Sphingosine-1-Phosphate and Estrogen Signaling in Breast Cancer

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ABSTRACT

Breast cancer remains the most common malignant disease in women. The estrogen receptor- α (ER α) and its ligand 17 β -estradiol (E₂) play important roles in breast cancer. E₂ elicits cellular effects by binding to ER α in the cytosol followed by receptor dimerization and translocation to the nucleus where it regulates gene expression by binding to ERE response elements. However, it has become apparent that E₂ also exerts rapid non-genomic effects through membrane-associated receptors. There is emerging evidence that this induces formation of the bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P). S1P in turn has been implicated in many processes important in breast cancer progression. One of the enzymes that produce S1P, sphingosine kinase 1 (SphK1), is upregulated in breast cancer and its expression has been correlated with poor prognosis. This review is focused on the role of the SphK/S1P axis in estrogen signaling and breast cancer progression and will discuss new therapeutic approaches targeting this axis for breast cancer treatment.

Keywords: sphingosine-1-phosphate, sphingosine kinase, estradiol, breast cancer, FTY720/Fingolimod.

Abbreviations: ER α , estrogen receptor α ; EGFR, epidermal growth factor receptor; ERE, estrogen response element; ERK, extracellular signal regulated kinase; E₂, 17 β -estradiol; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; MAPK, mitogen activated protein kinase; PHB2, prohibitin 2; SphK, sphingosine kinase; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; TNBC, triple negative breast cancer; TRAF2, TNF receptor-associated factor 2.

INTRODUCTION

The estrogen receptor- α (ER α) and its ligand 17 β -estradiol (E₂) play important roles in breast cancer. Most of the canonical genomic effects of binding of E₂ to ER α are mediated by nuclear transcriptional regulation. However, E₂ also exerts rapid non-genomic signaling through membrane-associated receptors many of them resulting from increased formation of the bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P). S1P and sphingosine kinases (SphKs) that produce it have been implicated in many processes important in breast cancer progression. In this review, we discuss the role of the SphK/S1P axis in estrogen signaling and breast cancer progression and also some new therapeutic approaches to potentially target this axis for breast cancer treatment.

Formation and metabolism of S1P

It has long been known that sphingolipid metabolism generates metabolites with important functions. The best characterized are ceramide, the backbone of all sphingolipids, its breakdown product sphingosine, and S1P. S1P metabolism has been discussed in many reviews (Hannun et al., 2008; Maceyka et al., 2014; Shamseddine et al., 2015) and is only briefly outlined here. Two sphingosine kinases, known as SphK1 and SphK2, catalyze the phosphorylation of sphingosine to S1P, which is irreversibly cleaved by S1P lyase to phosphoethanolamine and a fatty aldehyde or dephosphorylated back to sphingosine by several phosphatases which then can be reutilized for ceramide and sphingolipid formation. Tissue levels of S1P are thus determined by the balance between activity of SphKs and S1P lyase and phosphatases.

S1P signaling

S1P and its receptors

S1P has important roles in regulation of a wide variety of complex biological processes important for breast cancer progression (Carroll et al., 2015; Maceyka and Spiegel, 2014). Most of these actions are mediated by binding to a family of five specific cell surface receptors (S1PR1-5) (Maceyka and Spiegel, 2014). Numerous stimuli, including hormones such as estradiol (E_2), rapidly activate SphK1 and/or SphK2 to transiently increase intracellular S1P levels in specific pools. S1P produced mainly by activated SphK1 can then be secreted by Spns2, a member of the major facilitator superfamily of non-ATP-dependent transporters or by ABC transporters ABCA1, ABCC1, and ABCG2 (Nishi et al., 2014; Takabe et al., 2014). S1P in turn activates its receptors in an autocrine or paracrine manner known as ‘inside-out’ signaling of S1P (Hobson et al., 2001; Takabe et al., 2008). Physiological responses regulated by S1P depend on the spectrum of ubiquitously but differentially expressed S1PRs and the variety of G proteins they are coupled to. Thus, many signaling pathways downstream of S1PRs that have been linked to cancer progression have been shown to be activated depending on the cell type, including MAPKs, phospholipase C, adenylate cyclase, and Rac/PI3K/Akt, to name a few (Pyne et al., 2014; Takabe et al., 2008). Moreover, various types of cancer cells differentially express different sets of S1PRs, thus providing S1P with the ability to regulate numerous cellular processes important for breast cancer, including growth, survival, migration, invasion, inflammation, angiogenesis, and lymphangiogenesis (Nagahashi et al., 2014).

