Title: Correlating 3D Images of Organoids to the Invasive Potential of Tumors

What is the status of IRB approval? Initial work is being conducted using organoids generated from mouse mammary tissue. Work on human tissue is permitted under an existing IRB-approved protocol that permits creation of organoids from patient tissue but not generation of genomics data. A protocol and consent form for generation of organoids and genomics data from participants with a diagnosis of breast cancer and from non-cancerous participants undergoing surgical breast biopsy is under IRB review. The protocol, consent, and data security forms were pre-reviewed by a bioethicist and a genomics scientist who are themselves IRB members. Forms were submitted on 5/24/2018 and IRB-6, whose members have expertise in oncology. The IRB has been convened, with minor changes requested.

Background:

Metastasis is one of the main determinants of patient prognosis since mortality is often due to systemic spread of tumor and not in situ proliferation. For example, the 5-year survival rate for invasive breast cancer at diagnosis is 99% for patients with local tumors, 85% for those with regional spread, and only 26% for those with tumors that have spread to distant organs[1]. Currently, the molecular mechanism of metastasis is not well understood, and neoplastic drug interventions mostly target proliferation, not metastatic transformation.

It is important to understand the molecular mechanism of metastasis and have a reliable system to accurately and quantitatively characterize the invasive potential of different types of tumors to be able to develop therapeutic drugs that can improve outcomes for patients with metastatic carcinoma. Quantitative phenotypes are more amenable to systematic analysis and can be used in population genetics methods, where genetic variations and invasiveness can be studied by directly perturbing candidate gene. Such studies can lead to the discover of new targets for cancer therapy.

A quantitative model to study and characterize metastasis, or to predict the metastatic propensity of a tumor, has yet to be developed. On the other hand, there have been recent studies on the molecular basis for metastasis: Cheung et al. have demonstrated that tumor cells can invade and metastasize in clusters challenging the single-metastasis model, where a single tumor cell seeds a distant organ and begins to proliferate. Thus, we will develop and test an automated system that uses organoids, which are a cluster of 100’s of cells that are designed to serve as proxies for tumors in vivo, to model the invasive potential of tumors with different phenotypic profiles.

Research question or project aim:

1. Develop a robust technique for quantifying organoid invasiveness using genetically engineered mouse models.   
2. Apply the technique to organoids generated from human tumors and correlate model outputs with known molecular biomarkers of prognosis measured in the same sample,   
3. Correlate organoid invasiveness and molecular biomarkers with patient outcomes.

Hypothesis:

Hypothesis 1: Organoid invasiveness and organoid size are correlated with expression of Keratin 14, a known molecular biomarker whose protein expression is a known correlate with poor outcomes [2]. Thus, the first aim of the project will be to test the hypothesis that K14 is directly correlated to invasiveness versus the alternative hypothesis that organoid size drives both K14 expression and invasion.

Hypothesis 2: The second aim of the project will be to test the same hypothesis using organoids from human breast tumors.

Hypothesis 3: Determine whether organoid invasiveness correlates with poor patient outcomes versus the alternative hypothesis that patient outcomes are independent of organoid invasiveness.

Timeline and Additional Support Needs:  
  
My mentor has already put together a system that works, but he is also waiting for more organoid image data. I plan to become thoroughly familiarized with the system he has put together before the summer.

Project Method:

Sample Preparation:

Genetically engineered mouse models will be used to produce a supply of organoids with identical genetic background. Tumors will be removed from 3-5 mice, and a total of ~100 small organoids (<150 cells) and ~100 large organoids (>300 cells) will be generated per tumor. Organoids will be systematically generated at a range of size to permit a test of our hypothesis that the molecular biomarker for invasive tumors, K14, is directly correlated to invasiveness versus the alternative hypothesis that organoid size drives both K14 expression and invasiveness. The organoids will be imaged using differential interference contrast (DIC) microscopy. The images will be manually traced in ImageJ to define the organoid boundaries. Corresponding K14 images will also be available.

For the second part of the study, a total of human breast tumor specimens will be obtained from ~25 participants. The specimens will be processed and prepared in the same way as the mouse specimens. Again, the human organoids will be imaged using DIC and their boundaries traced manually using ImageJ. Corresponding K14 images will also be available for the human dataset.

Computer Model:

Let manually traced organoid boundaries be defined as a pair of points for , where P is the total number of points. These points will be mapped to a curve by using the length, L, as a parameter to a linear interpolator. The length, L, is equal to . A fast Fourier transform will be applied to this curve to generate a spectral power representation that has rotational and translational invariance.

The transformation is done separately for x and y coordinates, and the transform in x is given by , where M is the total number of points of the boundary after linear interpolation and is equal to 128 for all boundaries. is the frequency and is an element of {0, 1…, M-1}, and is an integer element of {0, 1..., M-1}. Similarly, the transform in y will be .

Furthermore, a normalization term is applied to achieve scale invariance. Additional spectral characterizations based on means and parametric derivatives will also be used to generate the final invasive score for an organoid.

The model is implemented in and will be used to test all the hypotheses set forth. For Hypothesis 1 (size vs. invasiveness), we will individually test for the correlation of K14 expression with invasion within the small organoid set and the large organoid set. These tests will be performed using a linear model. For Hypothesis 2 (same but for human samples), methods will be similar to Hypothesis 1. For Hypothesis 3 (survival outcomes), we will use a Cox proportional hazards model using the invasiveness and the K14 expression as factors.

Reference:

1. Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. "Cancer statistics, 2016." *CA: a cancer journal for clinicians*66.1 (2016): 7-30.
2. Cheung, Kevin J., et al. "Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters." *Proceedings of the National Academy of Sciences* 113.7 (2016): E854-E863.