Review

Somatostatin and Epidermal Growth Factor Receptors: Implications in Breast Cancer

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Abstract

Despite several advances, the underlying mechanism of complexity of breast cancer progression still remains elusive. In addition to the genetic predisposition, several growth factor receptors including insulin growth factor receptor (IGF), platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) relaying proliferative signals are accountable for disease progression. Epidermal growth factor receptors (EGFRs, or commonly known as ErbBs), members of the receptor tyrosine kinase family (RTKs), play a central role in tumor growth, progression and metastatic disease. Typically, agonist dependent activation of EGFR results in receptor phosphorylation, homo- and/or heterodimerization and modulation of signaling pathways leading to cell proliferation, survival and metastasis. Targeting one or multiple steps in EGFR-mediated tumor progression may serve as a better approach in drug therapies. Unlike EGFRs, G-protein coupled somatostatin receptors (SSTRs) have been recognized as negative regulators of breast tumors. The activation of SSTRs modulates downstream signaling responsible for tumor growth and consequent cytostatic or cytotoxic effects on tumor proliferation. SSTR subtypes are well characterized to form homo- and/or heterodimers within the same family as well as with other GPCRs. Clinically, the chimeric molecule targeting both SSTR5 and dopamine receptors (specifically dopamine receptor 2) is in use for the treatment of pituitary tumors. This review describes the interplay between SSTRs and EGFR and the potential role of such cross talk in attenuation of EGFR-mediated signaling pathways involved in tumorigenesis. Furthermore, recent findings supporting the role of SSTR in EGFR-mediated signaling in tumor biology are discussed in detail.

Introduction

Breast cancer is a complex heterogeneous form of cancer affecting 1 in 9 women worldwide. Each year, more than a million new cases of breast cancer and ~400,000 deaths are globally reported. While 90-95% are sporadic only 5-10% of all breast cancer cases are hereditary (Rosen et al. 2003). Breast cancer progression is often manifested by excessive cell proliferation, genetic mutations, angiogenesis and metastasis. More than 20-30% of the total hereditary breast tumors are due to inherited genetic mutations in breast cancer 1-susceptibility genes (BRCA1 and BRCA2 (Easton et al. 1995, Rosen et al. 2003, Wooster et al. 1995). The amplification of the cmyc gene is observed in 20-30% of breast tumors and linked with aggressive metastatic tumors of high grade (Deming et al. 2000). In normal breast tissue, p53 and phosphatase and tensin homolog (PTEN) function as tumor suppressor genes; however, mutations in p53 and loss of PTEN are associated with a high risk of breast cancer (Tsutsui et al. 2005). Current studies are focused on defining and identifying prognostic biomarkers including BRCA1 and BRCA2 genetic mutations, estrogen/progesterone (E/P) status and expression of p53/PTEN. The identification of such new biomarkers and their implication in prognosis and diagnosis has enhanced the understanding of the etiology of breast tumors and the application of individualized targeted therapies against tumor progression while reducing death rates (Weigel & Dowsett 2010). Despite such advances, classical markers including E, P and epidermal growth factor receptors (ErbBs) are routinely assessed for diagnostic and pathological examinations in breast cancer. So far, an extensive amount of research has been directed to the factors responsible for tumor progression, including EGFR; however, the potential significance of certain receptor proteins such as somatostatin receptors...
(SSTRs), which are responsible for tumor suppression, has not been studied in detail. More importantly, the physiological significance and pharmacological interaction between such receptor proteins remains to be elucidated.

The role of ErbB1 (commonly known as EGFR) in human malignancies including neck, head, colon and breast has been investigated extensively and thus remains the major target for anti-neoplastic drug discovery (Nicholson et al. 2001, Yarden 2001, Zimmermann et al. 2006). Interestingly, the EGFR and ErbB2 subtypes are over-expressed in > 30% of tumors with poor survival (Abd El-Rehim et al. 2004, Bo et al. 2008). Hyperactivity due to autocrine secretion in the ErbB network leads to over-production of ligands and receptors by the breast tumor cells. EGFR-mediated breast tumor progression is manifested by (i) over-expression, (ii) EGFR phosphorylation and (iii) homo and/or heterodimerization, preferentially with ErbB2, leading to aberrant downstream signaling pathways (Bo et al. 2008, Earp et al. 1995, Kallergi et al. 2008, Kraus et al. 1987, Martin & Philippe 2008, Olajoye et al. 2000, Ulrich et al. 1984, Yarden 2001). Numerous therapies including tyrosine kinase inhibitors (e.g., Lapatinib, Gefitinib and erlotinib) and monoclonal antibodies (e.g. trastuzumab, cetuximab) are clinically available, however, targeting EGFR alone has been deemed insufficient as a means of controlling the progression of breast tumors (Alvarez et al. 2010).

