REVIEW

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MicroRNAs and Treatment with Somatostatin Analogs in Gastro-Entero-Pancreatic Neuroendocrine Neoplasms: Challenges in Personalized Medicine

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ABSTRACT

Molecular predictive biomarkers represent an essential tool for the future of personalized oncotherapy. Gastro-entero-pancreatic neuroendocrine neoplasms are a heterogeneous group of epithelial tumors with a steady increase in incidence and prevalence. Their effective management depends on early diagnosis, personalized risk stratification, and monitoring response to therapy. A crucial element is identifying accurate biomarkers to predict/monitor therapeutic responses, assess drug resistance, and quantify residual disease in a reproducible and less invasive way. Taking into consideration their role in cell differentiation, cell proliferation, apoptosis and tumor development, microRNAs have gained interest as potential prognostic markers and treatment response predictors in neuroendocrine neoplasms. This review is the first to summarize the available data on the possible role of microRNAs in evaluating the efficacy of somatostatin analogs treatment in gastro-entero-pancreatic neuroendocrine neoplasms. Although the literature is scarce, the let-7 family targeting phosphoinositide 3 kinase – protein kinase B 1 – mammalian target of rapamycin signaling pathway might represent a promising biomarker with potential clinical benefit, but further research is required before their eventual clinical application. Furthermore, the ambiguous molecular mechanisms of neuroendocrine proliferation and the undefined signaling pathway of somatostatin analogs should encourage future research in this field that may lead to a different clinical approach to neuroendocrine disease.

Key words: neuroendocrine neoplasms – somatostatin analogs – microRNAs – predictive biomarker – let-7 family.

Abbreviations: AKT: protein kinase B; BACH1: transcription factor BTB and CNC homology 1; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; EMT: epithelial-mesenchymal transition; GEP-NENs: gastro-entero-pancreatic neuroendocrine neoplasms; GH: growth hormone; HMGA2: high mobility group A2; HOXA9: homeobox protein A9; HOXB7: homeobox protein B7; IGF1: insulin-like growth factor; IGF1R = insulin-like growth factor receptor; IGFBP-7: insulin-like growth factor binding protein 7; IRS1: insulin receptor substrate 1; IRS2: insulin receptor substrate 2; LRP4: low-density lipoprotein receptor-related protein 4; MAPK: mitogen-activated protein kinase; miRNAs: microRNAs; miRs: microRNAs; MMP1: matrix metalloproteinse-1; mTOR = mammalian target of rapamycin; mTORC1: mammalian target of rapamycin complex 1; mTORC2: mammalian target of rapamycin complex 2; NEN: neuroendocrine neoplasm; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; PDK1: phosphoinositide-dependent kinase 1; PI3K: phosphoinositide 3 kinase; PI3KCD: phosphatidylinositol -4,5-biphosphate 3 kinase catalytic subunit delta; PIP 3 -phosphatidylinositol (3,4,5)–triphosphate; PTP: protein tyrosine phosphatases; pNEN: pancreatic NEN; RSPO2: roof-plate specific spondin-2; SI-NEN: small intestinal NEN; SSAs: somatostatin analogs; SST: somatostatin; SSTR: somatostatin receptor; TSC1: tuberous sclerosis complex 1; TSC2: tuberous sclerosis complex 2; WNT: wingless-type MMTV integration site family; WNT2B: wingless-type MMTV integration site family, member 2B; ZEB1: zinc finger E-box binding homeobox 1; ZEB2: zinc finger E-box binding homeobox 2.

INTRODUCTION

Nearly 50 years have passed since President Nixon declared war on cancer [1]. Although momentous achievements have been made in understanding carcinogenesis, struggles still exist when transposing this new, valuable information into clinical practice. Genetic and epigenetic alterations that lead to carcinogenesis occur at various levels, from depletion or gain of an entire chromosome to dysregulating a single microRNA that controls hundreds of genes.

Gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) represent a heterogeneous group of epithelial tumors arising from neuroendocrine cells of the digestive tract. With a steady increase in prevalence and incidence [2-5], they now hold second place in prevalence among gastrointestinal tumors (after colon cancer) [6]. The prognosis and survival of patients diagnosed with GEP-NENs are influenced by the location of the primary neoplasm, functional status of the patient, tumor differentiation and stage, as well as treatment response [7]. All GEP-NENs have malignant potential and actually, most patients have metastatic disease at diagnosis. However, the molecular mechanisms linking neuroendocrine proliferation and tumor progression are not yet fully understood. The histopathologic diagnosis remains the gold standard and the surgical resection is the first choice treatment, but it depends on size, location and secondary disease. As most patients are diagnosed with advanced forms and the recurrence rate is high, surgery can rarely be curative. Targeted medical treatments for cytoreduction are limited with most of them being palliative as well [8]. One of GEP-NENs' key features is somatostatin receptor (SSTR) expression [9]. This makes them targets for therapy with somatostatin analogs (SSAs), which have been demonstrated to exert both antisecretory and antiproliferative effects [7].

Besides correct diagnosis and prompt treatment, a crucial measure in the management and monitoring of GEP-NENs is identifying a biomarker that may be able to predict response to SSAs therapy, peptide receptor radionuclide therapy or detect recurrence after surgery. Thus far, no predictive molecule has been found [9]. Among recent diagnostic, prognostic and predictive biomarkers, microRNAs (miRNAs/miRs) could play a major role in monitoring GEP-NENs.

