Clinical Impact of Sphingosine-1-Phosphate in Breast Cancer

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Breast cancer metastasizes to lymph nodes or other organs, which determine the prognosis of patients. It is difficult to cure the breast cancer patients with distant metastasis due to resistance to drug therapies. Elucidating the underlying mechanisms of breast cancer metastasis and drug resistance is expected to provide new therapeutic targets. Sphingosine-1-phosphate (S1P) is a pleiotropic, bioactive lipid mediator that regulates many cellular functions, including proliferation, migration, survival, angiogenesis/lymphangiogenesis, and immune responses. S1P is formed in cells by sphingosine kinases and released from them, which acts in an autocrine, paracrine, and/or endocrine manner. S1P in extracellular space, such as interstitial fluid, interacts with components in the tumor microenvironment, which may be important for metastasis. Importantly, recent translational research has demonstrated an association between S1P levels in breast cancer patients and clinical outcomes, highlighting the clinical importance of S1P in breast cancer. We suggest that S1P is one of the key molecules to overcome the resistance to the drug therapies, such as hormonal therapy, anti-HER2 therapy, or chemotherapy, all of which are crucial aspects of a breast cancer treatment.

1. Introduction

Breast cancer is the most common cancer diagnosis and the second most common cause of cancer death among women in the United States [1]. The majority of deaths due to breast cancer occur after metastasis, and it has become a systemic disease [2]. Breast cancer is known to metastasize to the lymph nodes first, which are a major determinant of prognosis, and forms the basis of the American Joint Commission on Cancer (AJCC) Cancer Staging [2]. A number of signaling proteins, such as vascular endothelial growth factor C and angiopoietins, have been reported to mediate lymphangiogenesis, formation of new lymphatic vessels from the pre-existing ones, which plays an important role in lymphatic metastasis [3–5]. In addition to these well-described signaling proteins, we have discovered that a bioactive lipid mediator, sphingosine-1-phosphate (S1P), also plays critical roles in lymphangiogenesis and lymph node metastasis, providing new insights into the mechanisms of cancer progression [6].

Breast cancer is a heterogeneous disease with distinct pathological features and clinical implications [7]. DNA microarray profiling studies on breast cancer have identified distinct intrinsic subtypes: luminal A, luminal B, human epidermal growth factor receptor 2- (HER2-) enriched, and triple-negative [8]. These subtypes are associated with different prognoses and resistance to drug therapy [9–13]. While hormone therapy or anti-HER2 therapies for breast cancer patients with estrogen receptor- (ER-) positive or HER2-enriched subtype are the most successful systemic therapies, only limited therapies are available for patients with the triple-negative subtype. Increasing number of evidence suggested that S1P has different roles in each subtype of breast cancer [14–17].

In this review, we introduce the lipid mediator, S1P, as a new player in breast cancer progression. We will also discuss
the potential roles of S1P in breast cancer and the possibilities of S1P signaling as a therapeutic target.

2. A Bioactive Lipid Mediator, S1P

S1P is a pleiotropic, bioactive lipid mediator that regulates a number of biological processes, including cell migration, survival, proliferation, angiogenesis, and immune and allergic responses [18–22]. S1P is generated from sphingosine inside the cell [23–27]. Ceramide and sphingosine, the metabolic precursors of S1P, are known to induce apoptosis, thus the balance between them and S1P in the cell has been suggested to function as a rheostat that determines the survival or death of the cell [28].

S1P is generated inside the cells by two sphingosine kinases, SphK1 and SphK2. SphK1 is located in the cytosol close to the cell membrane where its substrate sphingosine resides [29], while SphK2 is localized in specific organelles, such as the nucleus and mitochondria [30] (Figure 1). S1P produced inside cells by SphK1, but not SphK2, is secreted to the extracellular space by transporters and signals through its receptors on the outside of cells, a process referred to as “inside-out” signaling [31–33]. S1P produced by SphK2 in the nucleus regulates transcription of various genes expressed in the brain, liver, and kidneys [34–36]. SphK2 in mitochondria produces S1P that interacts with prohibitin 2 to regulate complex IV assembly and respiration [37].

Given its structure, S1P generated inside the cells is unable to freely pass through plasma membrane. S1P export requires transporters such as ATP-binding cassette (ABC) transporters or sphingolipid transporter 2 (Spns2) [14, 38–42]. S1P is exported from mast cells via ABCA1 [43], from astrocytes via ABCA1 [44], from endothelial cells via ABCA1 and ABCC1 [45], and from thyroid carcinoma cells via ABCC1 [46]. Spns2, a member of the major facilitator superfamily of non-ATP-dependent transporters, has been recently discovered to export S1P from cells [40–42]. Importantly, Spns2 was the first S1P transporter discovered to be physiologically functional in vivo [42].

After intracellular production by SphK1, S1P is released from the cell and can stimulate any of the five specific G protein-coupled receptors (S1PR1-5), which display tissue-specific expression patterns [14, 38], with each S1P receptor (S1PR) coupled to different G proteins, in an autocrine, paracrine, and/or endocrine manner [47]. The S1PRs are coupled to various G proteins, enabling them to regulate a broad spectrum of downstream signaling pathways and numerous biological processes [48–51].