In retrospect, the anti-proliferative role of somatostatin (SST), a multifunctional endogenous regulatory neuropeptide has been employed for the treatment of tumors of different origins (Ben-Shlomo & Melmed 2008, Buscail et al. 1995, 2002). The biological effects of SST are mediated by five membrane bound SSTR1-5 belonging to the G-protein coupled receptor family (Patel 1999). SSTRs are also known to regulate secretion of most, if not all, endocrine/exocrine hormones and growth factors. SSTRs activate various downstream targets and negatively regulate cell proliferation (Bousquet et al. 2004, Florio et al. 1999, 2000, Hagemeister & Sheridan 2008, Lahlou et al. 2004). The activation of SSTRs promotes homo- and/or heterodimerization within the same family and with other GPCRs and results in the modulation of downstream signaling cascades more efficiently compared to the native receptors (Grant et al. 2004, Grant & Kumar 2009, Pfeiffer et al. 2002, Rocheville et al. 2000a, b, Saveanu et al. 2002, Somvanshi et al. 2011). SSTRs have been clinically proven effective in suppressing pituitary and pancreatic tumor growth (Ben-Shlomo & Melmed 2008, Bousquet et al. 2004, Jaquet et al. 2005). A recent study from the authors’ laboratory showed a receptor-specific colocalization between SSTRs and ErbB2 in human breast cancer cells (Watt & Kumar 2006). These observations indicate the possibility of a potential functional interaction between SSTRs and ErbB2 in breast cancer. Nevertheless, the mechanistic role of SSTRs in the modulation of EGFR homo- and/or heterodimerization, phosphorylation and consequent inhibition of downstream signaling pathways remains elusive. The main emphasis of this review is to define the mechanisms that might be associated with the interaction of SSTR and ErbB subtypes and their pronounced impact in the modulation of signaling pathways which are critical in tumor progression and inhibition.

**Epidermal Growth Factor Receptors**

Epidermal growth factor (EGF) regulates normal as well as neoplastic cell growth. EGF mediates its biological effects via ErbBs. Ulrich et al. (1984) first identified EGF as the cell surface receptor in malignant cells and characterized it using molecular cloning techniques. The ErbB family is comprised of four transmembrane receptors (EGFR-4) that belong to the receptor tyrosine kinase (RTK) family (Carpenter et al. 1978, Yarden 2001). ErbBs are commonly comprised of three components: (i) the ligand-binding extracellular (EC) domain, (ii) the hydrophobic transmembrane region and, (iii) the intracellular cytoplasmic domain that is linked with the former and contains the tyrosine kinase domain (Harris et al. 2003, Savage et al. 1972). The extracellular domain is comprised of four subdomains designated as large domains (L1 and L2) and cysteine rich domains (C1 and C2) (Bajaj et al. 1987, Garrett et al. 2002, Ogiso et al. 2002). The intracellular domain of ErbBs consists of a highly conserved tyrosine kinase and C-terminal domain, involved in phosphorylation and transmission of downstream signaling (Garrett et al. 2002, Ogiso et al. 2002). There is a 53% structural homology within all the ErbB subtypes, not accounting for the differences in the tyrosine kinase domains (Jorissen et al. 2003). EGFR upon binding to EGF interacts with other ErbBs to activate the tyrosine kinase residues. However, ErbB2 is the only subtype which does not bind to any ligands and depends on other ErbBs, preferentially EGFR and ErbB3, for its activation and functionality. ErbB3 uniquely lacks inherent receptor kinase activity and relies on other ligand-activated ErbBs for its function (Guy et al. 1994). The expression of ErbB4, in general, is relatively less than of other ErbB subtypes. ErbB4, although having a tyrosine kinase domain, requires cleavage by membrane proteases to activate the intracellular tyrosine and its translocation to the cell surface (Rio et al. 2000).
The ligands for ErbBs are classified into three major groups depending on the receptor binding specificity. The first class consists of EGF and EGF-like binding ligands, tumor growth factor-α (TGF-α) and amphiregulin (AR) that specifically bind to EGFR (Gullick 2001, Suo et al. 2002, Yarden 2001). The second class is composed of betacellulin (BCT), heparin-binding-EGF and epiregulin that bind to EGFR and ErbB3. The third is the neuregulins (NRGs) family that is further sub grouped into NRG1 and NRG2 that bind to ErbB3 and ErbB4 whereas NRG3 and NRG4 bind only to ErbB4 (Yarden 2001). Of the four receptors, ErbB2 is the only receptor subtype that does not bind to any known ligand and relies on other ligand activated ErbBs for its physiological functions (Suo et al. 2002).

Prior to ligand binding, EGFR exists as a dormant monomer within the cell membrane. Receptor dimerization leads to conformational changes and exposure of the dimerization loop (Gadella & Jovin 1995). These alterations bring two EGF molecules in close proximity allowing receptor dimerization, provided there is a 1:1 ligand receptor complex. Binding of the two EGF molecules to EGFR stabilizes this complex formation (Lemmon et al. 1997). Binding of EGF to EGFR not only promotes homodimerization but also heterodimerization with other ErbBs (Earp et al. 1995).

**EGFR and Breast Cancer**

ErbBs are expressed in tissues of epithelial, mesenchymal and neuronal origin and involved in embryonic development through adulthood. Preponderance of data from transgenic and knockout models has indicated the role of EGFR in the development and normal functioning of tissues, most importantly in the brain and mammary gland (Alroy & Yarden 1997, Chryso-gelos & Dickson 1994, Gospodarowicz 1981, Herbst 2004).