The purpose of this review is to summarize the existing information on miRNAs and their role in monitoring treatment with SSAs in GEP-NENs. We distinctively focused on identifying those miRNAs which may serve as predictive biomarkers in GEP-NENs and may be, thus, helpful in matching targeted therapies with patients. Exploring and understanding their mechanism of action was another objective of the paper.

Somatostatin and somatostatin analogs - an overview

Somatotropin release-inhibiting hormone or somatostatin (SST) is a very important endocrine regulator of neurotransmission and secretion. Somatostatin has two active forms, one consisting of 14 amino acids and the other consisting of 28 amino acids. It is predominantly found in the peripheral and central nervous system, in the gut and in the endocrine pancreas [10]. Apart from its inhibitory functions (the inhibition of pituitary hormones [11, 12], the regulation of gastrin and gastric acid secretion [13] and the inhibition of other hormones in the gastrointestinal tract and pancreas [14-17]), SST can also control cell growth and tumor development [18]. Furthermore, it seems to exert anti-inflammatory and anti-nociceptive effects [19].

These effects depend in part on the type of SSTRs expressed on the cell's surface [7]. Somatostatin acts through binding to five different G protein-coupled membrane receptors: SSTR1 to SSTR5, of which SSTR2 presents with 2 isoforms: SSTR2a and SSTR2b [20, 21]. The receptors are widely, but varyingly distributed throughout all tissues in the human body. Moreover, SSTRs are also expressed in human cancers, including GEP-NENs, with characteristic receptor profiles being described in certain tumors. However, the SSTR profiles vary considerably between tumor types and also between tumors of the same type, with 70-90% of GEP-NENs most frequently expressing SSTR2, followed by SSTR5 [22-24].

A multitude of intracellular pathways following activation of the SSTRs have been described [7]. Inhibition of exocytosis, therefore the antisecretory function, is possible by altering the levels of second messengers, such as cyclic adenosine monophospate (cAMP) or by activating ion channels, and thus altering intracellular calcium levels. The antiproliferative role is exerted directly by inducing cell cycle arrest or apoptosis or by inhibiting the release of growth factors and indirectly by inhibition of angiogenesis [18, 19]. It seems that SSTR2 and SSTR3 were related to increased apoptosis, SSTR1 and SSTR2 to suppressed cell migration and invasion while all five receptors were related to reduced cell proliferation [25]. Actually, all five SSTRs can induce cell cycle arrest by activating the protein tyrosine phosphatases (PTPs) and subsequent modulation of different intracellular second messengers and pathways including cyclic guanosine monophosphate (cGMP) (SSTR2 and SSTR5), mitogen-activated protein kinase (MAPK) (SSTR1, SSTR2, SSTR4, SSTR5) and/or phosphoinositide 3 kinase – mammalian target of rapamycin (PI3K–mTOR) or PI3K– nulear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (SSTR2, by binding to PI3K's p85 part) [25]. Of these, the PI3K pathway, in particular, is known to work as a signal transduction mediator in GEP-NENs [26]. Activation of PTPs also results in inhibition of IGF-1 receptor (IGF1R) signaling (SSTR1–SSTR5) (and probably also of other receptors such as insulin receptor) [25].

These SSTR properties form the molecular basis for various clinical applications, including symptomatic therapy in hormone-secreting GEP-NENs, in vivo diagnostic examination with Octreoscan to evaluate the extent of the disease, and 90Y-DOTA, Tyr3-octreotide (90Y-DOTATOC) radiotherapy [26]. However, because of SST's incredibly short half-life (approximately 3 minutes), synthetic compounds have been developed [8]. If the natural ligands of SSTR1-5 (SST-14, SST-28 and cortistatin) are bound to these receptors with a high affinity, the synthetic peptide analogues, specifically Octreotide (MS201-995), Vapreotide (RC-160), Lanreotide (BIM 23014) and Seglitide (MK 678), however, demonstrate a variable affinity for SSTR, binding mostly to SSTR2 and showing lower affinity for SSTR5 [27]. By contrast, Pasireotide (SOM 230), a novel multireceptor-targeted SST displays a broader spectrum and additionally binds SSTR 1, 3, and 5 [27]. The preclinical data gave rise to the hope that Pasireotide represents a more effective antiproliferative tool in the treatment of patients with NENs but results from clinical trials have, so far, not been as positive as expected [7]. Nevertheless, with more than thirty years of gathered information [28], SSAs have confirmed their role in GEP-NENs' treatment, mitigating symptoms linked with functioning tumors – PROMID study [29], as well as inhibiting tumor growth – CLARINET study [30], although the exact underlying mechanisms of action of the SSAs and the signaling pathway targeted by this therapy need to be further clarified.

MicroRNAs in human pathology

MicroRNAs are part of a family of short, non-coding RNAs (less than 30 nucleotides) that act post-transcriptionally, inhibiting translation of or degradation of messenger RNA. In essence, the role of miRNAs is to optimize gene expression involved in cell differentiation, cell proliferation, apoptosis and tumor development [31]. It appears that 60% of protein-encoding genes are controlled by miRNAs, with one miRNA being able to influence the transcription of several genes, and with one gene being influenced by various miRNAs [32, 33].

