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REVIEW

Somatostatin receptors and the associated intracellular machinery: the two sides of the coin

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Abstract

Somatostatin receptors (SSTs) are widely expressed in pituitary tumors and neuroendocrine neoplasms (NENs) of different origins, i.e. the gastrointestinal tract and the thorax (lungs and thymus), thus representing a well-established target for medical treatment with SST ligands (SRLs). However, the response to SRLs is highly heterogeneous between tumors. Two main factors can contribute to this variability: (i) the differential SST expression among tumor types and (ii) the differential expression/modulation of the SST-related intracellular machinery. In this literature review, we provide an overview of available data on the variable expression of SSTs in pituitary tumors and NENs, together with the resulting clinical implications. Moreover, we aim to describe the complex intracellular machinery involved in SST signaling and trafficking. Particularly, we will focus on β-arrestins and describe their role in receptor internalization and recycling, as well as the various functions of these scaffold molecules in tumor pathogenesis and progression. This review highlights the interplay between membrane receptors and intracellular machinery, together with its role in determining the clinical behavior of the tumor and the response to treatment in patients with pituitary tumors or NENs.

Keywords: somatostatin receptors; pituitary tumors; neuroendocrine neoplasms; beta-arrestins; filamin A

Introduction

Somatostatin receptors (SSTs) belong to the family of seven transmembrane G protein-coupled receptors (GPCRs) (Günther *et al.* 2018). Five different SST subtypes have been identified, named SST_1 to SST_5 (Günther *et al.* 2018). Different splice variants have been identified for SST subtype 2 (SST_2) and subtype 5 (SST_5). The two variants described for SST_2 are named SST_{2A} and SST_{2B}; only SST_{2A} has been detected in human tissues. In this review, we will use the denomination SST_2 to refer to the 2A isoform (Günther *et al.* 2018). SST_5, on the other hand, presents with two truncated forms with four and five transmembrane domains (TMD), named SST_5TMD4 and SST_5TMD5, respectively (Durán-Prado *et al.* 2009). SSTs are widely expressed in the endocrine system, and in physiological conditions, their activation by naïve somatostatin (SRIF) leads to the inhibition of hormone secretion, including growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), insulin, glucagon, gastrin, cholecystokinin, and ghrelin. This effect is mainly mediated by the reduction of intracellular cAMP and Ca^{2+} levels (Theodoropoulou & Stalla 2013, Günther *et al.* 2018). Furthermore, different SST subtypes mediate the inhibition of cell proliferation by interacting with various intracellular pathways (e.g. activation of protein tyrosine phosphatases (PTP), consequential inhibition of extracellular signal-regulated kinase (ERK), and AKT phosphorylation), and can induce cell apoptosis

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(Theodoropoulou & Stalla 2013, Günther *et al.* 2018). Following ligand binding and the activation of associated signaling cascades, SSTs are usually desensitized, internalized, and targeted either to lysosomes for further degradation or recycled back to the cell membrane (Gatto & Hofland 2011). Receptor phosphorylation by GPCR kinases (GRKs) and β-arrestin (ARRB) recruitment to the cell membrane play a crucial role in the desensitization and internalization of SST_2, SST_3, and SST_5 (Tulipano *et al.* 2004).

The expression of SSTs is usually retained, or even increased, in tumors originating from the endocrine system, particularly in pituitary tumors and neuroendocrine neoplasms (NENs). In these tumor types, SSTs represent a valuable tool for the diagnosis, a target for treatment, as well as a potential prognostic factor.

The aim of our review is to provide a comprehensive overview of available data on the differential expression of SSTs in endocrine tumors, with a particular focus on pituitary tumors and NENs, and to underline the role of the intracellular machinery in the regulation of SST signaling in the different tumor types. Moreover, the emerging dual roles of ARRBs, i.e. GPCR-dependent and independent actions, are discussed.

Expression and impact of the differential SST expression in pituitary and neuroendocrine neoplasms

SSTs are widely expressed in pituitary tumors and NENs, and the different SST subtypes are often co-expressed in the same lesion (Reubi & Waser 2003, de Bruin *et al.* 2009, Song *et al.* 2016). Over the last 20 years, it has been largely demonstrated that SSTs can form homo- and heterodimers on the cell membrane, resulting in the modulation of the downstream signaling of the receptor (Rocheville *et al.* 2000, Pfeiffer *et al.* 2001, Milligan 2007, Grant *et al.* 2008, Gatto & Hofland 2011, Günther *et al.* 2018). The pattern of SST expression differs between tumor types (Table 1 and Supplementary Table 1, see the section on supplementary materials given at the end of this article), and it can be associated with the tumor grade and differentiation, as well as other factors (e.g. presence of overt hypercortisolism) (van der Pas *et al.* 2013, Herrera-Martinez *et al.* 2018). The variable expression of SST subtypes reported in different studies is likely affected by the technique used to identify the receptors (e.g. qRT-PCR vs Western blot/immunohistochemistry), and when immunohistochemistry (IHC) is performed, by the specific antibody and the quantification method used (Gatto *et al.* 2020*a*). To date, clinically available somatostatin receptor ligands (SRLs) mainly target SST_2 and SST_5. Novel SRLs designed to specifically target SST_1 and SST_3 are under investigation in preclinical studies (Zatelli *et al.* 2003, Fusco *et al.* 2008, Vazquez-Borrego *et al.* 2020).

Somatostatin receptor subtype 1, 3, and 4 (SST_1, SST_3, and SST_4)

SST_1 is frequently expressed in pituitary tumors. Particularly, SST_1 mRNA is consistently expressed in prolactin (PRL)-secreting pituitary tumors (Jaquet *et al.* 1999, Fusco *et al.* 2008), while frequent expression is also observed in GH-secreting pituitary tumors, both at mRNA and protein level (Table 1) (Zatelli *et al.* 2003, Lupp *et al.* 2011). The expression of SST_1 in a GH-secreting rat cell line was shown to be directly regulated by Pit-1, the transcription factor characterizing somatotroph, lactotroph, and thyrotroph lineage of pituitary cells (Asa *et al.* 2022, Melmed *et al.* 2022). Of note, a recent study by our group showed a significantly higher SST_1 mRNA expression in pituitary tumors co-secreting GH and PRL, compared to the pure GH-secreting one. This finding suggests a potential role of SST_1 as a marker of this peculiar tumor phenotype, which shows a preferential response to the dopamine receptor type 2 (D2R) agonist cabergoline *in vitro* (Gatto *et al.* 2023). In preclinical studies, the selective SST_1 agonist BIM-23926 failed to inhibit hormone secretion and cell proliferation in PRL-secreting pituitary tumors, whereas the compound inhibited GH and PRL secretion in GH/PRL co-secreting pituitary tumor primary cultures (Zatelli *et al.* 2003, Fusco *et al.* 2008). A significant inhibitory effect of BIM-23926 on chromogranin A secretion and cell viability was observed in nonfunctioning pituitary tumor cells, supporting the hypothesis of a cell type specific role of SST_1 signaling (Zatelli *et al.* 2004). In gastroenteropancreatic (GEP) and thoracic NENs, SST_1 mRNA expression is increased compared to healthy controls (Sampedro-Nunez *et al.* 2016, Herrera-Martinez *et al.* 2017, 2018). Particularly, SST_1 is the most represented SST subtype in lung NENs (Table 1), showing a potential prognostic role, whereby higher SST_1 expression is associated with a better overall survival (OS) (Kaemmerer *et al.* 2015*a*, Herrera-Martinez *et al.* 2017). In both lung and GEP-NENs, well-differentiated tumors have higher SST_1 expression compared to the poorly differentiated ones (Zamora *et al.* 2010, Kaemmerer *et al.* 2015*a*,*b*). SST_1 is also expressed in various other nonendocrine tumors (sarcomas, pancreatic adenocarcinomas, etc). Of note, in prostate cancer, SST_1 expression has been associated with metastatic disease (Lupp *et al.* 2013, Pedraza-Arévalo *et al.* 2017). Moreover, *in vivo* studies on gliomas showed that SST_1 agonists, as well as SST_2 and SST_5 agonists, inhibit tumor growth through both direct cytostatic and antiangiogenic activity (Barbieri *et al.* 2009).

