcinoma, and 15 cases of pancreatic ductal adenocarcinoma. These authors found the nuclear expression of INSM1 in all pure pancreatic neuroendocrine tumors (100% sensitivity). In 2 cases of mixed tumor the neuroendocrine carcinoma component was positive for INSM1, while the adenocarcinoma component was negative for INSM1. All control cases were negative for INSM1, while they were positive for synaptophysin.

NEUROENDOCRINE NEOPLASMS OF SKIN

In one study, the authors assessed INSM1 staining on 56 cases of Merkel cell carcinoma (47 primary tumors and 9 nodal metastases) (Lilo, 2018). All 56 cases of Merkel cell carcinoma showed expression of INSM1 (100% sensitivity). In contrast, synaptophysin, cytokeratin and chromogranin in the same material had expressions of 96%, 92% and 32%. In the control group (50 cases included various non-Merkel cell carcinoma neoplasms), no positive staining for INSM1 was found in any case.

In turn, other researchers developed their own dual immunohistochemistry protocol for INSM1/ cytokeratin 20 to detect dual expression of keratin and INSM1 on 15 small samples taken from Merkel cell carcinoma (Rush, 2018). They detected INSM1 in 14 of 15 specimens carrying a diagnosis of Merkel cell carcinoma (93% sensitivity). On the other hand, one specimen that was negative for INSM1 was also negative for cytokeratin and chromogranin, with only focal positivity for synaptophysin. Moreover, they checked the sensitivity of INSM1 in three other specimens of cutaneous neuroendocrine carcinoma (non-Merkel cell carcinoma) and obtained 100% sensitivity for INSM1. However, of the 8 cutaneous non-neuroendocrine neoplasms tested, only one tested positive for INSM1.

NEUROENDOCRINE NEOPLASMS OF THE HEAD AND NECK

Researchers performed INSM1 immunohistochemistry on 97 neuroendocrine tumors and 626 non-neuroendocrine tumors across all histologic grades and anatomic subsites of the head and neck (Rooper, 2018). These authors obtained the sensitivity of INSM1 99.0%, with a positive result for INSM1 they observed in all types of head and neck neuroendocrine tumors (middle ear adenoma, pituitary adenoma, paraganglioma, medullary thyroid carcinoma, olfactory neuroblastoma, small cell carcinoma, large cell neuroendocrine carcinoma, and sinonasal teratocarcinosarcoma). These authors obtained 97.6% specificity for INSM1 in almost all non-neuroendocrine tumors.

NEUROENDOCRINE NEOPLASMS OF THE UTERINE CERVIX

In one study, the authors made an immunohistochemical assessment of conventional neuroendocrine markers (chromogranin, synaptophysin and neural cell adhesion molecule) and INSM1 by analyzing 37 cases of high-grade neuroendocrine carcinoma of the uterine cervix (Kuji, 2017). These authors obtained the highest sensitivity (95%) for INSM1, while sensitivity for both chromogranin and synaptophysin was 86% and for neural cell adhesion molecules only 68%.

In turn, other authors, examining malignant tumors with neuroendocrine differentiation from the gynecologic organs, assessed the expression

of INSM1, synaptophysin, chromogranin, CD56, orthopedia homeobox and achaetescute homolog 1 in 2 cases in the uterine cervix (Roy, 2019). They obtained 100% sensitivity for INSM1, 100% for synaptophysin, 100% for CD56, 50% each for chromogranin and achaetescute homolog 1, and negative for orthopedia homeobox.

NEUROENDOCRINE TUMORS OF THE PROSTATE

In this study, the authors checked the expediency of the use of INSM1 in the diagnostics of neuroendocrine tumors of the prostate (Xin, 2018). They performed immunohistochemical tests on 13 needle biopsies of primary small cell carcinoma of the prostate, 5 samples of mixed small cell neuroendocrine carcinoma-acinar adenocarcinoma obtained from prostatectomy and 2 cases of metastatic small cell carcinoma. These authors obtained positive results for INSM1 in 12 cases of primary small cell carcinoma (92.3%), while the reactions for synaptophysin (84.6%) and chromogranin (53.8%) were weaker. In the remaining 5 cases of mixed tumors and 2 metastatic tumors sensitivity of INSM1 was 100%, similarly for synaptophysin, while the sensitivity of chromogranin (80%) was weaker. The test of the specificity of INSM1 was performed on the material including benign prostatic hyperplasia and prostate adenocarcinoma, in most cases they did not find nuclear reactivity for INSM1.

In turn, Roy et al. assessed the usefulness of INSM1 in immunohistochemical diagnostics – 32 cases included malignant tumors with neuroendocrine differentiation from the gynecologic organs, including prostate gland (n = 6) (Roy, 2019). Out of 4 examined cases of prostate adenocarcinoma with neuroendocrine differentiation, they obtained a positive result for INSM1 in 25%. However, for synaptophysin and Cd56 they obtained a positive result in 50%, and chromogranin was negative in all cases.

