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REVIEW

Insulinoma-associated protein 1 (INSM1): a potential biomarker and therapeutic target for neuroendocrine tumors

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Abstract

Background Insulinoma-associated protein 1 (INSM1), a transcriptional regulator with a zinc-finger DNA-binding domain, has been validated as a cytoplasmic marker for neuroendocrine differentiation of tumor cells. Next to its abundant expression in the fetal pancreas, it is expressed in brain tumors, pheochromocytomas, medullary thyroid carcinomas, insulinomas and pituitary and small-cell lung carcinomas. INSM1 is not expressed in normal adult tissues and/or most non-neuroendocrine tumors. It regulates various downstream signaling pathways, including the Sonic Hedgehog, PI3K/AKT, MEK/ERK1/2, ADK, p53, Wnt, histone acetylation, LSD1, cyclin D1, Ascl1 and N-Myc pathways. Although INSM1 appears to be a subtle and specific biomarker for neuroendocrine tumors, its role in tumor development has remained unclear.

Conclusions Here, we highlight INSMI expression, as well as its diagnostic significance and use as a therapeutic target in various neuroendocrine tumors. Targeting signaling pathways or gene expression alterations associated with INSM1 expression may be instrumental for the design of novel therapeutic strategies for neuroendocrine tumors.

Keywords Neuroendocrine tumor · Insulinoma-associated protein 1 · Tumor marker · Chromogranin A · Synaptophysin 1

1 Introduction

Neuroendocrine tumors (NETs) are epithelial neoplasms exhibiting neuroendocrine differentiation characteristics. Immunohistochemical markers are used to diagnostically evaluate these tumors [1]. As such, the transcription factor insulinoma-associated protein 1 (INSM1) is of relevance. Previously, Goto et al. constructed a human insulinoma cDNA library (ISL-153) and, by screening this library, identified a novel insulinoma-associated cDNA, i.e., insulin-associated antigen-1 (IA-1), which is now known as insulin-associated protein 1 (INSM1) [2]. The *INSM1* gene encodes a 58 kDa protein encompassing five zinc-finger DNA-binding motifs and dibasic amino acid pro-hormone conversion sites [2, 3]. Its N-terminus exhibits repressor activity [4]. The *INSM1* gene is located on chromosome 20p11.2 (Fig. 1a). An amino acid region between positions 167 and 262 at the N-terminus is responsible for its transcriptional activity [5–9]. Reactivation of INSM1 has been observed in tumors of neuroendocrine origin, including insulinomas, pituitary tumors, pheochromocytomas, medullary thyroid carcinomas, small-cell lung carcinomas, medulloblastomas, neuroblastomas and retinoblastomas.

Different regulatory elements upstream of the *INSM1* gene have been found to act in different NETs [10]. The 5′-upstream region (2,090 bp) of *INSM1* contains several tissue-specific regulatory elements that appear to account for its unique tumor-associated expression pattern [11]. Since INSM1 is highly expressed in tumors of neuroendocrine

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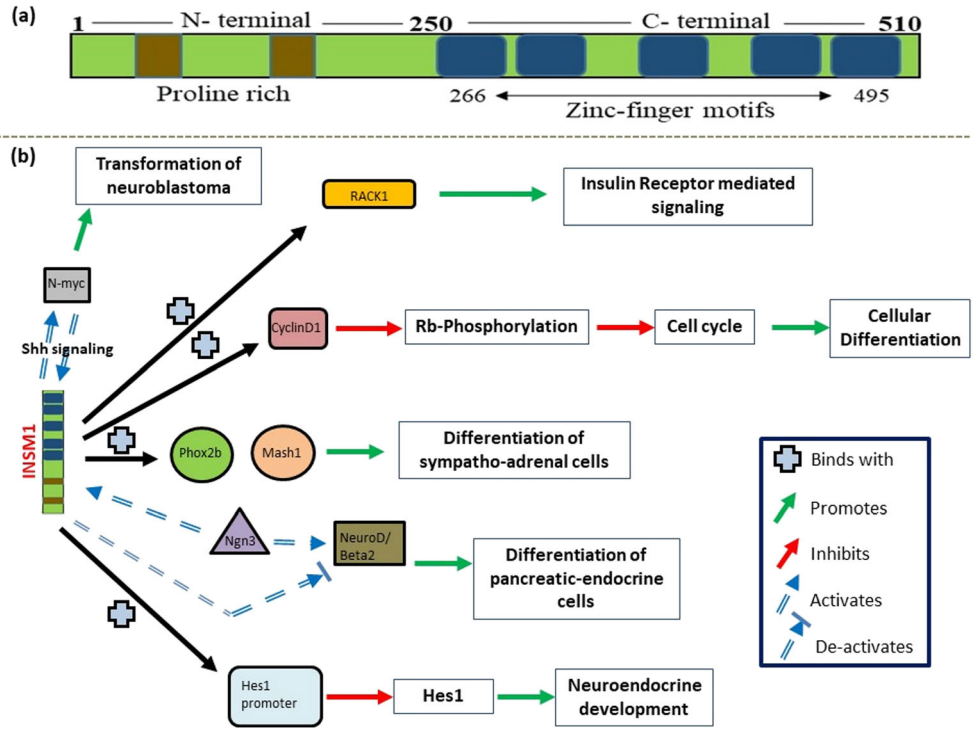

