Epithelial-mesenchymal plasticity in circulating tumour cells from prostate cancer PDX models

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Introduction

Monitoring circulating tumour cells (CTCs) for epithelial mesenchymal plasticity (EMP) could extend their clinical utility beyond simple enumeration, and may lead to gains in their prognostic power and further establish their role in guiding therapy. We hypothesise that patient-derived xenograft (PDX) models of prostate cancer will mimic dissemination of CTCs in human cancer patients, and allow us to further understand differences between EMP status of CTCs and primary tumours.

Research objectives

To conduct a molecular analysis and comparison of circulating tumour cells and primary tumours in human prostate cancer mouse xenograft models.

Methods

Paired blood and tumour samples were collected from four prostate cancer PDX models (BM18, LuCaP70, LuCaP96, LuCaP105) and nucleated cells/tissue collected to assess using an EMP-focused, 42 gene human-specific, nested RT-qPCR assay (n=10-11/model) and immunocytochemistry (n=11)

Results

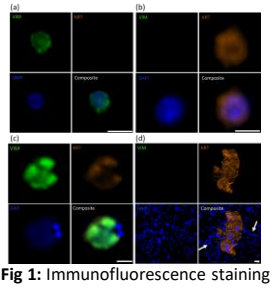
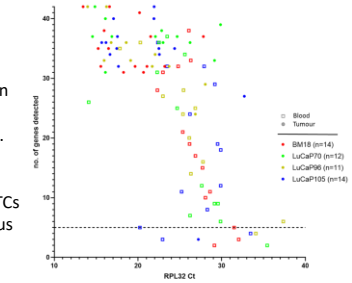


Fig 1: Immunofluorescence staining for vimentin (VIM; mesenchymal; green) and cytokeratins 8/18/19 (KRT; epithelial; red) in PDX-derived CTCs. Representative images of LuCaP70 CTCs staining for (a) VIM only, (b) KRT only or (c) both VIM and KRT; (d) KRT-positive CTC cluster surrounded by murine peripheral blood mononuclear cells (PBMCs; arrows). Cell nuclei stained with DAPI (blue). Scale bar, 5 µm.



PDX	total blood samples (n)	RPL32 positive samples (n)	percentage positive (%)
BM18	26	11	42
LuCaP70	16	11	69
LuCaP96	15	10	67
LuCaP105	20	10	50

Fig 2: The 42 gene human-specific nested RT-qPCR assay was performed on L32-positive blood samples and their paired tumour. The number of genes detected in each sample was plotted against their raw Ct value of RPL32 housekeeper gene.

The number of genes detected sharply decreased with lower expression of RPL32.

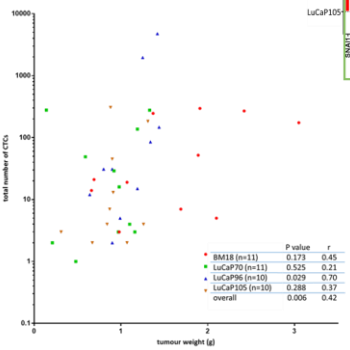


Fig 3: The tumour weight for each mouse was compared to the estimated total number of CTCs in that mouse's whole blood volume. Each symbol represents a sample from an individual mouse (n=1 per mouse).

Overall a significant correlation between CTC number and primary tumour weight was observed.

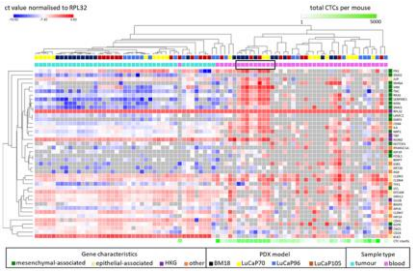


Fig 4: Heat map showing 42 gene panel expression profiles in blood and matched tumour samples as Ct values normalised to RPL32, with global normalisation. Hierarchical unsupervised clustering was performed using one minus Pearson correlation and global normalisation. The final row indicates estimated CTC counts for each blood sample. Black box indicates cluster of blood samples showing evidence of a hybrid phenotype.

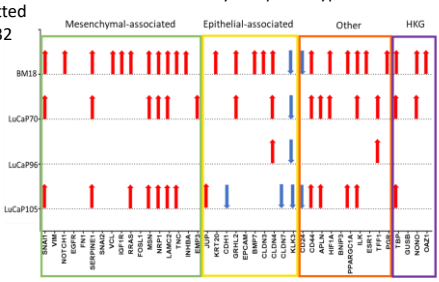


Fig 5: Summary of gene expression differences between CTC and primary tumour samples across PDX models. HKG, house keeping gene.

Discussion

> RNA analysis of enriched CTC fractions is feasible in prostate cancer PDX models
> CTC enumeration correlates with tumour size
> Heterogeneity across the EMP axis is observed in CTC s
> CTCs vary significantly in cell size

Limitations

Immunocytochemical analysis of CTCs for EMP markers was critical as pooled RNA-based CTC analysis cannot distinguish hybrid cells from epithelial and mesenchymal sub-populations.

2021 Embracing diversity in healthcare
Princess Alexandra Hospital Health Symposium
24–27 August 2021
Queensland Government