Expression of SST_3 is reported in approximately 85% of NENs, albeit the amount of receptor is usually not very high (Kaemmerer *et al.* 2015*b*, Qian *et al.* 2016). In a recent study focusing on insulinomas, SST_3 protein expression was positively correlated with tumor size and, by univariate analysis, associated with impaired patient survival (Peltola *et al.* 2023). SST_3 is frequently expressed in all pituitary tumor types, but predominantly in clinically nonfunctioning tumors,

Table 1 Expression of somatostatin receptors subtypes in pituitary and neuroendocrine tumors (NETs).

	SST_1 (%)		SST_2 (%)		SST_3 (%)		SST_4 (%)		SST_5 (%)	
Tumor type	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**	**Protein**	**mRNA**
Pituitary tumors (NF)	NA	97.36 ± 11.79	37.86 ± 11.60	98.70 ± 5.79	78.85 ± 21.68	95.20 ± 21.43	NA	80.53 ± 19.03	6.86 ± 5.19	64.89 ± 11.16
Pituitary tumors (PRL)	NA	100.00 ± 0.00	NA	100.00 ± 0.00	NA	NA	NA	NA	NA	75.00 ± 5.14
Pituitary tumors (ACTH)	NA	83.11 ± 14.75	31.80 ± 44.83	77.61 ± 14.29	100 ± 0	NA	NA	NA	54.45 ± 34.70	96.67 ± 5.53
Pituitary tumors (GH)	43.55 ± 25.77	91.61 ± 8.67	93.99 ± 5.49	100 ± 0.00	69.73 ± 34.51	67.94 ± 31.21	NA	NA	81.42 ± 18.79	95.54 ± 6.22
GEP-NENs	55.80 ± 19.35	90.28 ± 8.38	72.61 ± 14.02	91.61 ± 6.85	43.46 ± 23.08	71.98 ± 14.04	31.68 ± 21.72	26.41 ± 23.35	51.11 ± 21.38	71.47 ± 19.34
Lung NENs	69.95 ± 7.03	NA	51.15 ± 11.68	NA	2.53 ± 1.05	NA	9.53 ± 6.78	NA	43.68 ± 15.00	NA

Summary of the studies reported in Supplementary Table 1 (when at least 2 studies were available). Data are reported as weighted mean ± weighted S.D.
ACTH, adrenocorticotropic hormone-secreting tumor; GH, growth hormone-secreting tumor; GEP, gastroenteropancreatic; mRNA, evaluation of mRNA expression by qPCR; NF, non-functioning; NENs, neuroendocrine neoplasms; NA, not available; protein, evaluation of protein expression by immunohistochemistry; PRL, prolactin-secreting tumor.

where it is expressed in 88% of cases (Table 1) (Lupp *et al.* 2012, Casar-Borota *et al.* 2013, Flores-Martinez *et al.* 2020). Of note, SST_3 selective agonists have been recently shown to reduce cell viability in 62% of nonfunctioning pituitary tumor primary cultures *in vitro*, and SST_3 expression (evaluated both as mRNA and protein) significantly discriminated between responders and nonresponders (Vazquez-Borrego *et al.* 2020). These results have been further validated in a preclinical mouse model of pituitary tumors, where treatment with an SST_3 agonist significantly reduced tumor volume after 8 weeks of treatment (Vazquez-Borrego *et al.* 2020). As concerns nonendocrine cancers, SST_3 has been shown to be frequently expressed in meningiomas (Silva *et al.* 2015). In colon cancer, the stimulation of SST_3 reduces COX-2 activity and consequently reduces tumor growth (Colucci *et al.* 2008).

SST_4 is frequently expressed in the central nervous system, but is rarely detected in pituitary tumors and NENs (Günther *et al.* 2018, Ehms *et al.* 2022). Among different NEN sites, lung tumors show the highest SST_4 expression levels, with a significant overexpression compared to normal tissue (Herrera-Martinez *et al.* 2017).

Somatostatin receptor subtypes 2 and 5 (SST_2 and SST_5)

SST_2 is by far the most studied SST subtype, due to its broad and well-known expression reported in multiple tumor types, particularly in pituitary tumors and NENs. Furthermore, first-generation SRLs (fg-SRLs), mainly targeting SST_2, are available in clinical practice for more than three decades (Reubi & Waser 2003, Chinezu *et al.* 2014). In NENs, SST_2 is the predominant receptor both in terms of quantitative expression and frequency of positive cases (70–100%, Table 1 and Supplementary Table 1). For this reason, SST_2 represents an important target for diagnostic procedures. The latest guidelines on the management of patients with NENs recommend the use of radiolabeled SRLs (mainly ^{68}Ga-DOTATATE PET/CT) to stage the disease, considering the high sensitivity (92%, range 64–100%) and specificity (95%, range 83–100%) of this technique (Geijer & Breimer 2013, Pavel *et al.* 2020). Several studies showed a significant direct correlation between SST_2 protein expression, detected by IHC, and the uptake of radiolabeled SRLs, while a correlation with other SST subtypes was lacking (Brunner *et al.* 2017, Majala *et al.* 2022). Of note, SST_2 expression in NENs is highly variable, showing heterogeneity between different cases, as well as within the same tumor tissue sample (Charoenpitakchai *et al.* 2017). Multiple variables determining SST_2 expression in NENs have been identified, and one of the most important seems to be the primary localization of the tumor. Small intestine (SI)-NENs have been often reported to have higher SST_2 expression compared to the pancreatic counterpart. Other authors showed higher SST_2 expression in foregut

