**Computer vision-based analysis of tumor cell invasion and adhesion**

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**Abstract:**

**Innovation and Impact Statement:**

This is a new collaborative project between the Bear and Gomez groups that brings together expertise in the cell biology of tumor cell behavior and computer vision-based analysis of massive image data sets. With the advent of multicolor live-cell imaging and gene manipulation, there is an increasing need to find new methods to extract quantitative information from images and movies. The cellular structures that are the focus of this project, invadopodia and focal adhesions, are critical for tumor cell metastasis. Yet the standard in the field for analyzing these structures is to hand select a small number (<10) and perform limited measurements of area and some dynamic properties. The method under development here will allow unbiased analysis of *all* the invadopodia and/or focal adhesions (~50-1000 per cell) over 12-16 hours. This level of data analysis is required to identify subtle or combinatorial effects of genetic and/or pharmacological manipulation. Beyond method development, this approach will allow us to understand the effects of genes known to be involved in tumor metastasis such as PTEN and LKB1 that have never been studied at this level. Furthermore, we will evaluate the effects of B-Raf inhibition on these structures using the new Plexicon compound in a B-Raf mutant melanoma cell line. Thus, this project will have a significant impact on the field of cancer metastasis by allowing the inter-related dynamics of extracellular matrix degradation and cellular adhesion to be studied at an unprecedented level.

**Specific Aims:**

In this proposal, we seek to develop methods to analyze tumor cell behavior using computer vision-based techniques. We will use these methods to understand the role of known pro-invasive genes and test the role of metastasis-implicated genes such as PTEN and LKB1 whose role in matrix degradation and adhesion control is unknown. To accomplish this, we propose two specific aims:

**Aim 1. Computer vision-based analysis of invadopodia:** In this aim, we will develop an automated, computer vision-based assay to quantify invadopodia population statistics and dynamics from live-cell movies. Using this assay, we will evaluate gene knockdowns of candidate metastasis genes such as PTEN and LKB1. In addition, we will evaluate the use of this tool with pharmacological inhibitors such as the B-Raf inhibitor.

**Aim 2. Multiplex analysis of invadopodia and focal adhesions:** In this aim, we will combine or multiplex the established method for quantification of focal adhesion dynamics with the new method of invadopodia quantification from Aim 1 using 3-color live-cell imaging. By simultaneously and quantitatively examining these structures, we can begin to ask questions about how cells manage to dynamically balance adhesion and matrix degradation to achieve invasive motility.

**Background:**

Advanced melanoma is one of the most feared human cancers {Sharpless, 2003 #5122; Chin, 1998 #5123}. Although curable through surgery when diagnosed at early stage, melanoma is characterized by its therapeutic resistance, aggressive clinical behavior, and proclivity for early metastasis. Early metastasis is a key clinical feature of melanoma, and in perhaps no other malignancy is the ability to metastasize more closely correlated with clinical outcome {Cochran, 1997 #5243; Ahmed, 1997 #5244}. Metastasis is the process by which tumor cells leave the primary lesion and move to other parts of the body, thereby initiating tumor formation at multiple sites. During this process, cells detach from their adhesive contacts, degrade barriers such as the basement membrane, migrate to other locations within the body, invade new organs, and regain adherence to re-initiate growth {Kopfstein, 2006 #6767}. It is therefore not surprising that metastatic tumor cells have high *in vitro* cell motility and invasive capabilities.

Through the combined work of many labs, a conceptual framework for understanding cell migration has emerged {Lauffenburger, 1996 #304}. Motile cells display a characteristic cycle of steps leading to translocation. First, cells must become polarized, thereby defining a front-to-rear axis for directed movement. Second, once a cell is polarized, it protrudes a structure such as lamellipodia at the leading edge. Third, after the lamellipodia has extended forward, it must become stabilized by attachment to the underlying substratum. These attachment points are known as focal contacts or focal adhesions and contain clustered integrin receptors that create a bridge between the extracellular matrix and the actin cytoskeleton. Fourth, the cell body is then squeezed forward from the rear in an actomyosin-based contraction event. Finally, cells must lose substratum attachment at the rear or trailing edge. In the case of tumor cells, these general steps of migration must be preceded by the local degradation of extracellular matrix using spot-like structures called invadopodia that are heavily enriched with matrix metaloproteases (MMPs). The coordination of matrix degradation by invadopodia and adhesion to matrix required for movement is poorly understood.

Computer vision approaches have been applied in cellular biology settings to analyze large collections of both still and time lapse microscopy to quantitatively assess cellular phenotypes. The applications of computer vision approaches to analyzing microscopy images have ranged from large siRNA-based screens designed to discover proteins involved in mitosis (Neumann 2010) to tracking the effect of camptothecin on protein localization in individual cells (Cohen 2008). In the field of cellular motility, studies have demonstrated, using computer vision techniques, the subtle connection between cell edge movement and the Rho GTPases (Machacek 2009). These applications of computer vision techniques demonstrate the power of combining computational image processing techniques to determine the connection between perturbations cells and phenotype. By applying computer vision methods to examine Invadopodia and the interplay between Invadopodia and focal adhesions, this proposal seeks to determine the effects of several candidate genes on metastasis and develop sets of image processing tools that can be widely applied.

**Preliminary Studies:**

In collaboration with the Hahn lab at UNC, the Gomez lab has developed a software suite which automates the analysis of time-lapse movies of fluorescently tagged focal adhesion. This work has been submitted to PLOS Biology and is currently in revision. The publication describes a set of programs that automatically finds the focal adhesions in each frame of a provided movie, tracks those focal adhesions through time and then collects various sets of properties that describe the overall properties of the focal adhesions (Figure 1). To These automated methods are comparable in technique to the prior methods used to assess focal adhesion properties (Webb 2004), but the computer vision approaches allow all the detectable adhesions to be analyzed. Thus, while manual methods are able to quantify small number of adhesions (10-25), the automated methods we have developed can scale to collect hundreds of data points (Figure 2).

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| graphic_workflow.png  Figure 1: Summary of Focal Adhesion Analysis Methods | kinetics.png  Figure 2: (A) Sample tracking of single adhesion (green), surrounding adhesions (blue) and the cell edge (red) (B) The intensity of the adhesion in A over time (C-E) Summaries of the assembly rates and positions at birth and death of the adhesions. |

**Research Plan:**

**Aim 1. Quantitative analysis of invadopodial dynamics:**

Rationale

Experimental plan [WM2664 as a model, get some input from Dave here]

Potential pitfalls

**Aim 2. Combined imaging of invadopodia and focal adhesions:**

Rationale

Experimental Plan

Potential pitfalls