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REVIEW

Cross-talk and modulation of signaling between somatostatin and growth factor receptors

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Abstract The process of homo- and/or heterodimerization of G-protein coupled receptors (GPCRs) and receptor tyrosine kinase (RTK) families are crucial for implicating the fundamental properties of receptor proteins including receptor expression, trafficking, and desensitization as well as signal transduction. The members of GPCR and RTK family constitute largest cell surface receptor proteins and regulate physiological functions of cells in response to external and internal stimuli. Notably, GPCRs and RTKs play major role in regulation of several key cellular functions which are associated with several pathological conditions including cancer biology, neurodegenerative and cardiovascular diseases. The focus of this review is to highlight the recent findings on the possible cross-talk between somatostatin receptors (members of GPCR family) and growth factor receptors like epidermal growth factor receptors (members of RTK family). Furthermore, functional consequences of such an interaction in modulation of signaling pathways linked to pathological conditions specifically in cancer are discussed.

Keywords G-protein coupled receptors · Receptor tyrosine kinase · Somatostatin receptors · Signaling · Dimerization

List of abbreviations

cAMP	Cyclic adenosine monophosphate
c-tail	Cytoplasmic tail
DR	Dopamine receptor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
GPCRs	G-protein coupled receptors
HEK-293	Human embryonic kidney-293
MAPK	Mitogen-activated protein kinase
pbFRET	Photobleaching fluorescence resonance energy transfer
PI3K	Phosphoinositide 3-kinase
PTP	Protein tyrosine phosphatase
RTK	Receptor tyrosine kinase
SST-14	Somatostatin-14
SSTR	Somatostatin receptor
wt	Wild type

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Introduction

The G-protein coupled receptors (GPCRs) constitute one of the largest families of receptor proteins and include more than 800 members in human genome. The predominant role of GPCRs is to mediate the cellular responses upon variety of internal and external stimuli. Initially, such responses of GPCRs were believed to be regulated by native receptors which exist in monomeric form. However, advances in technology in past decade revolutionized the concept that GPCRs exist and function as monomeric entity [1]. In recent year's structure–function studies using biophysical approaches such as fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) in combination with classical biochemical assay have provided mechanistic insight for the functional diversity in

receptor biology [2–4]. Today, the majority if not all GPCRs exist and function as homo- and/or heterodimers with distinct functional activities than their native monomers or dimers. Among several membrane proteins which display homo- and heterodimerization are the members of GPCR family which have been extensively studied with biochemical, pharmacological, and physiological significance. Like GPCRs, member of receptor tyrosine kinase (RTK) are the first cell surface proteins associated with several human diseases. The prominent members of RTK family are the epidermal growth factor receptors (EGFRs) commonly known as ErbBs (namely ErbB1-4). ErbBs play an important role in regulation of cell proliferation, growth, survival, migration, and differentiation as well as are associated with several human cancers [5, 6]. Unlike ErbBs, somatostatin receptors (SSTR1-5), the members of GPCR family are known to play an important anti-proliferative role in cells. The members of GPCR and RTK family are two different families of cell surface proteins with distinct structure and function. The activation of GPCRs have an ability of transactivating the member of RTK family specifically which are activated by growth factors including platelet-derived growth factor, fibroblast growth factor, and epidermal growth factors (EGF) and lead to the regulation of several signal transduction pathways [7–11]. These fascinating observations have served as an instrumental tool in elucidating the concept of possible cross-talk between GPCRs and RTKs. The majority of GPCRs which transactivate RTKs also have growth promoting activity. In contrast, ligands for SSTR subtypes which inhibit cell proliferation and involve in EGFR transactivation have not been studied yet. The illustration of SSTR and ErbB subtypes could contribute to our understanding of their interaction and subsequent changes in cell responses and signal diversification. Both the receptors subtypes have pathological importance, whether these receptors functionally interact with each other and involve in modulation of signaling pathways is largely elusive.

The role of ligand binding on receptor pharmacology and signal transduction is well established. There are many other factors associated with receptor organization and orientation at cell surface; however, cross-talk between two receptor proteins plays a key role in translating the functional diversity. This review mainly focuses on the heterodimerization of SSTR and ErbB subtypes and discusses the consequences of such cross-talk with relevance to certain pathological conditions specifically in cancer biology. Although, not all five SSTR subtypes have been studied yet, this review concluded with recent observations on SSTR1 and SSTR5 alone or in combination as a platform for future studies in different pathological conditions.

Somatostatin (SST) and SST receptors

SST was first discovered in hypothalamic extracts as a growth hormone inhibiting peptide [12, 13]. The two naturally occurring bioactive isoforms of SST namely SST-14 and SST-28 are derived from common precursor pro-SST. SST acts as a neurotransmitter or paracrine/autocrine regulator and elicits diverse physiological function such as inhibition of hormonal secretion, smooth muscle contractility, nutrient absorption, and cell proliferation [14, 15]. In last over three decades, significant progress has been made to understand the dynamic of SST in different aspects including endocrine, exocrine neurotransmission, and neuromodulator functions. In addition to the regulation of pituitary hormonal secretion, SST is also associated with the pathophysiology of many diseases such as neoplasia, inflammation, diabetes mellitus, epilepsy, Alzheimer's disease, Huntington's disease, multiple sclerosis, and AIDS [16–19]. The biological functions of SST are mediated through five distinct subtypes namely SSTR1-5 which binds to SST in nanomolar affinity and mediates its biological functions in receptor-specific manner (Fig. 1) [15, 20, 21].