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NENs (i.e. tumors originating from lung, stomach, duodenum, and pancreas), compared to hindgut NENs (i.e. tumors originating from colon and rectum) (Papotti *et al.* 2002, Reubi & Waser 2003, Yerci *et al.* 2015, Watanabe *et al.* 2022). Insulinomas have a lower SST_2 expression (18 up to 83% of positive cases in different series) compared to gastrinomas (100% of positive tumors), showing a lower intensity of SST_2 staining by IHC compared to nonfunctioning pancreatic NENs (Kulaksiz *et al.* 2002, Papotti *et al.* 2002, Andreassen *et al.* 2019, Popa *et al.* 2021, Watanabe *et al.* 2021, Peltola *et al.* 2023). Another important variable known to impact SST_2 expression is the proliferation rate and, therefore, the grade of the tumor. In general, low-grade tumors (well-differentiated, less aggressive) display higher SST_2 expression compared to high-grade lesions (Kaemmerer *et al.* 2011, 2015*b*, Wang *et al.* 2017, Popa *et al.* 2021). However, Watanabe and colleagues reported a positive correlation between SST_2 mRNA expression and the percentage of Ki67-positive cells in hindgut NENs (Watanabe *et al.* 2022). Beyond the role in NEN diagnosis, SST_2 expression has been demonstrated to be a predictor of patient prognosis independently of tumor grade, i.e. higher SST_2 expression is associated with longer OS (Song *et al.* 2016, Wang *et al.* 2017). Furthermore, SST_2 is widely used as a target for treatment with fg-SRLs both in GEP-NENs and thoracic NENs, as recommended by the current guidelines (Pavel *et al.* 2020, Baudin *et al.* 2021). Two phase III trials showed the ability of fg-SRLs to inhibit hormone secretion (when present) and to reduce tumor progression (Rinke *et al.* 2009, Caplin *et al.* 2014), thus leading to the approval of these compounds for the treatment of low-grade, slow- progressing NENs and/or to control the hormonal hypersecretion. Peptide receptor radionuclide therapy (PRRT) using radiolabeled SRLs, with ^{177}Lu-DOTATATE as the established standard, is currently indicated as the second- or third-line treatment in progressive, low-grade, SST_2-positive NENs. Receptor binding of the 'carrier' SRL and its internalization results in radionuclide-induced DNA damage and eventual apoptosis of the tumor cells (Pavel *et al.* 2020, Baudin *et al.* 2021). In addition, fg-SRLs are recommended as first-line medical therapy in GH- and TSH-secreting pituitary tumors to control the associated hormonal syndrome and tumor growth due to the well-known high expression of SST_2 described for these tumor types (84–100% and 100% of cases, respectively; Table 1 and Supplementary Table 1) (Yoshihara *et al.* 2007, Lupp *et al.* 2011, Gatto *et al.* 2012, Casar-Borota *et al.* 2013, Chinezu *et al.* 2014, Beck-Peccoz *et al.* 2019, Giustina *et al.* 2020). Interestingly, while in NENs the use of fg-SRLs rarely leads to an objective tumor response (6.5% in lung-NENs and <5% in GEP-NENs), a significant tumor shrinkage (arbitrarily defined as >20% volume reduction) is found in more than half of the patients with GH-secreting pituitary tumors (Rinke *et al.* 2009, Caplin *et al.* 2014, Giustina *et al.* 2020, Lenotti *et al.* 2021). To date, the reason for this discrepancy has not been fully elucidated. However, no major differences in SST_2 expression between GEP-NENs and GH-secreting pituitary tumors have been reported, thus suggesting a possible differential modulation of the intracellular machinery (O'Toole *et al.* 2006). Nonetheless, SST_2 expression has been shown to be a reliable predictor for the biochemical response to fg-SRL treatment in GH-secreting pituitary tumors (Gatto *et al.* 2013*b*, Ilie *et al.* 2022). On the contrary, SST_2 is expressed at a lower level in pituitary tumors originating from the gonadotroph or corticotroph lineage. In this latter tumor type, a glucocorticoid-mediated downregulation of SST_2 has been demonstrated, both *in vitro* and *in vivo* (van der Hoek *et al.* 2005, Gatto *et al.* 2020*b*). SST_2 expression has also been described in multiple nonendocrine cancers, and the possibility of using SRLs or PRRT has been investigated. Frequent expression of SST_2 has been shown in thyroid cancer; however, SRLs did not show a consistent antitumoral effect, whereas PRRT is currently used only in experimental settings (Salavati *et al.* 2016). Similarly, in brain tumors, such as meningiomas and glioblastomas, SST_2 expression is detected (100% and 80–40% of cases, respectively (Silva *et al.* 2015, Tollefsen *et al.* 2021)). The use of both SRLs and PRRT in these tumors is, however, still limited to experimental settings (Mawrin *et al.* 2004, Kunikowska *et al.* 2022, Minczeles *et al.* 2023, Tollefsen *et al.* 2023). Several studies also investigated the possible use of SRLs in small cell lung cancer and hepatocarcinoma with varying results (Gomes-Porras *et al.* 2020). Recently, consistent SST_2 expression has been reported in multiple lymphoma subtypes, although the possible prognostic and therapeutic implications in this population have not been investigated yet (Juntikka *et al.* 2021).

SST_2 expression has been associated with a favorable prognosis in breast cancer, where it is overexpressed in 50–70% of cases (Gomes-Porras *et al.* 2020). Conversely, in prostate cancer, SST_2 is inversely associated to tumor differentiation, stage, and proliferation index (Hennigs *et al.* 2014).

SST_5 is another SST subtype representing a target for a clinically available second-generation SRL, pasireotide. Pasireotide, designed as a SST panligand, has preferential binding affinity for SST_5, and a high binding affinity for SST_1, SST_2, and SST_3 (within the nanomolar range). Of note, fg-SRLs also show moderate affinity to SST_5 (Gatto *et al.* 2019*b*). Due to the predominant expression of SST_5 in ACTH-secreting pituitary tumors (Table 1), pasireotide is currently the only pituitary-targeted drug approved for medical therapy in patients with Cushing's disease. In this setting, it has been shown to normalize urinary-free cortisol levels and to induce tumor shrinkage in about 40% of patients (Hofland *et al.* 2005, van der Hoek *et al.* 2005, Batista *et al.* 2006, Lupp *et al.* 2011, Behling *et al.* 2018, Fleseriu *et al.* 2021). Pasireotide is also suggested as a second-line medical therapy in patients with GH-secreting pituitary tumors partially resistant to fg-SRLs. A head-to-head comparison between pasireotide and octreotide in patients naive to medical treatment

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showed a similar efficacy of the two drugs in reducing GH secretion (48.3% and 51.6%, respectively), while pasireotide was superior in normalizing IGF-1 levels (38.6% vs 23.6%) (Colao *et al.* 2014). The superimposable efficacy of pasireotide and octreotide on GH inhibition, further confirmed by *in vitro* studies (reviewed in (Gatto *et al.* 2019*a*, Amarù *et al.* 2021)), suggests that in GH-secreting pituitary tumors, pasireotide effects are mainly driven by SST_2, whereas in ACTH-secreting pituitary tumors, SST_5 seems to be the preferential target (Muhammad *et al.* 2019).

Of note, SST_5 is the second most common SST subtype in GEP-NENs. Although not consistently, SST_5 expression has been associated with well-differentiated, low-grade tumors, and improved patient OS compared to SST_5-negative tumors (Zamora *et al.* 2010, Kim *et al.* 2011, Song *et al.* 2016, Wang *et al.* 2017, Popa *et al.* 2021, Peltola *et al.* 2023). On the contrary, some authors report that SST_5 expression in GEP-NENs can be associated with histopathological features related to a more aggressive behavior, e.g. vascular and nerve invasion (de Sa *et al.* 2006, Herrera-Martinez *et al.* 2018). Moreover, in both GEP-NENs and lung NENs, SST_5 is expressed at higher levels compared to the adjacent nontumoral tissue (Herrera-Martinez *et al.* 2017, 2018). The presence of the truncated form SST_5TMD4 has been associated with resistance to fg-SRL treatment in GH-secreting pituitary tumors, possibly due to the interaction between SST_5TMD4 and SST_2, resulting in the impaired localization of SST_2 at the cell membrane (Duran-Prado *et al.* 2010). Moreover, SST_5TMD4 has been associated with the presence of aggressive features in both GH-secreting pituitary tumors and GEP-NENs (Luque *et al.* 2015, Sampedro-Nunez *et al.* 2016). Similarly, tumoral SST_5TMD4 expression is associated with a worse prognosis in patients with breast and prostate cancer (Duran-Prado *et al.* 2012, Hormaechea-Agulla *et al.* 2017).

