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Development of a Bioinspired Stroma Model to Study the Role of Collagen Topology in Pancreatic Ductal Adenocarcinoma

Cole Drifka,[1,2] Agnes Loeffler,[3] Kevin Eliceiri,[1,2] W. John Kao[1,2]

[1.] Department of Biomedical Engineering, University of Wisconsin, Madison, USA
[2.] Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, USA
[3.] Department of Pathology, University of Wisconsin, Madison, WI, USA

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest human malignancies. It is currently the fourth-leading cancer killer in the U.S and projects to be second in cancer fatalities by 2030. The very poor prognosis for PDAC patients is largely attributed to a propensity for early and aggressive metastasis, late diagnosis, and resistance to current therapeutic regimens. Despite ongoing research, little significant progress in improving patient survival has occurred over the past decades. Traditionally, PDAC research and histopathological evaluation of tumor tissue has focused primarily on the genetics and behavior of transformed cells. There is growing evidence, however, that a dynamic interplay between cancer cells and the adjacent stroma influences tumor growth, angiogenesis, metastasis, and therapeutic resistance [1].

A mainstay tissue component of most tumor stromas, including PDAC, is the extracellular matrix protein collagen. Recently, a number of groups have shown that collagen fiber organization is uniquely altered in various cancer types and may carry biological and clinical significance [2,3]. To date, visualization of collagen-based changes has been greatly accelerated by Second Harmonic Generation (SHG) imaging, a laser scanning microscopy technique that can provide high-resolution, quantifiable images of discrete collagen fibers in intact tissues without the need for exogenous staining. For example, researchers have used SHG to identify unique collagen organizational patterns in breast cancer coined "tumor-associated collagen signatures" (TACS). One of these signatures, TACS-3, describes bundles of straightened, aligned collagen fibers oriented perpendicular to the tumor boundary [4]. Mechanistically, it is hypothesized that these fibers act as pathways that facilitate cancer cell migration away from the tumor and towards vasculature during the metastatic process. It has also been shown that the detection of TACS-3 in routine breast cancer histopathology slides can predict disease recurrence and patient survival [5]. Due to the emerging clinical significance of collagen reorganization, translational technologies have been developed to better detect and quantify changes [6].

Increased fibrillar collagen has long been clinically documented in PDAC, but its specific organization throughout the tumor has not been explored in the context of disease progression. Using SHG imaging and computational collagen fiber segmentation, we have demonstrated that robust collagen-based structural changes can be detected in the immediate vicinity of cancer cells in PDAC histopathology tissues (Figure 1). However, the underlying biological relevance of collagen remodeling in PDAC tumors and clinical manifestation remains unclear. Although histopathology is the gold standard for characterizing patient tissues, these samples are inherently 2D and static making it impossible to clearly elucidate what influence dynamic collagen changes have on cancer cells. Thus, a pairing between traditional histopathology and controllable 3D experimental *in vitro* models is needed to better study the role of collagen reorganization in PDAC.

Despite the emerging role of the stroma, experimental *in vitro* models that accurately recreate the heterogeneous PDAC microenvironment have been lacking. Realizing the importance of studying PDAC in the context of the stroma and the identifying key limitations of traditional 2D models, we previously developed a 3D *in vitro* microfluidic model of the PDAC microenvironment [7]. Microfluidic technology provides a unique platform to directly visualize live cell interactions with 3D collagen networks. Ongoing work is seeking to recapitulate key features of collagen topology at the PDAC cancer-stroma interface in viable *in vitro* tissue constructs by using SHG data obtained from clinical tissues as a blueprint. Individual fiber metrics (*i.e.* length, diameter) and bulk fiber metrics (*i.e.* concentration, alignment) are being assessed in the context of cancer and cancer-associated myofibroblasts. Experimental models that recreate collagen organization changes present in PDAC tissues are expected to provide mechanistic insight into the effect of collagen alterations on PDAC cell behavior during disease progression and the opportunity to assess novel therapeutics in a pathophysiologically relevant context.

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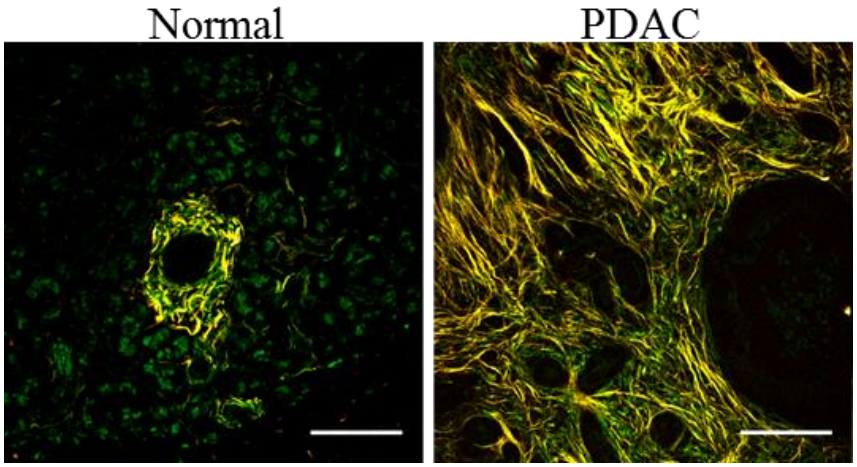

Figure 1. Collagen fibers are elongated and aligned around PDAC ducts compared to normal ducts. Cells are green, SHG signal from fibrillar collagen is yellow. Scale = 100 μm.