EGF and its cognate receptors play an important role in the normal development of the mammary gland. However, an imbalance in the regular cellular process of growth, repair and programmed cell death of the mammary gland leads to tumor formation. Aberrant functioning of EGFR is implicated in numerous human diseases including Alzheimer’s, cardiac dysfunction, psoriasis and skin lesions as well as psychological disorders including schizophrenia (Chaudhury et al. 2003, Hahn et al. 2006, King et al. 1990, Suzuki et al. 2002). However, the most studied role of EGFR is in tumorigenesis. EGFR and ErbB2 are the most studied prototype of ErbBs associated with the progression of breast cancer (Olayioye et al. 2000). A total of 40-50% of breast carcinomas express ErbBs (Abd El-Rehim et al. 2004, Normanno et al. 2006). Breast tumors expressing EGFR and ErbB2 are associated with poor clinical outcome (DiGiovanna et al. 2005, Toi et al. 1994). ErbB2 is likely to have a higher oncogenic transforming ability in comparison to EGFR. Overexpression, gene amplification and receptor mutations have been demonstrated in different tumor types. In addition, co-expression of ErbB subtypes enhances the transforming ability of breast cancer cells. An elegant study by DiGiovanna et al. (2005) reported that 15% of the 807 invasive breast tumors expressed EGFR and that the majority of these tumors (87%) co-expressed ErbB2 establishing a striking correlation between the expression of these two factors in breast cancer patients. Consistent with these observations, studies have also revealed that tumors with co-expression of EGFR/ErbB2/ErbB3 or ErbB2/ErbB3 have a more aggressive phenotype than tumors co-expressing ErbB3/ErbB4 (Abd El-Rehim et al. 2004).

The overexpression of EGFR and ErbB2 is often accompanied by elevated production of ligands such as EGF and transforming growth factor-β (TGF-β) as well as hyperactivated downstream signaling cascades (Normanno et al. 2006, Pilichowska et al. 1987). Immunohistochemical analysis of breast carcinomas revealed that more than 65% of cases were positive for EGF and TGF-a. In aggressive breast cancer, EGFR not only enhances mitogen activated protein kinase (MAPK) phosphorylation but is also associated with sustained and prolonged basal ERK1/2 expression (Thottassery et al. 2004). Kallergi et al. (2008) demonstrated that circulating tumor cells in blood samples from breast cancer patients expressed phosphorylated EGFR and ErbB2 in the early stages of the disease as well as in metastatic tumors. Additionally, these cells also displayed high levels of phosphatidylinositol-3-kinase (PI3K)/AKT phosphorylation. Any mutations in PI3K and AKT are associated with loss of PTEN and over-expression of ErbB2 (Kallergi et al. 2007a, b). Recently, nuclear translocation of EGFR was shown to exert a potential role in breast tumor cells associated with enhanced cell proliferation and with the induction of cyclin D1, a positive regulator of cell proliferation (Lo et al. 2005, Wang et al. 2010).

**Molecular Signaling of EGFR**

EGF binding to its cognate receptor induces dimerization, phosphorylation and internalization of the EGFR that triggers a network of intricate signaling. Among various signaling cascades, four major pathways that are regulated by EGFR include Janus kinase (JAK), signal transducers and activators of transcription (STAT), phospholipase C (PLC) and protein kinase C (PKC) pathways (Alroy & Yarden 1997, Citri &
Yarden 2006, Darnell et al. 1994, Jorissen et al. 2003, Katz et al. 2007). Of the multitude of signaling pathways, all ErbBs activate the Ras-MAPK upon ligand binding (Figure 1). EGFR targets several members of the MAPK family including extracellular regulated receptor kinases (ERK) ERK1/2, ERK5, janus kinases (JNK) and p38. Specifically, ERK1/2 is the most studied and well characterized pathway activated by growth factor receptors and associated with cell proliferation (Katz et al. 2007). MAPKs are serine/threonine kinases that orchestrate key cellular functions including cell growth, differentiation and proliferation. MAPK pathways are activated either by direct recruitment of the Src homology 2 (SH2) domain linked growth factor receptor-bound protein 2 (Grb2) or indirectly by the phosphotyrosine-binding (PTB) domain. Grb2 then recruits son of sevenless (SOS), a nucleotide exchange factor further activating Ras, upon exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). Activated Ras, in turn, phosphorylates Raf and results in activation of downstream kinases including MAP kinase kinases (MEK1/2). MEK1/2 subsequently phosphorylates ERK1/2 leading to the nuclear translocation of activated ERK where it initiates transcription of various genes including the specificity protein 1 (SP1), E2F, E twenty-six (ETS)-like transcription factor 1 (ElK-1) and activator protein 1 (AP-1). Gene transcription ultimately promotes cell growth including proliferation, differentiation, migration, invasion and anti-apoptosis. Recent studies have described a new isoform of ERK, ERK5 that is linked to tumorigenesis and associated with cell proliferation. The in vivo animal studies support a critical role of ERK5 in tumor growth due to the vasculogenesis and blood vessel homeostasis. Most importantly, tumor cells displaying high expression of ErbB2 also exhibit elevated basal expression of ERK5 (Montero et al. 2009).

**Figure 1.** Overview of the EGFR signaling pathway. Binding of EGF to EGFR leads to homo- and/or heterodimerization of EGFR, phosphorylation and activation of MAPK (ERK/p38) and cell survival (PI3K/AKT) pathways. These pathways consequently induce cell proliferation, invasion, and migration.
EGFR Directed Therapy in Breast Cancer

EGFR and ErbB2 over-expression, phosphorylation and heterodimerization are integral in tumor progression and therefore serve as important prognostic factors for the development of therapeutic targets (Normanno et al. 2003). The main approach to control tumor growth is targeting ErbBs and its signal transduction leading to inhibition of gene transcription. Two strategies are commonly used for the treatment of ErbBs positive breast cancer; monoclonal antibodies that block the membrane receptor upon binding to the EC domain and small molecule tyrosine kinase inhibitors (TKIs) that block the tyrosine kinase activity and modulate downstream signaling pathways (Ciardiello & Tortora 2001).