Intracellular actions of S1P

While it has long been suspected that S1P also has intracellular actions that are independent of S1PRs, only recently have several intracellular targets been identified that are likely to be important in the context of cancer. We found that S1P, but not dihydro-S1P, produced by SphK1 activated by TNF directly binds to and activates the E3 ubiquitin ligase activity of TNF receptor-associated factor 2 (TRAF2), an important component in NF- κ B signaling (Alvarez et al., 2010). NF- κ B regulates transcription of pro-survival or anti-apoptosis genes, thus identifying one of the mechanisms for the pro-survival actions of S1P in cancer progression. Interestingly, in contrast to SphK1, which is localized to the cytosol, SphK2 is mainly in the nucleus of most types of cells. We showed that nuclear S1P produced by ERK/MAPK-dependent activation of SphK2 is an endogenous inhibitor of histone deacetylases (HDACs) (Hait et al., 2009). Since SphK2 is present in repressor complexes together with HDACs in the nucleus of breast cancer cells (Hait et al., 2009), the S1P it produces inhibits HDAC activity resulting in enhanced transcription of specific target genes. This was the first indication that nuclear sphingolipid metabolism is involved in epigenetic regulation. Another link between nuclear S1P and gene expression was recently reported by Ogretmen and colleagues who discovered that S1P binds to hTERT and stabilizes telomerase at the nuclear periphery by allosterically mimicking hTERT phosphorylation. In murine xenografts, inhibitors of SphK2 decreased tumor growth and overexpression of wild-type hTERT in cancer cells, but not a hTERT mutant that was unable to bind S1P, restored tumor growth (Panneer Selvam et al., 2015). Their results suggest that S1P promotes telomerase stability and telomere maintenance important for cancer cell proliferation and tumor growth. In the mitochondria, SphK2 produces S1P that binds to prohibitin 2

(PHB2), a protein that regulates mitochondria assembly and function. Deleting SphK2 or PHB2 induced a mitochondrial respiration defect through cytochrome c oxidase (Strub et al., 2011) and may be important for the well-known Warburg metabolism of cancer cells. In this regard, it was suggested that SphK1, but not SphK2, functions to maintain the Warburg effect and cell survival (Watson et al., 2013).

Role of estrogen in breast cancer

Breast cancer is the most common cancer among women worldwide and occurs in about 1 in 8 women in the US (<http://www.cancer.org>, last accessed June 10, 2015). The estrogen receptor- α (ER α) plays an important role in breast cancer pathogenesis and progression (McDonnell et al., 2002). Patients with tumors that express ER α are termed “ER α -positive” and those lacking ER α are termed “ER α -negative”. The majority of human breast cancers start out as estrogen-dependent because they are derived from cells that express ER α (Saha Roy et al., 2012). The steroid hormone, E₂, interacts directly with estrogen-specific cytoplasmic/nuclear receptors, ER α 66 and ER α 46 (Marino et al., 2006). ER α 66 is the main ER α responsible for ER α -positive breast cancer responses to E₂. ER α 46 is a 46 kDa splice variant that also functions in association with ER α 66. The canonical pathway by which E₂ elicits cellular effects is initiated by binding to ER α in the cytosol followed by homo- or hetero-dimerization and translocation to the nucleus. In the nucleus, ER α dimers function as transcription factors by binding to specific response elements (ERE) on DNA, and either activating or repressing transcription (Mangelsdorf et al., 1995). These genomic responses are slow and take days to induce effects through translational events. E₂ also initiates rapid non-genomic responses, taking minutes to cause an effect through a membrane-associated

36 kDA splice variant (ER α 36) of ER α 66 (Marino et al., 2006), or through a G protein-coupled receptor (GPR30) (Filardo et al., 2007). Ample evidence has accumulated suggesting an important role for S1P in E₂-mediated signaling (Sukocheva et al., 2014).

Role of the SphK1/S1P axis in ER signaling

Our initial demonstration that overexpression of SphK1 in human breast cancer cells promoted tumorigenesis and neovascularization when implanted in nude mice (Nava et al., 2002) was followed by numerous reports confirming the importance of SphK1 and formation of S1P as anti-apoptotic and growth-promoting factors in breast cancer (Carroll et al., 2015; Newton et al., 2015; Pyne et al., 2014; Sukocheva and Wadham, 2014; Truman et al., 2014). Moreover, expression of SphK1 has been shown to correlate with poor prognosis in breast cancer patients (Pyne et al., 2014; Ruckhaberle et al., 2008).