Fig. 1 Structure and cellular activities of INSM1: The 510aa INSM1 protein has a N-terminal (aa 1-250) domain that contains two proline-rich regions (aa 43–58 and 183–205) and a C-terminal (aa 251–510) domain with five equally spaced zinc-finger motifs (**a**). The different cellular activities of INSM1, such as differentiation and neuroendocrine development, are depicted (**b**)

origin, it has been suggested that its promoter may be used to drive targeted therapy in NETs [12, 13]. In addition, due to its specific expression and reactivation characteristics under cancerous conditions, its clinical use as a diagnostic marker has been shown to be valuable [2, 4, 14]. Recently, Lilo et al. used, for example, INSM1 immunohistochemistry to improve the detection of sentinel lymph node metastases [15]. Also, the specificity and efficacy of *INSM1* promoter-driven gene therapy using adenoviruses for the treatment of neuroblastomas, retinoblastomas and medulloblastomas have been studied [16].

1.1 Function and mode of action of INSM1

INSMs are zinc-finger type transcription factors that are implicated in a wide range of biological processes [12]. Structurally, INSM1 is highly conserved in different species and predominantly expressed in developing mammalian neuroendocrine tissues and the nervous system. As such, it plays an important role in early embryonic neurogenesis [6]. INSM1 is also an islet transcription factor that is essential for the development of the pancreas and functions as a transcriptional repressor of NeuroD/β2 and the insulin gene [17]. Through binding to various cellular regulators, such as RACK1 and cyclin D1 [7], it additionally carries out extranuclear activities involved in a variety of cellular functions, such as the induction of differentiation and cell cycle arrest. Its ultimate effects are brought about by interactions with different molecules, depending on the type of tissue involved.

The human INSM1 protein has the ability to bind to both DNA and protein. By binding to cyclin D1, for example, it has been found that INSM1 can directly inhibit progression of the cell cycle [18]. Thus, INSM1 can functionally link transcriptional activity to cell cycle arrest. Additional cell signaling studies have revealed that INSM1 may contribute to cell cycle arrest/exit induction to facilitate cellular differentiation. Furthermore, it has been reported that interaction of INSM1 and RACK1 is necessary to enhance the insulin receptor (InR)-mediated signaling pathway [7]. The *INSM1* gene has been found to be reactivated in neuroendocrine tumors. This type of de-differentiation mimics normal embryonic development. Also, Zhang et al. suggested that INSM1 can interrupt the normal cell cycle, as it can bind to and compete with CDK4 for binding to cyclin D1. This, in turn, leads to inhibition of phosphorylation of the protein and, thus, to cell cycle arrest. Inhibition of cellular proliferation may lead to differentiation induction [7]. Lan and Breslin have suggested that INSM1 has many features that strongly support its role as an important regulator of neuroendocrine differentiation [12]. It is a direct target of Ngn3 and NeuroD1, which participate in endocrine and neuronal cell differentiation and survival and mature β-cell function, and are critical regulators of pancreatic β-cell development [5, 19-21]. First, INSM1 becomes activated by Ngn3, after which NeuroD/β2 is activated by Ngn3during pancreatic endocrine cell differentiation. Conversely, INSM1 may exert feedback activity to suppress NeuroD/β2 and its own expression [12]. Additionally, INSM1 may act as a downstream activator of Phox2b and Mash 1