SST receptors and dimerization

The dimerization is not common for all GPCRs and is rather receptor and ligand dependent [3, 22, 23]. Despite structural homology, receptors within the same family behave differently following ligand binding and some receptors are insensitive to the presence of ligand. In addition, some receptors remain as monomer or constitutive dimers whereas some receptor dimers dissociate to monomer upon ligand binding [4, 24]. Consistent with several other studies, dimerization is not always a necessary mechanism in GPCR activation [25, 26]. SSTR subtypes are the best examples for such diversity and have been studied extensively in past (Fig. 2) [4, 27–29]. Rocheville et al. [30] using combination of pharmacological, biochemical, and biophysical techniques elucidated the concept of SSTRs dimerization for the first time and demonstrated that SSTR5 exist as monomer in basal state and displayed dimerization upon agonist treatment in concentration dependent manner. SSTR1 is the only receptor subtype which enhanced at cell surface in response to ligand, inhibits cyclic adenosine monophosphate (cAMP) and cell proliferation despite the fact that receptor exist in monomeric state irrespective of ligand binding [30, 31]. SSTR2 is the most widely distributed and well-studied receptor subtype and serve as key regulator of all known SST functions in normal as well as pathological conditions. When expressed in HEK-293 cells, human

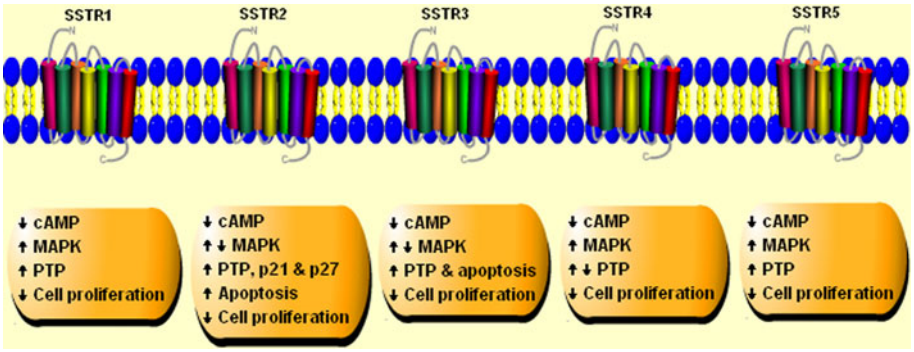

Fig. 1 Schematic diagram illustrating SSTR subtypes mediated cell signaling pathways and consequences on cell proliferation

SSTR2 exists as homodimer and dissociate into monomers in the presence of ligand, an event which is the prerequisite for receptor internalization [27]. Similar observation has also been made on porcine SSTR2, suggesting that hSSTR2 is not an isolated case and the dissociation of SSTR2 in response to ligands seems to be the common property of SSTR2 of different origins [27, 32]. In basal conditions, hSSTR3 exists as preformed homodimer at cell surface and homodimerization decreases in response to agonist [33]. Cells expressing cytoplasmic tail (c-tail) mutants also showed evidence of homodimerization albeit to the lesser degree than wt-hSSTR3 although the agonist-dependent inhibition of cAMP was lost [33]. In contrast to these observations, SSTR3 of rat origin forms dimers constitutively without any effect of ligand binding [28]. Somvanshi et al. [34] recently demonstrated that hSSTR4 exist as dimers in basal condition, dimerization was enhanced in the presence of ligand binding, inhibits cAMP, and displayed agonist-dependent changes in MAPK. Furthermore, c-tail deleted SSTR4 displayed loss of SSTR4 membrane expression and its ability to dimerize and inhibit cAMP. Taken together, these studies clearly show that within a family, SSTRs behave differently and the state of dimerization can also depend on the expression levels of receptor. Furthermore, dimerization is not the absolute requirement for receptors to be functionally active as shown for SSTR1.

Heterodimerization of SST receptors

The presence of multiple receptors in single cell with enhanced activities such as in neuronal, pituitary, and pancreatic cells are the supporting evidence that SSTR subtypes might function in heteromeric complex. SSTR subtypes not only heterodimerize within the family but does so with other members of GPCR family like dopamine, opioid, adrenergic, and growth factor receptors [28, 30, 31, 34–43]. In addition to displaying receptor-specific dimerization, SSTR subtypes have shown great diversity upon heterodimerization as well. HEK-293 cells cotransfected with SSTR2 and 3 of rodent origin exhibited constitutive homo- and heterodimers [28]. SSTR3-selective agonist L-796,778 showed a marked decrease in binding affinity in SSTR2/SSTR3 coexpressing cells, suggesting negative cooperativity between these receptor subtypes. The heteromeric complex of rat SSTR2/SSTR3 functions more or less like SSTR2 expressing cells. SSTR2 and SSTR5 heterodimerization is induced by selective SSTR2 agonist and not with endogenous pan-agonist SST-14 [36, 44]. Conversely, SST-14 has been demonstrated to enhance hSSTR1/hSSTR5 heterodimers formation [30, 31, 45].

Somvanshi et al. [34] described that chimeric SSTR4 with the c-tail of SSTR5 functions like *wt*-SSTR4, in contrast, with the c-tail of SSTR1 chimeric receptor functions like c-tail deleted hSSTR4. hSSTR4 dimerization is associated with increased expression of cyclin-dependent-kinase inhibitor $p27^{kip1}$ and inhibition of cell proliferation. Authors also reported that SSTR4 forms heterodimers with SSTR5 but not with hSSTR1 with significant changes in receptor function. Most importantly, hSSTR4 lacks coupling to G-proteins or to adenylyl cyclase in CHO-K1 cells due to the absence of the $G_{i\alpha 1}$ [46]. This study clearly defines a novel mechanism for the role of hSSTR4 in cell proliferation and modulation of signaling pathways in cell specific manner [34].

