Single-Cell Functional Biology in the Tumour-Bone Microenvironment: Identification of Individual Prostate Cancer Cells with High Metastatic Potential

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Background: Bone is a common site of prostate cancer (PCa) metastasis associated with pain and mortality. To treat advanced PCa, it is vital to understand which traits make prostate cancer cells more likely to spread to bone. While much research to date has studied the bulk population of tumour cells, only a small number of distinct tumour cells are responsible for successful bone metastasis. We are exploiting microfluidic technology to focus on these individual tumour cells with high metastatic potential. Our goal is to identify the functional biology and molecular changes in both individual tumour cells with high metastatic potential and their surrounding interactive niche cells that drive prostate cancer bone metastasis

Methods: Using fluid wall based microfluidic technology, we have successfully engineered and validated multiplexed, miniaturised coculture assay systems that facilitate the investigation of cancer cell dynamics and effectively model interactions between cells and the bone microenvironment. Specifically, we have used microfluidic technology to (1) design miniaturised multiplexed assays to identify and characterise rare but functionally significant cancer cell subpopulations and (2) probe individual cell metastatic potential by picking single PCa cells into bone microenvironment models,

Results: Miniaturised migration assays revealed subpopulations of ultra-migratory 'super-spreader' cells. A subset of superspreader cells exhibited 'polyaneuploid cancer cell state' characteristics (a transient state implicated in treatment resistance) including senescence markers (β-galactosidase, γH2Ax foci) and characteristic increases in cell and nuclear size and perinuclear granularity. Individual superspreader cells were isolated and although initially non-proliferative, we demonstrated that an PCa feeder bed restored superspreader proliferation after a 3-7 day quiescent period, reminiscent of the metastatic cascade. In separate studies, we have developed a robust in vitro workflow that screens the bone metastatic potential of hundreds of clones from a mixed population of cancer cells per experiment (with >98% single-cell picking success rates). Our microfluidic single-cell picking technology is unique in that it guarantees monoclonality while avoiding the high shear stress (and associated phenotypic changes) exerted by the alternative method, FACS. Single cells are picked into microplates containing in vitro bone microenvironment models. Single-cell picking demonstrated heterogeneity in individual PCa cell survival and proliferation in bone stromal cell lines and whole-murine marrow models. Microenvironment cells decreased heterogeneity in survival and proliferation relative to controls, suggesting a combination of microenvironment and clone drives differential cell fate. Higher proliferation was observed in aged murine marrow relative to young, recapitulating orthoptic xenograft tumour burden trends.

Conclusion: This study leveraged cutting-edge microfluidic technology to interrogate single-cell-level functional diversity, identifying distinct PCa cells with high metastatic potential, in order to better understand the pathogenesis of prostate cancer bone metastasis.

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