during differentiation of the sympathoadrenal lineage [8]. INSM1 also acts in epigenetic and transcriptional networks that control the differentiation of endocrine cells in the anterior pituitary gland, and it needs a SNAG domain to exert its function *in vivo* [22]. Furthermore, INSM1 has been found to be expressed in pulmonary neuroendocrine cells in mice, and that pulmonary neuroendocrinal cells depend on INSM1 for their differentiation [23]. INSM1 can bind to the *Hes1* promoter, leading to its repression, which is needed for neuroendocrine development. Thus, INSM1 is a key factor that regulates the differentiation of pulmonary neuroendocrine cells [23]. A decrease in INSM1 interferes with β-cell specification during the early postnatal period and impairs glucose homeostasis during metabolic stress in adults [24]. This suggests that INSM1 may affect diabetes development [24]. It has also been reported that INSM1 plays a critical role in the proliferation, basement membrane extract-coated invasion and soft-agar colony formation of neuroblastoma cells [25]. Furthermore, it has been proposed that a positive feedback loop involving Sonic Hedgehog (Shh) signaling is able to induce INSM1 through N-Myc, and that INSM1 may increase the stability of N-Myc, leading to the transformation of human neuroblastoma cells [25]. An overview of the different cellular activities of INSM1 is presented in Fig. 1b.

1.2 Role of INSM1 in neuroendocrine differentiation

INSM1 is a transcription factor that plays a central role in embryonic neuroendocrine cell differentiation. INSM1 autoregulates the expression of the *INSM1* gene by binding to its own promoter region [4]. Cyclin D1 and histone deacetylase-3 (HDAC-3) are target proteins of INSM1, which acetylates histones H3 and H4 [3]. INSM1 gene expression has predominantly been observed in cells of neuroendocrine tissues, including the retina, pancreas, adrenal gland, thymus, thyroid, gastrointestinal endocrine tissues and the cerebellum, and in fetal and neonatal forebrain, midbrain, hindbrain and olfactory epithelium [26, 27]. After birth, INSM1 expression decreases and completely vanishes in adults. During embryonic and adult neurogenesis, progenitors and nascent neurons express INSM1 [28]. A gene expression profiling study in mice by Farkas et al. revealed that INSM1 is expressed at embryonic days 9.5–11 in the dorsal telencephalon [29]. INSM1 expression in brain regions is strongly associated with neurogenesis. During cerebellar neurogenesis, the outer external granule layer of proliferating neuronal progenitor cells, the inner external granule layer of post-mitotic neurons and the dentate gyrus of the postnatal hippocampus express INSM1. Adult mature granule neurons lack INSM1 expression. Higher expression of INSM1 has also been observed in neuronal progenitors and developing sensory neurons [28]. Mouse embryos devoid of INSM1 expression exhibit a reduced cortical plate thickness and a decreased population of basal progenitor cells [29]. Wildner et al. reported that a homozygous INSM1 mutant mouse model exhibited fetal lethality due to catecholamine deficiency, which is crucial for the development of the sympatho-adrenal lineage [30].

1.3 INSM1 as a neuroendocrine marker

Neuroendocrine tumors originate from cells of the endocrine and nervous systems. The neuroendocrine (NE) system consists of pituitary, parathyroid, thyroid and pancreas cells and endocrine cells of the digestive and respiratory tracts. Generally, NE markers include chromogranin A (CgA), synaptophysin (Syn) and neuron-specific enolase (NSE) [31]. There exist also subtype-specific markers such as calcitonin, catecholamines, 5-hydroxy indole-acetic acid (5-HIAA), insulin, gastrin, pancreatic polypeptide and glucagon. High levels of these markers have been observed in both peripheral blood and immunohistochemical analyses of NETs. Considering the importance of INSM1, several findings suggest its involvement in different neuroendocrine activities and development. *INSM1* gene ablation in mice has, for instance, has been found to lead to impairment of pancreatic endocrine cell maturation and to severe impairment of catecholamine biosynthesis and secretion from the adrenal glands [12]. As already mentioned, this gene is highly expressed in neuroendocrine tumors and, as such, its significance as a marker for NETs is evident. An example in this regard is the recent finding that the sensitivity and specificity of INSM1 in diagnosing NETs in cytology samples were 99% and 97%, respectively, independent of the primary site [1]. The significance of INSM1 as a diagnostic marker for different forms of NET is discussed in the following section.