S1P levels are tightly regulated by the balance between its synthesis and degradation. S1P can be dephosphorylated back to sphingosine by two specific S1P phosphatases (SPP1 and SPP2), which belong to the family of magnesium-dependent, N-ethylmaleimide-insensitive type 2 lipid phosphate phosphohydrolases that reside in the endoplasmic reticulum [32, 52, 53]. S1P can also be irreversibly degraded by a pyridoxal phosphate-dependent S1P lyase to
hexadecenal and phosphoethanolamine, with the latter subsequently being reused for the biosynthesis of phosphatidylethanolamine [31].

3. S1P and Breast Cancer Progression

The tumor microenvironment (TME) is a determining factor for cancer biology and progression. The TME comprises not only cancer cells, but also the surrounding blood vessels, immune cells, other stromal cells with signaling molecules, and the extracellular matrix and interstitial fluid, which are now known to play important roles in tumor progression [54–56]. Determining the interaction between cancer and noncancer cells in the TME is essential to understanding the mechanisms underlying cancer progression and metastasis. Cancer cells provide bioactive molecules, such as cytokines, chemokines, and lipid mediators to the TME that influence cancer progression. Noncancer cells in the TME, such as blood vessels, lymphatic vessels, and inflammatory cells, also release bioactive molecules that influence cancer progression. S1P is now emerging as a key regulatory molecule in breast cancer through its ability to promote cell proliferation, migration, angiogenesis, and lymphangiogenesis (Figure 1). Further, S1P secreted to the extracellular spaces, including the interstitial fluid and lymphatic fluid, has been suggested to be important for metastasis by stimulating S1P signaling [30, 57]. In this section, we describe the roles of S1P in the TME and tumor progression (Figure 2).

Angiogenesis, the development of new blood vessels from preexisting vessels, determines the rate of growth and progression of cancer by providing oxygen, nutrition, and conduits for cancer cells for invading cells to metastasize [58, 59]. The neutralization of extracellular S1P with an anti-S1P antibody in vitro and in an animal model showed significant inhibition of angiogenesis, tumor growth, and metastasis, further confirming the dominant role of extracellular S1P in angiogenesis [60]. Tumors with upregulated SphK1 may themselves be a key source of S1P according to the data from mouse models and human patient samples [6, 61–64]. However, endothelial cells can also synthesize and release endogenous S1P [45].

In contrast to angiogenesis, few studies so far have examined the involvement of S1P signaling in lymphangiogenesis [65]. S1P can induce lymphatic endothelial cell (LEC) tube formation in an S1PR1-dependent manner [4, 66]. Moreover, angiopoietin-2-induced lymphangiogenesis in vitro was suppressed by an SphK1-specific pharmacological inhibitor, suggesting cross talk between angiopoietin-2 and S1P signaling pathways [6]. LEC-specific deletion of SphK1 in the SphK2 knockout mouse inhibited lymphatic vessel maturation, indicating that SphKs and S1P in LECs are required for proper development of lymphatic vessels [3].

Spns2 is an S1P transporter that plays a role in the regulation of the lymph node and lymphatic fluid S1P levels and consequently influences lymphocyte trafficking and lymphatic vessel network organization [67]. Spns2-deficient mice showed an aberrant lymphatic vessel network that appeared collapsed, with reduced numbers of lymphocytes. Levels of S1P were increased in the lymphatic fluid from Spns2-deficient mice as well as in specific tissues, including lymph nodes, and interstitial fluid.

Utilizing our newly established murine syngeneic orthotopic metastatic breast cancer model and mass spectrometry, we found that levels of S1P gradually increased both in tumors and in the circulation and correlated with tumor burden [6]. Further, treatment of tumor-bearing animals with the specific SphK1 inhibitor, SK1-I, reduced S1P levels in the tumor and in circulation and greatly reduced the tumor burden of the primary tumor, lymph node, and lung metastases. Importantly, inhibiting SphK1 significantly decreased S1P levels in serum and tumors in these mice, and both angiogenesis and lymphangiogenesis were suppressed, not only around the primary tumor but also in lymph nodes that were distant from the tumor [6]. Furthermore, lymph node and lung metastases were significantly suppressed. These results indicate that S1P plays a key role, not only in tumoral

![Figure 2: The roles of sphingosine-1-phosphate (S1P) in the interaction between tumor and tumor microenvironment (TME). S1P produced by tumor cells and the TME promote various pathological processes related to cancer progression.](image-url)
lymphangiogenesis, but also in lymph node lymphangiogenesis, which may actively promote metastasis via the lymphatics [6].

An S1P gradient with high S1P concentrations in the blood and lymphatic fluid circulation plays an important role in immune cell trafficking [67–71]. We established a convenient method for the collection and measurement of sphingolipids in the lymphatic fluid. The lymphatic fluid was absorbed onto filter paper after incision of cisterna chyli in murine models, and S1P levels were determined by mass spectrometry [72]. Levels of S1P in the lymphatic fluid are lower than those in blood and higher than those in lymph nodes in agreement with a previous study [72]. Levels of S1P in the lymphatic fluid as well as lymph node were significantly increased in SphK2 knockout mice compared to littermate controls. These results suggest that SphK2 and/or SphK1 regulate the levels of S1P in the lymphatic fluid.