Intracellular molecules involved in SST machinery

As mentioned earlier, the variable effects of SRLs are only partially related to the SST expression on cell membrane, and tissue-specific differences in the intracellular machinery could explain the differential treatment response observed in different tumors (Gatto & Hofland 2011). As briefly described in the introduction, ligand binding to SSTs leads to the decrease of hormone secretion mainly by two mechanisms: (1) inhibiting the activity of adenylate cyclase, therefore reducing intracellular cAMP levels and (2) inhibiting Ca^{2+} influx, thus reducing intracellular Ca^{2+} levels (Theodoropoulou & Stalla 2013, Günther *et al.* 2018). Furthermore, SST activation inhibits cell proliferation and induces cell apoptosis through different mechanisms. In particular, the activation of PTP leads to the inhibition of growth factor signaling, the regulation of ERK and AKT pathways, and the resulting cell cycle arrest (Theodoropoulou & Stalla 2013, Günther *et al.* 2018). SST_2 and SST_5 block cell cycle progression also by inducing the phospholipase C (PLC)-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (Theodoropoulou & Stalla 2013, Günther *et al.* 2018). DAG is involved in the regulation of ERK activation, while IP3 increases cytosolic Ca^{2+} levels (Theodoropoulou & Stalla 2013, Günther *et al.* 2018). Finally, SST_2 and SST_3 activation can lead to cell apoptosis through the upregulation of p53 and Bax, as well as the downregulation of Bcl2 (Theodoropoulou & Stalla 2013, Günther *et al.* 2018).

However, during the last decades, it has been demonstrated that several intracellular molecules are recruited after ligand binding to the various SST subtypes, modulating receptor desensitization, internalization, and recycling/degradation, thus potentially being involved in mediating tumor response to SRLs. These molecules include, among others, GRKs, ARRBs, and filamin A (FLNA) (Peverelli *et al.* 2021).

Moreover, other molecules have been also associated with resistance to SRL treatment (both *in vitro* and/or *in vivo*), although the exact mechanism by which they affect SST signaling remains elusive. A clear example of these complex interactions is the aryl hydrocarbon receptor-interacting protein (AIP), which is involved in the cell cycle regulation (Haworth & Korbonits 2022). Inactivating mutations of AIP, or low wild-type AIP expression, have been associated to fg-SRL treatment resistance in GH-secreting pituitary tumors. Multiple mechanisms leading to treatment resistance have been hypothesized, including the modulation of the SST–AIP–ZAC1 (zinc finger regulator of apoptosis and cell cycle arrest) pathway and the impairment of SST signaling due to the reduction of Gα protein expression (Ibanez-Costa & Korbonits 2017). Another proposed mechanism, although debated, is the presence of lower SST_2 expression in tumors with low wild-type AIP (Iacovazzo *et al.* 2016, Peverelli *et al.* 2021). Differently from fg-SRLs, the response to pasireotide does not seem to be affected by low AIP expression, suggesting a predominant action through SST_5 in case of impaired SST_2 signaling (Peverelli *et al.* 2021).

SST phosphorylation plays a pivotal role in determining the receptor trafficking. All SST subtypes, except SST_4, undergo agonist-stimulated phosphorylation (Liu & Schonbrunn 2001, Tulipano *et al.* 2004). To the best of our knowledge, molecules involved in SST_1 phosphorylation have not been investigated yet. As for SST_2, SST_3, and SST_5, phosphorylation seems to be mediated by GRK2 and GRK3 (Tulipano *et al.* 2004, Petrich *et al.* 2013, Lehmann *et al.* 2016). Moreover, the SST_2 phosphorylation pattern induced by GRK2, and the related intracellular trafficking of the receptor, varies depending on the agonists. Whereas octreotide induces robust SST_2 phosphorylation, followed by ARRB recruitment and receptor internalization, pasireotide results in partial

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receptor phosphorylation, with unstable binding to ARRBs, and consequent faster recycling of SST_2 (Pöll *et al.* 2010). In a cohort of GH-secreting pituitary tumors, higher GRK2 mRNA expression was correlated with a better antisecretory effect of octreotide (both *in vitro* and *in vivo*), suggesting that GRK2 may play a role in SST_2 signal transduction (Gatto *et al.* 2013*a*). To date, the role of GRK2 in mediating SST_2 activity has not been evaluated in other tumor types.

Recently, FLNA has emerged as an important player in SST pathophysiology. FLNA is a V-shaped cytoskeleton protein that exerts an essential role in anchoring transmembrane receptors to the actin cytoskeleton, thereby regulating the localization of receptors, their interaction with different intracellular molecules, as well as determining their postendocytic fate (Najib *et al.* 2012, Peverelli *et al.* 2014, Vitali *et al.* 2016, Treppiedi *et al.* 2018, 2021). Whereas, the role of FLNA has been extensively studied in relation to SST_2 and SST_5, to the best of our knowledge, no studies have been performed to investigate the relationship of FLNA with the other SST subtypes. FLNA can bind to the first intracellular loop of both SST_2 and SST_5, but the functional consequence seems to be different for the two receptors. FLNA does not affect basal receptor expression or membrane localization of SST_2, but it is necessary for SST_2 stability after prolonged agonist stimulation. This has been demonstrated in GH-secreting pituitary tumors and pancreatic NEN (panNEN) cell lines, where FLNA silencing (or the overexpression of a FLNA mutant specifically preventing the binding between FLNA and SST_2) leads to lower SST_2 protein expression after 72 h of treatment with a selective SST_2 agonist, likely by inducing lysosomal degradation (Peverelli *et al.* 2014, Vitali *et al.* 2016). Moreover, silencing of FLNA impairs the SST_2 agonist-induced antiproliferative and proapoptotic effect in GH-secreting pituitary tumor cell cultures and panNEN cell lines (Peverelli *et al.* 2014, Vitali *et al.* 2016). In contrast, FLNA silencing resulted in decreased basal SST_5 protein expression in ACTH-secreting pituitary tumors (Treppiedi *et al.* 2021). In line with the differential impact observed for FLNA on different SST subtypes, in a cohort of GH-secreting pituitary tumors, FLNA was positively correlated with SST_5 but not with SST_2 expression. However, in a subset of patients naive to medical treatment at the time of surgery, subsequently achieving biochemical control with fg-SRL treatment, a positive correlation between FLNA and SST_2 expression was also found (Coelho *et al.* 2019). In summary, several studies already demonstrated that FLNA is required by both SST_2 and SST_5 for the activation of multiple intracellular pathways in different tumor types (Peverelli *et al.* 2014, Vitali *et al.* 2016, Treppiedi *et al.* 2021). For example, in panNEN cell lines, FLNA seems to be crucial for the inhibition of cAMP accumulation, vascular endothelial growth factor (VEGF) secretion, and cell migration (Vitali *et al.* 2016). Furthermore, Treppiedi and colleagues showed that in ACTH-secreting pituitary tumors (both primary cultures and the murine cell model AtT-20), FLNA is required for the SST_5-mediated inhibition of cell proliferation, induction of apoptosis, and reduction of ACTH secretion (Treppiedi *et al.* 2021). Recently, it has been demonstrated that cell line models of GH-secreting pituitary tumor and melanoma not expressing FLNA show impaired SST_2/SST_5 heterodimerization in both basal condition and after pasireotide treatment, compared to cells expressing FLNA. However, the absence of FLNA increased SST_2/SST_5 heterodimerization after treatment with octreotide (Treppiedi *et al.* 2022). This finding suggests that FLNA plays a role in the modulation of SST_2/SST_5 heterodimerization in basal condition, while SST_2 activation by a full agonist leads to heterodimerization independently of FLNA. Finally, FLNA can be phosphorylated, modifying the interaction between G proteins and SST_2, thus interrupting the downstream signaling. The activation of SST_2 by naive SRIF decreases FLNA phosphorylation, suggesting the presence of an autoregulatory loop (Peverelli *et al.* 2018).