Rocheville et al. [30] described for the first time that SSTR1 and SSTR5 exist in heteromeric complex. Monomeric SSTR1 is resistant to internalization and rather upregulated at cell surface upon agonist treatment. On the contrary, SSTR5 exhibited time-dependant internalization. SSTR1 displayed internalization in cells cotransfected with SSTR1/SSTR5, suggesting that the trafficking of SSTR1 is modified by the receptor heterodimerization [30]. In parallel to these findings, homo- and heterodimerization between SSTR1 and SSTR5 was also observed in live cells using fluorescence correlation spectroscopy techniques [45]. In this study, although SSTR5 was demonstrated to form both homo- and heterodimers with SSTR1 in an agonist dependent manner, SSTR1 remained as a monomer when expressed alone despite its activation with agonist [45]. Interestingly, subsequent studies showed that SSTR5

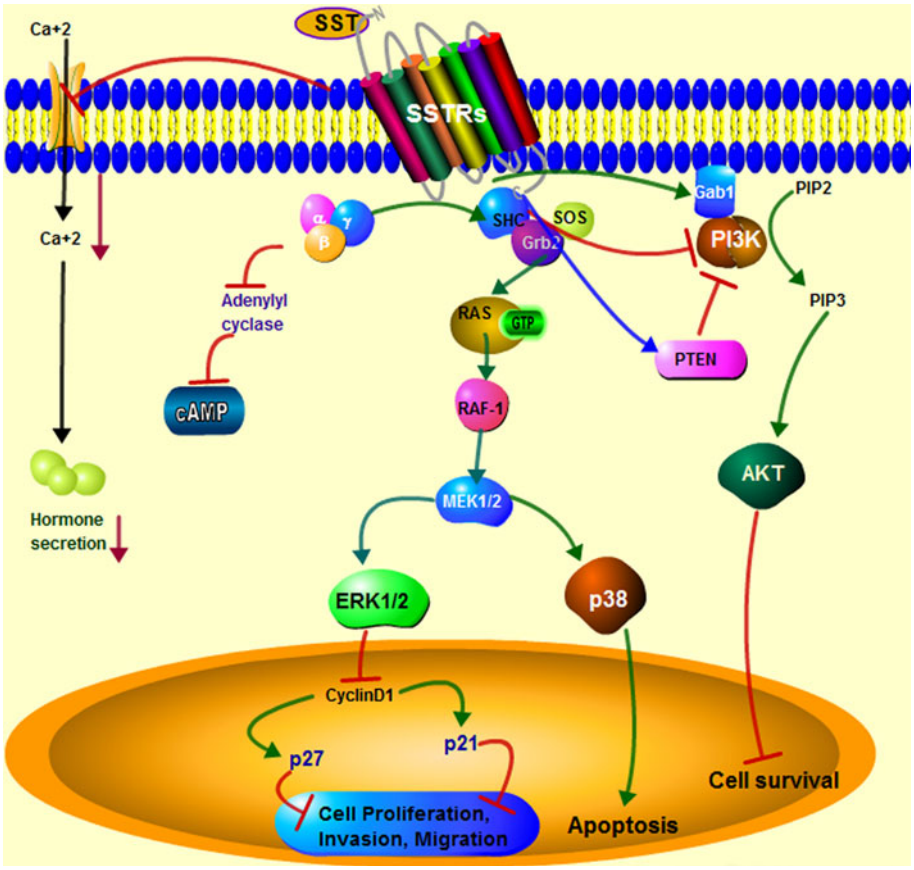

Fig. 2 Schematic illustration showing potential signaling targets mediated by SSTR subtypes. In the presence of SST or receptor specific agonist, SSTRs block Ca^{2+} influx and inhibit hormonal secretions. SSTRs inhibit cAMP via coupling to G-proteins specifically Gi and result in the activation of ERK in cell and receptor specific manner exhibiting inhibition of cell proliferation. SST mediated inhibition or activation of p38 in cell and receptor specific manner also induce apoptosis. As shown, SSTR subtypes-dependent activation of p27 and p21 is associated with cell cycle arrest (cytostatic effect). In contrast, inhibition of PI3K/AKT cell survival pathway and enhanced expression of PTEN upon SSTRs activation are involved in inhibition of cell proliferation

and SSTR1 heterodimerization was specifically induced upon activation of SSTR5 and not via activation of SSTR1 [31]. In addition, Grant et al. [31] described the mechanism for SSTR heterodimerization and elucidated that c-tail of SSTR like many other GPCRs is integral for G-protein coupling. The mechanism for internalization and dimerization is confined to the c-tail of SSTR5 was discovered by interchanging this segment to the SSTR1 [31]. SSTR5 homodimerization upon agonist activation was diminished in the presence of SSTR1 c-tail. Conversely, SSTR1 with the c-tail of SSTR5 displayed homodimerization in the presence of agonist, suggesting that c-tail is critical in receptor dimerization [31]. Most importantly, the ability of SSTR4 to form homodimer was not observed with the c-tail of SSTR1 [34]. SSTR1/SSTR5 and SSTR4/SSTR5 heterodimerization leads to greater efficiency in signaling and inhibition of cAMP [31, 34].