The interstitial fluid that bathes the tumor and stromal cells is an important part of the TME, not only as the initial route of metastasis, but also as a supplier of factors that promote tumor metastasis. The role of S1P in the TME, particularly in the interstitial fluid, has not been well studied due to a lack of efficient methods for collecting and quantifying levels in the interstitial fluid. We developed simple and reproducible methods to measure the levels of sphingolipids including S1P in small volumes of the interstitial fluid from healthy mammary glands and tumor using a modified centrifugation method combined with mass spectrometry [57]. In mice with a deletion of SphK1, but not SphK2, levels of S1P in the interstitial fluid from the mammary glands were greatly reduced. Levels of S1P in the interstitial fluid from the mammary tumors were reduced when tumor growth was suppressed by oral administration of the potent S1P receptor functional antagonist, FTY720 ( fingolimod; 2-amino-2-[2-(4-octylphenyl)ethyl]1,3-propanediol) [57]. Thus, S1P secreted from tumor cells to the interstitial fluid may play an important role of metastasis by stimulating S1P signaling in the TME [57]. Further, in breast cancer patients, we examined the levels of sphingolipids in the interstitial fluid from breast tumor tissue and normal breast tissue from two different areas in each patient and determined levels of sphingolipids in the fluid. We revealed that S1P levels were significantly higher in the human breast tumor tissue interstitial fluid than in the normal breast tissue interstitial fluid [57]. Thus, the observations made in animal models are applicable to human patients.

4. Clinical Impact of S1P in Human Breast Cancer Patients

The interaction between cancer cells and the TME is now considered key to understanding the mechanisms of how cancer progresses and metastasizes. Although numerous in vitro and in vivo studies have reported the importance of S1P in breast cancer progression, the evidence in human breast cancer patients had been limited until recently. We have determined the levels of S1P in breast cancer and normal breast tissues from surgical specimens and compared the difference in levels of S1P between those tissues [73]. S1P levels in the human breast cancer tissue are significantly higher than those in the normal breast tissue. Of note, there was a correlation in the levels of S1P between the breast cancer and normal breast tissues, which implies that S1P produced by tumor cells affect surrounding normal breast tissue. Taken together, S1P can be considered an important player in the interaction between cancer and the TME.

We also investigated ceramide metabolism in breast cancer patients and showed that ceramide levels in the human breast cancer tissue are significantly higher than those in the normal breast tissue [73]. Interestingly, however, there was no correlation in the ceramide levels between the breast cancer and normal breast tissues [73]. It has been reported that ceramide has a distinct role in cell survival and death. This finding indicates that ceramide production in tumor tissue occurs independently from the surrounding normal breast tissue. Further investigation is needed to determine the roles of ceramide in human breast cancer biology.

We have also quantified the levels of S1P in human blood and tissue samples from breast cancer patients utilizing mass spectrometry. Importantly, serum S1P levels were significantly elevated in stage IIIA breast cancer patients who had developed lymph node metastases, compared with age- and ethnicity-matched healthy volunteers [6]. More recently, we found that patients with lymph node metastasis showed significantly higher levels of S1P in breast cancer tissue than patients with negative nodes [74]. These findings revealed that S1P produced by breast cancer tissue affects lymph node metastasis in human patients, consistent with the observations in animal models. Research to clarify the role of S1P in cancer progression has now evolved from an experimental phase to a translational phase. More data from the clinical setting is now needed to translate the previous theories of how S1P promotes cancer progression based on in vitro and in vivo models to human breast cancer care.

5. S1P as a Therapeutic Target for Breast Cancer Patients

Breast cancer is treated based on the intrinsic subtype as described above. Pharmacological inhibition of 17β-estradiol (E2) production or E2 binding to the ER is an effective treatment for ER-positive patients. Although HER2-positive breast cancer is known to be aggressive, and patients with HER2-positive breast cancer had a poorer prognosis, anti-HER2 therapy, such as trastuzumab, pertuzumab, and TDM-1, which are recombinant antibodies that target HER2-positive cancer cells, have dramatically improved survival of patients with this subtype.

In contrast to ER-positive or HER2-positive breast cancers, ER-negative and HER2-negative breast cancers do not have specific therapies, which are one of the reasons that the latter type often demonstrates earlier disease recurrence and poorer prognosis. Any cancer subtype has a potential to gain resistance to a particular therapy. It has been reported that there is cross talk between the S1P signaling pathway and the E2 or HER2 signaling pathways. Thus, S1P may have a role in acquisition of drug resistance [75].
ER status is an important prognostic factor with ER-positive breast cancer patients having a better prognosis than ER-negative breast cancer patients. We reported the contribution of the S1P pathway to E2 signaling. Binding of E2 to ER stimulates release of S1P via ABC transporters, ABCC1 and ABCG2 [14]. This S1P in turn binds to and activates S1P receptors to stimulate ERK1/2 leading to downstream signaling events important for breast cancer proliferation, progression, and invasion. We showed that E2 induces export of S1P via ABCC1 and ABCG2 transporters, which may contribute to the nongenomic signaling of E2 important for breast cancer proliferation, progression, and invasion. We showed that E2 induces export of S1P via ABCC1 and ABCG2 transporters, which may contribute to the nongenomic signaling of E2 important for breast cancer pathophysiology [14]. Higher levels of SphK1 were found in ER-negative breast cancer, which are known for their higher proliferative activity [63]. It has also been shown that SphK1 expression correlates with poor prognosis of breast cancer patients [63]. Even in ER-positive breast cancer, higher expression of SphK1 correlated with poor patient survival rates and was associated with the development of tamoxifen resistance and earlier disease recurrence while on tamoxifen [76, 77]. Therefore, SphK1 expression levels in combination with ER status are proposed to be good biomarkers to predict response to tamoxifen [77] (Figure 3).