Interaction between somatostatin receptors and beta-arrestins

Arrestins constitute a small family of proteins, i.e. arrestin 1 to arrestin 4, involved in the regulation of GPCR desensitization, trafficking, and signaling. Arrestins 1 and 4 are only expressed in the retina (therefore called visual arrestins), whereas arrestin 2 and arrestin 3, better known as β-arrestin 1 (ARRB1) and β-arrestin 2 (ARRB2), are ubiquitously expressed. ARRB1 and ARRB2 show a high degree of sequence homology (sharing 78% of the amino-acid sequence) and functional overlap. However, the subcellular localization (nucleus and cytoplasm for ARRB1 and only cytoplasm for ARRB2) and the ability to interact with specific proteins differ between the two isoforms (DeWire *et al.* 2007). In addition to their pivotal role in GPCR desensitization and trafficking (including SSTs), ARRBs act as multifunctional scaffold proteins for a variety of protein–protein interactions (as depicted in Fig. 1) (Wess *et al.* 2023). Two classes of GPCRs have been identified based on the interaction with ARRBs: class A and B receptors (Oakley *et al.* 2000). Class A receptors have a higher affinity for ARRB2 compared to ARRB1, forming a complex that enables its internalization into clathrin-coated pits and subsequent dissociation near the cell membrane. Class B receptors have similar affinities for both ARRB1 and ARRB2, forming a stable receptor–ARRB complex that is targeted to endocytic vesicles (Oakley *et al.* 2000). SST_3 and SST_5 behave as class A receptors, SST_2 as a class B receptor, whereas trafficking of SST_1 and SST_4 does not seem to be dependent on ARRBs (Tulipano *et al.* 2004, Peverelli *et al.* 2008). However, unlike other class B receptors which are characterized by a slow recycling rate, SST_2 is efficiently recycled to the cell membrane after internalization (Tulipano *et al.* 2004, Cambiaghi *et al.* 2017). Of note, it has been demonstrated that SST_2, depending on the tested SRL, could behave both as a class A or a class

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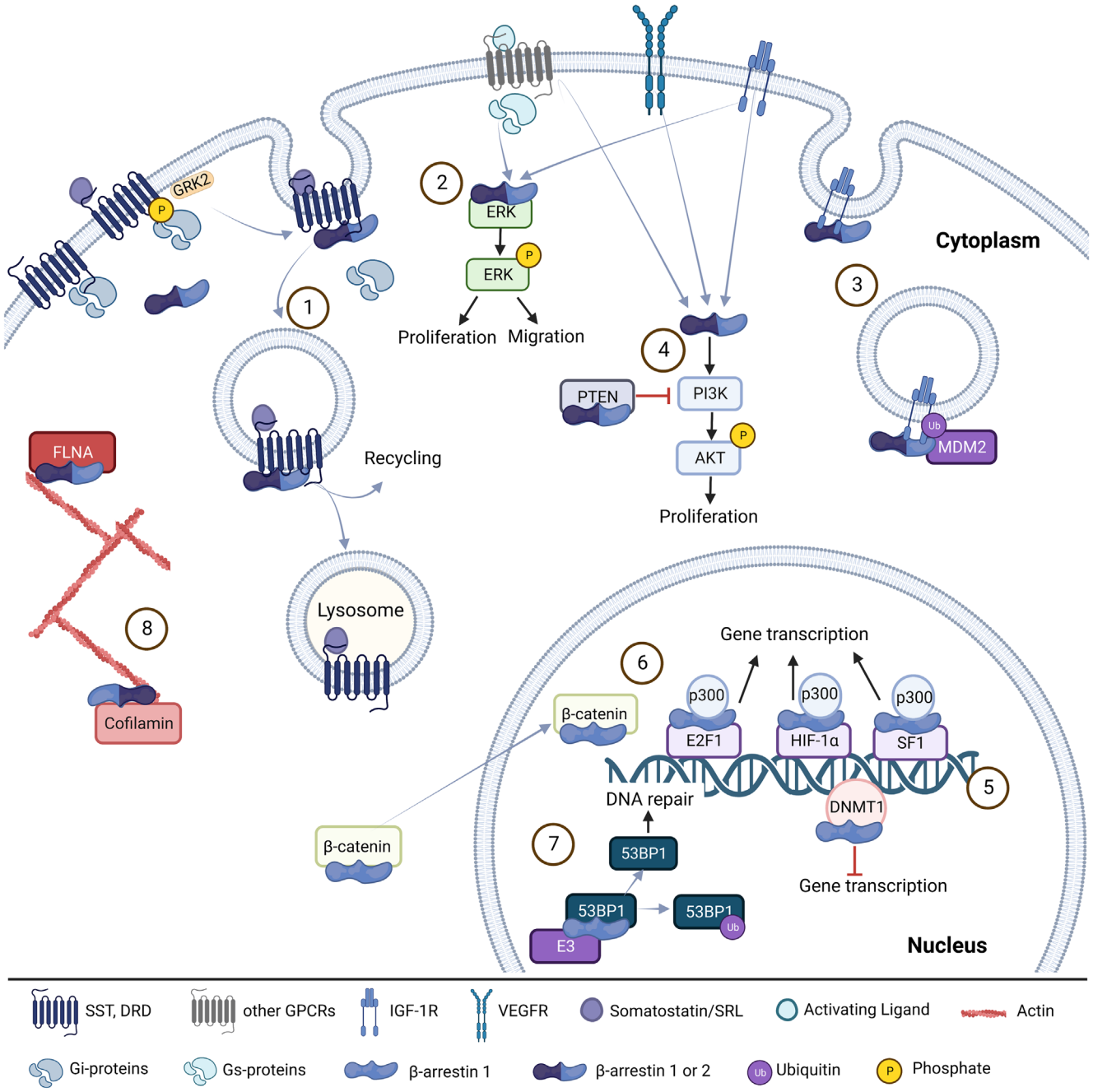

Figure 1

Schematic representation of main β-arrestin functions. Both β-arrestins are involved in G protein-coupled receptor (GPCR) desensitization after receptor phosphorylation, and GPCR internalization and downregulation (1). β-arrestins associate with ERK after GPCR or IGF-1R activation, leading to increased cell proliferation and migration (2). β-arrestins are involved in IGF-1R downregulation by mediating receptor ubiquitination (3). β-arrestins modulate AKT pathway both positively and negatively, resulting in the regulation of cell proliferation (4). β-arrestin 1 is involved in the modulation of epigenetic regulation in different ways: (i) forming complex with the histone acetylase p300 and multiple transcription factor (leading to increased gene transcription), (ii) forming a complex with the DNA methyl transferase 1 (DNMT1), and (iii) increasing DNA methylation and inhibiting gene transcription (5). β-arrestin 1 is involved in epithelial–mesenchymal transition (EMT) through the interaction with β-catenin or E2F1 (6). β-arrestin 1 has been linked to radiosensitivity as it can form a complex with the DNA repair protein 53BP1 and a E3 ubiquitin ligase, thus modulating 53BP1 levels (7). Both β-arrestins are involved in cytoskeleton rearrangement, and therefore cell migration (8). Figure created with Biorender.

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B receptor. In more detail, similarly to naive SRIF, treatment with octreotide induces co-internalization of SST_2 and ARRBs as a stable complex (class B receptor) (Cescato *et al.* 2006, Lesche *et al.* 2009, Cescato *et al.* 2010, Pöll *et al.* 2010). On the other hand, pasireotide induces a significantly lower SST_2 phosphorylation, leading to a less stable receptor–ARRB complex that dissociates near the plasma membrane, resembling the characteristics of a class A receptor (Lesche *et al.* 2009, Cescato *et al.* 2010, Pöll *et al.* 2010). Lastly, treatment with a SST_2 antagonist does not induce internalization of the receptor nor ARRB recruitment (Cescato *et al.* 2006, Song *et al.* 2014). Of note, SST recycling rate is not uniquely dependent on the interaction with ARRBs since other mechanisms such as receptor ubiquitination and dephosphorylation, as well as ARRB deubiquitination, also play an important role (Shenoy & Lefkowitz 2003, Kliewer & Schulz 2014, Tulipano *et al.* 2004).