The monoclonal antibody trastuzumab is the first line therapy for metastatic breast cancer and has been used clinically extensively (Goldenberg 1999). Trastuzumab binds to the EC domain of the ErbB2 receptor and inhibits the receptor phosphorylation, thereby abrogating the tumor proliferation with better outcome in breast cancer patients (Bozionellou et al. 2004). Randomized control trials have shown additive effects of trastuzumab with chemotherapy to reduce the recurrence of disease by 50% and mortality by >30%. An adjuvant therapy with paclitaxel (Taxol) in 60-80% of breast cancer patients showed a promising outcome. Trastuzumab, in an adjunct therapy with other anti-tumor agents such as aromatase inhibitor (anastrozole) have proven beneficial in ER/ErbB positive breast tumors (Kaufman et al. 2009). Unlike trastuzumab, which binds to EC domain of ErbB2, Pertuzumab, a newly discovered monoclonal antibody, prevents ErbB2 homo- and heterodimerization with other ErbBs, which is an important phenomenon seen in aggressive breast cancer tumors with shorter survival rates (Kristjansdottir & Dizon 2010). Cetuximab, a chimeric human-mouse monoclonal antibody also binds to EGFR (Harding & Burtness 2005). Furthermore, the complex of cetuximab-EGFR internalizes to cause defective downstream signaling and inhibition of cell proliferation leading to decreased invasiveness and metastasis (Harding & Burtness 2005).

In addition, several TKIs including gefitinib, erlotinib and lapatinib are approved for clinical use (Alvarez et al. 2010). Gefitinib and erlotinib are specific EGFR inhibitors that bind to EGFR extracellularly and terminate the downstream signaling, predominantly interfering with the ERK1/2 and PI3K/AKT signaling pathways (Campos 2008). Gefitinib is a potent inhibitor of cell proliferation in tumors over-expressing EGFR. In phase I trials, gefitinib was well-tolerated with limited toxicities, mainly dermal and...
gastrointestinal (Herbst et al. 2002; Nakagawa et al. 2003). In patients with tamoxifen resistant breast tumors, gefitinib showed anti-proliferative activity (Baselga et al. 2005). Lapatinib, a reversible TKI, is clinically used in breast tumors expressing both EGFR and ErbB2. Interestingly, lapatinib binds to the mutated or truncated forms of ErbB2 and exhibits an antitumor effect (Bouchalova et al. 2010). A newly discovered TKI, neratinib is an irreversible inhibitor of tumor effect (Bouchalova et al. 2010). The diverse biological effects of SST are mediated through the interaction with the five specific receptors SSTR1-5. SSTRs were initially identified in rodent pituitary cells as high affinity cell surface receptors (Schonbrunn & Tashjian 1978). The existence of more than one SSTR subtype was later proposed due to differential binding to SST-14 and SST-28 (Mandarino et al. 1981, Srikant & Patel 1981). Based on their molecular cloning and binding properties, SSTRs were classified into two subfamilies; somatotropin release-inhibiting factor (SRIF) -1 and SRIF-2 (Patel 1998). The SRIF-1 class was comprised of receptor subtypes sensitive to a specific ligand named OCT whereas receptors insensitive to this ligand constituted the SRIF-2 class (Reisine & Bell 1995, Tran et al. 1985). SSTRs belong to the heptahelical transmembrane GPCRs family and are high affinity cell surface receptors (Schonbrunn & Tashjian 1978). The sequence of human SSTRs was elucidated using molecular cloning techniques long after the identification of high-affinity plasma membrane SSTR binding sites (Yamada et al. 1992, 1993). SSTR subtypes have been cloned and are pharmacologically characterized in various species including humans (Bruno et al. 1992, Kluxen et al. 1992, O’Carroll et al. 1992). SSTR1 and SSTR2 were first cloned from human islets followed by cloning of SSTR3, SSTR4 and SSTR5 in human as well as rat tissues (Yamada et al. 1992, 1993). Except SSTR2, the genes encoding SSTRs are intronless (Patel 1999). SSTR2 gene expresses 2 splice variants; SSTR2A and SSTR2B, which differ in the number of amino acids in the C-terminus. The size of SSTRs ranges from 356-391 amino acid residues in length and exhibits 39-57% structural homology (Patel 1998, Reisine & Bell 1995). The transmembrane domains of SSTRs display greater sequence homology than the extracellular N-terminal and intracellular C-terminal domains (Patel 1998). The pharmacological and physiological properties of SSTR in target tissues are subtype-specific. All SSTRs bind to SST-14 and SST-28 with nanomolar affinities. The pharmacological profiles of receptors to ligand binding revealed that SSTR1-4 bind to SST-14 while SSTR5 binds to SST-28 with greater affinity (Patel 1998, 1999).