HER2 overexpression is a major determinant of breast cancer progression. S1PR4 stimulates the ERK1/2 pathway via a HER2-dependent mechanism in ER-negative MDA-MB-453 breast cancer cells [15]. High expression of S1PR4 and SphK1 is associated with shorter disease-specific survival in ER-negative breast cancer patients, highlighting the important role for S1PR4 and SphK1 in ER-negative breast cancer progression [78]. We found that breast cancer tissue S1P levels were lower in those with HER2 overexpression/amplification [74]. Considering that both HER2 and SphK1 are strong activators of survival signaling pathways such as MAPK, and HER2 signal is a strong autonomous signal, it is tempting to speculate that negative feedback suppresses activation of SphK1 in HER2-positive breast cancer. Further study is needed to determine the relationship between S1P signal and HER2 overexpression/amplification.

Patients with triple-negative breast cancer have a poor prognosis relative to other breast cancer subtypes. LM2-4 cells that gained lung metastatic phenotype from primary triple-negative MDA-MB-231 breast cancer cells showed a requirement on SphKs/S1P signaling for cell growth, survival, and cell motility. PF-543, a selective, potent inhibitor of SphK1, attenuated epidermal growth factor-mediated cell growth and survival signaling through inhibition of AKT, ERK, and p38 MAP kinase pathways in LM2-4 cells, but not in the parental MDA-MB-231 human breast cancer cells [16]. These observations highlight the importance of SphKs/S1P signaling in metastatic triple-negative breast cancers and targeted therapies [16].

The roles of ATP-binding cassette transporters, such as ABCC1, ABCG2, in breast cancer patients have been reported. For instance, ABCC1 and ABCG2 have been associated with chemoresistance in breast cancer [79]. Further, ABCC1 and ABCG2 are highly expressed in core basal subtype, which is one of the most aggressive breast cancer subtypes, while ABCG2 is highly expressed in the HER2 or core basal subtypes [80]. Moreover, ABCC1 or ABCG2 expression is associated with shorter disease-free survival [80]. Taken together, ABC transporters are highly expressed in aggressive breast cancer subtypes, and tumor ABC transporter expression is associated with poor prognosis.

Several agents targeting S1P signaling have been tested in preclinical models [81, 82]. FTY720 is a novel immunosuppressive agent that shows structural similarity to sphingosine and is intracellularly phosphorylated by SphK2, but not SphK1 [83, 84]. FTY720-P, the phosphorylated form of FTY720, binds to S1PR1 in lymphocytes and/or endothelial cells, which causes the inhibition of lymphocyte trafficking.
into the circulating blood and the accumulation of lymphocytes in secondary lymphoid tissues [85–87]. FTY720-P is transported through the same pathway as S1P [88]. Further, FTY720 has been approved by the US Food and Drug Administration (FDA) for multiple sclerosis [31]. SK1-I is a selective SphK1 inhibitor [89, 90]. We found that BML-258, one of the SK1 inhibitors, reduced S1P levels in the tumor and in circulation, and greatly reduced the size of the primary tumor, lymph node, and lung metastasis in animal model [6]. The anti-S1P mAb, sonepcizumab, and its murine counterpart, sphingomab, substantially reduced tumor progression, and, in some cases, eliminated measurable tumors [91, 92]. Antibody-mediated neutralization of extracellular S1P could result in a reduction of tumor volumes and metastatic potential, as a result of inhibition of new blood vessel formation [60, 93]. Further studies are needed if new therapies are to be developed for cancer patients based on S1P pathways.

6. Conclusion

In this review, we discussed the roles of S1P in breast cancer progression. S1P secreted from cells interacts with the TME, which has been suggested to be important for metastasis. Importantly, recent translational research has demonstrated an association between S1P levels in human breast cancer patients and clinical outcomes, which highlights the clinical importance of S1P in breast cancer. We suggest that the S1P pathway could be an important therapeutic avenue to overcome the resistance to drug therapies, such as hormonal therapy, anti-HER2 therapy, or chemotherapy, all of which are crucial aspects of breast cancer treatment.