SSTRs also physically interact with other members of GPCR family for example opioid, dopamine, and β-adrenergic receptors [35, 37, 38, 40]. Activation of SSTR2 in cells coexpressing SSTR2 and μ-opioid receptor resulted in increased receptor phosphorylation, desensitization, and endocytosis of both receptors. However, μ-opioid receptor-specific ligand DAMGO increased only phosphorylation and desensitization of both the receptors without any discernable changes in SSTR2 internalization [37]. Conversely, SSTR5 and D2R or SSTR2 and D2R exist as monomers and homodimers under basal condition and upon ligand binding resulted in formation of heteromeric complex [35, 38]. With respect to the formation of SSTR2 and D2R heterodimers, dopamine affinity was increased and SSTR2 functions remained unchanged [35]. Furthermore, recent studies have also described that SSTR1 and SSTR5 functionally interact with adrenergic and growth factor receptors with significant changes in receptor coupling to adenylyl cyclase and signaling in receptor specific manner [39–43]. Whether, the activation of two receptors in the presence of receptor-specific agonist also elicit similar effects in cells expressing these receptor endogenously is largely elusive. In support, previous studies using cultured striatal neurons have shown heterodimerization between D2R and SSTR2 in basal condition whereas receptor heterodimerization in neurons was completely diminished in the presence of the D2R antagonist eticlopride [35].

Growth factor receptors

Unlike SST, EGF plays fundamental role in cellular physiology including development, proliferation, differentiation, survival, and transformation. The biological effect

of EGF is mediated by ErbBs which belong to the type I family of RTKs and are commonly known as ErbB1/EGFR/HER1, ErbB2/HER2, ErbB3/HER3, and ErbB4/HER4. ErbBs are well expressed in tissues of epithelial, mesenchymal, and neuronal origin [47–49]. All four ErbBs display structural similarities, an extracellular domain containing two cysteine-rich regions (II and IV) that mediate ligand binding interspersed with two unique domains (I and III), a single membrane-spanning region, and a cytoplasmic region that contains multiple phosphorylation sites which respond to ligand binding and activation [47, 50–52].

Growth factor receptors homo- and/or heterodimerization

ErbBs exist and function in receptor-specific heteromeric complex. EGFR in the absence of agonist exist in monomeric state and upon activation in the presence of ligand display homo- and heterodimerization. ErbB2 is devoid of any known ligand, however, its conformation is such that the dimerization loop is constitutively extended, and receptor exists as constitutive dimers. Furthermore, homo- and heterodimerization of ErbB2 is linked to the levels of expression as frequently seen in highly proliferating tumors [53–55]. Moreover, in the process of heterodimerization within the family, ErbB2 is the most common and preferred protomer and plays an important role in modulation and potentiation of receptor signaling than the native receptors [47, 56]. Enhanced receptor signaling of ErbB2-containing dimers has been attributed to delayed internalization in conjunction with efficient recycling to the membrane. In contrast, ErbB3 contains mutations within the cytoplasmic domain that impede tyrosine kinase activity [5]. Therefore, heterodimerization of ErbB3 with other ErbBs is the prerequisite to elicit its signaling [57]. Similar to ErbB1, inactive ErbB4 is present in a monomeric state and forms homo- and heterodimers upon activation [58]. With respect to heterodimerization and modulation of signaling pathways, however, some controversies exist. The functional diversity of ErbBs in heteromeric complex has drawn great attention, despite significant structural homology ErbBs exhibit distinct nature for ligand binding as well as trafficking in receptor specific manner (Fig. 3). However, which combination of heteromeric complex is most mitogenic is still controversial. Despite the fact that ErbB2 is devoid of ligand and ErbB3 is with impaired tyrosine kinase, ErbB2 and ErbB3 in heteromeric complex are the most potent mitogenic and oncogenic complex [59–64]. This association leads to the activation of PI3K cell survival pathway via ErbB3 attributed to trafficking properties of the complex and prolonged signaling [65–67]. In contrast to these observations, heteromeric complex of EGFR–ErbB2 has also been shown as the potent inducer of mitogenic signaling in comparison to other various pair of homo- and heterodimers [5, 55, 68, 69]. As described earlier, neu differentiating factor (NDF) structural analog of EGF has shown to prompt homo- and heteromeric complex between ErbB2 and ErbB2 or ErbB4 [70–72]. These studies cumulatively emphasize that the presence of ErbB2 or ErbB4 is required component of NDF binding and signal transduction via ErbB2. Of note, in cells expressing EGFR and ErbB2, the formation of heteromeric complex is preferential than homodimers. Heterodimerization between EGFR and ErbB3 can also be induced by EGF. Most importantly, inactive heteromeric complex is also formed with ErbB3 which lacks signals via ErbB2 [73].