2 INSM1 as a diagnostic NET marker

Merkel cell carcinoma (MCC) is a rare, clinically aggressive, cutaneous neuroendocrine neoplasm. Rush et al. assessed the relevance of INSM1 as a nuclear marker and reported that it may offer additional diagnostic utility [32]. In addition, it has been reported that INSM1 expression identified via endoscopic ultrasound and fine pancreatic neuroendocrine tumor needle aspiration cytology (EUS-FNAC) may serve as an important diagnostic tool for assessing therapeutic strategies, including molecular-targeted therapy [33]. In primary lung neoplasms, INSM1 has been found to exhibit a sensitivity comparable to that of synaptophys and CD56, and a specificity equal to that of chromogranin [34]. Although not entirely sensitive or specific, INSM1 may also serve as a potential marker for the diagnosis of extra-skeletal myxoid chondrosarcoma in case access to molecular genetic testing is limited [35]. INSM1 also serves as a useful immunohistochemical marker for diagnosing pancreatic NETs [36] and exhibits an excellent

performance both individually and in combination with Syn, CgA and CD56 for diagnosing NETs of the thoracic cavity [37]. A previous immunohistochemistry-based study by Rosenbaum et al. revealed that the expression of INSM1was significantly increased in neoplastic tissues compared to non-neoplastic tissues. The researchers also showed that gastrointestinal neuroendocrine neoplasms and neoplasms with known metastases exhibited significantly higher expression levels than those without metastases [38]. A summary of evidence supporting the diagnostic utility of INSM1 in neuroendocrine tumors is provided in Table 1.

The dual role of INSMI in NE differentiation and cancer progression has only recently been recognized. The expression of INSM1 during adulthood is usually blocked, whereas its higher expression only in NETs contributes to cancer progression [43]. Since INSM1 acts as a transcription factor in fetal tissues, no extra-nuclear activity during NE cell differentiation is observed. In contrast in NETs, apart from its transcriptional role, INSM1 also exhibits extra-nuclear activity. By doing so, INSM1 triggers the insulin receptor signaling pathway-mediated PI3K/AKT pathway and induces pancreatic cell differentiation [7]. INSM1 binds to cyclin D1 and, thereby, inhibits CDK4 binding, which leads to inhibition of Rb protein phosphorylation (see above). Inhibition of Rb protein phosphorylation, in turn, induces cell cycle arrest in non-NE cells [6]. In neuroblastoma, a positive feedback loop of Shh signaling was found to be strongly associated with increased expression of INSM1 and N-Myc. This positive feedback loop triggers neuroblastoma cell proliferation and overexpression of INSM1, which exacerbates tumor growth and development. INSM1 increases the stability of N-Myc by activating the PI3K/AKT/GSK3β signaling pathway. Stabilized N-Myc, in turn, binds to the E2-box region of the *INSM1* promoter and activates INSM1 expression [44]. Re-expression of INSM1 in normal adult NE cells contributes to a tumor phenotype with an increased N-Myc expression. Thus, the expression of INSM1 in adult NE cells together with other oncogenes could help to delineate the role of INSM1 in NE differentiation and NET progression.

2.1 INSM1 and pancreatic cancer diagnosis

The two most common types of NETs are pancreatic neuroendocrine tumors (PanNETs) and nonfunctioning PanNETs (NF-PanNETs). One PanNET type is insulinoma, which is a small, rarely metastasizing tumor secreting a vast amount of insulin. In contrast, NF-PanNETs are larger, frequently metastasize and do not secrete hormones [45]. The expression of INSM1 in these tumors has been reported very recently and it helps in determining whether a particular PanNET is a focal insulinoma or a metastatic NF-PanNET [45]. In addition, expression of INSM1 along with that of CgA, Syn and neural cell adhesion molecule (NCAM) has been used as a biomarker for the diagnosis of PanNETs [40]. Tanigawa et al. suggested that INSM1 may serve as a useful immunohistochemical marker for diagnosing PanNETs based on the observed nuclear expression of INSM1 in all classic PanNET cases among 27 NETs investigated [36]. In pancreatic endocrine cell development, Ngn3, INSM1 and NeuroD/β2 form a tight transcriptional network that is essential for the development of β-cells [5]. The NeuroD/β2 and insulin encoding genes have been found to contain functional INSM1 binding sites in their