OTHER RARE LOCALIZATION OF NEUROENDOCRINE TUMORS

NEUROENDOCRINE NEOPLASMS OF THE URINARY BLADDER

In the presented study, the authors assessed the immunohistochemical expression of INSM1 on 32 whole sections of small cell neuroendocrine carcinoma of the urinary bladder and compared INSM1 expression with synaptophysin, chromogranin and CD56 (Kim Jr, 2020). In 28 cases these authors obtained a positive result for INSM1, in 24 cases for CD56, in 19 cases for synaptophysin, and in 14 cases for chromogranins.

NEUROENDOCRINE NEOPLASMS OF THE BREAST

In another study, the authors compared the expression of INSM1, orthopedia homeobox, chromogranin, synaptophysin, CD56 and achaetescute homolog 1 in invasive mammary carcinoma (Roy, 2019). In the material studied, they found the strongest expression for achaetescute homolog 1 and synaptophysin (85.7%) and weaker for INSM1, chromogranin, and CD56 (71.4%). In contrast, the expression of orthopedia homeobox was negative.

PERIPHERAL NEUROBLASTIC TUMORS

In another study, the authors assessed the immunohistochemical profile of INSM1 in cases of peripheral neuroblastic tumors and compared INSM1 expression in these tumors to that seen in other embryonal neoplasms (non-neuroblastic tumors) (Wang, 2019). Nuclear expression of INSM1 was 78% in peripheral neuroblastic tumors, including in neuroblastomas 84%, in ganglioneuroblastomas 100%, and in ganglioneuromas 33%. In the non-neuroblastic tumors control group, these authors found INSM1 expression in rhabdomyosarcomas (50%), in nephroblastomas (32%), and in Ewing sarcomas (20%).

PRIMARY CENTRAL NERVOUS SYSTEM NEOPLASMS

Other authors checked INSM1 expression in primary central nervous system neoplasms (Ames, 2018). They obtained nuclear immunostaining for INSM1 in medulloblastomas (87%), while diffuse nuclear INSM1 immunostaining was observed in all central neurocytomas and pituitary adenomas. However, they found rare staining with INSM1 in other high-grade embryonal tumors and high-grade gliomas. These authors observed nuclear INSM1 staining in normal brain tissue only in early neuronal development, while they did not find nuclear INSM1 staining in adult normal brains, including areas of gliosis.

In typical cases, when the diagnostics of neuroendocrine neoplasms is not difficult, it is based on standard histologic and cytologic stains and there is no need to perform immunohistochemical testing (Mukhopadhyay, 2019). In diagnostically difficult cases, when the clinical picture of the disease and the histologic features of the examined tumor are not typical and differ from the accepted norm, immunohistochemical reactions are performed, thanks to which it is possible to identify the neuroendocrine differentiation, enabling the classification of neuroendocrine tumors. Currently, three conventional markers of neuroendocrine differentiation (synaptophysin, chromogranin, and CD56) are used in the histopathological diagnostics of neuroendocrine neoplasms, but the test result does not always give an explicit answer to the type of tumor present. This is due to the fact that synaptophysin is sensitive, but not specific enough, chromogranin is highly specific, while its sensitivity is very weak, and CD56 is highly sensitive, but due to its limited specificity it may stain a variety of non-neuroendocrine tumors. Even the use of a combination of these markers on surgical specimens or cytology specimens gives negative results in 10% to 25% of high-grade neuroendocrine tumors (Hamanaka, 2014, Maleki, 2012, Nicholson, 2002, Travis, 2015, Zheng, 2013). There is therefore a need to find a new neuroendocrine marker that would demonstrate both high sensitivity and specificity. The use of INSM1 in histopathological diagnostics of neuroendocrine neoplasms, which is the only nuclear neuroendocrine marker with high sensitivity and specificity so far, gives hope for a more accurate diagnosis in diagnostically difficult cases. However, the results of studies on the usefulness of INSM1 in the diagnostics of neuroendocrine neoplasms are not conclusive.