Heterodimerization between SST receptor and growth factor receptors

SSTR subtypes like many other GPCRs are involved in variety of physiological functions and linked to several key downstream signaling cascades. Like SSTRs, ErbBs also associate with multiple signaling molecules. Although, both SSTR and ErbB subtypes regulate common signaling pathways with distinct cellular responses, however, the molecular mechanism for diversified signaling upon receptor activation are not well understood. A new role of SSTRs in concert with growth factor receptors is discussed here (Fig. 4). It is not an isolated case. Previous studies have shown the role of insulin like growth factor receptor modulated phosphorylation, desensitization, and trafficking of $\beta 2$ adrenergic receptor along with activation of signaling pathways [74]. In contrast, IGF1 counteracts the effects of β-adrenoceptors as well. There is evidence that ErbBs heterodimerization is not only restricted to the members of its own family but ErbBs have also been shown to interact with the members of GPCRs as well as receptors belonging to ionotropic receptor family such as NMDA receptors and certain adhesion molecules such as integrin [69, 75]. The concept of functional cross-talk between GPCR and ErbBs was first emerged from studies by Daub et al. describing agonist for GPCRs namely endothelin (ET-1), lysophosphatidic acid (LPA), and thrombin stimulate EGF release by autocrine and paracrine mechanism and are linked to several downstream signaling pathways via transactivation of EGFRs. Furthermore, transactivation of EGFR have also been described for significant changes in $\alpha 1$ and β-adrenergic receptors via involvement of GPCRs [76]. The functional consequences of released EGF which triggered EGFR transactivation are intimately associated with cancer growth via modulation of several tumor promoting

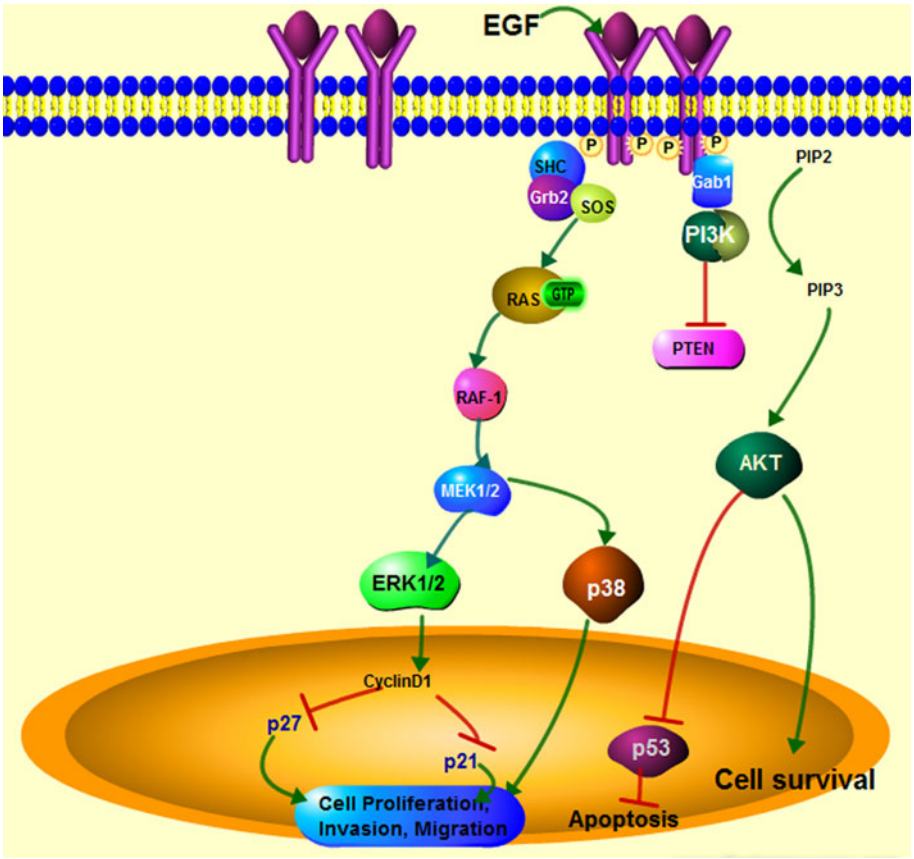

Fig. 3 Schematic presentation depicting the most common signaling pathways associated with EGF-induced cell proliferation. EGF via ErbBs induces cell proliferation by targeting signaling pathways and enhances cell proliferation and tumor promoting factors. In the presence of EGF, EGFR displays enhanced phosphorylation, homo- and/or heterodimerization with other ErbBs and modulate various integrated pathways. EGFR-mediated activation of MAPK (ERK/p38) and cell survival (PI3K/AKT) pathway induce cell proliferation, invasion, and migration. Activated ERK leads to the inhibition of p27 and p21 via involving cyclin D1 and results in cell proliferation. Activated PI3K and AKT suppress PTEN and p53 expression and promote cell proliferation

signaling pathways as well as suppression of tumor inhibiting signaling molecules. Whether such physiological response of cells is directly associated with EGFR and GPCR oligomerization or indirectly associated with the promoting of hormone secretion is unknown. There are many compelling reasons to contemplate the possible cross-talk between SSTR and ErbBs at least in two different directions such as central nervous system and tumor of different origins. SSTRs and ErbBs are well expressed in brain and breast tumor tissues [43, 77, 78]. Like SSTRs, growth factor receptors displayed functional homo- and heterodimerization or dissociation of preformed dimers upon ligand activation and created novel receptors with enhanced signaling and internalization [27, 30, 31, 34, 38, 45]. Expression of SSTRs is inversely related with ErbBs in breast tumor cells, however, in human glioma both SSTR2 and ErbBs are highly expressed [79]. In glioma cells, SSTR2 affects EGF-promoted cell proliferation and also exhibits significant inhibition of EGF-dependent ERK1/2 hyperphosphorylation. Most significantly, the biological outcome of ERK1/2 activation is dependent on cell type, extra-cellular factors, and their receptors. Additional studies on pancreatic tumor provide direct evidence for antagonizing effects of SST on cell proliferation and mitogenic action of EGF [79]. These studies directly support the possible interactions between ErbBs and SSTRs and its consequences on cell proliferation and signal transduction pathways [80]. With this concept in mind, we recently described that SSTR1 and SSTR5 functionally interact with EGFR in heteromeric complex in breast cancer cells including MCF-7 cells and MDA-MB231 cells [41–43]. Because, in tumor cells, all ErbBs and SSTR subtypes are expressed and respond to common ligands, it was difficult to delineate the role of individual receptors. Kharmate et al. [41, 42] recently described the cross-talk between SSTR and ErbB subtypes in HEK-293 cells expressing ErbBs endogenously and transfected with SSTR subtypes. As discussed in next section of this review SSTR1 and SSTR5 mono- or cotransfected cells displayed receptor-specific role on EGF-mediated signal transduction pathways. These studies provide a better explanation that SSTRs indeed antagonize EGF-mediated effect, the phenomenon associated with physical interactions between SSTR/EGFR along with dissociation of EGFR/ErbB2.