The dysregulation of miRNAs is a hallmark of human pathology. Diabetes, cardiovascular, lung, kidney, neurodegenerative diseases, polycystic ovary syndrome, all express aberrant miRNAs [34-36]. Several studies appraised miRNAs as biomarkers for neoplastic disease and explored their role as predictors of treatment response [37-39]. The specific miRNA expression pattern was described in hematological cancer [40], lung cancer [41], prostate cancer [42], gastrointestinal cancers [43-45] to name a few. While a subset of these miRNAs are overexpressed in cancers and have shown to target tumor suppressor genes, hence termed oncogenic miRNAs or oncomiRs, other miRNAs that target oncogenes are underexpressed in cancers and are often called tumor-suppressor-like miRNAs [46]. Certain miRNAs are known to have a dual role, both oncogenic and tumor-suppressive, depending on the cancer-specific context [47, 48]. Dysregulation of miRNAs has been reported to be involved in the pathogenesis of NENs, as well [31, 49-51].

Gastro-entero-pancreatic neuroendocrine neoplasms and microRNA dysregulation

Altered expression levels of miRNAs were determined in NENs of the lung, thyroid and prostate [51-54] as well as in insulinomas [55]. The scarcity and diversity of GEP-NENs have led to a small number of studies that evaluate miRNA expression pattern in this disease. Both upregulation and downregulation of microRNAs have been observed in GEP-NENs, but the lack of concordance of the study designs and methods and the heterogeneous, sometimes even conflicting results, indicate that their role in neuroendocrine carcinogenesis still needs to be consolidated. In addition, most studies provide tissue based results, the ones evaluating circulating miRNAs come in short. Nevertheless, let-7 family, along with miRNA-7, miRNA-148, miRNA-96, miRNA-196, miRNA-21, miRNA-133, miRNA-125 and miRNA-375 are the most frequently found to be dysregulated and seem to be promising biomarkers. The main findings identified regarding pancreatic and GEP-NENs (without a certain separation between the segments of the digestive tract) are presented in Table I, whereas the ones regarding the NENs of the small intestine are summarized in Table II. Two studies evaluated miRNA expression pattern in rectal and colorectal NENs. The first one singled out 10 miRNAs that were associated with lymphovascular invasion in rectal NENs, with miRNA-885-5p being the most up-regulated [56]. Wang et al. [57] identified miRNA-186 to be significantly downregulated when comparing blood, tumor and stool samples from colorectal NEN patients with healthy controls.

Table I. MicroRNA dysregulation in gastro-entero-pancreatic neuroendocrine neoplasms

Reference	miRNA	Dysregulation	Observations
Roldo et al. 2006 [58]	miR-103, miR-107	↑	Discriminated between pancreatic tumor tissue and normal pancreatic islets.
	miR-155	↓	
	miR-204	↑	Singled out insulinomas when compared to nonfunctioning tumors.
	miR-21	↑	Associated with tumor proliferation and metastasis.
	miR-99a, miR-99b, miR-100, miR-125a, miR-125b1, miR-125b2, miR-129-2, miR-130a, miR-132, miR-342	↑	Differentiated pNENs from acinar cell carcinomas and normal pancreas.
Thorns et al. 2014 [59]	miR-642, miR-210	↑	Differentiated pNEN tissue samples from the exocrine pancreas and pancreatic islets; correlated to MiB1 score and metastasis, respectively.
	miR-193b	↑	Differentiated pNEN patients (serum samples) from healthy controls.
Lee et al. 2015 [60]	miR-196, miR-142-5p, miR-27b	↑	High MiR-196 expression - aggressive behavior, poor prognosis, decreased disease-free and overall survival in pNENs.
Gill et al. 2019 [61]	miR-3653	↑	Discriminated patients with distant metastases from patients with locoregional disease following surgical resection in pNENs.
	miR-4417, miR-574-3p, miR-664b-3p	↓	
Zimmerman et al. 2018 [62]	miR-21, miR-30a-5p, miR-320, miR-331, miR-660	↑	Associated with the presence of metastases in GEP-NENs; a connection between metastatic disease and Ki-67 proliferation index through miR-150, miR-21 and miR-660 was found.
	let-7b, miR-150	↓	
Cavalcanti et al. 2020 [63]	miR-96-5p	↑	Demonstrated that miR-96-5p's levels increased with tumor grade in GEP-NENs.
Panarelli et al. 2019 [64]	miR-375, miR-143, miR-7, miR-21	↑	Expression of miR-615 and miR-92b can discriminate ileum and appendix NEN from rectum and pNEN, and ileum NEN can be differentiated from appendix NEN through miR-125b, miR-192 and miR-149, whereas miR-429 and miR-487b can separate rectal from pancreatic NEN.
	miR-615, miR-92b, miR-125b, miR-192, miR-149, miR-429, miR-487b, miR-328	↓	

miR: microRNA; GEP-NENs: gastro-entero-pancreatic neuroendocrine neoplasms, pNENs: pancreatic neuroendocrine neoplasms

