PROJECT SUMMARY

Epithelial-stromal interactions are critical determinants of epithelial cell behavior in a tumor. While the stromal microenvironment and alterations in the stromal extracellular matrix (ECM), influences invasive and migratory properties at the invasive front, the role of the epithelial matrix in influencing cancer cell behavior are less well understood. Extracellular matrix (ECM) perturbations in epithelial cancers contribute to metastatic spread accounting for 80% of the cancer-related deaths worldwide. The molecular cues that affect the ECM and precede stromal influences on epithelial cell metastasis, is a critical first step at identifying pathways that can be molecularly targeted in the future.

The overarching goal of this project is to determine whether alterations in the extracellular matrix of cancer epithelial cells can sufficiently alter cancer cell invasion, migration and metastatic propensity. Fibronectin (FN) matrix dysregulation in response to hypoxia using hypoxia mimetics and 1% O_2 will be investigated in the MCF10A progression series to determine whether cellular responses in the three cell lines alternate between premalignant and malignant phenotypes or preinvasive and invasive phenotypes. During low O_2 levels in the cells, Hypoxia-inducible factor (HIF)-1α and HIF2α proteins undergo stabilization and translocate to the nucleus to enable transcription of genes involved in cell survival, angiogenesis, and metabolism. An angiogenic transcriptional target of HIFα is VEGF (Vascular endothelial growth factor), which interacts with the C-terminal Heparin-II domain of the FN protein. Using non-contacting and contacting transwell assays, with endothelial cells, the role of FN fibrillogenesis in binding VEGF or releasing VEGF to promote angiogenesis will be determined. While breast cell lines are used in this study, the project goal applies to epithelial cancer cell invasion and migration and hypoxia-mediated angiogenesis that depend on FN fibrillogenesis.

The long-term goal of this study is to provide new information on unfavorable FN matrix changes that can be perhaps stabilized that in combination with existing therapeutic strategies will help alleviate cancer cell aggression.

SPECIFIC AIMS

Breast Cancer is one of the most common cancers found among women of every racial and ethnic group [1], however, significant disparities exist between, for example, African American Women compared to White Women [2]. Breast cancer heterogeneity, resistance to therapy, and later stage disease at diagnosis has contributed much to the disparity in cancer incidence and mortality in underrepresented communities [3-5]. While breast cancer incidence is often lower in minority populations, aggressiveness is often heightened and mortality often disproportionate [6]. The most common breast cancer is breast carcinoma that arises from the epithelial cells lining the lobules and ducts. Using a breast cell line progression series of the disease, the main goal of this proposal is to investigate whether epithelial phenotypes can be sufficiently manipulated to alleviate cancer aggression.

Extracellular matrix (ECM) perturbations in epithelial cancers contribute to metastatic spread accounting for 80% of the cancer-related deaths worldwide. Fibronectin (FN) is an ECM glycoprotein that in its polymeric form can prevent transformation and invasion. Hypoxia, a cellular adaptation in tumors enhances FN assembly, contributing to aligned FN fibrils in cancer cells. While the ECM remodeling in the cancer stroma and hypoxia are known, ***there is an gap in our understanding of how hypoxia affects epithelial cancer cell fibrils and whether FN fibril perturbations in these cells may either enhance or retard tumor invasion, migration and angiogenesis.*** A systematic analysis of the function of FN fibrillogenesis in epithelial cancer cells of varying invasive behavior is a critical first step in targeting early events in cancer spread.

To address this problem, we will perturb hypoxia-induced fibrillogenesis in untransformed MCF10A, premalignant MCF10AT and pre-invasive MCF10DCIS.com breast epithelial cells. Using the FUD peptide that specifically targets FN assembly, we can specifically ask whether altering the polymeric state of FN in response to hypoxia, is sufficient to drive changes in invasive, migratory and angiogenic potential of less invasive cells. *Our long-term goal is to determine whether less-favorable FN matrix assemblies in epithelial cancers can be stabilized to reverse a malignant phenotype*. Our study would establish that tweaking FN polymerization in epithelial cancer cells of varying invasiveness would either enhance or retard cancer aggression.

Specific Aim 1. ***Determine whether hypoxia alters FN fibrillogenesis in the MCF10A breast cancer progression series of cells***

This aim will be addressed in two parts. (1a) The effect of hypoxia on measuring FN fibrillogenesis, (1b) FN turnover in mediating FN fibrillogenesis. Using the MCF10A progression series of cell lines we will test whether hypoxic cellular environments affect FN matrix assembly in breast epithelial cells cultured on 2D and 3D matrices. Fibrillogenesis regulation will also be investigated using endocytosis assays of Rhodamine labeled FN and FN secretion in the media to establish whether hypoxia-mediated fibrillogenesis is coupled to uptake and reformation of fibrils

Specific Aim 2. ***Determine whether hypoxia-mediated fibrillogenesis alters invasion, migration and cell survival mechanisms***

This aim will investigate transwell migration and invasion of the MCF10A series of cells in collagen. Apoptotic, anoikis and autophagic mechanisms that play a role in hypoxia and the alteration of these responses when fibrillogenesis is abrogated will also be investigated.

Specific Aim 3. ***Determine the role of fibrillogenesis in angiogenesis.***

The angiogenic role of fibrillogenesis will be determined in this aim. VEGF binding to FN and VEGF secretion into the media will be determined using VEGF immunoassays. Co-culture of endothelial cells with the MCF10A series of cells will be used to determine whether VEGF secretion is enhanced. A non-contacting transwell assay with endothelial and epithelial cells will be used to carry out an in vitro angiogenesis assay to determine the biological readout of fibrillogenesis on angiogenic potential.

The proposed study will establish the role of fibrillogenesis in tumor epithelial cells, and provide new data on how changes in matrix FN can alter the cancer cell phenotype. Using critical mediators of metastasis such as invasion, migration, anoikis, autophagy and angiogenesis, our work will test whether cancer cells with varying degrees of invasion can acquire phenotypes that contribute to more aggressive cancers. This study will provide novel and useful insight on possible mechanisms that can be used to tweak FN assembly into their more unfavorable forms to inhibit cancer metastasis.