Abbreviations

ABC: ATP-binding cassette
E2: 17β-estradiol
ER: Estrogen receptor
HER2: Human epidermal growth factor receptor 2
LEC: Lymphatic endothelial cell
S1P: Sphingosine-1-phosphate
SphK: Sphingosine kinase
Spns2: Sphingolipid transporter 2
SPP: S1P phosphatase
TME: Tumor microenvironment.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2017," CA: A Cancer Journal for Clinicians, vol. 67, no. 1, pp. 7–30, 2017.
[2] V. Mumprecht, M. Honer, B. Vigl et al., "In vivo imaging of inflammation- and tumor-induced lymph node lymphangiogenesis by immuno-positrion emission tomography," Cancer Research, vol. 70, no. 21, pp. 8842–8851, 2010.
[3] T. H. Pham, P. Baluk, Y. Xu et al., "Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning," The Journal of Experimental Medicine, vol. 207, no. 1, pp. 17–27, 2010.
[4] C. M. Yoon, B. S. Hong, H. G. Moon et al., "Sphingosine-1-phosphate promotes lymphangiogenesis by stimulating S1P1/Gi/PLC/Ca2+ signaling pathways," Blood, vol. 112, no. 4, pp. 1129–1138, 2008.
[5] M. Nagahashi, S. Ramachandran, O. M. Rashid, and K. Takabe, "Lymphangiogenesis: a new player in cancer progression," World Journal of Gastroenterology, vol. 16, no. 32, pp. 4003–4012, 2010.
[6] M. Nagahashi, S. Ramachandran, E. Y. Kim et al., "Sphingosine-1-phosphate produced by sphingosine kinase 1 promotes breast cancer progression by stimulating angiogenesis and lymphangiogenesis," Cancer Research, vol. 72, no. 3, pp. 726–735, 2012.
[7] A. S. Coates, E. P. Winer, A. Goldhirsch et al., "Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015," Annals of Oncology, vol. 26, no. 8, pp. 1533–1546, 2015.
[8] C. M. Perou, T. Sorlie, M. B. Eisen et al., "Molecular portraits of human breast tumours," Nature, vol. 406, no. 6797, pp. 747–752, 2000.
[9] L. A. Carey, E. C. Dees, L. Sawyer et al., "The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes," Clinical Cancer Research, vol. 13, no. 8, pp. 2329–2334, 2007.
[10] R. Rouzier, C. M. Perou, W. F. Symmans et al., "Breast cancer molecular subtypes respond differently to preoperative chemotherapy," Clinical Cancer Research, vol. 11, no. 16, pp. 5678–5685, 2005.
[11] F. M. Blows, K. E. Driver, M. K. Schmidt et al., "Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies?," PLoS Medicine, no. 5, article e1000279, 2010.
[12] T. Sorlie, C. M. Perou, R. Tibshirani et al., "Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications," Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 19, pp. 10869–10874, 2001.
[13] T. Sorlie, R. Tibshirani, J. Parker et al., "Repeate observation of breast tumor subtypes in independent gene expression data sets," Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 14, pp. 8418–8423, 2003.
[14] K. Takabe, R. H. Kim, J. C. Allegood et al., "Estriodil induces export of sphingosine 1-phosphate from breast cancer cells...
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via ABCC1 and ABCG2,” The Journal of Biological Chemistry, vol. 285, no. 14, pp. 10477–10486, 2010.

[15] J. S. Long, Y. Fujiwara, J. Edwards et al., “Sphingosine 1-phosphate receptor 4 uses HER2 (ERBB2) to regulate extracellular signal regulated kinase-1/2 in MDA-MB-453 breast cancer cells,” The Journal of Biological Chemistry, vol. 285, no. 46, pp. 35957–35966, 2010.

[16] A. Maiti, K. Takabe, and N. C. Hait, “Metastatic triple-negative breast cancer is dependent on SphK/SIP signaling for growth and survival,” Cellular Signalling, vol. 32, pp. 85–92, 2017.

[17] M. Maczis, S. Milstien, and S. Spiegel, “Sphingosine 1-phosphate and estrogen signaling in breast cancer,” Advances in Biological Regulation, vol. 60, pp. 160–165, 2016.

[18] M. Aoki, H. Aoki, R. Ramanathan, N. C. Hait, and K. Takabe, “Sphingosine 1-phosphate signaling in immune cells and inflammation: roles and therapeutic potential,” Mediators of Inflammation, vol. 2016, Article ID 8606878, 11 pages, 2016.

[19] M. Aoki, H. Aoki, R. Ramanathan, N. C. Hait, and K. Takabe, “Corrigendum to “Sphingosine-1-phosphate Signaling in immune cells and inflammation: roles and therapeutic potential,” Mediators of Inflammation, vol. 2016, Article ID 2856829, 1 page, 2016.

[20] N. J. Pyne and S. Pyne, “Sphingosine 1-phosphate and cancer,” Nature Reviews Cancer, vol. 10, no. 7, pp. 489–503, 2010.

[21] M. Maceyka, S. Milstien, and S. Spiegel, “Sphingosine 1-phosphate: the Swiss army knife of sphingolipid signaling,” Journal of Lipid Research, vol. 50 Supplement, pp. S272–S276, 2009.

[22] S. Spiegel and S. Milstien, “The outs and the ins of sphingosine-1-phosphate in immunity,” Nature Reviews Immunology, vol. 11, no. 6, pp. 403–415, 2011.

[23] H. Liu, D. Chakravarty, M. Maceyka, S. Milstien, and S. Spiegel, “Sphingosine kinases: a novel family of lipid kinases,” Progress in Nucleic Acid Research and Molecular Biology, vol. 71, pp. 493–511, 2002.

[24] M. Maceyka, S. G. Payne, S. Milstien, and S. Spiegel, “Sphingosine kinase type 1 promotes estrogen-dependent tumorigenesis of breast cancer MCF-7 cells,” Experimental Cell Research, vol. 281, no. 1, pp. 115–127, 2002.

[25] H. Liu, R. E. Toman, S. K. Goparanju et al., “Sphingosine kinase type 2 is a putative BH3-only protein that induces apoptosis,” The Journal of Biological Chemistry, vol. 278, no. 41, pp. 40330–40336, 2003.