Table II. MicroRNA dysregulation in small intestinal neuroendocrine neoplasms

Reference	miRNA	Dysregulation	Observations
Ruebel et al. 2010 [65]	miR-183, miR-488, miR-19a+b	↑	Compared matching primary ileal NENs and metastases; miR-133a - putative prognostic biomarker or therapeutic target.
	miR-133a, miR-145, miR-146, miR-222, miR-10b	↓	
Li et al. 2013 [66]	miR-96, miR-182, miR-183, miR-196a	↑	Compared primary SI-NENs to their respective metastases and identified a correlation between tumor progression and miRNA dysregulation.
	miR-31, miR-129-5p, miR-133a, miR-215	↓	
Dossing et al. 2015 [67]	miR-129-5p, let 7 family	↓	(tumor tissue and cell lines): compared the expression profiles of primary tumors with their matched metastases and normal tissue; Transfection of miR-129-5p and let-7 family led to growth inhibition of pulmonary and intestinal cell lines;
Miller et al. 2016 [68]	miR-204-5p, miR-7-5p, miR-375	↑	The upregulated miRNAs distinguished between SI-NENs and healthy controls; the downregulated miRNAs - markers of metastatic disease.
	miR-1, miR-143-3p	↓	
Heverhagen et al. 2017 [69]	miR-7-5p, miR-96-5p	↑	Compared SI-NENs with control tissue sample; MiR-7-5p – dysregulated in the sera of patients as well, proposed as biomarker;
	miR-9-5p, miR-122-5p, miR-124-3p, miR-143-3p, miR-144-3p	↓	
Mandal et al. 2017 [70]	miR-96	↑	Differentiated metastases (both liver and lymph nodes) from the primary tumor.
	miR-133a	↓	
Bowden et al. 2017 [71]	miR-22-3p, miR-21-5p	↑	(tumor tissue and serum samples): the dysregulated miRNAs - putative biomarkers, associated with metastatic disease.
	miR-150-5p	↓	
Arvidsson et al. 2017 [72]	miR-375	↑	Singled out ileal-NENs when compared to normal small intestine.
Malczewska et al. 2019 [49]	miR-425-5p, miR-500a-5p, miR-125b-5p, miR-362-5p	↑	(serum samples): compared SI-NEN patients with healthy controls; MiR-425-5p and 500a-5p - diagnostic markers; MiR-125b-5p and miR-362-5p might predict residual or recurrent disease.

miR: microRNA; SI-NENs: small intestine neuroendocrine neoplasms.

Somatostatin analogs treatment and microRNA dysregulation in gastro-entero-pancreatic neuroendocrine neoplasms

As already mentioned, miRNAs might act as biomarkers or even as targets for tumor directed therapy [73]. It is difficult to ascertain an association between GEP-NENs and miRNAs (Tables I and II); the matter is even worse concerning the role miRNAs play in modulating the effects of SSAs (Table III). The provided results are unfortunately highly heterogeneous. Nevertheless, there are still a number of miRNAs that may play a role in both GEP-NEN pathogenesis and responsiveness to SSAs therapy.

Let-7 family

Bosch et al. [74] analyzed for the first time the individual effect of therapy with SSAs on the miRNA dysregulation pattern in small intestinal NENs (SI-NENs). Besides identifying a dysregulation in miRNA expression induced by treatment with SSAs (Table III), their analysis revealed that let-7c-5p was consistently upregulated whereas miRNA-3137 was consistently downregulated in every patient after SSAs therapy. As shown in Table I and Table II, two other studies investigated the role of let-7 family in NEN carcinogenesis and found that the downregulation of this miRNA family is involved in the metastatic process [62, 67]. Later, through fundamental research, Dossing et al. [23] demonstrated that SSAs therapy was responsible for the upregulation of 4 of the let-7 family members in lung and small intestine cells [23], which resulted in inhibition of the growth of the carcinoid cell lines. It seems that SSAs can reestablish let-7 family expression, thus probably overturning the pathways involved in NEN development. Let-7 miRNAs are involved in multiple biological processes including cell differentiation and it is well known that there is an association between let-7 dysregulation and different types of aggressive cancers. Specifically, they were widely identified as tumor suppressors that directly target miRNAs of genes involved in the cell cycle and in signal transduction pathways that lead to carcinogenesis. The loss of let-7 family members indicates poor survival in general [76]. However, the function of each let-7 family member is ill-defined, they seem to each have different functions, even in the same cell [76]. Moreover, let-7 levels are constantly changing due to genetic and epigenetic factors and it appears that both low and high levels of this family can lead to tumorigenesis, emphasizing the importance of its regulation [77]. Regarding NENs, they were found to target HMGA2, BACH1 and MMP1 and reduce the expression of these oncogenes that resulted in growth inhibition of carcinoid cell lines [67]. The let-7 miRNAs are fundamental performers in the insulin sensitivity and the glucose metabolic pathway through the inhibition of IGF-1R, a decisive target in the PI3K/mTOR signaling route [78, 79]. Let-7 was also found to induce autophagy by coordinately

Table III. Impact on miRNA expression of somatostatin analogs treatment in neuroendocrine tumors