Although some conflicting results are reported in the literature, ARRBs have been suggested as predictors of the response to fg-SRL treatment in GH-secreting pituitary tumors (Table 2). A study by Gatto and colleagues, evaluating 22 primary cultures, reported that ARRB1 mRNA expression was significantly lower in tumors considered as responder to octreotide compared to nonresponders, with the responder ones showing also higher SST_2 and GRK2 mRNA expression. The correlation between ARRB1 and the response to octreotide shown *in vitro* was confirmed in the same cohort *in vivo*, evaluating the response to the acute octreotide test (Gatto *et al.* 2013*a*). Moreover, ARRB1, ARRB2, and the combined evaluation of ARRBs with SST_2 (expressed as SST_2/ARRB ratio) were shown to be correlated with the response to long-term treatment with fg-SRLs in patients with GH-secreting pituitary tumors (Gatto *et al.* 2016). However, these data were not supported by a more recent study, which identified SST_2 mRNA expression as the sole predictive factor for the response to fg-SRL treatment among the different tested molecules (Coelho *et al.* 2018). The reason for this discrepancy remains unclear, warranting further investigations into the role of ARRBs in fg-SRL response.

Studies in panNEN cell lines demonstrated that the third intracellular loop (IC3) of SST_2 is essential for G-protein binding, whereas ARRB binding to the C-terminal domain (CT) results in the formation of stable SST_2–ARRB complexes and receptor recycling. However, both the integrity of IC3 and CT is essential to mediate the SST_2 antiproliferative and antiangiogenic effects, as well as to induce apoptosis, suggesting a crucial role of ARRBs in SST_2 signaling pathways (Cambiaghi *et al.* 2017).

The impact of ARRBs on treatment outcomes in ACTH-secreting pituitary tumors has not been investigated in detail. Recently, Kageyama and colleagues demonstrated in the murine corticotroph cell line AtT-20 that ARRB2 modulates SST expression, i.e. ARRB2 knockdown increases SST_2 and SST_5 expression at both mRNA and protein levels. On the other hand, ARRB1 knockdown does not impact SST protein expression (Kageyama *et al.* 2021). Moreover, it has been shown that hypercortisolism, besides reducing SST_2 levels, modulates ARRB expression. Several studies, carried out using multiple cell lines (including AtT-20 cells), have shown that treatment with dexamethasone results in the downregulation of ARRB2 and the upregulation of ARRB1, although data regarding ARRB1 modulation are conflicting (Oakley *et al.* 2012, Gatto *et al.* 2020*b*, Kageyama *et al.* 2021). Of note, ACTH-secreting pituitary tumor samples obtained from patients with overt hypercortisolism before neurosurgery had significantly higher ARRB1 mRNA expression compared to patients with normalized cortisol levels, while ARRB2 levels were similar in the two groups (Gatto *et al.* 2020*b*). Finally, the potential effect of pasireotide treatment on ARRB modulation is still debated, as no difference in ARRB expression levels was reported after 72 h pasireotide treatment in one study, while ARRB1 upregulation has been reported after 24 h treatment by another group (Gatto *et al.* 2020*b*, Kageyama *et al.* 2021). Of note, an elegant study by Oakley and colleagues showed that in a lung adenocarcinoma cell model, dexamethasone-induced modulation of ARRB expression was able to redirect the GCPR signaling from a G protein-dependent to an ARRB-dependent signaling (Oakley *et al.* 2012). Therefore, the regulation of ARRBs in ACTH-secreting pituitary tumors and their potential role in mediating treatment outcomes in this peculiar tumor type, tightly linked to various degrees of hypercortisolism, need further elucidation.

The ability of different molecules to differentially activate either G protein-mediated or ARRB-mediated pathways after binding with a GPCR is called biased agonism. As discussed earlier, treatment with fg-SRLs or pasireotide induces differential ARRB recruitment, suggesting that pasireotide may behave as a biased agonist for SST_2. Moreover, in both nonendocrine models (HEK293 and AR42J cells) and endocrine models (GH4 cells), it was shown that full agonists, such as octreotide, and biased agonists, such as pasireotide, result in differential signal transduction due to the activation of specific G proteins. Both compounds inhibit the adenylate cyclase activity through pertussis toxin-sensitive G proteins; however, octreotide and pasireotide act as agonist and antagonist, respectively, on pathways mediated by pertussis toxin-insensitive G proteins or the Gβ/γ proteins (Cescato *et al.* 2010, Rodriguez *et al.* 2018). Recently, Zhao and colleagues showed that paltusotine, a nonpeptide SST_2 agonist, has G protein-biased properties, inducing lower ARRB recruitment compared to octreotide (Zhao *et al.* 2023*a*,b).

The concept of designing compounds that preferentially trigger the activation of pathways depending on G proteins or ARRBs has been successfully employed for D2R, a GPCR frequently expressed in pituitary tumors (Allen *et al.* 2011, Free *et al.* 2014, Mangili *et al.* 2020, 2022). Recent studies conducted in rat GH-secreting

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Table 2 Overview of the studies evaluating the role of β-arrestins in pituitary and neuroendocrine tumors.

Ref	Tumor type	Main findings	ARRB1	ARRB2
Peverelli *et al.* (2008)	GH-secreting pituitary tumors	Recruitment by SST_5	No	Yes
		Co-localization with SST_5 in endocytic vesicles after 30 min stimulation	No	No
		Binding with SST_5 dependent on the integrity of the c-terminus	–	No[a]
		Binding with SST_5 dependent on the integrity of the third intracellular loop	–	Yes
Gatto *et al.* (2013*a*)	GH-secreting pituitary tumors	Correlation between:		
		ARRB1 and ARRB2 mRNA	Absent	Absent
		ARRBs mRNA and GRK2 mRNA	Inverse	Direct
		ARRBs mRNA expression and SST_2 mRNA	Absent	Absent
		ARRBs mRNA expression and SST_5 mRNA	Absent	Absent
		ARRBs mRNA levels and % GH suppression after acute octreotide test	Inverse	Absent
		ARRBs mRNA levels in primary cultures: responders vs nonresponders to octreotide	Lower in responders	No difference
		Effect of fg-SRL presurgical treatment on ARRBs mRNA expression	No difference	No difference
		Effect of octreotide treatment on ARRBs mRNA expression in GH3 cells	No difference	No difference
Gatto *et al.* (2016)	GH-secreting pituitary tumors	Correlation between:		
		ARRBs mRNA expression and SST_2 protein expression	Inverse	Inverse
		ARRBs mRNA expression and SST_5 protein expression	Absent	Absent
		fg-SRL long-term treatment:		
		ARRBs mRNA levels and IGF-1 normalization	Lower in responders	Lower in responders
		ARRBs mRNA levels and complete biochemical response	Lower in responders	Lower in responders
		ARRBs mRNA expression and GH normalization	No difference	No difference
		SST_2/ARRBs mRNA ratios and GH normalization	Higher in responders	Higher in responders
		ARRBs mRNA expression and stratified classification of responsiveness	Inverse trend	Inverse trend
		Effect of fg-SRL presurgical treatment on ARRBs mRNA expression	No difference	No difference
Cambiaghi *et al.* (2017)	Pancreatic NEN	Recruitment by SST_2 agonist and co-localization in endocytic vesicles	Yes	Yes
		Binding with SST_2 dependent on the integrity of the c-terminus	Yes	Yes
		Binding with SST_2 dependent on the integrity of the third intracellular loop	No	No
Coelho *et al.* (2018)	GH-secreting pituitary tumors	Correlation between:		
		ARRB1 and ARRB2 mRNA	Positive	Positive
		ARRBs mRNA expression and SST_2 mRNA and protein expression	Absent	Absent
		ARRBs mRNA expression and SST_5 mRNA and protein expression	Absent	Absent
		Effect of fg-SRL presurgical treatment on ARRBs mRNA expression	No difference	No difference
		fg-SRL long-term treatment:		
		ARRBs mRNA levels and biochemical control after	No difference	No difference
Gatto *et al.* (2020*b*)	ACTH-secreting pituitary tumors	Dexamethasone treatment (72 h) in AtT20 cells:		
		ARRBs mRNA expression	Upregulation	Downregulation
		ARRBs protein expression	Upregulation	Downregulation
		Ketoconazole treatment (72 h) and ARRBs mRNA expression in AtT20 cells	No difference	No difference
		Pasireotide treatment (72 h) and ARRBs mRNA expression in AtT20 cells	No difference	No difference
		Correlation between ARRBs mRNA expression and UFC levels at time of surgery	Positive	Absent

(Continued)

Table 2 Continued.