**Somatostatin and Somatostatin Receptors**

The role of SST in the negative regulation of normal and tumor cell growth as well as the modulation of growth factors and hormone mediated cell proliferation has emerged as a potential therapeutic approach for tumor treatment (Pyronnet et al. 2008, Susini & Buscail 2006). The diverse biological effects of SST are mediated through the interaction with the five specific receptors SSTR1-5. SSTRs were initially identified in rodent pituitary cells as high affinity cell surface receptors (Schonbrunn & Tashjian 1978). The existence of more than one SSTR subtype was later proposed due to differential binding to SST-14 and SST-28 (Mandarino et al. 1981, Srikant & Patel 1981). Based on their molecular cloning and binding properties, SSTRs were classified into two subfamilies; somatotropin release-inhibiting factor (SRIF) -1 and SRIF-2 (Patel 1998). The SRIF-1 class was comprised of receptor subtypes sensitive to a specific ligand named OCT whereas receptors insensitive to this ligand constituted the SRIF-2 class (Reisine & Bell 1995, Tran et al. 1985). SSTRs belong to the heptahelical transmembrane GPCRs family and are high affinity cell surface receptors (Schonbrunn & Tashjian 1978). The sequence of human SSTRs was elucidated using molecular cloning techniques long after the identification of high-affinity plasma membrane SSTR binding sites (Yamada et al. 1992, 1993). SSTR subtypes have been cloned and are pharmacologically characterized in various species including humans (Bruno et al. 1992, Kluxen et al. 1992, O’Carroll et al. 1992). SSTR1 and SSTR2 were first cloned from human islets followed by cloning of SSTR3, SSTR4 and SSTR5 in human as well as rat tissues (Yamada et al. 1992, 1993). Except SSTR2, the genes encoding SSTRs are intronless (Patel 1999). SSTR2 gene expresses 2 splice variants; SSTR2A and SSTR2B, which differ in the number of amino acids in the C-terminus. The size of SSTRs ranges from 356-391 amino acid residues in length and exhibits 39-57% structural homology (Patel 1998, Reisine & Bell 1995). The transmembrane domains of SSTRs display greater sequence homology than the extracellular N-terminal and intracellular C-terminal domains (Patel 1998). The pharmacological and physiological properties of SSTR in target tissues are subtype-specific. All SSTRs bind to SST-14 and SST-28 with nanomolar affinities. The pharmacological profiles of receptors to ligand binding revealed that SSTR1-4 bind to SST-14 while SSTR5 binds to SST-28 with greater affinity (Patel 1998, 1999).

**Homo and/or Heterodimerization of SSTRs**

The concept that GPCR exist and function in monomeric entities has recently been challenged. The presence of multiple SSTR subtypes in the same cells in different tissues suggests the potential for dimerization between different SSTRs. Homo and/or heterodimerization of GPCRs within the same family has been well documented (Baragli et al. 2007, Grant et al. 2004, Heldin 1995, Jaquet et al. 2005, Jordan et al. 2001, Rocheville et al. 2000a). Such protein-protein interactions are potential targets for new therapeutic agents. Rocheville et al. (2000b) were the first to report evidence of physical interactions between SSTRs in transfected cells. This study described that SSTR5 exists as a monomer in basal conditions and formed stable dimers upon SST treatment in a concentration depend-ent manner. Patel et al. (2002) demonstrated an agonist dependent heterodimerization between SSTR1 and SSTR5, whereas SSTR5 formed homo and heterodimers. Unlike SSTR5, SSTR1 remained as a monomer, irrespective of the agonist stimulation. Furthermore, the heterodimerization between SSTR1 and SSTR5 was subtype specific and was promoted by SSTR5 activation alone (Patel et al. 2002). The swapping of SSTR5 C-tail with the C-tail of SSTR1 abrogated the agonist mediated homodimerization and internalization of SSTR5. Conversely, replacing the SSTR1 with the SSTR5 C-tail, surprisingly, resulted in the chimeric receptor mimicking heterodimerization and internalization of SSTR5 upon agonist stimulation. Grant et al. (2004) described that SSTR2 exists as pre-formed dimers, which dissociate upon agonist treatment prior to internalization. The same authors in a separate study also reported that SSTR2 activation selectively promotes heterodimerization between SSTR2/5 whereas activation of SSTR5 alone or with SSTR2 failed to produce such heterodimerization. Furthermore, heterodimerization between SSTR2/5 modulates the signaling properties and was shown to have an enhanced anti-proliferative effect. War et al. (2011) demonstrated that SSTR3 exists as a pre-formed homodimer in the basal state whereas agonist treat-
ment decreases dimer formation. Additionally, C-tail deficient SSTR3 displayed homodimerization similar to wt-SSTR3 (War et al. 2011). Similarly, SSTR4 exists as a dimer in monotransfected cells, however, upon deletion of the C-tail, the receptor lost the ability to dimerize and displayed impaired internalization (Somvanshi et al. 2009). Moreover, SSTR4 exhibited receptor specific heterodimerization with SSTR5 but not with SSTR1 (Somvanshi et al. 2009). These studies established the critical role of the C-tail in receptor dimerization and internalization and suggested that activation of one protomer is sufficient to promote receptor dimerization. Furthermore, SSTR2/3 heterodimers displayed high binding affinity to SST-14 and SSTR2 specific agonist and resistance to agonist-induced desensitization. Interestingly, SSTR2/3 heterodimers were identified as new receptors, albeit with similar pharmacological properties as SSTR2 but with the loss of SSTR3-like properties (Pfeiffer et al. 2001).