Reference	MiRNA	Dysregulation	Observations
Bosch et al. 2019 [74]	let-7c-5p, miR-24-3p, and miR-215-5p	↑	The miRNAs profiles of tumor tissues from eight patients (two tumor samples of the same patient prior to and after the initiation of treatment) were explored and the dysregulation pattern induced by SSAs in SI-NENs was dentified.
	miR-3137, miR-10a-3p, miR-185-3p, miR-339-5p, miR-371a-5p, miR-4436b- 5p, mir-4653-3p, mir-4793-3p, miR-619-5p, miR-4455, and miR-4656, miR-1226-3p	↓	
Dossing et al. 2018 [23]	let-7 family, miR-7, miR-148a	↑	Evaluated the regulative role of SSAs on miRNA expression profiles in two cell lines – lung (NCI-H727) and small intestine (CNDT2).
Li et al. 2015 [75]	miR-96, miR-182, miR-183, miR-196a, miR-200a	↑	Explored the expression pattern of 9 miRNAs previously identified by them as having a role in tumor progression[66]. They first compared both untreated and SSAs-treated SI-NEN patients with healthy donors and then further explored this therapy's role in miRNA level regulation by comparing untreated with SSAs-treated patients. Apart from mir-200, the other 4 miRNAs showed a significant upregulation in the SSAs-treated group; the previously identified downregulated miRNAs remained essentially unaltered.

suppressing components of an amino acid sensing pathway to repress mTORC1 and prevent its activation [80] (Fig 1). Others reinforced these results and have demonstrated that the let-7 family can directly target and inhibit IGF-1 and IGF-1R. This leads to the inhibition of PI3K, and thus abolishes cell division, differentiation and survival [81, 82]. To sum up, the positive modulation of the let-7 family by SSAs targets inhibits the IGF-1/PI3K signaling pathway, in addition to potentially inhibiting glucose nutrients in aiding the rapid growth of cancer cells. Through this mechanism, cancer cells are targeted on both survival signaling as well as their supply of nutrients [23].

MicroRNA-3137 and microRNA-185

As shown in Table III, besides let-7 family, Bosch et al. also identified that the downregulation of miRNA-3137 and miRNA-185 is induced by SSAs therapy in SI-NENs [74]. Concerning miRNA-3137, there is no distinct literature about its specific effects, but target analyses showed that it interacts with SSTR2, its downregulation induced by SSAs treatment being a possible mechanism through which this therapy exerts its antitumor properties. Regarding miRNA-185, in a rat pituitary adenoma GH3 cell line, it appears to function as an oncogene. Fan et al. [83] observed reduced expression of miRNA-185 and increased expression of SSTRs in SSAs responder growth hormone (GH)- secreting pituitary adenomas pituitary adenomas when compared to SSAs non-responder adenomas and normal pituitary glands. Their study also found that miRNA-185 targeted SSTR2 mRNA to downregulate SSTR2 protein expression, promote proliferation, and inhibit apoptosis of tumor cells in the rat pituitary adenoma GH3 cell line [83]. This suggests that miRNA-185 might be involved in drug resistance to SSAs and pituitary adenoma tumorigenesis. However, most studies investigating miRNA-185 and its role in carcinogenesis, depicts it as a tumor suppressor, by blocking protein kinase B (AKT1) in non-small cell lung carcinoma [84], by targeting vascular endothelial growth factor in breast cancer [85], or through influencing the WNT2B pathway in nasopharyngeal carcinoma in vitro [86]. Thus, downregulation of miRNA-185, such as downregulation of miRNA-3137, might be a direct effect of treatment with SSAs, or the antitumor properties of this therapy are mediated at least in part via miRNA-185 downregulation. Further studies are required to confirm the effects of biotherapy with SSAs on miRNA-185 and miRNA-3137 and to explore the underlying mechanisms as well.

MicroRNA-7 and microRNA-148a

In addition to the let-7 family and miRNA-3137, miRNA-7 and miRNA-148a might be of interest as well. It is known that miRNA-7 is endocrine specific [87] and, as shown in Table I and Table II, highly present in NENs [23, 64, 68, 69, 72]. MiRNA-7 has been involved as tumor suppressor in multiple cancer types, targeting numerous oncogenic signaling pathways. In glioblastoma, miRNA-7 inhibits both epidermal growth factor receptor (EGFR) and the AKT-mTOR signaling, by targeting upstream regulators, namely insulin receptor substrate 1and 2 (IRS1 and IRS2) [88]. In addition, in hepatocellular carcinoma, it has been shown to inhibit cellular growth, invasion and migration in vitro and more importantly tumorigenesis and metastasis in vivo, through blocking PIK3CD. This structure is a decisive item of the PI3K/AKT pathway and functions downstream of EGFR [89]. Another essential aspect of the induction of miRNA-7 expression in cancer lies in the fact that miRNA-7, through its inhibitory actions on central cancerous signaling pathways, can increase sensitivity and overturn the otherwise chemo- or radiotherapy resistant tumor cells [90]. MiRNA-7 also directly targets IGF-1R in gastric cancer with significant inhibition of the metastatic potential being observed [91]. Regarding NENs, miRNA-7 appears to act mainly as an oncogene, as it is upregulated in several studies performed on GEP-NEN tissues. Dossing et al. [23] revealed an interesting and surprising finding, namely that miRNA-7 was further up-regulated by SSAs and able to inhibit the proliferation of NCI-H727 and CNDT2 carcinoid cells, indicating that it could mediate some of the growth inhibitory effect induced by SSAs therapy [23]. On the same note, inhibiting miRNA-7 led to the increased growth of the carcinoid cell lines. Both miRNA-7 and SSAs target the PI3K/AKT/mTOR pathway and influence IGF-1 and IGF-1R, highlighting the significance of the up-regulation