Neuroendocrine neoplasms are mainly located in the respiratory system and digestive system, with 25% of primary lung neoplasms being neuroendocrine tumors, 75% of which are mixed neuroendocrine tumors containing also a non-neuroendocrine component (Gustafsson, 2008). These tumors are characterized by very high mortality (Friedberg, 1997, Travis, 1998). Investigating all primary lung neuroendocrine neoplasms on surgical specimens Rooper et al. and Mukhopadhyay et al. obtained high sensitivity of INSM1 (96.4% and 95%) (Mukhopadhyay, 2019, Rooper, 2017). Similar results were also obtained by Doxtader et al. and Viswanathan et al. comparing the sensitivity of INSM1 of primary lung neuroendocrine neoplasms in cytology cell blocks (92%), (92.3%) with surgical specimens (100%), (89.8%) (Doxtader, 2018, Viswanathan, 2019). In contrast, the study completed by Staaf et al. on surgical specimens found much weaker sensitivity of INSM1 (72%), which may be due to the fact that they had a much smaller number of cases than the other authors (Doxtader, 2018, Mukhopadhyay, 2019, Rooper, 2017, Staaf, 2020, Viswanathan, 2019). In turn, Rooper et al. found a significantly higher sensitivity of INSM1 for neuroendocrine lung neoplasms *as a group* compared to each individual neuroendocrine marker (synaptophysin, chromogranin and CD56) (Rooper, 2017). None of the other authors observed statistically significant differences when comparing the sensitivity of INSM1 with the sensitivity of individual neuroendocrine markers (Doxtader, 2018; Mukhopadhyay, 2019; Staaf, 2020; Viswanathan, 2019). In their research, both on cytology specimens and surgical specimens, sensitivity of INSM1 for neuroendocrine lung neoplasms *as a group* was similar to synaptophysin and CD56, and statistically higher than chromogranin (Doxtader, 2018, Mukhopadhyay, 2019, Staaf, 2020. Viswanathan, 2019). Rooper et al. also found a significantly higher sensitivity of INSM1 compared to all three markers (synaptophysin, chromogranin and CD56) treated *as a group* (Rooper, 2017), while in the study by Mukhopadhyay et al. and Kriegsmann et al. sensitivity of INSM1 (95%, 76%) was weaker than the sensitivity of the traditional three neuroendocrine markers treated *as a group* (100%, 97%) (Kriegsmann, 2020, Mukhopadhyay, 2019). The observed differences may be due to the fact that the study by Rooper et al. excluded mixed neuroendocrine tumors from their material, while the other authors also examined mixed primary lung neoplasms with non-neuroendocrine component (Doxtader, 2018, Kriegsmann, 2020, Mukhopadhyay, 2019, Rooper, 2017, Staaf, 2020. Viswanathan, 2019).

Results obtained by Mukhopadhyay et al. regarding sensitivity of INSM1 for small cell lung carcinoma (98%) are comparable to the data reported by Rooper et al. (94.9%), Rosenbaum et al. (100%) and Fujino et al. in surgical specimens (100%), Doxtader et al. on cytology cell blocks (93%), Nakra et al. and

Rodriguez et al. on small biopsies (97%) and on cytology specimens (91%) (Fujino, 2015; Mukhopadhyay, 2019; Nakra, 2019; Rodriguez, 2018; Rooper, 2017; Rosenbaum, 2015). In material derived from carcinoid tumors Mukhopadhyay et al. observed a sensitivity of INSM1 of 98% (Mukhopadhyay, 2019). This result is similar to the result obtained by Fujino et al., Rooper et.al. and Rosenbaum et al. (100%) (Fujino, 2015; Rooper, 2017, Rosenbaum, 2015). In contrast, the sensitivity of INSM1 in relation to large cell neuroendocrine carcinoma described by Mukhopadhyay et al. (75%) was significantly lower than the results obtained by Rooper et al. (91.3%) (Mukhopadhyay, 2019; Rooper, 2017). It is difficult to explain the reason for such a large disparity between the two studies, as both authors used the same clone (A8) from the same company (Santa Cruz). Perhaps the reason may be the slight difference in methodology. The dilution of INSM1 (1:250) used by Mukhopadhyay et al. was weaker than in the Rooper study (1:200). In Mukho-padhyay's study, the antibody was dispensed manually. Mukhopadhyay et al. used Ventana's Optiview detection kit with the optional amplifer, while Rooper et al. used Ventana's UltraView detection kit.

Doxtader et al. observed that the specificity of INSM1 for pulmonary neuroendocrine neoplasms in cytology cell blocks was similar to the specificity of chromogranin (100%) and higher than the specificity of synaptophysin (95%) and CD56 (95%) (Doxtader, 2018). Similarly, Viswanathan et al. in cytology specimens found the same specificity for INSM1, synaptophysin and chromogranin (100%) and weaker specificity for CD56 (Viswanathan, 2019). In surgical specimens, Rooper et al. and Mukhopadhyay et al. observed high values of the specificity of INSM1 (96.2%), (97%), synaptophysin (96.8%), (90%) chromogranin (99.4%), (98%), and CD56 (93.7%), (87%) (Mukhopadhyay, 2019; Rooper, 2017). On the other hand, the specificity of INSM1 (87%) for primary neuroendocrine neoplasms was significantly higher compared to the three traditional neuroendocrine markers *as a group* (61%) (Mukhopadhyay, 2019). In addition, Rooper et al. found an upward trend in the specificity of INSM1 compared to the traditional panel of neuroendocrine markers, but it was not a statistically significant difference (Rooper, 2017).

The second most common site of neuroendocrine neoplasms is the digestive system, with primary neuroendocrine neoplasms having a different digestive tract localization that strongly influences the expression of INSM1. Gonzalez et al. found 100% sensitivity of INSM1 in primary gastroenteropancreatic neuroendocrine neoplasms and 94% sensitivity of INSM1 in metastatic gastroenteropancreatic neuroendocrine neoplasms (Gonzalez, 2019). Similarly, Rodriguez et al. observed 99% sensitivity of INSM1 in neuroendocrine tumors with neuroendocrine features in the digestive tract (Rodriguez, 2018). On the other hand, Rosenbaum et al. found significantly higher expression of INSM1 of midgut gastrointestinal neuroendocrine neoplasms with known metastases compared to those that had not yet metastasized (Rosenbaum, 2015). In turn, McHugh et al. who included the material from the appendix in the primary gastroenteropancreatic neuroendocrine neoplasms, obtained much weaker sensitivity of INSM1 (80.9%) compared to the results of Gonzalez et al. and Rodriguez et al. (Gonzalez, 2019; McHugh, 2020; Rodriguez, 2018). Also, Yang et al. who tested only primary appendiceal adenocarcinoma of ex-goblet cells obtained very poor sensitivity of INSM1 (62%) showing no differences compared to chromogranin, synaptophysin, and CD56 (Yang, 2019).