[26] Y. A. Hannun and L. M. Obeid, “Principles of bioactive lipid signalling: lessons from sphingolipids,” Nature Reviews Molecular Cell Biology, vol. 9, no. 2, pp. 139–150, 2008.

[27] O. Cuvelier, D. S. Rosenthal, M. E. Smulson, and S. Spiegel, “Sphingosine 1-phosphate inhibits activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes,” The Journal of Biological Chemistry, vol. 273, no. 5, pp. 2910–2916, 1998.

[28] S. M. Pitson, P. Xia, T. M. Leclercq et al., “Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling,” The Journal of Experimental Medicine, vol. 201, no. 1, pp. 49–54, 2005.

[29] M. Nakajima, M. Nagahashi, O. M. Rashid, K. Takabe, and T. Wakai, “The role of sphingosine-1-phosphate in the tumor microenvironment and its clinical implications,” Tumour Biology, vol. 39, no. 4, 2017.

[30] K. Takabe, S. W. Paugh, S. Milstien, and S. Spiegel, “Inside-out signaling of sphingosine-1-phosphate: therapeutic targets,” Pharmacological Reviews, vol. 60, no. 2, pp. 181–195, 2008.

[31] K. Takabe and S. Spiegel, “Export of sphingosine-1-phosphate and cancer progression,” Journal of Lipid Research, vol. 55, no. 9, pp. 1839–1846, 2014.

[32] L. A. Heffernan-Stroud and L. M. Obeid, “Sphingosine kinase 1 in cancer,” Advances in Cancer Research, vol. 117, pp. 201–235, 2013.

[33] N. C. Hait, J. Allegood, M. Maceyka et al., “Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate,” Science, vol. 325, no. 5945, pp. 1254–1257, 2009.

[34] N. C. Hait, L. E. Wise, J. C. Allegood et al., “Active, phosphorylated fngolimod inhibits histone deacetylases and facilitates fear extinction memory,” Nature Neuroscience, vol. 17, no. 7, pp. 971–980, 2014.

[35] M. Nagahashi, K. Takabe, R. Liu et al., “Conjugated bile acid-activated SIP receptor 2 is a key regulator of sphingosine kinase 2 and hepatic gene expression,” Hepatology, vol. 61, no. 4, pp. 1216–1226, 2015.

[36] G. M. Strub, M. Paillard, J. Liang et al., “Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration,” FASEB Journal, vol. 25, no. 2, pp. 600–612, 2011.

[37] R. H. Kim, K. Takabe, S. Milstien, and S. Spiegel, “Export and functions of sphingosine-1-phosphate,” Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, vol. 1791, no. 7, pp. 692–696, 2009.

[38] N. Kobayashi, T. Nishi, T. Hirata et al., “Sphingosine 1-phosphate is released from the cytosol of rat platelets in a carrier-mediated manner,” Journal of Lipid Research, vol. 47, no. 3, pp. 614–621, 2006.

[39] A. Kawahara, T. Nishi, Y. Hisano, H. Fukui, A. Yamaguchi, and N. Mochizuki, “The sphingolipid transporter Spns2 functions in migration of zebrafish myocardial precursors,” Science, vol. 323, no. 5913, pp. 524–527, 2009.

[40] S. Fukuhara, S. Simmons, S. Kawamura et al., “The sphingosine-1-phosphate transporter Spns2 expressed on endothelial cells regulates lymphocyte trafficking in mice,” The Journal of Clinical Investigation, vol. 122, no. 4, pp. 1416–1426, 2012.

[41] Y. Hisano, N. Kobayashi, A. Yamaguchi, and T. Nishi, “Mouse Spns2 functions as a sphingosine-1-phosphate transporter in vascular endothelial cells,” PLoS One, vol. 7, no. 6, article e38941, 2012.

[42] P. Mitra, C. A. Oskeritzian, S. G. Payne, M. A. Beaven, S. Milstien, and S. Spiegel, “Role of ABCC1 in export of sphingosine-1-phosphate from mast cells,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 44, pp. 16394–16399, 2006.

[43] K. Sato, E. Malchinkhuu, Y. Horiuichi et al., “Critical role of ABCC1 transporter in sphingosine 1-phosphate release from astrocytes,” Journal of Neurochemistry, vol. 103, no. 6, pp. 2610–2619, 2007.

[44] Y. M. Lee, K. Venkataraman, S. I. Hwang, D. K. Han, and T. Hla, “A novel method to quantify sphingosine 1-phosphate
by immobilized metal affinity chromatography (IMAC),” *Prostaglandins & Other Lipid Mediators*, vol. 84, no. 3–4, pp. 154–162, 2007.

[46] N. Bergelin, T. Blom, J. Heikkila et al., “Sphingosine kinase as an oncogene: autocrine sphingosine 1-phosphate modulates ML-1 thyroid carcinoma cell migration by a mechanism dependent on protein kinase C-alpha and ERK1/2,” *Endocrinology*, vol. 150, no. 5, pp. 2055–2063, 2009.

[47] S. E. Alvarez, S. Milstien, and S. Spiegel, “Autocrine and paracrine roles of sphingosine-1-phosphate,” *Trends in Endocrinology and Metabolism*, vol. 18, no. 8, pp. 300–307, 2007.