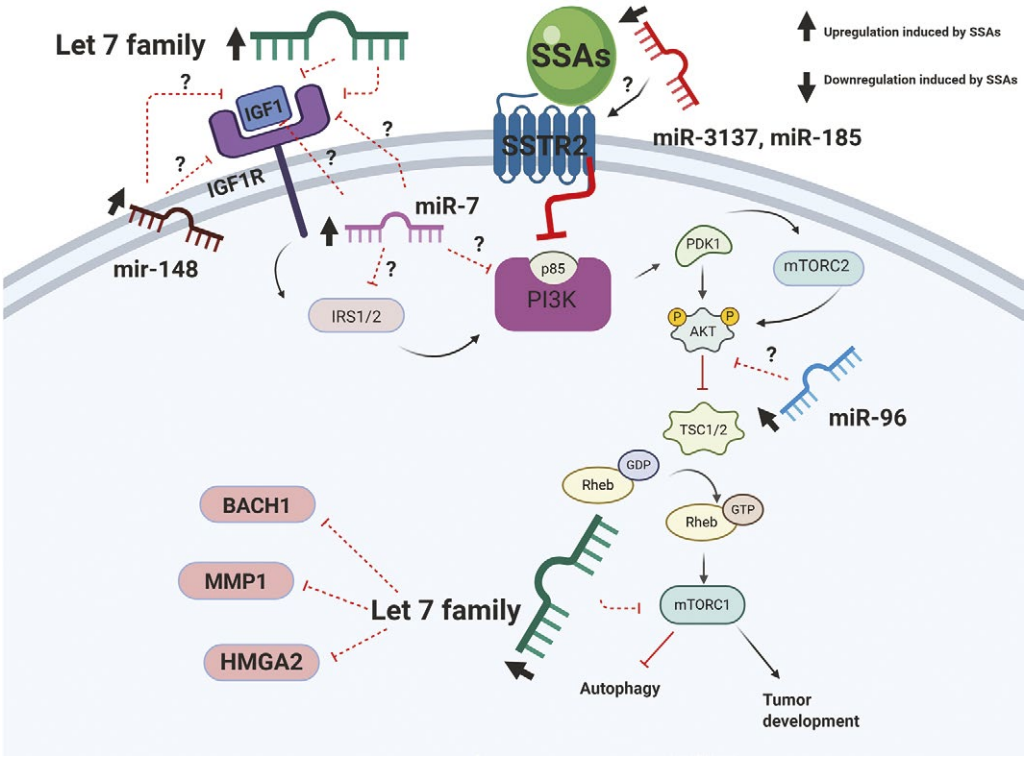

Fig. 1 (created with BioRender.com). The mechanisms and pathways through which therapy with SSAs by modulating miRNAs expression, might control tumor growth. As illustrated in the figure, let-7 family, but also other miRNAs, were found to be dysregulated by SSAs therapy and might act mainly by targeting different key components of the PI3K/AKT/mTOR pathway to inhibit GEP-NENs progression. Normally, activation of mTOR through a cascade of activating or inhibiting processes, leads to increased tumor development and decreased autophagy. The upregulation of let-7 family (or the reestablishment of let-7 family expression), miR-7, miR-148 and miR-96, as well as the downregulation of miR-3137 and miR-185 induced by SSAs seems to interfere with this signaling pathway and lead to its inhibition. Let-7 family targets IGF1, IGF1R and components of an amino acid sensing pathway upstream of mTORC1. MiR-7 inhibits IGF1, IGF1R, IRS1&2 and PI3K (the latter through PI3KCD). Mir-148 targets IGF1 & IGF1R. MiR-96 normally directly targets AKT mRNA, leading to its downregulation and thus induction of mTOR pathway, but its upregulation possibly inverses its role and mir-96 might act as a tumor suppressor, inhibiting this pathway. By interacting directly with SSTR2, miR-3137 and miR-185 normally downregulate SSTR2 protein expression, but their downregulation possibly reverses this process. IGF1R: insulin-like growth factor receptor; IGF1: insulin-like growth factor; PI3K: phosphoinositide 3 kinase; IRS1: insulin receptor substrate 1; IRS2: insulin receptor substrate 2; PIP3: phosphatidylinositol 3; PDK1: phosphoinositide-dependent kinase 1; AKT: protein kinase B; mTORC1: mammalian target of rapamycin complex 1; mTORC2: mammalian target of rapamycin complex 2; TSC1: tuberous sclerosis complex 1; TSC2: tuberous sclerosis complex 2; SSAs: somatostatin analogs; SSTR2: somatostatin receptor 2; PI3KCD: phosphatidylinositol -4,5-biphosphate 3 kinase catalytic subunit delta; arrow: triggers; red line: inhibits; oncogenes: HMGA2, BACH1 and MMP1.

of this miRNA by the SSAs treatment. Although it seems that miRNA-7 mostly acts as an oncogene in GEP-NENs, its upregulation by SSAs suggests that it may be able to invert its role to tumor suppressor at higher levels of expression. Other miRNAs have demonstrated a dual role in the same tumor model, such as miRNA-375 and the miRNA-191/245 cluster. In prostate cancer, depending on the cellular context and disease stage, miRNA-375 was found to act either as an oncomiR or tumor-suppressor miRNA [92]. The MiRNAs from the miRNA-191/245 cluster were shown to function as oncogenes in estrogen receptor positive cells and to impair tumor growth in estrogen receptor negative cells in breast cancer [93].