The results of tests by three independent teams on pure pancreatic neuroendocrine neoplasms showed 100% sensitivity of INSM1 (Kim, 2020, Takase, 2018, Tanigawa, 2018). Such high sensitivity concerned both cell blocks and surgical specimens, and it was higher than the 3 traditional neuroendocrine markers (synaptophysin 97%, chromogranin 92%, and CD56 85%). However, the disadvantage of INSM1 was the fact that in the case of pancreatic non-neuroendocrine tumors Kim et al. obtained positive staining in pancreatic solid pseudopapillary neoplasms, while Tanigawa et al. in the same tumor type observed no positive staining for INSM1 (Kim, 2020, Tanigawa, 2018). On the other hand, Takase et al. demonstrated the presence of INSM1 in pancreatic non-neuroendocrine tumors (pancreatic ductal adenocarcinoma within adenocarcinoma cell clusters) (Takase, 2018).

A rarer localization of neuroendocrine neoplasms is skin. When studying Merkel cell carcinoma, Lilo et al. observed 100% sensitivity and

specificity for INSM1 (Lilo, 2018). Similarly, high sensitivity for INSM1 (93%) in Merkel cell carcinoma was obtained by Rush et al. (Rush, 2018). Also, neuroendocrine neoplasms of the head and neck showed high sensitivity and specificity for INSM1 (99.0%) (97.6%) (Rooper, 2018). Similarly, in the uterine cervix Kuji et al. observed sensitivity for INSM1 (95%), and Roy et al. obtained 100% sensitivity for INSM1 (Kuji, 2017, Roy, 2019).

On the other hand, the results of research on the usefulness of INSM1 in the diagnostics of neuroendocrine neoplasm of the prostate, the urinary bladder, the breast, peripheral neuroblastic tumors or primary central nervous system neoplasms require further study, as they were based on single scientific reports.

CONCLUSION

The results of the analysed studies indicate that INSM1 is a strong nuclear marker of neuroendocrine differentiation with high sensitivity and specificity. In addition, the great advantage of nuclear staining with INSM1 is that it can be performed even on very small material samples containing a few cells, and at the same time it is easy to interpret the results both in surgical specimens and cytology specimens. INSM1 can be very useful in the diagnostics of neuroendocrine lung neoplasms as the firstline marker of neuroendocrine differentiation or in combination with synaptophysin or CD56. INSM1 also appears to be very useful in the diagnostics of pure pancreatic neuroendocrine neoplasms, neuroendocrine neoplasms of the digestive system (excluding tumors from the appendix), Merkel cell carcinomas, neuroendocrine neoplasms of the head and neck and the uterine cervix. The remaining locations of neuroendocrine neoplasms, due to the very small number of cases studied, require further research. Finally, INSM1 cannot be used to differentiate neuroendocrine neoplasms, because it stains both tumor cells in small cell lung carcinoma, large cell neuroendocrine carcinoma, typical carcinoid, atypical carcinoid and mediastinal paraganglioma.

Literature

Amelung J.T., Bührens R., Beshay M., Reymond M.A. **Key genes in lung cancer translational research: a meta-analysis.** Pathobiology. 2010; 77(2):53-63.

Ames H.M., Rooper L.M., Laterra J.J., Eberhart C.G., Rodriguez F.J. **INSM1 expression is frequent in primary central nervous system neoplasms but not in the adult brain parenchyma.** J Neuropathol Exp Neurol. 2018; 77(5):374-382.

Amin M.B., Edge S., Greene F., Byrd D.R., Brookland R.K., Washington M.K. et al. (Editors). In: **AJCC cancer staging manual.** 2017; Springer International Publishing (Chicago, USA); ISBN: 978-3-319-40617-6.

Breslin M.B., Zhu M., Lan M.S. **NeuroD1/E47 regulates the E-box element of a novel zinc finger transcription factor, IA-1, in developing nervous system**. J Biol Chem. 2003; 278(40):38991-38997.

Breslin M.B., Zhu M., Notkins A.L., Lan M.S. **Neuroendocrine differentiation factor, IA-1, is a transcriptional repressor and contains a specific DNA-binding domain: identification of consensus IA-1 binding sequence**. Nucleic Acids Res. 2002; 30(4):1038-1045.

Burgart L.J., Shi C., Adsay N.V., Fitzgibbons P., Frankel W.L., Klimstra D.S. et al. **Protocol for the examination of specimens from patients with carcinoma of the pancreas, version: 4.1.0.0.** 2020; College of American Pathologists.

Chen C., Breslin M.B., Lan M.S. **Sonic hedgehog signaling pathway promotes INSM1 transcription factor in neuroendocrine lung cancer**. Cell Signal. 2018; 46:83-91.

