Tissue Clearing as a Mechanism to Identify Changes in Fibronectin Structure During Breast Cancer Metastasis
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ABSTRACT
With metastasis accounting for approximately 90% of breast cancer deaths and an alarming number of over 300,000 new breast cancer cases to be diagnosed by the end of 2018, there is growing need to understand the process of breast cancer. Changes in the extracellular matrix (ECM) of the tumor microenvironment play an essential role in this deadly tumor progression. Specifically, the glycoprotein fibronectin (FN), has been identified to be up-regulated in patients with worse clinical outcomes. During tumor progression fibronectin undergoes conformational changes that aid in metastatic dissemination. In order to analyze the dynamic changes in FN expression and evaluate architectural changes within the metastatic niche, electrophoretic tissue clearing was used to image changes in whole murine lungs that have undergone breast cancer metastasis. The X-CLARITY tissue clearing system utilizes electrophoresis which removes the lipid compounds from tissues to make them optically transparent. Coupling this with the c-PRESTO immuno-labelling technique and confocal light microscopy allows imaging of FN expression and structure in whole murine organs. In this study, a tissue clearing, and staining protocol was developed and used to visualize the 3D FN structure in whole murine lungs. Future studies will evaluate changes in FN expression and structure over time during cancer progression. This would contribute to on-going research on the development of more effective breast cancer drugs that aim to combat metastasis.

KEYWORDS
Tissue clearing, X-CLARITY, Fibronectin, Breast cancer metastasis, whole organ clearing, 3D tissue imaging