[48] N. J. Pyne, F. Tonelli, K. G. Lim, J. S. Long, J. Edwards, and S. Pyne, “Sphingosine 1-phosphate signalling in cancer,” *Biochemical Society Transactions*, vol. 40, no. 1, pp. 94–100, 2012.

[49] A. L. Parrill, S. Lima, and S. Spiegel, “Structure of the first sphingosine 1-phosphate receptor,” *Science Signaling*, vol. 5, no. 225, article pe23, 2012.

[50] T. Sanchez and T. Hla, “Structural and functional characteristics of S1P receptors,” *Journal of Cellular Biochemistry*, vol. 92, no. 5, pp. 913–922, 2004.

[51] M. J. Kluk and T. Hla, “Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G-protein-coupled receptors,” *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, vol. 1582, no. 1–3, pp. 72–80, 2002.

[52] H. L. Stunff, C. Peterson, R. Thornton, S. Milstien, S. M. Mandala, and S. Spiegel, “Characterization of murine sphingosine-1-phosphate phosphohydrolase,” *The Journal of Biological Chemistry*, vol. 277, no. 11, pp. 8920–8927, 2002.

[53] C. Ogawa, A. Kihara, M. Gokoh, and Y. Igarashi, “Identification and characterization of a novel human sphingosine-1-phosphate phosphohydrolase, hSPP2,” *The Journal of Biological Chemistry*, vol. 278, no. 2, pp. 1268–1272, 2003.

[54] H. Wiig, O. Tenstad, P. O. Iversen, R. Kalluri, and R. Bjerkgv, “Interstitial fluid: the overlooked component of the tumor microenvironment?,” *Fibrogenesis & Tissue Repair*, vol. 3, p. 12, 2010.

[55] H. Haslene-Hox, E. Oveland, K. C. Berg et al., “A new method for isolation of interstitial fluid from human solid tumors applied to proteomic analysis of ovarian carcinoma tissue,” *PloS One*, vol. 6, no. 4, article e19217, 2011.

[56] H. Wiig and M. A. Schwart, “Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer,” *Physiological Reviews*, vol. 92, no. 3, pp. 1005–1060, 2012.

[57] M. Nagahashi, A. Yamada, H. Miyazaki et al., “Interstitial fluid sphingosine-1-phosphate in murine mammary gland and cancer and human breast tissue and cancer determined by novel methods,” *Journal of Mammary Gland Biology and Neoplasia*, vol. 21, no. 1–2, pp. 9–17, 2016.

[58] K. Takabe, A. Yamada, O. M. Rashid et al., “Twofer anti-vascular therapy targeting sphingosine-1-phosphate for breast cancer,” *Gland surgery*, vol. 1, no. 2, pp. 80–83, 2012.

[59] W. C. Huang, M. Nagahashi, K. P. Terracina, and K. Takabe, “Emerging role of sphingosine-1-phosphate in inflammation, cancer, and lymphangiogenesis,” *Biomolecules*, vol. 3, no. 3, 2013.

[60] B. Visentin, J. A. Vekich, B. J. Sibbald et al., “Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages,” *Cancer Cell*, vol. 9, no. 3, pp. 225–238, 2006.

[61] S. Ponnusamy, S. P. Selvam, S. Mehrotra et al., “Communication between host organism and cancer cells is transduced by systemic sphingosine kinase 1/sphingosine 1-phosphate signalling to regulate tumour metastasis,” *EMBO Molecular Medicine*, vol. 4, no. 8, pp. 761–775, 2012.

[62] T. Kawamori, T. Kaneshiro, M. Okumura et al., “Role for sphingosine kinase 1 in colon carcinogenesis,” *FASEB Journal*, vol. 23, no. 2, pp. 405–414, 2009.

[63] E. Ruckhaberle, A. Rody, K. Engels et al., “Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer,” *Breast Cancer Research and Treatment*, vol. 112, no. 1, pp. 41–52, 2008.

[64] S. Pyne, J. Edwards, J. Ohotski, and N. J. Pyne, “Sphingosine 1-phosphate receptors and sphingosine kinase 1: novel biomarkers for clinical prognosis in breast, prostate, and hematological cancers,” *Frontiers in Oncology*, vol. 2, p. 168, 2012.

[65] T. Aoyagi, M. Nagahashi, A. Yamada, and K. Takabe, “The role of sphingosine-1-phosphate in breast cancer tumor-induced lymphangiogenesis,” *Lymphatic Research and Biology*, vol. 10, no. 3, pp. 97–106, 2012.

[66] V. Anelli, C. R. Gault, A. J. Snider, and L. M. Obeid, “Role of sphingosine kinase-1 in paracrine/transcellular angiogenesis and lymphangiogenesis in vitro,” *FASEB Journal*, vol. 24, no. 8, pp. 2727–2738, 2010.

[67] M. Nagahashi, E. Y. Kim, A. Yamada et al., “Spsn2, a transporter of phosphorylated sphingoid bases, regulates their blood and lymph levels, and the lymphatic network,” *FASEB Journal*, vol. 27, no. 3, pp. 1001–1011, 2013.

[68] J. G. Cyster and S. R. Schwab, “Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs,” *Annual Review of Immunology*, vol. 30, pp. 69–94, 2012.

