Public Abstract

Metastasis, the process by which a tumor spreads throughout the body, is responsible for the majority of cancer-associated deaths, and while prognoses have steadily improved for early stage breast cancers, stage IV metastatic breast cancer remains an incurable disease. Over the past decade, oncology has undergone a renaissance in the form of tumor immunotherapy, culminating in the 2018 Nobel Prize in Medicine. Immunotherapy refers to therapeutic approaches that seek to instruct the body's own immune system to attack the cancer. Our immune systems are in a constant state of balance wherein they remain sufficiently active as to eliminate pathogens (e.g. bacteria, viruses, parasites, etc.) but not so overactive that they attack the body itself (e.g. autoimmune diseases). Eliciting immune responses to cancers is a challenge because, while our immune systems frequently are able to recognize malignancies as foreign and eliminate them, they often view the cancer as "self" and do not attack it. Immunotherapies act by tipping this balance in favor of an activated immune state, and in doing so, cause the immune system to attack and eliminate the cancer. These therapies have demonstrated remarkable successes in patients with stage IV melanoma, lung cancer, renal cell carcinoma, and more, yet stage IV breast cancer has remained unresponsive to these therapies. The rationale for why these therapies have been ineffective in breast cancer is thought to surround mechanisms that suppress the immune response to keep immune cells from accessing and infiltrating tumors.

Before breast cancers spread to distant sites such as the bone, lungs, or brain, they first spread to lymph nodes (LNs). The lymphatic system drains fluid and debris from tissues and collects them in intermittent depots referred to as LNs. LNs contain vast numbers of immune cells, known as lymphocytes. These lymphocytes traffic to LNs where they are exposed to various molecules, referred to as "antigens" that have drained from nearby tissues through the lymphatics. The context in which these antigens are presented to lymphocytes within LNs dictates whether the lymphocytes should respond to or ignore them when they encounter them in other tissues. After undergoing this education process in LNs, lymphocytes traffic to distant tissues, and if they encounter those antigens in those tissues, they respond according to how they were educated in LNs, either attacking the cells that express the antigens or ignoring them.

Traditionally, the occurrence of LN metastases prior to distant metastases has been attributed to plumbing: tumor cells that break off from a primary tumor may find themselves within lymphatics and the first site that they encounter is a LN. Nonetheless, LNs harbor a vast array of lymphocytes with the potential to react to these tumor cells, so it would be reasonable to expect that tumor cells that arrive in LNs would be rapidly eliminated. Yet, LN metastases are frequent in breast cancer and precede dissemination to distant tissues. Recently, **we discovered that when tumor cells metastasize to LNs, they act locally upon the lymphocytes therein to reeducate them to be tolerant of the tumor. Then, when those lymphocytes traffic to distant tissues, they bring that tolerance with them in a manner that allows tumors to seed and grow in those distant sites.** This proposal seeks to revert this education process to stop the tolerance and render the lymphocytes reactive to the tumors so that they can traffic to distant tissues and eliminate the metastases.

The first portion of this proposal focuses on understanding the biological mechanisms that allow LN metastases to condition the immune system. We will develop a mouse model of LN metastasis (Aim 1) and use this model to discover the biology behind this process (Aim 2). Our approach employs a variety of advanced technological approaches including next-generation sequencing of tumor cells, single-cell sequencing of immune cells, computational algorithms that we have developed to generate maps of the immune system, and a platform for tracking cells as they traffic throughout the body of a mouse using a fluorescent protein. These toolsets will not only be useful for our own studies surrounding LN metastasis, but will also serve as resources to the breast cancer metastasis research community.

The second portion of this proposal seeks to use the insights gleaned from the first portion to develop new therapies that switch the immune system from being tolerant of the tumor to reactive against it. We will generate therapies consisting of LN metastasis-targeting antibodies linked to molecules that disrupt the ability of the tumors to induce immune tolerance. This therapy represents a novel approach to targeting cancer by specifically inhibiting the effects of LN metastases. We will also combine these therapies with classic immunotherapies with the expectation that after disrupting the tolerance program in the LNs, the immunotherapies will be able to activate anti-tumor immune responses against distant metastases. This approach represents a novel treatment strategy with the potential to turn stage IV breast cancer into a disease that is curable.