Heterodimerization of SSTRs within the same family and with other related GPCRs is a well-established notion. SSTR2 functionally interact with µ-opioid receptor in HEK-293 cells (Pfeiffer et al. 2002). Furthermore, heterodimerization between SSTR5 and dopamine receptor subtype 2 (D2R) and SSTR2/D2R opened an opportunity for the development of chimeric molecules targeting SSTR5/D2R that have been successfully applied in the treatment of pituitary tumors (acromegaly) (Baragli et al. 2007, Jaquet et al. 2005, Saveanu et al. 2002). Recent studies showed that synergistic activation strengthened the pre-existing SSTR5 and β-adrenergic heterodimers whereas activation of individual receptor subtypes leads to the dissociation of the heteromeric complex (Somvanshi et al. 2011). The heterodimerization of SSTRs has been shown to enhance the signaling properties and such functional consequences may have potential therapeutic implications in different pathological states.

Molecular Signaling of SSTRs

Ligand binding to SSTRs initiates complex signal transduction pathways (Figure 2). Agonist mediated activation of SSTRs leads to conformational changes in the receptor prior to coupling with the G-proteins comprised of a trimeric complex of three tightly bound subunits (α, β and γ). Upon activation, G-proteins convert GDP to GTP by nucleotide exchange and consequently relay downstream signals via dissociation of the α subunit from the βγ complex (Pierce et al. 2002). Adenylyl cyclase (AC) was among the first identified enzyme effectors regulated by GPCRs, including SSTRs (Patel et al. 1994). All SSTR subtypes bind to pertussis toxin (PTX) sensitive G-proteins that are Gi/o type and negatively regulate AC to inhibit cAMP formation, which further downregulates the protein kinase A (PKA) pathway (Meyerhof 1998). The inhibitory effect of SSTRs on the cAMP/PKA pathway has been demonstrated in human pituitary adenomas, rat cortex and hippocampus, pancreatic islets as well as ovine retina, in a receptor specific manner (Meyerhof 1998, Patel 1999). SSTRs alter cGMP in a receptor and tissue dependent manner, by modulating the activity of guanylyl cyclase, which also regulates nitric oxide mediated oxidative stress (Lahlou et al. 2004). Earlier studies on rat pancreatic islets, human pituitary adenomas and various other cell types have also demonstrated that SSTRs modulate ion channels (Ca²⁺ and K⁺) as well as phospholipase A (PLA) and phospholipase C (PLC) pathways (Cervia & Bagnoli 2007, Csaba & Dournaud 2001, Lahlou et al. 2004, Reisine & Bell 1995). Additionally, SSTRs, via Gaα2, regulate high-voltage gated Ca²⁺ channels and also inhibit intracellular Ca²⁺ entry in human pituitary adenomas, cardiac fibroblasts and cortical astrocytes as well as in rat sympathetic neurons, hippocampus and pancreatic cells (Ikeda & Schofield 1989; Kleuss et al. 1991; Zhu & Yakel 1997). Concerning the specificity of the receptor subtype, the involvement of SSTR2 has been studied in modulation of cAMP and Ca²⁺ whereas limited information is available on the roles of other subtypes in this regard. Furthermore, SST has also been suggested to activate conductance of different K⁺ channels via SSTR4, leading to hyperpolarization of the cell membrane in human and rat brain regions as well as pituitary and pancreatic cells (de Weille et al. 1989). The effects of SST on the Na⁺/H⁺ pump have been studied in rat hepatocytes as well as breast cancer cells of different origins and are mainly mediated via SSTR2 and SSTR5.

SSTRs and Breast Cancer

SST and SSTRs are highly expressed by breast cancer cells and autopsied breast tissue. SSTI immunoreactivity has been demonstrated in approximately 30% of breast tumor tissues as well as in most breast cancer cell lines (Albérini et al. 2000, Kumar et al. 2005, Reubi 1990, Weckbecker et al. 1994). As discussed above, there are direct and indirect mechanisms for the SST effects on breast tumor cells. The direct effect of SST or its analogs is exerted by binding to SSTRs, resulting in inhibition of cell proliferation and/or induction of apoptosis. Studies have demonstrated that 15-66% of primary breast tumors are positive for SSTRs by binding analysis whereas 75% were positive when imaged in vivo using [111In-DTPA-DPhe1]-octreotide scintigraphy (Prevost et al. 1994, Weckbecker et al. 1994). Pfeiffer et al. (2002) demonstrated
that SSTR2 and SSTR5 were the predominant subtypes expressed in these tumors. Several previous studies have also reported that SSTR2 is the most abundant SSTR subtype expressed in breast tumors (Evans et al. 1997, Kumar et al. 2005, Reubi et al. 1990, Watt & Kumar 2006). In addition, SSTR2 expression has been found to be ubiquitous (Evans et al 1997). Vikic-Topic et al. (1995) described that the SSTR2 transcript is predominantly expressed in all breast tissue samples, followed by SSTR1, SSTR3 and SSTR4. Moreover, SSTR1 was detected along with SSTR2 transcripts in 96% of breast tissues examined. Furthermore, the expression of mRNA and protein levels of all SSTR subtypes was shown in a cumulative study of 98 ductal not otherwise specified (NOS) breast tumor cases (Kumar et al. 2005). Additionally, it was suggested that the SSTRs are variably distributed at the tumor site and adjacent tumor regions (Kumar et al. 2005). In contrast to observations by Vikic-Topic et al., the findings by Kumar et al. (2005) established the correlation of SSTRs with the tumor grade and the levels of ER and PR. SSTR1 and 4 were correlated with ER whereas SSTR2 was correlated with PR in addition to ER.

