Tumor–Stroma Interactions Drive 3D Growth and Self-Organization in PDAC Spheroids: A Quantitative Study in Microfluidic Models

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Summary

We present a microfluidic-based 3D model of pancretic cancer that reveals how tumor–stroma interactions and matrix mechanics govern spheroid self-organization. Using quantitative imaging, we uncover cell type–specific growth patterns and stromal responses. This platform advances *in vitro* modeling of pancretaic cancer heterogeneity and reveals biomechanical drivers of tumor architecture.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer marked by an intense desmoplastic response [1,2]. This fibrotic reaction leads to the formation of a dense and mechanically abnormal extracellular matrix (ECM) that plays a crucial role in supporting tumor growth, invasion, and resistance to therapy. The biomechanical properties of this ECM—particularly its stiffness—are key modulators of tumor architecture and behavior [3,4], yet their specific impact remains insufficiently quantified across different tumor phenotypes. Central to the desmoplastic microenvironment are pancreatic stellate cells (PSCs), a major fibroblastic population responsible for producing and remodeling the ECM. Upon activation by tumor signals, PSCs acquire a myofibroblast-like phenotype that contributes to ECM deposition, organization, and altered mechanics [5]. To investigate how these stromal elements influence PDAC morphogenesis, we developed a 3D microfluidic model that integrates tumor cells with defined epithelial–mesenchymal traits, collagen-based ECM, and activated PSCs.
Using advanced imaging and computational analysis, we provide a quantitative characterization of tumor–stroma interactions that drive cell type–specific spheroid growth and self-organization [6].

Materials and methods

We developed a 3D PDAC-on-a-chip model by embedding human pancreatic tumor cells within collagen type I hydrogels inside microfluidic devices. The system included four PDAC cell lines—two classical (Capan-2, BxPC-3) and basal-like (Panc-1, MIA PaCa-2)—to capture phenotypic diversity. To explore how ECM mechanics influence tumor behavior, we varied collagen concentration (2.5, 4, and 6 mg/ml), modulating not only matrix stiffness but also its structural organization. This allowed us to assess how changes in ECM physical properties affect the growth and self-organization of tumor spheroids. Aditionally, to further simulate the stromal compartment, we incorporated activated human pancreatic stellate cells (HPSCs) in a 2:1 ratio relative to tumor cells, enabling direct coculture within the ECM.

Spheroid growth was monitored over ten days using phase-contrast microscopy. We quantified spheroid area over time to evaluate growth dynamics under different matrix and coculture conditions. To analyze the 3D spatial organization, we used Lattice LightSheet microscopy, which provided high-resolution volumetric reconstructions of spheroids. Nuclear segmentation was performed to characterize intercellular organization within the spheroids, extracting quantitative metrics such as nuclear connectivity and compactness using custom image analysis scripts in MATLAB.

Results

We first analyzed how the mechanical and structural properties of the extracellular matrix influence spheroid formation in PDAC. By varying collagen concentration in the microfluidic device, we observed that ECM physical properties modulates both growth dynamics and 3D architecture in a cell type–dependent manner. Epithelial-like lines (Capan-2, BxPC-3) formed spheroids across all tested conditions, with larger structures appearing at higher collagen concentrations. In contrast, mesenchymal-like lines (Panc-1, MIA PaCa-2) required stiffer matrices (≥4 mg/ml) to initiate self-organization, generating smaller but more compact spheroids. Lattice LightSheet microscopy and nuclear segmentation revealed that increased stiffness promoted higher nuclear density and tighter intercellular organization in epithelial-derived spheroids. These findings confirmed that ECM mechanics alone can differentially regulate PDAC morphogenesis based on tumor subtype.

Building upon these observations, we introduced activated HPSCs to investigate stromal influences on spheroid development. Coculture in dense (6 mg/ml) collagen matrices revealed contrasting effects. While Capan-2 spheroids significantly increased in size and structural cohesion in the presence of HPSCs, BxPC-3 spheroids were reduced in both growth and organization. For mesenchymal-like cell lines, the impact of HPSCs was minimal, suggesting limited responsiveness to stromal modulation under these conditions. 3D imaging showed that HPSCs did not integrate into spheroids but remained in the matrix, exerting paracrine and mechanical effects depending on the tumor phenotype. Quantitative analysis of nuclear connectivity confirmed that stromal effects on spheroid architecture were strongly cell type–specific.

Together, these results demonstrate that both ECM properties and stromal interactions play essential and complementary roles in shaping PDAC spheroid self-organization. Our microfluidic model enables a detailed, quantitative dissection of these biomechanical and cellular interactions, offering a powerful tool to study tumor heterogeneity and the mechanobiology of PDAC progression.

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