Chen C., Notkins A.L., Lan M.S. **Insulinoma-associated-1: From neuroendocrine tumor marker to cancer therapeutics**. Mol Cancer Res. 2019; 17(8):1597-1604.

Choi J.H., Choi Y.H., Kang J., Paik W.H., Lee S.H., Ryu J.K. et al. **Natural history of small pancreatic lesions suspected to be nonfunctioning pancreatic neuroendocrine tumors**. Pancreas. 2018; 47(10):1357-1363.

Dasari A., Shen C., Halperin D., Zhao B., Zhou S., Xu Y. et al. **Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States.** JAMA Oncol. 2017; 3(10):1335-1342.

de Caestecker M.P., Yahata T., Wang D., Parks W.T., Huang S., Hill C.S. et al. **The Smad4 activation domain (SAD) is a proline-rich, p300-dependent transcriptional activation domain.** J Biol Chem. 2000; 275(3):2115-2122.

De Smaele E., Fragomeli C., Ferretti E., Pelloni M., Po A., Canettieri G. et al. **An integrated approach identifies Nhlh1 and Insm1 as Sonic Hedgehog-regulated genes in developing cerebellum and medulloblastoma**. Neoplasia. 2008; 10(1):89-98.

Delaloge S., Pautier P., Kerbrat P., Castaigne D., Haie-Meder C., Duvillard P. et al. **Neuroendocrine small cell carcinoma of the uterine cervix: what disease? What treatment? Report of ten cases and a review of the literature**. Clin Oncol (R Coll Radiol). 2000; 12(6):357-362.

Doxtader E.E., Mukhopadhyay S. **Insulinoma-associated protein 1 is a sensitive and specific marker of neuroendocrine lung neoplasms in cytology specimens**. Cancer Cytopathol. 2018; 126(4):243-252.

Duggan A., Madathany T., de Castro S.C., Gerrelli D., Guddati K., García-Añoveros J. **Transient expression of the conserved zinc finger gene INSM1 in progenitors and nascent neurons throughout embryonic and adult neurogenesis**. J Comp Neurol. 2008; 507(4):1497-1520.

Egashira A., Morita M., Kumagai R., Taguchi K.I., Ueda M., Yamaguchi S. et al. **Neuroendocrine carcinoma of the esophagus: Clinicopathological and immunohistochemical features of 14 cases.** PLoS One. 2017; 12(3):e0173501.

Farkas L.M., Haffner C., Giger T., Khaitovich P., Nowick K., Birchmeier C. et al. **Insulinoma-associated 1 has a panneurogenic role and promotes the generation and expansion of basal progenitors in the developing mouse neocortex**. Neuron. 2008; 60(1):40-55.

Friedberg J.S., Kaiser L.R. **Epidemiology of lung cancer.** Semin Thorac Cardiovasc Surg. 1997; 9(1):56-59.

Fujino K., Motooka Y., Hassan W.A., Ali Abdalla M.O., Sato Y., Kudoh S. et al. **Insulinoma-Associated Protein 1 Is a Crucial Regulator of Neuroendocrine Differentiation in Lung Cancer.** Am J Pathol. 2015; 185(12):3164-3177.

Gierl M.S., Karoulias N., Wende H., Strehle M., Birchmeier C. **The zinc-finger factor Insm1 (IA-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells**. Genes Dev. 2006; 20(17):2465-2478.

Gilbertson R.J. **Medulloblastoma: signalling a change in treatment.** Lancet Oncol. 2004; 5(4):209-218.

González I., Lu H.C., Sninsky J., Yang C., Bishnupuri K., Dieckgraefe B. et al. **Insulinoma-associated protein 1 expression in primary and metastatic neuroendocrine neoplasms of the gastrointestinal and pancreaticobiliary tracts**. Histopathology. 2019; 75(4):568-577.

Goto Y., De Silva M.G., Toscani A., Prabhakar B.S., Notkins A.L., Lan M.S. **A novel human insulinoma-associated cDNA, IA-1, encodes a protein with "zinc-finger" DNA-binding motifs.** J Biol Chem. 1992; 267(21):15252-15257.

Gustafsson B.I., Kidd M., Chan A., Malfertheiner M.V., Modlin I.M. **Bronchopulmonary neuroendocrine tumors.** Cancer. 2008; 113(1):5-21.

Hallet J., Law C.H., Cukier M., Saskin R., Liu N., Singh S. **Exploring the rising incidence of neuroendocrine tumors: a population-based analysis of epidemiology, metastatic presentation, and outcomes**. Cancer. 2015; 121(4):589-597.

Hamanaka W., Motoi N., Ishikawa S., Ushijima M., Inamura K., Hatano S. et al. **A subset of small cell lung cancer with low neuroendocrine expression and good prognosis: a comparison study of surgical and inoperable cases with biopsy**. Hum Pathol. 2014; 45(5):1045-1056.

Hauso O., Gustafsson B.I., Kidd M., Waldum H.L., Drozdov I., Chan A.K. et al. **Neuroendocrine tumor epidemiology: contrasting Norway and North America.** Cancer. 2008; 113(10):2655-2664.