E₂ activates SphK1 in breast cancer cells and increases formation and export of S1P (Sukocheva and Wadham, 2014; Takabe and Spiegel, 2014). Increased SphK1 activity correlates with enhanced cell growth, and also is required for E₂-dependent activation of MAPK and intracellular Ca²⁺ mobilization in ER α -positive MCF-7 breast cancer cells (Sukocheva et al., 2006).

Anti-estrogen therapy with tamoxifen or aromatase inhibitors is the treatment of choice for ER α -positive breast cancer. Unfortunately, loss of ER α expression leading to resistance to hormonal therapies is common and hormonal therapies are not effective in ER α -negative breast cancers. One of the mechanisms by which breast cancer cells become resistant is a switch to growth factor-dependent growth. Intriguingly, activation of ER α by E₂ activates EGFR by a "criss-cross" transactivation process important for

E₂-dependent growth that requires ER α , activation of SphK1, production and secretion of S1P that activates S1PR3, leading to enhanced processing of pro-EGFR and activation of EGFR (Sukocheva et al., 2006) (Figure 1). This also caused increased localization of EGFR in endosomes to delay its degradation and direct it for recycling for continuous proliferative signaling (Sukocheva et al., 2009). Interestingly, SphK1 mRNA is also increased after E₂ treatment, suggesting that SphK1 is transcriptionally regulated by ER α . Transactivation to EGFR was also detected in T47D ER α -positive cells in response to E₂ treatment, but not in ER α -negative SK-BR-3; whereas, S1P was able to transactivate the EGFR in both ER α -positive and ER α -negative cells (Sukocheva et al., 2013). This suggests that both E₂ and S1P are critical components in the transactivation of EGFR in the transition from E₂-dependent growth to growth-factor-dependent growth. Moreover, SphK1 was required for EGF-induced breast cancer migration, proliferation, and cell survival and both ER α and GPR30 have been implicated in initiation of this signaling (Sukocheva and Wadham, 2014). However, only E₂ and not EGF stimulated export of S1P via ABCC1 and/or ABCG2 from breast cancer cells in an ER α -dependent manner (Takabe et al., 2010). Although Spns2, another bona fide S1P transporter, has been shown to export S1P from cells (Nishi et al., 2014; Takabe and Spiegel, 2014) and to be important in inflammatory and autoimmune diseases in mouse models (Donoviel et al., 2015), its involvement in breast cancer has not yet been investigated.

S1P in development of tamoxifen resistance

Tamoxifen is an anti-estrogen drug that binds to ER α , preventing estrogen binding, thereby causing cell growth arrest in breast cancer cells that are estrogen-dependent. Previous studies have shown that when patients with ER α -positive breast cancer are

treated with tamoxifen for 5 years, the rate of cancer recurrence is reduced by 39 percent and breast cancer mortality is decreased by about one-third throughout the first 15 years (Davies et al., 2011). Unfortunately, half of these patients will ultimately fail therapy due to acquired resistance. Moreover, breast cancer in patients whose tumors do not express ER α , progesterone receptor, and human epidermal growth factor receptor 2 (HER2, also known as ErbB-2), termed triple-negative breast cancer (TNBC), is aggressive with high recurrence, metastatic, and mortality rates (Bayraktar et al., 2013). These patients do not respond to hormonal therapy due to *de novo* (intrinsic) resistance and have limited treatment options.

The SphK1/S1P/S1PRs axis has been implicated in the development of tamoxifen resistance or acquired (extrinsic) chemoresistance. SphK1 expression and activity were shown to be elevated in acquired-tamoxifen resistant ER α -positive MCF7 cells, and SphK1 inhibition or downregulation restored the anti-proliferative and pro-apoptotic effects of tamoxifen (Sukocheva et al., 2009; Watson et al., 2010). In a cohort of 304 ER α -positive breast cancer patients, SphK1 expression correlated with tamoxifen resistance (Pyne et al., 2012). Moreover, high SphK1 and ERK1/2 expression in tumors of ER α -positive breast cancer patients, high S1PR1, but not S1PR2, expression, and higher expression of S1PR1/3 and ERK1/2 were all found to be associated with shorter time to recurrence on tamoxifen (Watson et al., 2010). These correlations suggest that the SphK1/S1P/S1PR1/3 axis and ERK1/2 may cooperate to promote ER α -positive breast cancer progression and resistance to anti-estrogen therapies.

