Conversations in the tumor microenvironmentasion: experimental models of fibroblast driven cancer cell inv

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Summary

This thesis examines how CAFs regulate tumor invasion through coordinated mechanical, biochemical, and spatial mechanisms. Using 3D in vitro models, time–lapse imaging, and pharmacologic perturbations, the findings reveal several interconnected principles that reshape stromal control paradigms in cancer biology.

Chapter 2 demonstrates that CAF – tumor spatial proximity is a primary determinant of invasion onset and organization. This observation complements in vivo studies showing that stromal architecture and fibroblast distribution define invasive niches[1], [2]. By co–embedding CAF and tumor spheroids at controlled distances, we show that CAFs remodel collagen I through fiber alignment, compaction, and tension propagation, consistent with prior demonstrations of fibroblast driven ECM contractility [3], [4], [5], [6]. This distance dependent ECM remodeling forms anisotropic fiber tracks that direct collective cancer cell migration, supporting earlier mechanobiology findings on ECM anisotropy and migration guidance [4], [7], [8]. Importantly, pharmacologic inhibition of MMPs reduced collagen degradation and spheroid expansion but did not abolish CAF migration or low level dissemination, echoing recent work distinguishing “mechanical remodeling” from “proteolytic remodeling” [9]. Heterospheroid models confirmed that CAFs generate similar ECM dynamics even when spatial order differs, demonstrating the robustness of matrix directed invasion.

Chapter 3 focuses on methods to investigate EMT and its dynamic regulation by TGF–β signaling. EMT is now recognized as a spectrum rather than a binary switch[10]. Using live cell imaging, we systematically outline molecular and cellular techniques to monitor TGF–β induced EMT in normal and cancer cell lines. It describes how to assess SMAD activation, changes in epithelial and mesenchymal marker expression and localization, alterations in cell morphology and polarity, dynamic reorganization of the actin cytoskeleton and stress fibers, as well as migration assays. Together, these methods provide a practical toolbox for probing EMT dynamics and epithelial plasticity in vitro and are well suited to interrogate heterogeneous or metastable EMT states described in recent literature [11].

In chapter four we found that conditioned media of lung fibroblasts supports invasion of lung adenocarcinoma spheroids in a TGF–β receptor signaling dependent manner, but I the absece of detectable TGF–β ligand. While this thesis does not establish the molecular basis of this activation, the findings are consistent with broader literature suggesting that receptor signaling strength can be shaped by the biochemical context of secreted factors. These results underscore the possibility that fibroblast derived cues can potentiate signaling pathways even when ligands are not detectable, revealing an additional layer of stromal influence on tumor cell behavior.

Finally, the microfluidic review highlights an ongoing shift toward engineered models capable of imposing spatial, temporal, and mechanical control over tumor – stroma interactions. Together, the findings across Chapters 2 – 5 support an integrated view in which tumor invasion emerges from the interplay of CAF driven matrix mechanics, ECM architecture, EMT plasticity, and noncanonical modes of stromal signaling. Collectively, this thesis shows that CAF mechanics, ECM architecture, EMT dynamics, and paracrine signaling operate as an integrated regulatory system that shapes tumor invasiveness.