Inzani F., Petrone G., Fadda G., Rindi G. **Cyto-histology in NET: what is necessary today and what is the future?** Rev Endocr Metab Disord. 2017; 18(4):381-391.

Jacob J., Storm R., Castro D.S., Milton C., Pla P., Guillemot F. et al. **Insm1 (IA-1) is an essential component of the regulatory network that specifies monoaminergic neuronal phenotypes in the vertebrate hindbrain**. Development. 2009; 136(14):2477-2485.

Jiang S.X., Kameya T., Shoji M., Dobashi Y., Shinada J., Yoshimura H. **Large cell neuroendocrine carcinoma of the lung: a histologic and immunohistochemical study of 22 cases.** Am J Surg Pathol. 1998; 22(5):526-637.

Kaufmann O., Georgi T., Dietel M. **Utility of 123C3 monoclonal antibody against CD56 (NCAM) for the diagnosis of small cell carcinomas on paraffin sections**. Hum Pathol. 1997; 28(12):1373-1378.

Kawaguchi A., Ikawa T., Kasukawa T., Ueda H.R., Kurimoto K., Saitou M. et al. **Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis.** Development. 2008; 135(18):3113-3124.

Kim D., Viswanathan K., Goyal A., Rao R. **Insulinoma-associated protein 1 (INSM1) is a robust marker for identifying and grading pancreatic neuroendocrine tumors**. Cancer Cytopathol. 2020; 128(4):269-277.

Kim I.E.Jr, Amin A., Wang L.J., Cheng L., Perrino C.M. **Insulinoma-associated protein 1 (INSM1) expression in small cell neuroendocrine carcinoma of the urinary tract.** Appl Immunohistochem Mol Morphol. 2020; 28(9):687-693.

Kim J.Y., Hong S.M. **Recent updates on neuroendocrine tumors from the gastrointestinal and pancreatobiliary tracts**. Arch Pathol Lab Med. 2016; 140(5):437-448.

Klimstra D.S., Modlin I.R., Adsay N.V., Chetty R., Deshpande V., Gönen M. et al. **Pathology reporting of neuroendocrine tumors: application of the Delphic consensus process to the development of a minimum pathology data set**. Am J Surg Pathol. 2010; 34(3):300-313.

Klöppel G. **Neuroendocrine neoplasms: dichotomy, origin and classifications.** Visc Med. 2017; 33(5):324-330.

Kriegsmann K., Zgorzelski C., Kazdal D., Cremer M., Muley T., Winter H. et al. **Insulinoma-associated protein 1 (INSM1) in thoracic tumors is less sensitive but more specific compared with synaptophysin, chromogranin A and CD56**. Appl Immunohistochem Mol Morphol. 2020; 28(3):237-242.

Kuji S., Watanabe R., Sato Y., Iwata T., Hirashima Y., Takekuma M. et al. **A new marker, insulinoma-associated protein 1 (INSM1), for high-grade neuroendocrine carcinoma of the uterine cervix: Analysis of 37 cases**. Gynecol Oncol. 2017; 144(2):384-390.

Lan M.S., Breslin M.B. **Structure, expression, and biological function of INSM1 transcription factor in neuroendocrine differentiation**. FASEB J. 2009; 23(7):2024-2033.

Lan M.S., Li Q., Lu J., Modi W.S., Notkins A.L. **Genomic organization, 5'-upstream sequence, and chromosomal localization of an insulinoma-associated intronless gene**, IA-1. J Biol Chem. 1994; 269(19):14170-14174.

Lan M.S, Russell E.K, Lu J., Johnson B.E., Notkins A.L. **IA-1, a new marker for neuroendocrine differentiation in human lung cancer cell lines.** Cancer Res. 1993; 53(18):4169-4171.

Lilo M.T., Chen Y., LeBlanc R.E. **INSM1 Is More Sensitive and Interpretable than Conventional Immunohistochemical Stains Used to Diagnose Merkel Cell Carcinoma**. Am J Surg Pathol. 2018; 42(11):1541-1548.

Lloyd R.V., Osamura R.Y., Kloppel G., Rosai J. (Editors). **WHO classification of tumours of endocrine organs. WHO classification of tumours, 4th edition, volume 10.** 2017; International Agency for Research on Cancer (Lyon, France); ISBN: 978-92-832-4493-6.

Maleki Z. **Diagnostic issues with cytopathologic interpretation of lung neoplasms displaying high-grade basaloid or neuroendocrine morphology**. Diagn Cytopathol. 2011; 39(3):159-67.

McHugh K.E., Mukhopadhyay S., Doxtader E.E., Lanigan C., Allende D.S. **INSM1 is a highly specific marker of neuroendocrine differentiation in primary neoplasms of the gastrointestinal tract, appendix, and pancreas**. Am J Clin Pathol. 2020; 153(6):811-820.

Mellitzer G., Bonné S., Luco R.F., Van De Casteele M., Lenne-Samuel N., Collombat P. et al. **IA1 is NGN3-dependent and essential for differentiation of the endocrine pancreas.** EMBO J. 2006; 25(6):1344-1352.