Table 1 Evidences for diagnostic utility of INSM1 in case of neuroendocrine cancers

S.No	NE cancer type	Methodology used	Sample size	No. or % of positive samples	Remarks	Reference
1	Pancreatic cancer	Immunohistochemistry	27	27 positives	-	[37]
2	Lung cancer	Cytological study	32	31 positives	Sensitivity equal to that of CD56 marker for small cell carcinoma	[39]
3	Breast Cancer	Immunohistochemistry	129	88.3% positives (neuroendocrine neoplasms)	INSM1 expression found significantly increased in neoplastic as compared to non-neoplastic tissue.	[40]
4	Prostate cancer	Immunohistochemistry	-	92.3% positives (prostatic small cell carcinomas)	High sensitivity and specificity for detection of small cell carcinoma of prostate	[41]
5	Head and neck cancer	Immunohistochemistry	-	94.9% positives (small cell lung carcinomas) and 91.3% positives (large cell neuroendocrine carcinomas)	96.4% sensitivity across all grades of thoracic NETs	[38]
6	Cervical cancer (high-grade neuroendocrine carcinoma of uterine cervix)	Immunohistochemistry	37	95% cases positive	Higher expression of INSM1 than other markers such as NCAM and CgA	[42]

proximal promoter regions. INSM1 transcriptional repressor activity occurs through the recruitment of histone deacetylase-3 (HDAC-3), leading to histone H3/histone H4 modification.

Studies in INSM1 mutant mice have revealed a defect in insulin-positive β-cell formation, but no impairment in α-cell generation. It has also been shown that in INSM1-mutant mice, pancreatic endocrine cell differentiation is hampered. Endocrine precursor cells were generated in the pancreas and intestine, but failed to differentiate. In established β-cells, INSM1 deletion in mice resulted in decreased insulin gene expression and secretion. The gene expression and functional characteristics of mature mutant β-cells mimicked those of immature β-cells [23].

2.2 INSM1 in lung cancer diagnosis

INSM1 has been found to serve as a sensitive marker for NE differentiation in human lung tumors [10]. Serum levels of NSE and CgA are regularly used as markers for the diagnosis of NE lung cancers [46]. Lan et al. reported that INSM1 was expressed in 97% of SCLC-derived cell lines, with a high degree of concordance with the NE markers CgA and L-3,4-dihydroxyphenylalanine decarboxylase [47]. Rodriguez et al. recently reported cytological findings from 32 specimens and found that INSM1 was positive in the majority of SCLC cases, with a high sensitivity equivalent to that of CD56 [48]. INSM1 has also been identified as a prominent differential marker for SCLC along with Hash1 and gastrin-releasing peptide. About10 to 15% of all lung cancers are small-cell lung carcinomas (SCLCs), an aggressive type of NET. Elevated levels of INSM1 have been observed in SCLC [49]. In addition, it has been found that exogenous expression of INSM1 in bronchiolar epithelial cells results in poor alveolarization and alveolar space expansion at the final phase of lung development. It also prevented cyclin D1 expression in INSM1/rtTA bi-transgenic mouse bronchiolar epithelia and delayed cell cycle progression. Chen and colleagues reported that INSM1 not only plays a role in alveolar septation, but also affects proliferation and club cell regeneration during pulmonary epithelium injury [39]. They further found that exogenous expression of CCSP promoter-driven INSM1 impaired normal lung development, especially postnatal alveologenesis [39]. Another group reported that in NE lung cancer cells, Shh signaling induces INSM1 expression via N-Myc. Shh signaling and INSM1 expression also increased N-Myc protein stability. INSM1 functions as a key player in NE lung cancer via Shh signaling that induces crosstalk with the PI3K/AKT and MEK/ERK1/2 pathways to enhance N-Myc stability [50]. It has been reported that in adenocarcinoma cell lines (H358 and H1975) INSM1 is regulated through the Notch1-Hes1 signaling pathway, and that Notch1-Hes1 signaling suppresses INSM1 expression along with achaete-scute homolog-like 1 (ASCL1) and brain 2 (BRN2) expression [50]. All these studies underscore that INSM1 serves as a highly sensitive and specific diagnostic marker for neuroendocrine lung tumors [6, 51].