Functional consequences of SST and growth factor receptors heterodimerization

The existing literature support the notion that GPCRs and ErbBs are associated with the uncontrolled tumor growth specifically breast, prostate, colon, lung, and ovarian

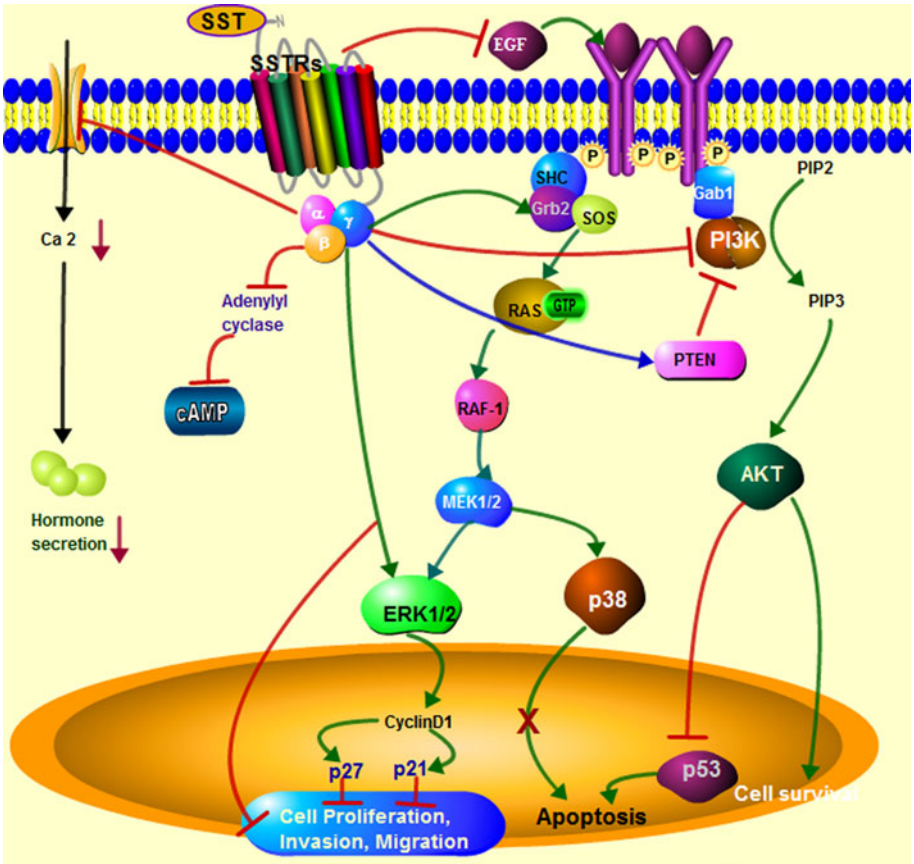

Fig. 4 SSTRs antagonize EGF-mediated effects on cell survival pathways. SSTRs inhibit the EGF-mediated action of EGFR and block receptor phosphorylation. Cell survival pathways including PI3K and AKT are inhibited in SSTR subtypes specific manner and leads to the inhibition of cell proliferation. Subsequent activation of PTEN and p53 are associated with the inhibition of cell proliferation and induction of apoptosis, respectively. SSTRs activation also enhances p27 and p21 and promotes inhibition of cell proliferation. This illustration is constructed from references [40–42]

cancer [10, 81, 82]. Some of the key signal transduction pathways are stimulated by GPCRs and ErbBs with distinct physiological responses of cells. GPCRs-mediated transactivation of RTK uses EGF for activation is well established via binding to specific adaptor proteins. For instance, Shc is associated in regulation of signaling cascades linked to PI3K/AKT and MAPK pathways. Importantly, Shc is also linked to the suppression of signaling molecules which inhibits tumor growth (Fig. 4). EGFR phosphorylation via transactivation in the presence of GPCR agonists such as ET-1, LPA, and thrombin are associated with MAPK activation in both transformed and non-transformed cells [83–85]. The recruitment and activation of key adaptor proteins such as Shc, Grb, son of sevenless (SOS), and SH2 domain-containing phosphatases (SHP1), as well as EGF-induced EGFR phosphorylation is SSTR1 or 5 dependent [43]. This study provides first direct evidence that SSTRs and ErbB subtypes linked with each other. The oncogenic effects of ErbBs are tightly regulated by enhanced receptor expression at cell surface and colocalization with other ErbBs, receptor phosphorylation and importantly homo- and/or heterodimerization. Most significantly, the imbalance and dysregulation of ErbBs in cancer is predominantly associated with uncontrolled tumor growth. Accordingly, antagonizing these crucial integrated steps might serve not only to divert ErbBs signaling but also provide potential therapeutic intervention in cancer treatments. The role of SSTR subtypes in modulation of aforesaid ErbBs actions/functions are largely elusive but has been partially addressed in three recent studies either using breast cancer cells or cells transfected with SSTR subtypes [41–43]. These studies addressed whether the presence or activation of SSTR altered EGF-induced signaling pathways which promote tumor progression and are responsible for treatment failure. This review further focuses on these questions to highlight how SSTR subtypes via functional cross-talk divert the deleterious effects of ErbBs.