Mukhopadhyay S., Dermawan J.K., Lanigan C.P., Farver C.F. **Insulinoma-associated protein 1 (INSM1) is a sensitive and highly specific marker of neuroendocrine differentiation in primary lung neoplasms: an immunohistochemical study of 345 cases, including 292 whole-tissue sections**. Mod Pathol. 2019; 32(1):100-109.

Nakra T., Nambirajan A., Guleria P., Phulware R.H., Jain D. **Insulinoma-associated protein 1 is a robust nuclear immunostain for the diagnosis of small cell lung carcinoma in cytology smears**. Cancer Cytopathol. 2019; 127(8):539-548.

Nicholson S.A., Beasley M.B., Brambilla E., Hasleton P.S., Colby T.V., Sheppard M.N., et al. **Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens.** Am J Surg Pathol. 2002; 26(9):1184-97.

Oberg K., Castellano D. **Current knowledge on diagnosis and staging of neuroendocrine tumors.** Cancer Metastasis Rev. 2011; 30 Suppl 1:3-7.

Oronsky B., Ma P.C., Morgensztern D., Carter C.A. **Nothing but NET: A review of neuroendocrine tumors and carcinomas.** Neoplasia. 2017; 19(12):991-1002.

Osipovich A.B., Long Q., Manduchi E., Gangula R., Hipkens S.B., Schneider J. et al. **Insm1 promotes endocrine cell differentiation by modulating the expression of a network of genes that includes Neurog3 and Ripply3**. Development. 2014; 141(15):2939-2949.

Parent A.S., Matagne V., Westphal M., Heger S., Ojeda S., Jung H. **Gene expression profiling of hypothalamic hamartomas: a search for genes associated with central precocious puberty**. Horm Res. 2008; 69(2):114-123.

Pomeroy S.L., Tamayo P., Gaasenbeek M., Sturla L.M., Angelo M., McLaughlin M.E. et al. **Prediction of central nervous system embryonal tumour outcome based on gene expression.** Nature. 2002; 415(6870):436-442.

Ramalingam S., Eisenberg A., Foo W.C., Freedman J., Armstrong A.J., Moss L.G. et al. **Treatment-related neuroendocrine prostate cancer resulting in Cushing's syndrome.** Int J Urol. 2016; 23(12):1038-1041.

Rindi G., Klimstra D.S., Abedi-Ardekani B., Asa S.L., Bosman F.T., Brambilla E. **A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal**. Mod Pathol. 2018; 31(12): 1770-1786.

Rodriguez E.F., Chowsilpa S., Maleki Z. **Insulinoma-associated protein 1 is a robust nuclear immunostain for the diagnosis of small cell lung carcinoma in cytology smears**. Acta Cytol. 2018; 62(5-6):333-338.

Rooper L.M., Bishop J.A., Westra W.H. **INSM1 is a sensitive and specific marker of neuroendocrine differentiation in head and neck tumors**. Am J Surg Pathol. 2018; 42(5):665-671.

Rooper L.M., Sharma R., Li Q.K., Illei P.B., Westra W.H. **INSM1 demonstrates superior performance to the individual and combined use of synaptophysin, chromogranin and CD56 for diagnosing neuroendocrine tumors of the thoracic cavity**. Am J Surg Pathol. 2017; 41(11):1561-1569.

Rosenbaum J.N., Duggan A., García-Añoveros J. **Insm1 promotes the transition of olfactory progenitors from apical and proliferative to basal, terminally dividing and neuronogenic**. Neural Dev. 2011; 6:6.

Rosenbaum J.N., Guo Z., Baus R.M., Werner H., Rehrauer W.M., Lloyd R.V. **A novel immunohistochemical and molecular marker for neuroendocrine and neuroepithelial neoplasms.** Am J Clin Pathol. 2015; 144(4):579-591.

Roy M., Buehler D.G., Zhang R., Schwalbe M.L., Baus R.M., Salamat M.S. et al. **Expression of insulinoma-associated protein 1 (INSM1) and orthopedia homeobox (OTP) in tumors with neuroendocrine differentiation at rare sites.** 2019; 30(1):35-42.

Rush P.S., Rosenbaum J.N., Roy M., Baus R.M., Bennett D.D., Lloyd R.V. **Insulinoma-associated 1: A novel nuclear marker in Merkel cell carcinoma (cutaneous neuroendocrine carcinoma).** J Cutan Pathol. 2018; 45(2):129-135.

Sandgren J., Andersson R., Rada-Iglesias A., Enroth S., Akerstrom G., Dumanski J.P. et al. **Integrative epigenomic and genomic analysis of malignant pheochromocytoma.** Exp Mol Med. 2010; 42(7):484-502.

Shi C., Woltering E., Beyer D.T., Klimstra D., Mallin K., Bergsland E. et al. **Neuroendocrine tumors of the colon and rectum.** In: Amin M.B., Edge S., Greene F., Byrd D.R., Brookland R.K., Washington M. K. et al. (Editors). **AJCC cancer staging manual.** 2017; Springer International Publishing (Chicago, USA); ISBN: 978-3-319-40617-6.

Shi C., Adsay V., Bergsland E.K., Berlin J., Branton P.A., Fitzgibbons P.L. et al. **Protocol for the examination of specimens from patients with tumors of the endocrine pancreas, version: 4.0.0.2.** 2020; College of American Pathologists.