MiRNA-148a was identified to be endocrine specific as well, playing a major role in endocrine tumorigenesis [23]. Furthermore, therapy with SSAs was found to up-regulate miRNA-148a's expression in endocrine cells, which makes this miRNA a possible predictive biomarker in GEP-NENs [23]. MiRNA-148a is aberrantly expressed in different types of malignant diseases, acting mostly as a tumor suppressor. Its upregulation, on the other hand, is linked only with glioma and osteosarcoma [94]. Xu et al. [95] investigated through fundamental research whether miRNA-148a is involved in regulating IGF-1R signaling activity in breast cancer. The study proved that miRNA-148a could halt the proliferation of breast cancer cells by downregulating IGF-1R through binding directly to its 3'UTR unit [95]. Therefore, the upregulation of miRNA-148a induced by SSAs therapy [23] might inhibit the PI3K signaling pathway through influencing growth factors like IGF-1 and its receptor IGF-1R [26, 96], which might result in the induction of cell cycle arrest and apoptosis [26] in NENs.

MicroRNA-200

The miRNA-200 family consisting of miRNA-141, miRNA-200a, miRNA-200b, miRNA-200c and miRNA-429, is one of the most studied regulators of the epithelial-mesenchymal transition (EMT) process [97, 98]. The miRNA-200 family has also been studied regarding endocrine carcinogenesis and SSAs therapy. Specifically, it was shown that miRNA-200a was independently upregulated in the liver metastases of SI-NEN patients, irrespective of SSAs treatment [75]. Firstly, Li et al. [66] investigated potential differences in miRNA expression between primary tumors, mesenteric and liver metastases in SI-NEN tissue specimens and identified 9 miRNAs that may have an impact on SI-NEN tumor progression [66] (Table II). Later on, by extending their analyses from tissue specimens to serum samples, they demonstrated that SSAs have a regulative role on miRNA levels [75]. The QRT-PCR analysis revealed that miRNA dysregulation pattern is also found when investigating blood samples and when comparing SSAs-treated and untreated patients to healthy controls. In this respect, downregulation of miRNA-31, miRNA-129-5p, miRNA-133a, and miRNA-215 was also identified in patients' serum at all stages of disease. On the other hand, sustained upregulation of miRNA-196a, miRNA-182 and miRNA-200a was identified in patients with liver metastases alone. Moreover, when the panel of 9 miRNAs was evaluated in SSAs-treated versus untreated patients, the levels of miRNA-96, miRNA-182, miRNA-183, miRNA-196a were even higher in the SSAs-treated patients, regardless of stage of disease, as compared with untreated or healthy donors. Of note, they showed that in patients with liver metastases, miRNA-200a was upregulated irrespective of SSAs treatment. MiRNA-200 family directly targets the zinc finger E-box binding homeobox 1 and 2 (ZEB1 and ZEB2) transcription factors, which normally act as transcriptional repressors of E-cadherin, and inhibit the metastasis process [97, 98]. They act as important regulators of breast cancer progression, being responsible for maintaining mammary epithelial identity. Their downregulation is seen in more aggressive tumors, whereas their loss results in tumor cells acquiring mesenchymal characteristics including enhanced metastatic capacity [99]. Fundamental research showed that when treating medullary thyroid carcinoma cells with antagomiRs for miRNA-200b and miRNA-200c, the cells failed to express E-cadherin and gained an invasive profile [100]. In anaplastic thyroid carcinoma, miRNA-200a, -b and -c were shown to be downregulated, as a consequence of overexpression of EGF, indicating that this family may play a regulatory role in modulating EMT, but this time by EGF/EGFR [101]. Using a mouse model of pNENs, Title et al. [102] demonstrated that, in a dosage-dependent regulation, even small changes in ZEB1 expressions are plentiful to produce a great impact on the EMT process. By removing or reexpressing members of the miR-200 family, the EMT process was promoted or halted, respectively, thus proving that they are important coordinators of the EMT axis [102]. However, there are discrepancies in literature and some studies showed that miRNA-200 family members can, on the contrary, enhance mammary tumor cell migration and/or metastasis [103]. It seems they act pleiotropically, fulfilling both the tumor suppressor and oncogene roles. Regarding SI-NENs, aberrant miRNA-200a expression has been detected in tissue specimens as well in patients' serum [75]. However, contrary to what had been described before, miRNA-200a levels were exclusively and significantly higher in patients who developed liver metastases when compared to healthy patients. Therefore, unexpectedly, miRNA-200a exhibited an atypical behavior, meaning that it was upregulated only in the liver metastases patients, with no significant difference between the untreated patients with primary tumors or lymph node metastases and healthy donors. Furthermore, this phenomenon was independent of SSAs treatment, with no considerable disparity between the treated and untreated patients. Yet, when comparing untreated patients with primary tumors or lymph node metastases with SSAs - treated patients with primary tumors or lymph node metastases, miRNA-200a showed significant upregulation induced by treatment. It seems as if miRNA-200a respects its anti-proliferative role up to the stage of metastasis (idea supported by the fact that it did not show upregulation in primary and lymph node metastases compared to healthy donors and by its upregulation induced by SSAs treatment in the aforementioned stages of disease) where its role is reversed. This suggests that, just like miRNA-7, miRNA-375 [92] and the miRNA-191/245 cluster [93], miRNA-200a might act as a tumor suppressor in the initial phases of SI-NEN progression, probably in relation to the EMT pathway, but, as the disease progresses and as a result of genetic and epigenetic changes, we experience an overturn of its role.

