DEPARTMENT OF DEFENSE
BREAST CANCER RESEARCH PROGRAM (BCRP)
BREAKTHROUGH AWARD PRE-APPLICATION NARRATIVE FORM

PI or Initiating PI Last Name: _____ Log Number: _____

Application Title: Biomechanics of the Stromal Regulation of DCIS

1. What BCRP overarching challenge(s) will the proposed research address?

- ☐ Eliminate the mortality associated with metastatic breast cancer
- ☐ Prevent breast cancer (primary prevention)
- ☑ Distinguish aggressive breast cancer from indolent cancers; overcome the problems of overdiagnosis and overtreatment
- ☐ Revolutionize treatment regimens by replacing drugs that have life-threatening toxicities with safe, effective interventions
- ☑ Identify what drives breast cancer growth and metastasis; identify why some breast cancers become life threatening metastases
- ☐ Identify what makes the breast susceptible to cancer development
- ☐ Determine why some, but not all, women get breast cancer
- ☐ Determine why/how breast cancer cells lay dormant for years and then re-emerge (recurrence); determine how to eliminate dormant cells early
- ☐ Other (provide justification below)

If "Other" is selected, please provide justification within the context of the breast cancer landscape [*http://cdmrp.army.mil/bcrp/pdfs/bc_landscape13.pdf*] in the text box below. (140 character limit, including spaces)

2. How will the proposed research lead to a solution for the overarching challenge(s)? (2,000 character limit, including spaces)

This application directly addresses one of the problems identified in the "Breast Cancer Landscape" – that it is not possible to distinguish the ductal carcinoma in situ (DCIS) that will develop into invasive cancer (IC) from the DCIS that will not invade. As a result, many women are treated with redundant interventions that are associated with potential short-term and long-term morbidities. Most attempts to define morphologic or molecular indicators of DCIS progression have focused on the DCIS epithelial cells themselves. However, the stromal microenvironment surrounding DCIS is also altered, but the implications and origins of this stromal response have not been extensively studied. In the proposed project we will study a novel topic – the biomechanical forces exerted by DCIS, their consequences in the surrounding stroma, and the effect of the resulting stromal changes on the progression of DCIS to IC. One of our major goals is to identify stromal biomarkers that will distinguish aggressive DCIS that is likely to invade from indolent DCIS and thereby overcome the problem of overtreatment (overarching challenge #3). In addition, our studies will provide insight into those mechanisms that drive the progression of DCIS to IC and, potentially, to life threatening metastases (overarching challenge #5). In this proposal, we hypothesize that the intralumenal accumulation of neoplastic epithelial cells in DCIS imparts a tensile force on the surrounding stroma that activates resident fibroblasts to remodel the extracellular matrix (ECM). The extent and specific topography of the remodeled ECM will encourage the invasion of DCIS cells and the progression to IBC. Furthermore, we propose that specific features of the topography of the re-organized ECM can serve as biomarkers to identify DCIS that are most likely to progress to IC. Our hypothesis involves two major, interacting components – the biomechanical forces exerted by DCIS and the stromal changes that surround DCIS.

3. How does the proposed research move beyond an incremental advancement? (1,000 character limit, including spaces)

The role of biomechanical forces in cancer progression is an emerging area of study which has focused on invasive cancers. The biomechanical forces present in DCIS have not been measured and their effect on the resident fibroblasts and surrounding ECM has not been previously analyzed. A limited number of transcriptomic analyses of DCIS stroma have shown many gene expression changes at the transition from in situ to invasive carcinoma. Yet, analyses of the prognostic significance of these stromal gene expression changes are relatively few, and detailed analyses of the topography (i.e., organization, density, and orientation of matrix fibers) of the ECM surrounding DCIS and the prognostic significance of this topography, to our knowledge, do not exist. Therefore, the proposed project is breaking new ground by studying 1) the biomechanical forces in DCIS, 2) a translation of these forces into remodeled ECM, and 3) the prognostic significance of the topography of remodeled ECM.

4. What funding level (direct costs) is requested for the proposed research? Level 1 (up to $350K)

   Provide justification for the funding level. (140-character limit, including spaces)

This application is innovative, high-risk/high-reward research that is in the early stages of idea development and appropriate for level 1.