Ref	Tumor type	Main findings	ARRB1	ARRB2
Mangili *et al.* (2020)	NF and PRL-secreting pituitary tumors	Involvement in DRD2-mediated AKT dephosphorylation in MMQ	No	Yes
		Involvement in DRD2-mediated antiproliferative effect in NF pituitary tumors	No	Yes
Kageyama *et al.* (2021)	ACTH-secreting pituitary tumors	Dexamethasone treatment (72 h) in AtT20 cells:		
		ARRBs mRNA expression	No difference	Downregulation
		ARRBs protein expression	No difference	Downregulation
		Pasireotide treatment (24 h) and ARRBs mRNA expression in AtT20 cells	Upregulation	No difference
		Silencing of ARRB1[b]	–	No difference
		Silencing of ARRB2[c]	Upregulation	–
Mangili *et al.* (2022)	PRL-secreting pituitary tumors	Silencing of ARRB2		
		DRD2-mediated cell proliferation inhibition	ND	Yes
		DRD2-mediated AKT phosphorylation inhibition	ND	Yes

[a]Mutation at the C-terminus of SST_5 enhanced SST_5 internalization mediated by ARRB2; [b]Silencing of ARRB1 also induced upregulation of POMC, reversed by treatment with pasireotide, and SST_5 mRNA downregulation; [c]Silencing of ARRB2 also induced increase in basal POMC expression, reversed by treatment with pasireotide or dexamethasone, SST_2 and SST_5 upregulation (mRNA and protein).

ACTH, adrenocorticotropic hormone; ARRB, β-arrestin; fg-SRL, first-generation somatostatin receptor ligand; GH, growth hormone; GRK2, G protein-coupled receptor kinase 2; NEN, neuroendocrine neoplasms; NF, nonfunctioning; ND not determined; PRL, prolactin; SST2, somatostatin receptor subtype 2A; SST5, somatostatin receptor subtype 5; UFC, urinary free cortisol.

(GH3) and PRL-secreting (MMQ) pituitary tumor cell lines showed that the antiproliferative effect of D2R is mainly ARRB2-dependent. Knockdown of ARRB2 abrogated the antiproliferative effect of treatment with a D2R selective agonist, while inhibition of Gαi/o protein with pertussis toxin did not attenuate the effect of the D2R agonist on cell viability (Tan *et al.* 2021). In agreement with these observations, treatment with the ARRB2-biased agonist UNC9994 showed an antiproliferative effect in both *in vitro* experiments in GH3 and MMQ cells, and in *in vivo* MMQ xenografts, whereas the G_i-protein biased agonist MLS1547 showed minimal effect in MMQ cells (Tan *et al.* 2021, Di Muro *et al.* 2023). Interestingly, MLS1547 showed a stronger antiproliferative effect in GH3 cells compared to MMQ cells, suggesting a differential interplay of the intracellular machinery based on the cell lineage, the G-protein expression levels, as well as GRKs and ARRBs (Tan *et al.* 2021, Di Muro *et al.* 2023).

However, specific ARRB-dependent pathways activated after SST stimulation have not been identified yet. Of note, it has been reported that the recruitment of ARRBs following GPCR phosphorylation by GRK2 or GRK3 promotes receptor internalization, whereas the recruitment of ARRBs by GRK5- or GRK6-phosphorylated GPCRs assembles a signalome, leading, for example, to the phosphorylation of ERK or the activation of the Src pathway (Crepieux *et al.* 2017). Therefore, in view of the fact that SST phosphorylation is mainly mediated by GRK2 and GRK3, it could be speculated that the interaction between SSTs and ARRBs does not activate an independent downstream signal.

GPCR-dependent and -independent actions of beta-arrestins

In addition to their well-characterized role in the regulation of GPCR trafficking, as well as in hampering G protein signaling, ARRBs can act as scaffolding molecules, modulating multiple intracellular pathways in GCPR-dependent and/or -independent manners (Fig. 1) (Wess *et al.* 2023). ARRBs interact with hundreds of proteins (almost 300 for ARRB1 and almost 400 for ARRB2, of which 171 proteins interact with both ARRB isoforms) (Crepieux *et al.* 2017). In this context, the involvement of ARRBs in cell proliferation, migration, and survival, as well as in the regulation of the stem cell phenotype, has been consistently demonstrated. These data support the important role of ARRBs in the pathogenesis and/or progression of different tumors (Sobolesky & Moussa 2013, Song *et al.* 2018, Rosano & Bagnato 2019, Kallifatidis *et al.* 2020).

One of the better-characterized interactions involves ARRBs and ERK1/2 pathway, which can be induced in a GPCR-dependent and independent manner (Fig. 1). This results in the activation of the downstream ERK targets involved in the proliferation, migration, and invasion of tumor cells (Sobolesky & Moussa 2013, Song *et al.* 2018,

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Rosano & Bagnato 2019). An example of a GPCR-dependent mechanism has been shown in breast cancer cells, where ERK activation by protease-activated receptor-2 (PAR-2, belonging to the GPCR family) requires both ARRB1 and ARRB2, resulting in increased cell migration (Ge *et al.* 2004). In Ewing's sarcoma, it was shown that ARRB1 recruitment by insulin-like growth factor 1 receptor (IGF-1R), belonging to the receptor tyrosine kinase (RTK) family, leads to ERK phosphorylation and cell proliferation in a GPCR-independent manner (Girnita *et al.* 2007). Notably, ARRBs can modulate IGF-1R receptor downregulation by functioning as adaptors between the receptor and the ubiquitin ligase MDM2, but they can also activate the phosphoinositide 3-kinase (PI3K)/AKT pathway, thereby stimulating proliferation (Fig. 1) (Povsic *et al.* 2003, Girnita *et al.* 2005). ARRB-mediated activation of the PI3K/AKT pathway has been observed for other receptors as well, such as VEGFR and chemokine receptor type 7 (CXCR-7) (Song *et al.* 2018). However, inhibition of the AKT pathway through the interaction of ARRBs with the tyrosine phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10) and the resulting enhancement of the lipid phosphatase activity has also been reported (Lima-Fernandes *et al.* 2011). The modulation of the AKT pathway by ARRBs is thus complex, acting both in a stimulatory and inhibitory manner (Fig. 1).

Furthermore, ARRBs are involved in cytoskeletal reorganization, influencing cellular migration. In this context, the main function of ARRBs is to coordinate cytoskeletal signaling driven by GPCR pathways, as well as to induce RTK transactivation after GPCR activation (Fig. 1) (Song *et al.* 2018, Rosano & Bagnato 2019).