MicroRNA-96

Not unexpectedly, the expression pattern of miRNA-96 is heterogenous as well and it functions either as an oncomiR by promoting cellular growth, invasiveness and metastasis in breast cancer [104], pancreatic cancer [105] and hepatocarcinoma [106] or as a tumor suppressor in renal cell carcinoma [107], colorectal cancer [108] and lymphoma [109]. Regarding NENs, it seems that miRNA-96 mostly acts as an oncogene; its levels increase with tumor grade [63] and its regulation is influenced by SSAs therapy. It was shown to directly target at the 3'UTRs of AKT1S1 mRNA, leading to its downregulation and thus induction of mTOR activities [110, 111]. In addition, miRNA-96 also targets insulin-like growth factor binding protein 7 (IGFBP-7), a gene involved in regulating the availability of IGFs in tissue and in supervising IGF's binding to its receptors. The IGFBP-7 protein is active in the vascular endothelium. Its interactions with IGFs and their receptors are thought to help stop the BRAF signaling pathway, which is involved in directing cell growth [112]. As previously shown in Tables I and II, miRNA-96 plays a prognostic role in GEP-NENs as it is correlated with tumor progression [66, 69, 70]. Regarding biotherapy with SSAs, Mao et al. [112] noted a down-regulation of miRNA-96 in GH-secreting pituitary adenomas samples treated with lanreotide [112]. Similarly, after having demonstrated that miRNA-96 is involved in SI-NEN progression and metastasis, since its levels were significantly higher in primary tumor and liver metastases patients vs. healthy donors, Li et al. [75] showed that SSAs therapy further upregulated mir-96 in SI-NEN at all stages. This may imply that miRNA-96, when expressed at sufficiently high levels, might inverse its role and act as a tumor suppressor, following

the expression pattern of miR-7, miR-200, miR-375 and miR-191/245 cluster [75, 92, 93].

MicroRNA-196a

Aberrant miRNA-196a expression has been observed in a broad spectrum of malignancies, with increasing evidence that it may serve as a diagnostic, prognostic or predictive biomarker, both tissue and blood based, in digestive tract cancers [113]. As indicated by Table I, the expression of miRNA-196a is correlated with increased proliferation in pNENs, as well as with aggressive behavior and metastasis. It has been demonstrated that miRNA-196a displays complementarity to the homeobox (HOX) gene cluster [114-116] and that it targets genes that play important roles in the WNT signaling pathway [117-119]. MiRNA-196 is highly expressed in NENs when compared to healthy controls both in tissue and serum specimens [66, 75]. Li et al. [75] studied the biological role of miRNA-196a target genes in midgut and lung carcinoid cells, demonstrating the importance of miRNA-196a in regulating HOXA9 and HOXB7 together with LRP4 and RSPO2, both at the transcriptional and translational levels [120]. This hints towards a possible implication of the WNT signaling pathway in NEN carcinogenesis. Although a regulatory effect on cell proliferation was not found, miRNA-196's relationship with the HOX gene cluster and the WNT signaling pathway raised interest and deserves recognition. Concerning SSAs therapy, miRNA-196a's expression was high in SI-NENs and SSAs therapy further upregulated its levels at different disease stages, similar to miRNA-96's behavior, but the underlying mechanisms remain uncertain.

CONCLUSIONS

There is an increasing interest in using miRNAs as predictive biomarkers in GEP-NENs. As far as we are aware, this is the first study that aimed at organizing the existing information on miRNAs and their role in mediating the antiproliferative effects of SSAs therapy in GEP-NENs. Unfortunately, the small number of studies and the highly heterogeneous results prevent us from drawing a definite conclusion. However, a possible association between let-7 and SSAs has been unveiled. Future challenges remain to reinforce this association or even illustrate the mechanisms through which, therapy with SSAs, by modulating let-7 family, can control tumor growth. The putative dual role of miRNA-7, miRNA-148, miRNA-96, and miRNA-200 (tumor suppressor or oncogene depending on the stage of the tumor or even their expression levels) strengthens the idea that more research on these miRNAs and a better understanding of the underlying mechanisms of action should be sought out before their eventual clinical usage. The fact that these miRNAs and SSAs therapy seem to target mTOR signaling pathway takes the clinician a step forward to understanding how and when to administer this treatment to obtain the best possible outcome and paves the way toward new targeted therapy.

We know now that cancer, specifically neuroendocrine neoplasms, is far more complex, heterogeneous and intricate than imagined in 1971. The dream of using miRNAs as predictive tools in GEP-NENs is achievable only by understanding their biological function and overcoming the shortcomings through more validation studies on a greater number of patients and with standardized methods. Moreover, the miRNA field will need to further focus on whether different miRNAs indeed have specific activities in a particular cancer type or whether an algorithm based on a panel of miRNAs that will provide a multifaceted view of the disease, is required.

Conflicts of interest: None to declare.

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