The Characterisation of Tumour Self-Seeded Cells in Liver Cancer

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Introduction

Recent studies indicated a multi-directional seeding of circulating tumour cells (CTCs). Apart from seeding to distant tissues (metastases), CTCs can also reinfiltrate and colonise already established tumours. This process of "tumour self-seeding" provides new insights into the dynamics of tumour progression, and has been indicated to promote tumour growth, angiogenesis and invasion.

However, tumour self-seeded cells (TSCs) have not been well identified and characterised due to unsuitable animal models. Therefore, this study aims to develop a novel animal model to recapitulate the process of tumour self-seeding and characterise the transcriptional and functional profiles of TSCs in liver cancer.

Methods

Cell transfection: Human colorectal cancer cell line HCT116 was knocked-in with the KikGR plasmid. KikGR HCT116 cells could be photoconverted from green (KikGreen) to red (KikRed) fluorescence by violet light irradiation.

Model development: KikGR HCT116 cells were injected into the liver of nude mice to mimic colon cancer liver metastasis. CTCs were irradiated in blood vessels and KikRed⁺ TSCs could be differentiated from un-photoconverted primary tumour cells (PCs) (Figure 1).

TSC isolation and analysis: KikRed⁺ TSCs and KikGreen⁺ PCs were isolated using flow cytometry or imaged by confocal microscopy. Isolated TSCs and PCs were investigated using RNA sequencing and functional assays.

scRNA-seq analysis: Potential TSC population in cancer patients was identified using single-cell RNA sequencing (scRNA-seq) data.

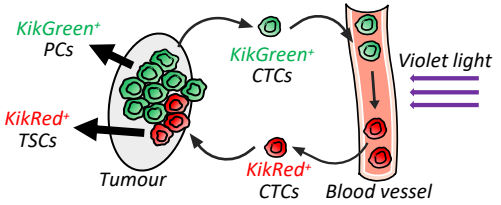

Figure 1. Concept of tumour self-seeding model.

Results

TSC isolation and visualisation: KikRed⁺ TSCs presented as a clear population using flow cytometry (Figure 2A). Fluorescent imaging showed TSCs formed a tumour mass within the primary tumour (Figure 2B).

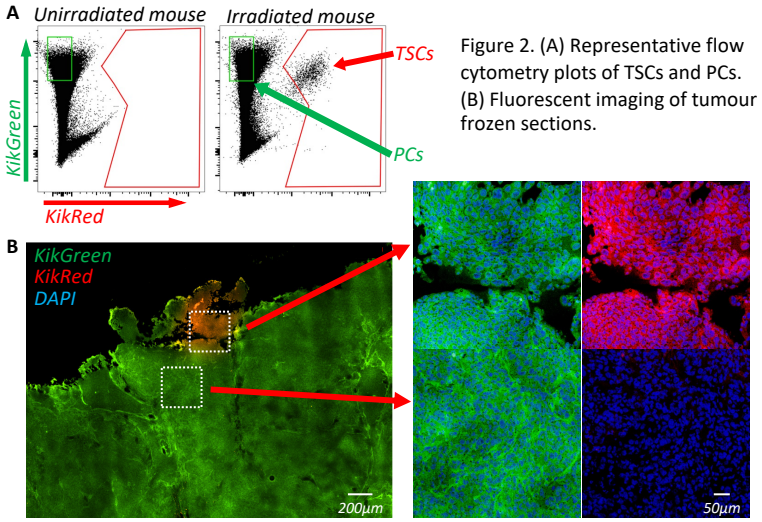

Figure 2. (A) Representative flow cytometry plots of TSCs and PCs. (B) Fluorescent imaging of tumour frozen sections.

RNA sequencing:

- Identified 114 differentially expressed genes between TSCs and PCs (fold change > 2 or < -2; adjusted p value < 0.05) (Figure 3).
- Potential TSC markers were identified: TM4SF1, EMP1, LCK, SH3BGRL3.
- TSCs were enriched with biological processes relating to cell migration and cell proliferation.

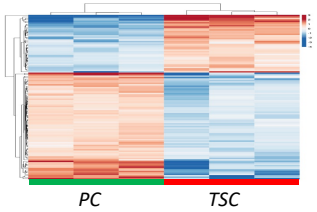

Figure 3. Heatmap showing differentially expressed genes between TSCs and PCs (fold change > 2 or < -2; adjusted p value < 0.05).

Functional assays:

- In invasion assay, a significantly higher number of TSCs invaded through trans-well membrane than PCs.
- Significantly higher number of colonies were formed by TSCs than PCs using colony-formation assay.

scRNA-seq analysis: using scRNA-seq data, all TSC markers were highly expressed in a unique cluster of cancer cells, indicating the presence of TSC population in cancer patients (Figure 4AB).

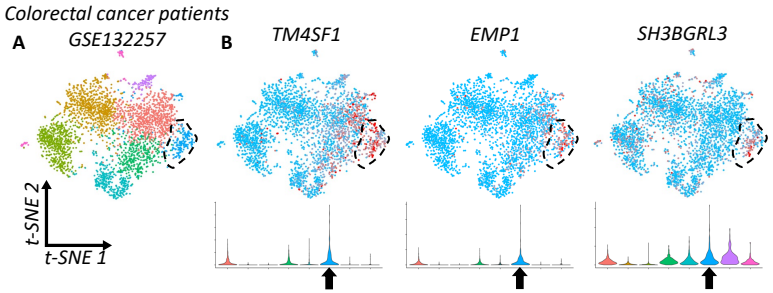

Figure 4. (A) t-SNE projection of tumour cells from dataset GSE132257. The Cluster expressing TSC markers is circled. (B) Expression of TSC markers presented in t-SNE and violin plots.

Conclusions

- A novel tumour self-seeding model was successfully developed.
- Potential TSC markers were identified.
- TSCs are more invasive and tumorigenic than PCs.
- Potential TSC population could be identified in cancer patients.

Acknowledgements

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