5. What period of performance is requested? 3 Years

6. Will the proposed research include a clinical trial? No

Rationale:

Ductal carcinoma in situ (DCIS) constitutes 20% of all newly diagnosed breast cancers and 30-40% of breast cancers identified mammographically. DCIS lesions are believed to be direct precursors of invasive breast carcinoma (IC), but are confined to the breast duct by a basement membrane. Only 15% of DCIS recur, as either non-invasive, invasive or metastatic disease. Yet, all DCIS, when detected, are treated. Current treatment for DCIS is typically breast conserving surgery followed by radiation therapy. Therefore, many DCIS are over-treated. There is a need for molecular or pathologic features (prognostic indicators) that reliably identify those DCIS that will recur or progress. Identification of these prognostic indicators will require a better understanding of the mechanisms underlying the progression of DCIS to IC.

It is well-established in ***IC*** that the tumor stroma and microenvironment are critical determinants of cancer progression and therapeutic response. Breast cancer stroma is composed of multiple cell types, but principal among them is the fibroblast and the extracellular matrix (ECM) that it produces. We, and others, have shown that fibroblasts in IC differ from fibroblasts in normal breast in their ability to regulate epithelial cell growth and in their gene expression profiles. In cancer, fibroblasts are activated to remodel ECM and up-regulate expression of a variety of growth factors, and thereby, promote cancer growth, invasion, and metastasis. However, fibroblast activation and matrix remodeling are not limited to IC, but are also found to varying degrees surrounding DCIS. Several investigators have performed gene expression profiling of stromal and epithelial compartments in DCIS and IC and found few gene expression changes in the epithelium during the transition from in situ to invasive disease, but found significant expression changes in the stromal compartment. These data suggest that the stroma in DCIS plays a functional role in driving the progression of DCIS.

The role of biomechanical forces in cancer development and progression is an emerging area of study which has focused primarily on IC. Compared with normal breast, IC are stiffer, with a higher elastic modulus. The increased stiffness is largely a result of fibroblast activation and ECM remodeling. In turn, the increased tension in IC stroma causes cancer epithelial cells to up-regulate multiple signaling pathways, resulting in cancer growth, invasion, and metastasis. The biomechanical forces present in DCIS have not been specifically measured and the effect of these forces on the surrounding stroma has not been previously studied. In this proposal, we will use innovative in vitro models to measure the forces exerted by DCIS on surrounding stroma and determine the response of stromal fibroblasts. We will also investigate the use of a novel “biomarker” of recurrence and progression of DCIS to IBC – the topography of the remodeled ECM surrounding DCIS.

The experience of the initiating and partnering PIs is an ideal melding of capabilities to pursue the important and understudied topic of the biomechanical regulation of breast cancer development and progression. Dr. Andra Frost is an anatomic pathologist and breast cancer researcher with diagnostic expertise in breast cancer and a research background in fibroblast-epithelial interactions in breast cancer. Dr. Joel Berry is a bioengineer with experience in tissue and vascular modeling, bioreactor design and construction, and image analysis.

Approach:

We will test several components of our overall hypothesis, as stated in the Pre-application Narrative Form. Specific Aim 1 will focus on quantifying the biomechanical forces in DCIS. First, we will establish the presence of circumferential forces surrounding DCIS, using an in vitro model of DCIS tissue. Second, we will determine whether the circumferential forces activate stromal fibroblasts to begin ECM remodeling (Fig 1) and assess the impact of this fibroblast activation on the growth and invasion of DCIS cells. Specific Aim 2 will focus on the influence of the resulting reorganized ECM topography on the DCIS cells. We will also determine the feasibility of utilizing the topography of the remodeled collagen, specifically matrix fiber density, fiber alignment and orientation, and fiber spacing (“porosity”), to identify those DCIS most likely to progress to invasive, and potentially, metastatic carcinoma. These ECM parameters will be measured in DCIS tissues with and without IC using quantitative image analysis after laser scanning microscopy to highlight ECM arrangement.

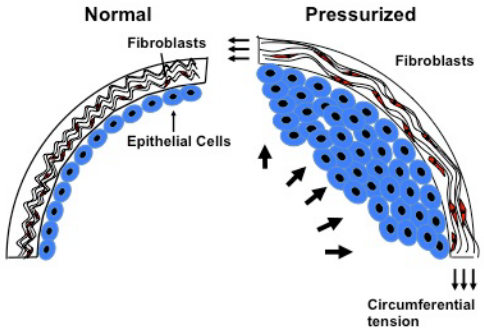

Fig 1. Depiction of lumenal (internal) pressure in DCIS extending to interstitial stroma. Two states are represented. In the first normal pressure state (left), there is normal lumenal pressure and tension in the interstitial stroma. In the second state (right), increased lumenal pressure from proliferating DCIS cells causes circumferential tension in the interstitial stroma, ECM and fibroblasts.