Changes in ErbBs expression and colocalization at cell surface in the presence of SSTR subtypes

The first indication of cross-talk between two receptor proteins is their colocalization at cell surface or intracellularly. The presence of ErbB2 and ErbB3 impedes the internalization of EGFR and resulted in EGFR phosphorylation. Furthermore, prolonged membrane expression of EGFR and blockade of degradation leads to enhanced signaling due to the formation of a heteromeric complex of

EGFR with ErbB2 or ErbB3 [86, 87]. Whether the presence of SSTR subtypes interfere in the process of ErbBs trafficking is not known. Recent studies have provided new insights for the role of SSTR subtypes in this direction [41–43]. Watt et al. [43] recently described that the treatment of breast cancer cells with SST alone or in combination with EGF had no effect on SSTR1 whereas upregulated SSTR5 expression and induced loss of EGFR at cell surface. Interestingly, EGFR loss at cell surface was not accompanied by receptor accumulation intracellularly, suggesting receptor degradation and termination of EGFR mediated mitogenic signaling. Cells transfected with SSTR1 and SSTR5 alone or in combination also displayed similar results [41, 42].

EGFR phosphorylation is inhibited by SST receptors

Receptor phosphorylation is modulated by the dimerization partner; however, even within the context of the same heterodimer, distinct ligands can differentially affect a receptor's signaling properties [47, 88]. EGFR expression and the duration of EGFR phosphorylation are linked with tumor progression and antagonizing phosphorylation of EGFR is the first step in the intervention on cell growth and significant therapeutic approach in tumors of various origins. EGFR homodimerization is most effective in inducing EGFR phosphorylation in the presence of EGF than the heterodimerization with ErbB3 following activation by neuregulin [86]. We recently described that SSTRs block EGFR phosphorylation and homodimerization as well as heterodimerization with ErbB2 [41, 42]. Taken together these results implicate a competing action of SSTR subtypes in EGFR dimerization and phosphorylation and subsequently the modulation of signaling cascades as discussed next.

Activation of SST receptors antagonize EGFR-mediated MAPK signaling pathways

The conclusive statement from several previous studies suggest that inadequate balance between receptor activation and trafficking exert detrimental role on activation of signaling pathways via EGFR or other members [89]. Furthermore EGFR trafficking, receptor internalization, targeting receptor for degradation, and receptor recycling back to cell surface are linked to the receptor mediated-mitogenic signaling. Therefore, any interference in receptor expression at cell surface will play pivotal role on cell response. Consistent with these informations, sustained and prolonged activation of ErbBs and blocking its degradation leads to the extracellular signal-regulated kinase (ERK) activation as well as resulted in cellular transformations [89].

Ligand-dependent activation of ErbBs and their functional interactions as homo- and heterodimers activate downstream pathways such as MAPK (ERK1/2), consequently leading to well-defined and characterized role on cell proliferation, tumor growth, and resistance to apoptosis. ErbBs also activate the MAPK pathway in conjunction with several other intermediate proteins [88, 90]. In contrast, SSTR subtypes upon agonist regulate ERK in receptor and cell dependent manner [91–93]. Whether ERK is activated or inhibited in the presence of SSTR subtypes, the ultimate cellular response is the inhibition of cell proliferation. For instance, ERK inhibition is required for the anti-proliferative activity of SSTR5. Furthermore, activation of ERK pathways is also involved in SSTR2-mediated anti-proliferative effects [41]. While, EGF activated ERK phosphorylation in *wt* HEK-293 cells in time dependent manner, SST with or without EGF induces comparable activation of ERK1/2 in *wt* and SSTR5 expressing cells but displayed inhibition of EGF induced ERK phosphorylation seen in *wt* cells. These observations suggest that prolonged and sustained phosphorylation of ERK1/2 upon activation of SSTRs is a prerequisite for anti-proliferative effect [91–93]. Recently, ERK5 has also been described in regulating cellular mechanisms associated with ErbBs in tumors [94, 95]. Consistent with these observations our recent study suggests that SSTR1 might function differently in mono-and/or cotransfected cells in modulation of ERK5 in the presence of tumor promoting effect of EGF [41, 42]. Importantly, cells cotransfected with SSTR1 and 5 exhibited greater responses to SST treatment in blocking EGF-mediated changes in ERK phosphorylation [42].

Activation of SST receptors antagonize EGFR-mediated tumor promoting signaling pathways

Tumor progression and loss of trastuzumab responsiveness in tumor treatment associated with activated PI3K/AKT cell survival pathway and suppression of PTEN [96]. Inhibition of PI3K/AKT serves as potential therapeutic target. The inhibitors of EGFR and ErbB2 in addition to exert anti-tumor effects should have an ability to inhibit PI3K/AKT [97, 98]. In line with these observations, recent studies have shown the inhibition of PI3K in the presence of SSTR activation [41, 42]. Whether inhibition of PI3K upon SSTRs activation plays any role on trastuzumab treatment in cancer is not known. The inhibition of PI3K/AKT, phosphorylation in the presence of SSTRs provide first evidence that the gradual loss of SSTR subtypes might be responsible for the loss of trastuzumab responsiveness being associated with enhanced PI3K and loss of PTEN as the tumor progresses [99].

