NOVEL QUANTITATIVE APPROACH FOR INVESTIGATING TISSUE STRUCTURE-FUNCTION RELATIONSHIPS OF BREAST CANCER METASTATIC POTENTIAL

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Abstract

Cancer is the second leading cause of death after cardiovascular disease in the United States. In 2010, 1.5 million new cancer cases were reported in the United States alone and over 550,000 cancer related deaths, which resulted in more than 1500 deaths per day. Of particular interest is breast cancer, which is the second leading cause of cancer related deaths in women with over 40,000 deaths and over 200,000 new cases of invasive carcinoma in 2010.

Detection of breast cancer begins with a breast examination or mammogram. If a lump is identified, a biopsy is sent to a pathologist to analyze the tissue architecture. While pathologists are highly trained to identify qualitative features of a tissues structure and to subsequently assign those features to a particular cancer grade, this method has a high degree of subjectivity and variability. In 2000, a Swedish study reported only 30% consistency of breast cancer grading between ten independent pathology labs utilizing the standard staging system. Unfortunately, misdiagnosis may result in a lack of treatment as well as in administration of unnecessary treatments and procedures. Misdiagnosis is estimated to affect as high as 12% of cancer cases according to some reports. For example, in 1999 The Johns Hopkins Hospital in Baltimore published the results of a study that showed a 1.4% error rate in pathology tests in patients referred for cancer treatment.

The Scarff-Bloom-Richardson (SBR) tumor grading system is the most frequently used grading system to classify breast cancer. The SBR system grades a tissue based on cell division, tubule formation, cell size, and uniformity. A major limitation of this system is that it is dependent on the visual inspection of biopsied tissue sections by trained pathologists. Unfortunately, this semi-quantitative approach can result in a high degree of variability. Furthermore, since the majority of these tissue sections are analyzed with standard dyes (i.e. hematoxylin and eosin) one may miss the presence of additional structural features (i.e. localized membrane proteins) that hold the potential to aid in the correct classification of biopsied tissue samples. Thus, the development of a method to quantify unique structural features specific to breast cancer tumorigenesis is needed for generating a more rigorous diagnostic system for breast cancer detection and subsequent treatment.

In order to identify and quantify unidentified complex acinar structural changes along the metastatic cascade, we first developed a three dimensional culture system to

mimic the microenvironment of the normal breast tissue. More specifically, we utilized six related breast epithelial cell lines that represented the various stages of breast cancer tumorigenesis ranging from a normal to invasive phenotype. We subsequently monitored the localization of two integrin membrane markers, α3 and α6, over the course of two weeks using fluorescent confocal microscopy. From these microscopic images we developed (in collaboration with Dr. Bülent Yener, Department of Computer Science, RPI) several metrics that captured the key acinar structural changes along the metastatic cascade including cell polarity, hollow lumen, size, and shape. A machine-learning algorithm was then trained to distinguish between cancer grades based on our pre-defined metrics. Furthermore, we tested the ability of our metric set to successfully detect structural changes by manipulation of the TGF-β signaling pathway. Our metrics confirmed structural changes associated with administration of TGF-β2, which initiated the acquisition of invasive cell morphologies in Ras transformed pre-cancerous cells; whereas treatment with the TGF-β receptor caused the loss of acquired invasive cell morphologies in malignant cells.

From these studies, we concluded our classification analysis is better suited and more accurate for 14 day samples relative to 7 day samples. Specifically, our metrics accurately differentiate between pre-cancerous, non-invasive carcinomas, and invasive carcinomas with an 82.3% classification accuracy when cultured for 14 days. In addition, we measured significant structural and cell polarity changes induced by altered TGF-β signaling in both pre-cancerous and invasive carcinoma acinar structures. Our conclusions suggest that cell polarity as well as acinar structures can be quantified and used to distinguish between ranging metastatic potentials. This quantitative method holds promise for future use as a clinical diagnostic tool. In addition, the development of our *in vitro* system has the ability to analyze structure-function relationships and to test potential therapeutic treatment's capabilities to reduce cell metastatic abilities, prevent cancer progression, or induce cancer cell death programs.