2.3 INSM1 in breast cancer diagnosis

In women, breast cancer represents the most common cancer and major cause of cancer-related death [52]. Early diagnosis and thorough characterization of tumors may significantly improve its prognosis. A limited number of markers is in use for the diagnosis of breast cancer, and most of them are ineffective for early-stage detection [53]. CgA and Syn are regularly used markers for NE differentiation, and the outcome associated with their detection in breast cancer is poor [54]. The breast cancer and salivary gland expression (BASE) gene, which encodes a putative secreted protein, has recently been identified as a breast cancer marker with a restricted expression in breast cancer cells and the salivary gland [55]. As indicated above, INSM1 has been shown to repress the NeuroD/β2 gene along with cyclin D1, an eminent ERα target, by employing histone deacetylases [19]. Until 2008, no studies were available explaining the link between INSM1 expression and tumors of the breast [56]. Preliminary ChIP experiments in MCF7 cells revealed the presence of INSM1 at the *BASE* gene promoter [57–59]. Rosenbaum and colleagues found that INSM1 could be detected in breast carcinomas when there was no histologic sign of NE differentiation [45]. Recently, Roy et al. confirmed the diagnostic utility of INSM1 as a sensitive and novel marker of neuroendocrinal/neuroepithelial differentiation in gynecologic tumors, including breast cancers [60].

2.4 INSM1 and prostate cancer diagnosis

A proper diagnosis of small-cell carcinoma (SCC) of the prostate, which exhibits an aggressive behavior and a poor prognosis, is challenging [61]. In general clinical practice, the markers used to identify prostate cancer are serum levels of NSE, CD56, Syn and CgA [62]. Also, higher INSM1 mRNA levels have been observed in NE prostate carcinoma samples compared to normal samples. In a comparative study of commonly used markers, INSM1 was found to be positive in 92.3% of prostatic SCCs, while chromogranin A was positive in 53.8% and synaptophysin was positive in 84.6% of SCCs. For the detection of SCC of the prostate, INSM1has turned out to be a highly sensitive and specific marker. A study of composite and metastatic NE prostate tumors showed that most of the normal NE prostate tissues did not show INSM1 expression [63]. Thus, INSM1 is now considered to be a novel, sensitive and specific marker for the detection of SCC of the prostate [63]. Recently, Roy et al. confirmed the diagnostic utility of INSM1 as a sensitive and novel marker of neuroendocrinal/neuroepithelial differentiation at rare sites such as the prostate [60].

2.5 INSM1 and head and neck cancer diagnosis

A wide variety of neuroendocrine tumors is found in the head and neck. Syn, CgA and CD56 are commonly used markers for the diagnosis of NE head and neck (HN) cancer [64]. Rooper et al. reported that INSM1 was positive in all types of HN NE tumors tested, including pituitary adenoma, middle ear adenoma, paraganglioma, medullary thyroid carcinoma, small-cell carcinoma and olfactory neuroblastoma, and exhibited a sensitivity of 99% [37]. The authors stated that INSM1 was sufficiently sensitive and specific to serve as a standalone first-line marker for tumors of the head and neck.

2.6 INSM1 and cervical cancer diagnosis

The most sensitive markers for NE cervical cancer are Syn, CgA, NSE and CD56 [65]. In addition, Kuji et al. recommended INSM1 as a useful immunohistochemical marker for diagnosing NE cervical cancer [41]. High-grade NE carcinoma of the uterine cervix (HGNCUC) is a malignant tumor, and INSM1has been found to be closely related to the development of HGNCUC. INSM1 is frequently expressed in NE tumors [41] and in HGNCUC it has been detected in 95% of the cases, whereas other markers, such as chromogranin A, synaptophysin and neural cell adhesion molecule (NCAM) were found to be expressed to a lesser extent. The percentages of samples expressing these latter markers were 86%, 86% and 68%, respectively [41].