The role of SSTRs to inactivate EGF-mediated effect is further strengthened with the changes in $p27^{Kip1}$ expression and membrane translocation of PTP. The activation of $p27^{Kip1}$ and membrane translocation of PTP is associated with SST-induced anti-proliferative effect. We also observed increased expression of $p27^{Kip1}$ in the presence of SST treatment in cells expressing SSTR1 or SSTR5 and both receptors together [41, 42]. In agreement with previous studies, results described by Kharmate et al. suggest a combined cytostatic (increased expression of $p27^{Kip1}$) and cytotoxic/apoptotic (increased membrane expression of PTP1C) effect of SSTR subtypes [100, 101].

The presence of specific ErbBs ligands dictates the diversity in signal transduction and cell responses in the formation of receptor complexes [5, 47, 51, 53, 72, 88, 102]. Importantly, prolonged and sustained association of ligand to the receptor may be a critical component in regulating the biological activities such as internalization, desensitization, and degradation [47, 88]. Furthermore, the changes described here in MAPK and PI3K/AKT as well as in PTP translocation and expression of $p27^{Kip1}$ in response to SST or SSTR-specific agonist further enhanced in the presence of EGFR inhibitor AG1478 and knocking down EGFR in the presence of siRNA [41, 42].

The presence of SST receptors lead to the dissociation of homo- and heterodimerization of ErbBs

The formation of homo- and/or heteromeric complex among ErbBs is best characterized for potent signal transduction and effective mitogenic and oncogenic signaling. Most importantly, the changes in receptor stoichiometry, conformational dynamics and receptor orientation at the cell surface, and the key regulatory mechanisms for receptor heterodimerization might play critical role in receptor specific manner. Whether SSTR subtypes impede ErbBs homo- and heterodimerization has not been explored in detail. Our recent study has shown that SSTR5 induced the dissociation of heteromeric complex between EGFR/ErbB2. In addition, we also provided the evidence that SSTR1 and SSTR1/5 also interfere with the formation of ErbBs heteromeric complex in receptor specific manner [41, 42]. SSTR1 transfected cells exhibited SSTR1/EGFR heteromeric complex and subsequent dissociation of EGFR/ErbB2 heterodimerization that possibly resulted in the inhibition of EGFR phosphorylation. Most importantly, cells coexpressing SSTR1/5 responded differently than the monotransfected cells. Cotransfected cells showed heterodimerization between SSTR5/EGFR with the dissociation of SSTR1/EGFR and EGFR/ErbB2 heterodimerization [41, 42]. The potential interference of SSTRs in ErbBs homo- and/or heterodimerization may serve as a regulatory mechanism for the role of SSTR subtypes in the modulation of EGF-mediated mitogenic effects. Such speculation is in general agreement with previous studies where it has been demonstrated that the presence of ErbB2 and ErbB3 impedes the internalization of EGFR and resulted in EGFR phosphorylation due to prolonged membrane expression [86]. In such conditions, EGFR degradation is reduced and resulted in enhanced signaling due to the formation of a heteromeric complex with EGFR and accounts for signaling modulation [87]. Hence, a competing action of SSTR subtypes in EGFR dimerization, inhibition of EGFR phosphorylation, and subsequently the modulation of signaling cascades might lead to a novel therapeutic approach in cancer biology.

Conclusions

The cross-talk between SSTR and ErbB subtypes in modulation of cancer-related signaling pathways govern clinical implications. The members of GPCR and RTK family represent potential target in several pathological conditions and have drawn great attention of pharmaceutical industries. GPCR constitute 60% of drugs used today in clinical setup. Understanding the role of ErbB1 and ErbB2 in certain diseases has led to the development of therapeutic intervention that targets these receptors and their downstream pathways [102]. Taken together, ErbBs could be targeted by different mechanism in tumors over expressing these receptors. As a consequences of different array of functions in human biology and pathological conditions, GPCR and EGFRs are the most attractive molecules which can be explored in development of new therapeutic drugs for the treatment of tumor of different origin where SSTR and ErbB subtypes play critical role. It could be possible that inactivation and blocking of homo- and heterodimerization of ErbBs along with the activation of SSTR subtypes may prevent tumor growth and cell proliferation. There is need to refine the use of predictive markers such as incorporating screening of key components of the MAPK and PI3K/AKT pathways in addition to defining ErbBs expression levels and mutations. Furthermore, combining cytostatic EGFR inhibitors with cytotoxic compounds should prove beneficial. However, there are many other combinations of heteromeric complexes between SSTR and ErbB subtypes remained to be investigated and the molecular mechanism associated with the cross-talk are not yet well understood. Therefore, it is worth investigating other combinations and their functional consequences in cancer biology. Since, growth factor receptors are prominent players in normal cell physiology hence, complete ablation of these receptors may not be highly appreciable. Therefore, in human breast cancer and pancreatic cancer

whereby ErbBs play crucial role in tumor progression and treatment failure, a combined approach targeting ErbBs over expression and activation of SSTR will provide most effective therapeutic approach with less untoward effects than existing treatment choices.

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Conflict of interest The author declares no conflict of interests.

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