[69] A. Ben Shoham, G. Malkinson, S. Krief et al., “S1P1 inhibits sprouting angiogenesis during vascular development,” *Development*, vol. 139, no. 20, pp. 3859–3869, 2012.

[70] K. Gaengel, C. Niaudet, K. Hagikura et al., “The sphingosine-1-phosphate receptor S1P1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2,” *Developmental Cell*, vol. 23, no. 3, pp. 587–599, 2012.

[71] B. Jung, H. Obinata, S. Galvani et al., “Flow-regulated endothelial S1P receptor-1 signaling sustains vascular development,” *Developmental Cell*, vol. 23, no. 3, pp. 600–610, 2012.

[72] M. Nagahashi, A. Yamada, T. Aoyagi et al., “Sphingosine-1-phosphate in the lymphatic fluid determined by novel methods,” *Heliyon*, vol. 2, no. 12, article e00219, 2016.

[73] M. Nagahashi, J. Tsuchida, K. Moro et al., “High levels of sphingolipids in human breast cancer,” *The Journal of Surgical Research*, vol. 204, no. 2, pp. 433–444, 2016.

[74] J. Tsuchida, M. Nagahashi, M. Nakajima et al., “Breast cancer sphingosine-1-phosphate is associated with phospho-sphingosine kinase 1 and lymphatic metastasis,” *The Journal of Surgical Research*, vol. 205, no. 1, pp. 85–94, 2016.

[75] O. Cuviiller, I. Ader, P. Bouquerel, L. Brizuela, C. Gsaltder, and B. Malavaud, “Hypoxia, therapeutic resistance, and sphingo-sine 1-phosphate,” *Advances in Cancer Research*, vol. 117, pp. 117–141, 2013.

[76] J. S. Long, J. Edwards, C. Watson et al., “Sphingosine kinase 1 induces tolerance to human epidermal growth factor receptor 2 and prevents formation of a migratory phenotype in response to sphingosine 1-phosphate in estrogen receptor-positive breast cancer cells,” *Molecular and Cellular Biology*, vol. 30, no. 15, pp. 3827–3841, 2010.
C. Watson, J. S. Long, C. Orange et al., “High expression of sphingosine 1-phosphate receptors, S1P1 and S1P3, sphingosine kinase 1, and extracellular signal-regulated kinase-1/2 is associated with development of tamoxifen resistance in estrogen receptor-positive breast cancer patients,” The American Journal of Pathology, vol. 177, no. 5, pp. 2205–2215, 2010.

J. Ohotski, J. S. Long, C. Orange et al., “Expression of sphingosine 1-phosphate receptor 4 and sphingosine kinase 1 is associated with outcome in oestrogen receptor-negative breast cancer,” British Journal of Cancer, vol. 106, no. 8, pp. 1453–1459, 2012.

M. Dean, “ABC transporters, drug resistance, and cancer stem cells,” Journal of Mammary Gland Biology and Neoplasia, vol. 14, no. 1, pp. 3–9, 2009.

A. Yamada, T. Ishikawa, I. Ota et al., “High expression of ATP-binding cassette transporter ABCC11 in breast tumors is associated with aggressive subtypes and low disease-free survival,” Breast Cancer Research and Treatment, vol. 137, no. 3, pp. 773–782, 2013.

R. L. Proia and T. Hla, “Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy,” The Journal of Clinical Investigation, vol. 125, no. 4, pp. 1379–1387, 2015.

S. Müllten and S. Spiegel, “Targeting sphingosine-1-phosphate: a novel avenue for cancer therapeutics,” Cancer Cell, vol. 9, no. 3, pp. 148–150, 2006.

V. Brinkmann, “Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology,” Pharmacology & Therapeutics, vol. 115, no. 1, pp. 84–105, 2007.

V. Brinkmann, J. G. Cyster, and T. Hla, “FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function,” American Journal of Transplantation, vol. 4, no. 7, pp. 1019–1025, 2004.

K. Chiba, Y. Yanagawa, Y. Masubuchi et al., “FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing,” Journal of Immunology, vol. 160, no. 10, pp. 5037–5044, 1998.

K. Budde, M. Schutz, P. Glander et al., "FTY720 (fingolimod) in renal transplantation," Clinical Transplantation, vol. 20, Supplement 17, pp. 17–24, 2006.

S. Thangada, K. M. Khanna, V. A. Blaho et al., "Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines lymphocyte egress kinetics," The Journal of Experimental Medicine, vol. 207, no. 7, pp. 1475–1483, 2010.

Y. Hisano, N. Kobayashi, A. Kawahara, A. Yamaguchi, and T. Nishi, "The sphingosine 1-phosphate transporter, SPNS2, functions as a transporter of the phosphorylated form of the immunomodulating agent FTY720," The Journal of Biological Chemistry, vol. 286, no. 3, pp. 1758–1766, 2011.

S. W. Paugh, B. S. Paugh, M. Rahmani et al., "A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia," Blood, vol. 112, no. 4, pp. 1382–1391, 2008.

D. Kapitonov, J. C. Allegood, C. Mitchell et al., “Targeting sphingosine kinase 1 inhibits Akt signaling, induces apoptosis, and suppresses growth of human glioblastoma cells and xenografts,” Cancer Research, vol. 69, no. 17, pp. 6915–6923, 2009.