Regulatory and phenotypic effects of metastatic genes and environments

Overview/Abstract

Almost all primary breast cancers are treatable by surgery and/or radiation, but that unfortunately is rarely the case upon metastasis. On average more than 70% of invasive breast cancer (BC) metastasize to other organs. Most cancer patients die of metastatic breast cancers (MBC) due to the emergence of aggressive cellular phenotypes. Knowing the evolutionary paths of these aggressive phenotypes would enable us to predict MBC from non-metastatic disease, which would lead to better patient stratification and treatment design. As all cellular alterations, aggressive phenotypes can result from genetic, microenvironmental or stochastic causes. Genetic alterations driving MBC evolution have been extensively studied. Accumulating data indicate a limited role of single genetic mutations in the emergence of aggressive phenotypes, whereas microenvironmental conditions that select for cancer cells with those phenotypes are less studied. On the one hand, we have shown that the harsh tumor microenvironment (TME) of breast cancer including acidosis, starvation, and hypoxia can cause cancer cells to acquire aggressive phenotypes such as local invasion and metastasis (*1*–*3*). Besides external microenvironmental factors, internal molecular levels are major contributors to phenotypes at the cellular and cell-population levels. On the other hand, we showed that gene regulatory networks play a crucial role in driving metastasis (*4*, *5*) . Our recent work highlighted the nonmonotone effects of regulatory transcription factors (TFs) in metastasis, specifically through the study of BACH1, a metastasis activator. Using synthetic gene circuits to tune BACH1 levels up in metastatic MDA-MB-231 breast cancer cells revealed a nonmonotonic response of invasiveness to BACH1 expression (*6*). Despite these advances, *the joint effects of precisely controlled external stress factors and internal protein levels have not been investigated*. This is what we propose to initiate in this seed grant application.

In this proposal we seek to address this critical challenge by integrating synthetic biology and evolutionary OMICS approaches to conduct quantitative investigations of regulatory genetic and microenvironmental factors related to metastasis in breast cancer. By leveraging advanced genome engineering techniques alongside single-cell multi-omics analyses, we aim to dissect the regulatory networks and phenotypic adaptations that enable cancer cells to survive, evolve, and thrive in harsh microenvironments selecting for metastatic phenotype. For this pilot study we will focus on acidosis as one of the main TME stressors, shown to drive metastatic phenotype(*7*, *8*). *Our hypothesis is that adaptation to acidic microenvironment can synergize with BACH1 expression to cause gene regulatory perturbation leading to phenotypic heterogeneity/plasticity evolvable to metastatic disease.* With the long-term goal is to understand and control the causes of breast cancer metastases, here we focus on the regulatory and phenotypic effects of precisely controlled gene expression combined with cellular adaptation to metastasis-promoting acidosis, in 3 Aims.

Aim1: Engineer cell lines with synthetic gene circuits for precise up or down-regulation of BACH1 and other genes.
Aim2: Determine the molecular-regulatory responses to precise internal and external perturbations relevant to metastasis.
Aim 3: Determine the phenotypic responses to precise internal and external perturbations relevant to metastasis in 2D and 3D cell culture models.

We have synergistic expertise in co-engineering genomes with synthetic gene circuits and in conducting multi-omics examinations upon evolutionary adaptation to environmental conditions promoting metastasis. This project will generate preliminary data crucial for joint R01 and U01 submissions in the near future.