A recent review summarized the diverse roles of the two ARRB isoforms on stem cell phenotype regulation in several tumor types (Kallifatidis *et al.* 2020). In particular, ARRB1 was shown to promote cell proliferation and induce stem cell phenotype in bladder cancer, non-small cell lung cancer, and myeloproliferative disorders, while it inhibits stem cell renewal in medulloblastoma. Interestingly, the two ARRB isoforms showed an opposite effect in bladder cancer, with ARRB1 functioning as an oncogene, while ARRB2 acts as a tumor suppressor (Kallifatidis *et al.* 2020).

ARRB1 is the sole isoform capable of translocating to the nucleus and influencing gene expression by directly interacting with transcription factors, as well as modulating the epigenetic profile (Fig. 1). As an example, ARRB1 can stabilize hypoxia-induced factor-1α (HIF-1α) by forming complexes with the histone acetyltransferase p300 and HIF-1α, leading to increased transcription of angiogenetic factors, such as VEGF and endothelin 1 (Shenoy *et al.* 2012, Cianfrocca *et al.* 2016). Furthermore, complex formation with p300 and the transcription factor SP1 (specific protein 1) can mediate the transcription of human telomerase reverse transcriptase (hTERT), thus preventing cell senescence (Liu *et al.* 2017). Activation of the delta-opioid receptor, leading to the interaction of ARRB1 with p300 and the subsequent acetylation of histone H4, was previously reported to increase the transcription of p27 and c-fos. These studies suggest that the ARRB1–p300 complex can be activated by different mechanisms, resulting in the stimulation of various transcriptional pathways (Kang *et al.* 2005). ARRB1 may also alter the epigenetic profile by binding to the DNA methyl transferase 1 (DNMT1), facilitating the methylation of different genes (such as *PTEN*), thus leading to the inhibition of their expression (Shu *et al.* 2015).

Involvement of ARRB1 in the epithelial–mesenchymal transition (EMT) has been reported in various cancers (Fig. 1), e.g. promoting the Wnt/β-catenin pathway in prostate and colorectal cancer, and through epigenetic modulation in non-small cell lung cancer (Rosano *et al.* 2013, Pillai *et al.* 2015, Duan *et al.* 2016, Tan *et al.* 2020, Song *et al.* 2021). In more detail, ARRB1 can promote the translocation of β-catenin to the nucleus and the subsequent transcription of target genes responsible for EMT, cell migration, and invasion in colorectal cancer, without affecting the expression of β-catenin (Song *et al.* 2021). On the other hand, in prostate cancer, ARRB1 has been shown to increase both the transcriptional activity and the expression of β-catenin (Duan *et al.* 2016). In non-small cell lung cancer, ARRB1 is involved in EMT upon nicotine treatment, which induces the translocation of ARRB1 into the nucleus, where it forms a complex with p300 and the transcription factor E2F1. This leads to the transcription of fibronectin, vimentin, Zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2, all typically upregulated during the EMT (Pillai *et al.* 2015).

Emerging evidence points to the role of EMT in pituitary tumors and NENs. In particular, markers of EMT have been associated with an invasive phenotype in pituitary tumors, and they seem to be correlated with fg-SRL resistance in GH-secreting pituitary tumors (Qian *et al.* 2007, Wang *et al.* 2018, Gil *et al.* 2021, 2023). A recent transcriptomic study on GH-secreting pituitary tumors identified three subgroups, each showing different proportions of invasive tumors. Interestingly, EMT markers, including ARRB1, were differentially expressed in the three groups. In particular, ARRB1 was significantly higher in group three, displaying the highest rate of invasive growth (Rymuza *et al.* 2022). EMT is also involved in panNEN progression, and it has been shown to correlate with poor prognosis. However, the involvement of ARRB1 in the EMT of NENs has not been evaluated in detail yet (Zhou *et al.* 2021, Venugopal *et al.* 2022).

Finally, ARRB1 seems to be related to radiosensitivity in both *in vitro* and *in vivo* studies. In particular, ARRB1 has been reported to form a complex with the DNA repair protein 53BP1, promoting its ubiquitination and degradation (Fig. 1). Therefore, lower ARRB1 expression results in higher 53BP1 expression, eventually leading

to a more effective DNA repair following exposure to radiation (Nieto *et al.* 2020). However, in non-small cell lung cancer, both overexpression and knockdown of ARRB1 were shown to suppress cell viability after radiation through different mechanisms, i.e. increased DNA damage and apoptosis when ARRB1 was overexpressed, and inhibition of cell proliferation by ERK and NF-κB pathway suppression with ARRB1 knockdown. Intriguingly, the same study showed that cells overexpressing ARRB1 showed reduced apoptosis 10 hours after radiation, possibly due to an increase of the DNA repair (Wang *et al.* 2019). Nevertheless, studies on the impact of ARRBs on the response to PRRT in both pituitary tumors and NENs are lacking.

Outlook and conclusion

As discussed earlier, SRLs have a pivotal role in the treatment of pituitary tumors and well-differentiated NENs due to the widely recognized high expression of SSTs in these tumor types. However, the response to SRLs is not only affected by the mere presence/absence of a single receptor subtype or the ability of the receptors to form homo- and heterodimers, but also by the differential expression of the components of the intracellular signaling machinery and their interaction with the involved receptors. These components of the intracellular machinery allow a fine-tuning of GPCR signaling and trafficking, as well as the cross-talk with other membrane receptor families, such as RTKs. In particular, ARRBs play a crucial role in SST signaling and vesicular trafficking, being involved at multiple levels in the pathogenesis/clinical behavior of different tumors. However, the exact role of ARRB expression in pituitary tumors and NENs has not been fully elucidated yet. As previously mentioned, different studies, mainly carried out in GH-secreting pituitary tumors, evaluated ARRB expression as a potential marker of fg-SRL response, with conflicting results (Gatto *et al.* 2013*a*, 2016, Coelho *et al.* 2018). In this light, the development of new compounds with biased agonist properties for GPCRs could allow us to better elucidate the impact ARRB-dependent and ARRB-independent pathways on SST signaling. Furthermore, a drug able to fully activate the G protein-dependent inhibitory pathways of SSTs (e.g. SST_2) on hormone secretion and cell proliferation, without recruitment of ARRBs (thus leading to reduced receptor desensitization and internalization), could improve the response rate in patients expressing the targeted receptor. Recently, a small molecule (i.e. barbadin) able to inhibit the interaction of ARRBs with AP2 and consequently impair the formation of clathrin-coated vesicles was shown to interfere with the internalization of multiple $G\alpha_s$-coupled GPCRs and to affect the activation of specific intracellular pathways (Beautrait *et al.* 2017). However, the effect of this molecule on SST signaling needs further investigation.

An alternative strategy applied to modulate ARRB function, in the perspective of a novel treatment approach, is the development of aptamers that selectively bind ARRBs. Aptamers are oligonucleotides whose secondary and tertiary structure enables binding with specific protein targets, inhibiting protein–protein interaction, as well as altering ARRBs signaling (Wess *et al.* 2023). A preliminary study showed that treatment with a chimera composed of an aptamer targeting ARRB2 and one aptamer binding nucleolin can inhibit colony formation in leukemic cells, showing the potential of this strategy also in other tumors (Kotula *et al.* 2014). A caveat to the approach of directly targeting ARRBs, however, is the impact on the interaction of these molecules with multiple GPCRs and RTKs, as well as on the epigenetic machinery, considering their ubiquitous expression and the involvement in diverse signal transduction systems. Combination of this strategy with a tumor-targeted delivery may provide a viable alternative.

In conclusion, there is growing evidence of the importance of the intracellular regulatory machinery in modulating SST signaling. However, an in depth characterization of these mechanisms is necessary to better understand their differential role in the specific tumor types, thus favoring the development of novel targeted pharmacological approaches.

Supplementary materials

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Declaration of interest

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