Inhibition of the ER/S1P axis

Inhibitors that effectively target the ER α /S1P axis could also potentially be useful as new therapies for breast cancer. Numerous studies have shown the inhibitors of SphK1 decrease cancer cell growth and survival and also sensitize them to chemotherapeutics. For example, the non-selective SphK1/2 inhibitor, SKI-II, has been shown to abrogate ER α signaling, likely acting both as a SphK inhibitor and in a similar manner as tamoxifen by directly binding to the ER α and blocking binding of E₂ (Antoon et al., 2011a). Moreover, SphK1 inhibition by siRNA knockdown or treatment with SKI-5C sensitizes TNBC cells to chemotherapeutic drugs (Datta et al., 2014). However, fewer studies have shown the utility of SphK1 inhibitors *in vivo*. We found that treatment mice bearing syngeneic breast tumors with the specific SphK1 inhibitor SK1-I not only suppressed S1P levels in the tumor and circulation, but importantly reduced tumor burden and metastases to lymph nodes and lungs (Nagahashi et al., 2012). Growth of MDA-MB-468 xenograft tumors in mice was significantly inhibited by the SphK1/2 inhibitor SKI-II and the tyrosine kinase inhibitor gefitinib when used in combination, but not as single agents (Martin et al., 2014). Although inhibition of SphK2 has also been reported to reduce tumorigenesis (Antoon et al., 2011b; Liu et al., 2013), further studies are needed to insure that these effects are solely dependent on inhibition of SphK2 activity.

We and others found that the multiple sclerosis pro-drug FTY720/fingolimod, a sphingosine analog that is phosphorylated mainly by SphK2 to a S1P mimetic *in vivo*, has pleiotropic anti-cancer actions in breast cancer cells and in animal models. First, FTY720 has anti-proliferative actions in many types of cancer cells without affecting normal cells (Romero Rosales et al., 2011). Moreover, FTY720 is a substrate and thus

a competitive inhibitor of SphK1 and SphK2, decreasing levels of pro-survival S1P and increasing levels of pro-apoptotic sphingosine (Pyne et al., 2014). Phosphorylated FTY720 (FTY720-P) is also a functional antagonist of and downregulates S1PR1, which interferes with activation of NF- κ B and STAT3, and inhibits neovascularization in B cell-derived tumors (Deng et al., 2012; Lee et al., 2010; Liu et al., 2012) and colorectal cancer (Liang et al., 2013; Nagahashi et al., 2014). Furthermore, in breast cancer cells, FTY720-P produced in the nucleus by nuclear SphK2 is a potent inhibitor of class 1 HDACs that enhances histone acetylations and regulates expression of a restricted set of genes important for cancer progression, independently of its known effects on canonical signaling through S1PR1 (Hait et al., 2015). Importantly, in ER α -negative human and murine breast cancer cells and in ER α -negative syngeneic breast tumors, FTY720 activated re-expression of silenced ER α , which restored the ability of the anti-estrogen drug tamoxifen to block breast cancer proliferation and enhance apoptosis (Hait et al., 2015). Because a high fat diet and associated obesity are now endemic and associated with worse prognosis in breast cancer, we also investigated the effect of FTY720 administration on increased tumorigenesis in high fat diet fed mice. FTY720 significantly impaired development, progression and aggressiveness of spontaneous breast tumors in MMTV-PyMT transgenic mice and also reduced HDAC activity and restored expression of estrogen and progesterone receptors induced by the high fat diet (Hait et al., 2015). Taken together, these results provide further support the notion that FTY720 deserves consideration as a new therapeutic for treatment of both hormonal therapy-resistant breast cancer and triple-negative breast cancer.

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Competing interests statement

The authors declare no competing financial interests.

FIGURE LEGEND

Figure 1. Role of the SphK/S1P axis in signaling pathways initiated by E₂. Binding of E₂ to cytosolic ER α induces its dimerization and translocation to the nucleus where it associates with estrogen response elements (ERE) to regulate gene expression. E₂ can also signal through cell surface E₂ receptors to initiate rapid non-genomic effects that include activation of SphK1 and production of S1P. After export of this S1P by transporters, it activates S1PRs (such as S1PR3) leading to downstream signaling that regulates many processes important for breast cancer progression including processing of pro-EGFR by metalloproteinases. EGF then stimulates EGFR-mediated signaling important for cell growth (Sukocheva et al., 2013). S1P produced in the nucleus by SphK2 is an endogenous inhibitor of HDACs (Hait et al., 2009).

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