Although the diagnostic utility of INSM1 is undisputed, the significance of INSM1 as a stand-alone diagnostic marker for different types of cancer is still under question. For instance, a recent study reported that for thoracic tumors, all conventional markers and their combinations had a higher sensitivity (97%), but lower specificity (78%), for NE differentiation than INSM1. Hence, INSM1 can act as a meaningful adjunct in the differential diagnosis of NE neoplasias. As yet, however, replacing traditional markers by INSM1 is not justified [66]. Švajdler et al. reported that a composite marker panel of p16, CD56 and TTF1 exhibited a higher sensitivity than INSM1 for diagnosing pulmonary small-cell carcinoma, as it could classify 100% of the SCLC cases correctly, whereas INSM1 exhibited a sensitivity of 81% [67]. Leblebici et al. reported that INSM1 is less effective in highlighting Merkel cells within nevus sebaceous lesions than CK20 [68].

3 INSM1 as a therapeutic target for NET

The *INSM1* gene is expressed exclusively during embryonal development and is re-expressed at high levels in NE tumors. Therefore, its regulatory region is considered a potential candidate for the design of targeted therapies [9]. Several studies have tried to establish the therapeutic utility of INSM1 in NE cancers, such as the combination of different Shh signaling pathway inhibitors targeting INSM1 and N-Myc to inhibit lung cancer cell growth. Such combinations may indeed be used as new treatment options for SCLC [42]. Also, INSM1 promoter-driven conditionally replicating adenovirus therapies may serve as new tools for the treatment of insulinoma. Next to its sensitive detection of NE tumors [42], intratumoral adenoviral delivery has shown that the *INSM1* promoter may directly enhance herpes simplex virus type 1 thymidine kinase (HSV-tk) gene expression in a nude mouse tumor model. This expression effectively represses tumor growth in response to ganciclovir treatment and is specific and effective for targeted therapy in PNETs [16]. Attempts have been made to use a 1.7 kbp *INSM1* promoter region in adenoviral HSV-tk gene therapy to treat NE tumors and to modify INSM1 to protect it from the influence of essential adenoviral sequences that interfere with its specificity and to further enhance the tissue specificity of the *INSM1* promoter region [69]. A potent therapeutic approach for chemo-resistant SCLC patients was found to be INSM1-driven suicide gene therapy using YCD-YUPRT/5FC or NTR/SN27686 constructs, which achieved high levels of cytotoxicity in both chemo-sensitive and chemo-resistant SCLC cells. This form

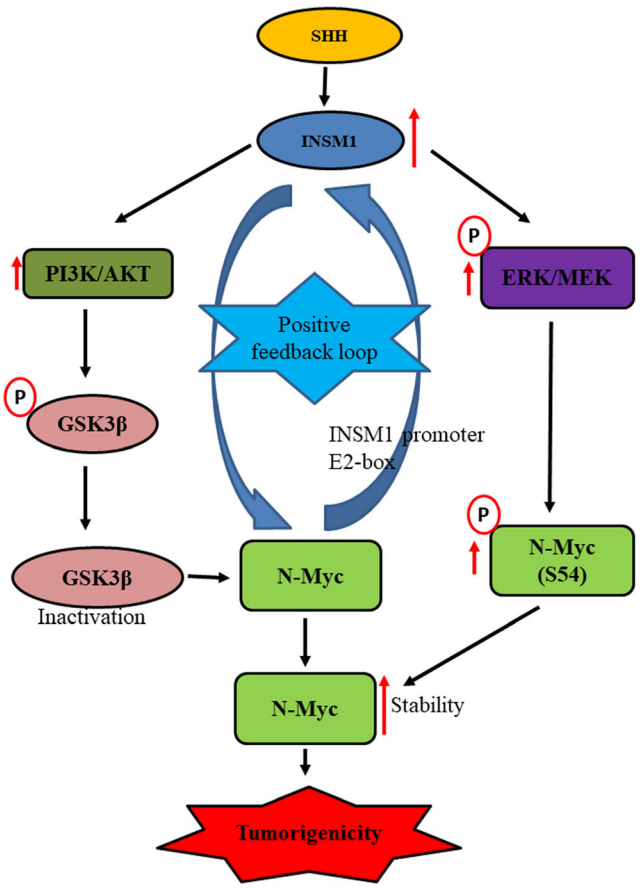

Fig. 2 Diagram representing the mechanisms by which INSM1 promotes tumorigenicity in neuroendocrine tumors