In the past few years, various SST analogs have been developed and used as anti-proliferative agents in the treatment of breast cancer. Unlike SST that has a short plasma half-life of 3 minutes, newly synthesized SST analogs have better efficacy, therapeutic index and are free from major side effects (Lamberts et al. 1991, Schally 1988). Setyono-Han et al. (1987) showed the inhibitory effects of Sandostatin (an analog of SST) on proliferation of MCF-7 cells in a concentration and time dependent manner. Interestingly, Sandostatin had an antagonizing effect on estradiol and growth hormones in MCF-7 cells suggesting that SST and SST analogs directly act as potential anti-proliferative agents on human breast cancer cells.

**Figure 2.** Schematic illustration of SSTR signaling. Activation of SSTRs by SST or receptor-specific agonists inhibits $\text{Ca}^{2+}$ influx and hormonal secretions. SSTRs couple to Gi proteins and commonly inhibit cAMP. SSTRs modulate the MAPK and PI3K pathways in a receptor specific manner and result in inhibition of cell proliferation, survival and migration.
Vapreotide, another SST analog, was evaluated and it was found that prolonged administration was well tolerated in cases of pre-treated metastatic patients, resulting in diminished levels of IGF-1 during the entire length of the treatment (O'Byrne et al. 1999). Similarly, Canobbio et al. (1995) indicated that the SST analog Lanreotide significantly suppressed the levels of IGF-1 in postmenopausal breast cancer patients previously untreated for the tumor.

Amongst all SST analogs, octeriotide (OCT) has been studied extensively for the treatment of different types of tumors. As an anti-hormonal drug, OCT has been used in combination with tamoxifen for the treatment of breast cancer as well as in DMBA-induced rat mammary carcinoma. OCT also effectively increased the anti-neoplastic effect of ovariectomy in these rat models (Weckbecker et al. 1994). Sharma et al. (1996) demonstrated that SST had a cytotoxic effect on MCF-7 cells in a receptor-specific manner. In this regard, it should be noted that SSTR3 is the only receptor subtype that uniquely participates in the induction of apoptosis. Furthermore, OCT induced apoptosis through activation of tumor suppressor proteins, namely wild-type 53 and Bcl-2–associated X protein (Bax) in MCF-7 cells, suggesting a potential antitumor role of SST analogs (Sharma & Srikant 1998). Paclitaxel, known for its excellent anti-tumor activity lacks cell specificity. Huang et al. (2000) synthesized an OCT conjugated with paclitaxel that internalized into the cytoplasm of SSTR positive tumor cells and induced apoptosis in MCF-7 cells by promoting tubule formation, while retaining paclitaxel's biological properties.

Cross-talk between ErbBs and SSTRs
EGFR has been associated with cell proliferation, survival and transformation (Normanno et al. 2006). In pathological conditions such as breast cancer, ErbBs are highly expressed in higher grade and aggressive tumors. SSTRs are known to be negative regulators of cell proliferation and have been acknowledged for the treatment of various tumors (Bousquet et al. 2004, Cameron Smith et al. 2003, Patel 1990). Unlike ErbBs, SSTRs are well expressed in lower grade and less aggressive breast tumors. These observations suggest an inverse relation between SSTR and ErbB subtypes in breast cancer. Finding that activation of GPCRs leads to the phosphorylation of EGFR resulting in enhanced and diversified signaling established the first paradigm of inter-receptor crosstalk. Daub et al. (1996) were the first to describe the concept of EGFR transactivation by GPCRs in rat fibroblasts. There is compelling evidence that could substantiate the possible crosstalk between SSTRs and ErbBs. All SSTR and ErbB subtypes are extensively expressed in breast tissues and cell lines (Kumar et al. 2005, Rivera et al. 2005, Watt & Kumar 2006). SSTRs and ErbBs are co-expressed in breast cancer cells and display colocalization in a receptor, cell line and ER-dependent manner. SSTR subtypes are highly expressed in ER cells, whereas these cells expressed relatively low levels of ErbBs in comparison to ER cells (Watt and Kumar 2006). SST also inhibits the effects of EGF in pancreatic tumors, indicating that activation of SSTR subtypes may impede ErbBs heterodimerization and diminish its tumor promoting effects (Liebow et al. 1986). In addition, SSTRs and ErbBs regulate the MAPK and PI3K/AKT pathway in a receptor specific manner; albeit, with opposite outcomes on cell proliferation.

SSTRs Modulate EGFR Functions
SSTR1 and SSTR5 modulate EGFR heterodimerization and tumor promoting downstream signaling in breast cancer as well as HEK-293 cells (Watt et al. 2009, Kharmate et al. 2011a, b) (summarized in Figure 3). In breast cancer cells, agonist treatment resulted in the dissociation of SSTR5/EGFR and the association of SSTR1/EGFR. The agonist dependent association/dissociation between SSTRs/EGFR consequently led to the modulation of ERK1/2 phosphorylation. Watt et al. (2009) demonstrated that there is a synergistic activation of SSTR and EGFR upon treatment with SST and EGF which delayed the phosphorylation of ERK1/2 in MCF-7 cells, suggesting a mechanism whereby SST can block EGF-induced proliferation. These results further strengthen the concept that SSTRs and ErbBs functionally interact in cancer.

