SPECIFIC AIMS
Approximately 40,000 women die from breast cancer every year in the United States (1). Several processes determine breast cancer progression, including growth, invasion, hemangiogenesis, and metastasis. Sphingosine-1-phosphate (S1P), a pleiotropic bioactive lipid mediator and a ligand for five specific G-protein coupled receptors (S1PR1-5), regulates these critical processes (2,3). SphK1, one of the two sphingosine kinases that generates S1P, is up-regulated in breast cancer and is associated with tumor angiogenesis (2,4), resistance to chemotherapy (5), and correlates with a poor prognosis (6). We have reported that the elevated S1P in breast cancer cells is exported by ATP-binding cassette transporters and subsequently activates its receptors in an autocrine/paracrine manner (7), defined as “inside-out signaling” (2). We also found that circulating S1P levels are elevated with cancer progression that correlates with poor survival (8).

The extent of lymph node metastasis is a major determinant for staging and prognosis of breast cancer (9). Clinical and experimental evidence suggests that migration of tumor cells into lymph nodes is also mediated by lymphangiogenesis (10), a process that generates new lymphatic vessels from pre-existing ones. Nevertheless, the molecular mechanisms of tumor-induced lymphangiogenesis are not well understood (11). Our recent study suggests that S1P also plays an important role in tumor-induced lymphangiogenesis in vivo (8). In agreement with this notion, S1P has been shown to induce lymphangiogenesis in vitro via the S1PR1 receptor (12,13), and animals that lack SphK1 and S1P specifically in lymphatic endothelial cells (LEC) have a defect in maturation of lymphatic vessels (14). S1P also stimulates LECs to release angiopoietin 2 (Ang2) ((15) and Fig. 2D), a critical regulator of lymphangiogenesis (16), whose levels in breast cancer correlate with metastases to lymph nodes and poor prognosis (17).

Our overarching hypothesis is that S1P produced by SphK1 is a previously uncharacterized cancer-induced lymphangiogenic factor that is critical for breast cancer progression and metastasis. We further suggest that intricate crosstalk between S1P released from the primary tumor and/or LECs and Ang2 released from LECs via S1PR1 play important roles in breast cancer-induced lymphangiogenesis (Fig. 1), resulting in lymph node metastasis and reduced survival. To evaluate these hypotheses, we propose the following specific aims:

AIM 1. Determine the role of the SphK1/S1P/S1PR1 axis and Ang2/Tie2 amplification loop in lymphangiogenesis in vitro: Based on our preliminary results, we will interrogate the novel concept that SphK1/S1P/S1PR1 axis is essential for Ang2 driven lymphangiogenesis. According to this paradigm, binding of Ang2 to Tie2 on LECs stimulates SphK1 leading to formation and subsequent release of S1P that in turn binds to S1PR1, leading to lymphangiogenesis and further release of Ang2 (Fig. 1). Using pharmacological and molecular approaches we will demonstrate that this feed-forward amplification loop is critical for lymphangiogenesis in vitro. We will also determine how Ang2 activates SphK1, and how S1P is released from LECs, focusing on ABC transporters (7) and Spns2 (18).

AIM 2. Determine the in vivo role of SphK1/S1P/S1PR1 axis in breast cancer-induced lymphangiogenesis: Murine breast cancer cells with varying metastatic potential will be orthotopically implanted into immune competent mice as we described (19) and cancer progression including lymph node metastasis will be monitored in live animals by bioluminescence. Concomitantly, effects on regional lymph node lymphangiogenesis and peri-tumoral lymphangiogenesis and levels of S1P in tumors, blood, and lymph will be determined and the effects of pharmacological agents targeting the SphK1/S1P/S1PR1 axis and Ang2 examined. Complementary genetic approaches with SphK1 null mice (low blood and lymph S1P), mice lacking S1P only in blood (20) or in lymph (14), and Spns2 null mice will also be utilized to examine the potential contribution of host S1P to cancer-induced lymphangiogenesis. Furthermore, we will seek additional clinical evidence in support of our hypothesis by analyzing SphK1, S1PR1, S1P, and Ang2 in tumors, regional lymph nodes, and blood samples from breast cancer patients.

Aim 3. Determine the sources of S1P involved in lymphangiogenesis and metastasis of spontaneous breast tumors: We will generate mammary epithelium-specific sphingosine kinase-deficient mice and LEC-specific S1PR1 knockout mice. These mice devoid of S1P production or signaling in specific tissues will be bred with the MMTV-Her2/neu spontaneous breast tumor model. These genetic approaches will not only delineate the pathophysiological role of S1P in lymphangiogenesis and metastasis of spontaneous breast cancer, but will also provide mechanistic insights into the cellular sources of S1P involved in these processes.

These studies will lead to better understanding of the role of SphK1/S1P/S1PR1 axis in breast cancer progression and lymphangiogenesis that could lay the foundation for addition of a novel S1P-targeted modality to the armamentarium of anti-breast cancer treatment.