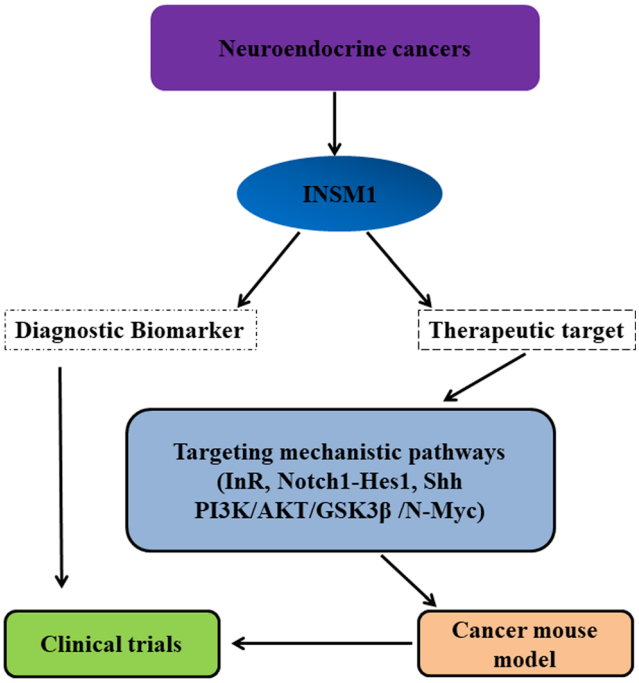

Fig. 3 The use of INSM1 as a specific biomarker and therapeutic target may lead to an improved diagnosis and treatment of neuroendocrine tumors

of therapy was found to be superior to that of single-agent therapy in chemo-resistant SCLC cells [70]. Cramer et al. used the endogenous nuclear-shuttling activity of the NFκB system, which is very prominent in many types of cancer including lung cancer, and found that insertion of a DNA nuclear targeting sequence (DTS) recognized by NFκB was able to improve plasmid nuclear delivery and enhance the therapeutic activity of a validated transcriptional cancer-targeted suicide gene therapy system [71].

4 Conclusions and future perspectives

The application of biomarkers in basic and clinical research has become common practice, and is well-documented in clinical trials [72]. Biomarkers play a significant role in improving drug development processes [72]. The US FDA is promoting the use of potential biomarkers in basic and clinical research. In most NE cancers, particularly SCLC, INSM1 has been shown to serve as a more sensitive and specific biomarker than other neuroendocrine markers. In this review, we focused on INSM1 as a potential biomarker and therapeutic target for neuroendocrine tumors. Although INSM1 has evolved as a subtle and specific biomarker for neuroendocrine tumors, its role in tumor development is not fully resolved yet. INSM1 has been reported to provoke tumorigenicity in NE tumors through its extranuclear activity. INSM1 modulates the Shh signaling pathway, as well as PI3K/AKT, MEK/ERK1/2, ADK, p53, cyclin D1, β-catenin, LSD1 and N-Myc protein expression (Fig. 2). However, INSM1 also acts as a transcription factor, and targeting a transcription factor directly for tumor growth suppression is difficult. Strategies targeting INSM1-associated signaling pathways have, however, been shown to be effective from the perspective of INSM1 expression in NETs [73, 74]. Fusing suicide or reporter genes targeting NETs to the *INSM1* promoter may be used for an effective diagnosis and therapy of NETs [43].

Since INSM1 expression in NETs is high, a small biopsy sample may be sufficient for detecting its expression by IHC staining for diagnosing particularly pancreatic and SCLC tumors [36]. Owing to its novelty and the involvement of putative unknown factors, the efficacy of INSM1 as a therapeutic target has not been tested yet in NET patients. Although several studies have investigated its use as a diagnostic, prognostic and/or predictive biomarker, no clinical trials have been performed so far. It would be useful to initiate a clinical trial in SCLC patients to analyze the efficacy of chemotherapy based on INSM1 expression. A further understanding of the role of INSM1 expression in the development and progression of NETs would be helpful for its use as a specific biomarker and therapeutic target, leading to an improved diagnosis and treatment of NETs. Also, more clinical studies are required to prove the sensitivity and specificity of INSM1 as a diagnostic marker in different NETs (Fig. 3). All the above sections highlight the potential diagnostic and therapeutic utility of INSM1 in different types of NET. Further clinical studies are required to firmly establish its utility in mainstream diagnostics.

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Compliance with ethical standards

Conflict of Interest None declared.

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