Staaf J., Tran L., Söderlund L., Nodin B., Jirström K., Vidarsdottir H. et al. **Diagnostic value of insulinoma-associated protein 1 (INSM1) and comparison with established neuroendocrine markers in pulmonary cancers: a comprehensive study and review of the literature**. Arch Pathol Lab Med. 2020; 144(9):1075-1085.

Takase Y., Naito Y., Okabe Y., Ishida Y., Yamaguchi T., Abe H. et al. **Insulinoma-associated protein 1 expression in pancreatic neuroendocrine tumours in endoscopic ultrasound-guided fine-needle aspiration cytology: An analysis of 14 patients**. Cytopathology. 2019; 30(2):194-200.

Tanigawa M., Nakayama M., Taira T., Hattori S., Mihara Y., Kondo R. et al. **Insulinoma-associated protein 1 (INSM1) is a useful marker for pancreatic neuroendocrine tumor.** Med Mol Morphol. 2018; 51(1):32-40.

Taniwaki M., Daigo Y., Ishikawa N., Takano A., Tsunoda T., Yasui W. et al. **Gene expression profiles of small-cell lung cancers: molecular signatures of lung cancer.** Int J Oncol. 2006; 29(3):567-575.

Travis W.D., Brambilla E., Burke A.P., Marx A., Nicholson A.G. (Editors). **WHO classification of tumours of the lung, pleura, thymus and heart. WHO classification of tumours, 4th edition, volume 7.** 2015; International Agency for Research on Cancer (Lyon, France); ISBN: 978-92-832-2436-5.

Travis W.D., Rush W., Flieder D.B., Falk R., Fleming M.V., Gal A.A., et al. **Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid**. Am J Surg Pathol. 1998; 22(8):934-944.

Viswanathan K., Siddiqui M.T., Borczuk A.C. **Insulinoma-associated protein 1 is a sensitive and specific marker for lung neuroendocrine tumors in cytologic and surgical specimens**. J Am Soc Cytopathol. 2019; 8(6):299-308.

Wang H., Krishnan C., Charville G.W. **INSM1 expression in peripheral neuroblastic tumors and other embryonal neoplasms**. Pediatr Dev Pathol. 2019; 22(5):440-448.

Weed J.C.Jr, Graff A.T., Shoup B., Tawfik O. **Small cell undifferentiated (neuroendocrine) carcinoma of the uterine cervix.** J Am Coll Surg. 2003; 197(1):44-51.

Welcker J.E., Hernandez-Miranda L.R., Paul F.E., Jia S., Ivanov A., Selbach M. et al. **Insm1 controls development of pituitary endocrine cells and requires a SNAG domain for function and for recruitment of histone-modifying factors**. Development. 2013; 140(24):4947-4958.

Wildner H., Gierl M.S., Strehle M., Pla P., Birchmeier C. **Insm1 (IA-1) is a crucial component of the transcriptional network that controls differentiation of the sympatho-adrenal lineage**. Development. 2008; 135(3):473-481.

Xie J., Cai T., Zhang H., Lan M.S., Notkins A.L. **The zinc-finger transcription factor INSM1 is expressed during embryo development and interacts with the Cbl-associated protein**. Genomics. 2002; 80(1):54-61.

Xin Z., Zhang Y., Jiang Z., Zhao L., Fan L., Wang Y. et al. **Insulinoma-associated protein 1 is a novel sensitive and specific marker for small cell carcinoma of the prostate**. Hum Pathol. 2018; 79:151-159.

Yang C., Gonzalez I., Zhang L., Cao D. **Neuroendocrine markers insulinoma-associated protein 1, chromogranin, synaptophysin, and CD56 show rare positivity in adenocarcinoma ex-goblet cell carcinoids**. Gastroenterology Res. 2019; 12(3):120-127.

Yoshida A., Makise N., Wakai S., Kawai A., Hiraoka N. **INSM1 expression and its diagnostic significance in extraskeletal myxoid chondrosarcoma**. Mod Pathol. 2018; 31(5):744-752.

Zhang T., Saunee N.A., Breslin M.B., Song K., Lan M.S. **Functional role of an islet transcription factor, INSM1/IA-1, on pancreatic acinar cell trans-differentiation**. J Cell Physiol. 2012; 227(6):2470-2479.

Zheng G., Ettinger D.S., Maleki Z. **Utility of the quantitative Ki-67 proliferation index and CD56 together in the cytologic diagnosis of small cell lung carcinoma and other lung neuroendocrine tumors**. Acta Cytol. 2013; 57(3):281-90.

Zhu M., Breslin M.B., Lan M.S. **Expression of a novel zinc-finger cDNA, IA-1, is associated with rat AR42J cells differentiation into insulin-positive cells**. Pancreas. 2002; 24(2):139-145.

Zilfou J.T., Hoffman W.H., Sank M., George D.L., Murphy M. **The corepressor mSin3a interacts with the proline-rich domain of p53 and protects p53 from proteasome-mediated degradation**. Mol Cell Biol. 2001; 21(12):3974-3985.