APCRC-Q
Australian Prostate Cancer Research Centre Queensland

Epithelial-mesenchymal plasticity contributes to therapy resistance in prostate cancer

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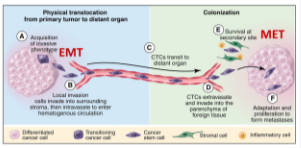
1. Epithelial-Mesenchymal Plasticity (EMP) and the Mesenchymal-Epithelial reverting Transition (MErT) in Metastasis

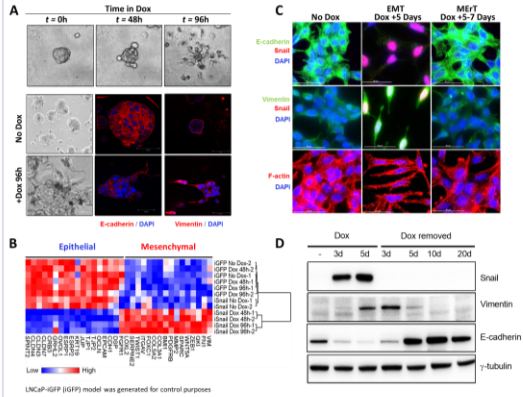
The epithelial-mesenchymal transition (EMT) endows carcinoma cells with the phenotypic traits required to escape the primary site and seed distant metastases[1]. Recent studies in preclinical models show that the transient induction of EMT and subsequent reversion of tumor cells to their epithelial phenotype (termed as MErT) is required for the formation of overt metastases[2].

Metastasis is the main cause of prostate cancer related mortality[3]. To investigate the role of MErT in human carcinoma metastasis, we characterized MErT in prostate adenocarcinoma (LNCaP) using a reversible model of Snail-mediated EMT.



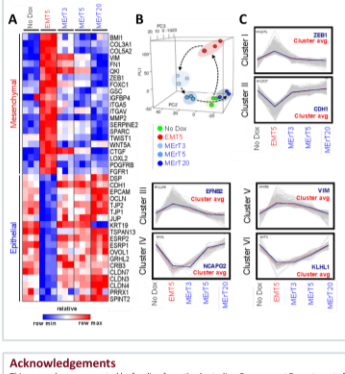
2. Dynamic EMP Model

The LNCaP-iSnail model is a dynamic model of EMP allowing for the timely transition of cells through an EMT with acquisition of an invasive phenotype upon doxycycline (Dox) induction of Snail expression (A and B). The subsequent removal of Dox and shutdown of Snail expression leads to activation of MErT and re-expression of epithelial markers following EMT (C and D).



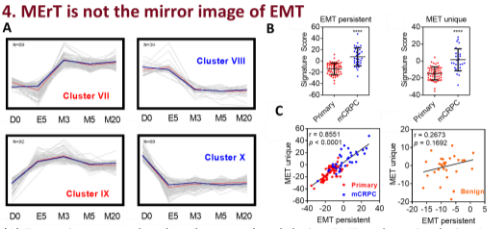
LNCaP-iGFP (iGFP) model was generated for control purposes

3. The temporal complexity of the MErT transcriptional program



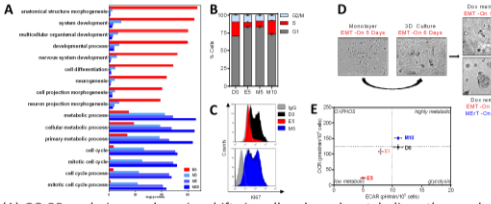
(A) Heatmap of the expression of known epithelial and mesenchymal markers. Heatmap colour gradient refers to z-score of the gene expression within each row. (B) Principal Component Analysis (PCA) as cells transition from an epithelial (No Dox) to a mesenchymal (EMT5) and back to an epithelial (MErT3-20) state. (C) Transcriptional clusters generated through pattern matching of select genes (blue) and classified by temporal reversion pattern. Clusters I and II reverting expression by day 3 of MErT, Clusters III and IV by day 5 and Clusters V and VI by day 20.

4. MErT is not the mirror image of EMT



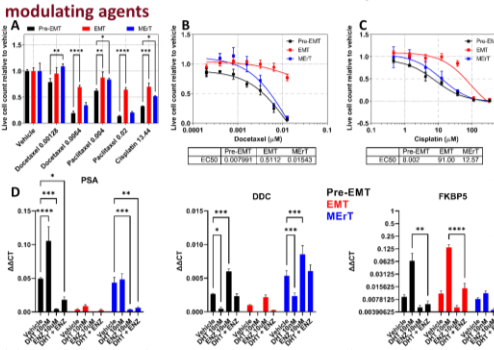
(A) Transcripts upregulated or downregulated during EMT and persist during MErT; Transcripts upregulated or downregulated at 3 days of MErT and persist over the 20 days. (B) EMT persistent and MET unique signatures in primary PCa and mCRPC. (C) Correlation plot of the EMT persistent and MET unique signatures in primary PCa and mCRPC, and benign prostate tissue from Grasso et al., 2012. Error bars indicate SEM, n = 3 biological replicates, unpaired t-test, p-value: **** < 0.0001. Cluster avg: the average transcriptional pattern of the cluster.

5. MErT reawakens EMT-induced invasive dormant-like cells



(A) GO BP analysis reveals major shifts in cell cycle and metabolic pathways during EMT/MErT. MErT reverses (B) EMT-induced cell cycle arrest and (C) impaired proliferative ability, which is required to (D) reinitiate tumour spheroid growth in 3D Matrigel cultures. (E) Seahorse metabolic assays confirm the metabolic plasticity of tumour cells undergoing EMT and MErT.

6. MErT cells are more resilient to chemotherapeutic and AR modulating agents



(A) Live cell count of Pre-EMT, EMT, and MErT cells treated with indicated chemotherapeutic agents over 48 hours, relative to vehicle control. Sigmoidal dose-response curves and calculated IC50 values of Pre-EMT, EMT, and MErT cells treated with serial dilutions of (B) Docetaxel or (C) Cisplatin over 72 hours. (D) ΔΔCT values of the expression of AR-regulated genes PSA, DDC, and FKBP5 following 48 hours of indicated treatments. Two-way ANOVA, n=3, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Acknowledgements

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