The concept that SSTR and ErbB receptors associate as heterodimers or possibly display ligand-dependent dissociation of preformed heteromeric complexes with significant changes in signaling molecules has enormous implications for receptor biology in cancer and in drug development. Kharmate et al. (2011a, b) demonstrated that the presence of SSTR1 or 5 altered EGFR membrane expression, phosphorylation and heterodimerization of EGFR/ErbB2. EGFR heterodimerization with ErbB2 and receptor phosphorylation are critical steps in stimulating and sustaining the downstream cell proliferating signals linked to tumor growth. The activation of SSTR 1 or 5 in transfected HEK-293 cells significantly diminished the membrane expression of EGFR, which was consistent with the observations in breast cancer cells. SSTR5 alone and in combination with SSTR1 partially blocked EGFR phosphorylation (Kharmate et al. 2011a, b). In comparison, SSTR1 monotransfected cells completely abolished EGFR phosphorylation. Furthermore, in wt-
HEK-293 cells, while EGF enhanced the ERK1/2 phosphorylation in a time dependent manner, SST alone or in combination with EGF showed comparable ERK1/2 phosphorylation. Interestingly, in SSTR1 or SSTR5 expressing cells, EGF induced ERK1/2 phosphorylation was significantly less, whereas upon concomitant treatment of SST and EGF, ERK1/2 phosphorylation was prolonged. Furthermore, activation of SSTR1 or 5 in mono- and/or cotransfected cells modulate EGF mediated ERK5 phosphorylation. Of note, SST displayed a much greater inhibitory effect on EGF mediated ERK1/2 and ERK5 phosphorylation in SSTR1/5 cotransfected cells. Similarly, SSTRs inhibit EGF mediated p38 phosphorylation in a receptor specific manner with pronounced inhibition in the presence of SSTR1 alone. Furthermore, these results were corroborated with the changes in the expression levels of p27$^{kip1}$, an index of cell proliferation and PTP membrane translocation. These results suggest that SSTR1 and 5 specifically induced cytostatic rather than cytotoxic effects (Kharmate et al. 2011a, b).

PI3K/AKT cell survival pathways play an important role in tumor progression. Aggressive tumor growth is frequently associated with the loss of PTEN, a hyperactivated PI3K pathway and the failure of Trastuzumab therapy (Kallergi et al. 2008). Furthermore, the activation of SSTR1 or 5 lead to the inhibition of PI3K and AKT phosphorylation. Moreover, this inhibition was shown to be more pronounced in cells expressing SSTR1/5 indicating that SSTRs activation might play a role in response to Trastuzumab treatment in cancer. It is highly possible that the gradual loss of SSTR subtypes as the tumor progresses might, in part, be responsible for the loss of Trastuzumab responsiveness, being associated with enhanced PI3K and loss of PTEN. Kharmate et al. (2011a, b) demonstrated that cells expressing SSTR1, SSTR5 and SSTR1/5 promote the dissociation of the EGFR/}

Figure 3. SSTRs modulate EGF mediated signaling pathways. Activation of SSTRs inhibits the EGF-mediated EGFR homodimerization, receptor phosphorylation as well as the MAPK and PI3K/AKT pathways, resulting in inhibition of cell proliferation.
ErbB2 heteromeric complex. SSTR1 and SSTR5 transfection of cells exhibited SSTR1/EGFR or SSTR5/EGFR heteromeric complex formation, resulting in the inhibition of EGFR phosphorylation. More importantly, SSTR1/5 cotransfected cells displayed SSTR5/EGFR heterodimerization whereas there was no SSTR1/EGFR complex formation. These observations show that the interference of SSTRs in the ErbB homo- and/or heterodimerization, the consequent inhibition of EGFR phosphorylation and the regulation of EGF-mediated downstream signaling might serve as novel therapeutic targets in EGFR positive tumors. Most importantly, inhibition of EGFR using AG1478 and knocking down EGFR in the presence of siRNA enhanced SSTR1 and SSTR5-mediated inhibition of cell proliferation via blocking of the tumor-promoting signaling cascades (Kallergi et al. 2008, Kharmate et al. 2011a, b)

Conclusions and Future Directions

Since ErbBs represent a prominent class of cell surface proteins in tumors and are linked to the regulation of cell proliferation, interference in ErbB membrane functions and inhibition of tumor regulatory pathways may serve as an instrumental tool in drug design for the treatment of breast tumors. It is worth investigating whether SSTRs might be exploited therapeutically in combination with the inhibition of ErbBs for cancer treatment. This review underscores the unappreciated role of SSTRs that contribute to the inhibition of EGFR induced changes that may significantly advance our understanding of tumor progression, patient prognosis and future drug development in EGFR positive breast tumors.

Given the wide spread distribution of EGFR in breast cancer and its effects, particularly in tumor cell proliferation, it is not surprising that its modulation has been the subject of great interest in tumor cell biology, including breast cancer. Unfortunately, the regulation of EGFR alone to date has been insufficient in controlling tumor growth. This review addresses a new dimension regarding the role of SSTR subtypes, which are also present in tumor cells and are potential targets to prevent tumor progression. Therapeutic application of SSTR activation along with inhibition of EGFR may provide a new clinical approach in the treatment of breast cancer and lay the foundation for rational drug design, in order to maintain normal function of EGFR in tumor cells and spare cells from aggressive proliferation. Nonetheless, SSTR subtypes appear to gain a prominent and unique role for therapeutic implication in tumor cell biology.

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Author’s Contributions

GK and UK designed and drafted the article. Both authors read and approved the final manuscript.

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