Targeting EMT to Modulate Prostate Cancer Cell Chemoresistance

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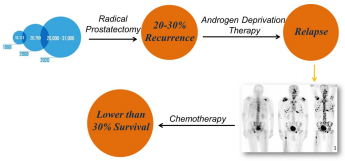
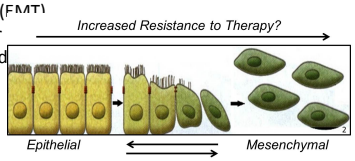
Introduction

- Metastatic prostate cancer (PC) is one of the leading causes of cancer related male deaths in Australia



- Epithelial to mesenchymal transition (EMT) is a process that can facilitate tumour growth, progression to metastasis and has also been associated with chemoresistance.



- To determine how EMT and resultant reversal of this state (MET) influences the chemoresistance profile of PC cells and further determine if and how specific genetic regulators are involved in this process

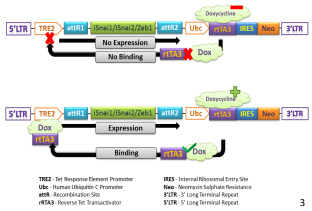
Aims

- To validate Snai1, doxycycline hyclate (dox) inducible LNCaP EMT model and determine whether EMT-MET status of prostate cancer (PC) cells affect their chemoresistance profiles in a 2D monolayer model
- To determine if EMT-MET status of PC cells affect their chemoresistance profiles in a 3D spheroid model

Project Hypothesis: ***Dysregulation of functional regulators of EMT alters prostate cancer cell chemosensitivity and modulates disease progression.***

Methods

- LNCaP inducible Snai1 EMT model where overexpression of Snai1 is induced by Doxycycline Hyclate (dox) is used and validated using qRT-PCR and western blotting
- Concentration response of doxorubicin on PC cells in EMT and No dox control cells was carried out using live/dead cell staining. Cell tracker CMFDA (*Life Technologies*; *Ex/Em* 492/517) was used to pre-stain live cells before seeding and DRAQ7 (*BioStatus; Ex/Em* 600/488) was used for staining dead cells which is maintained in the culture media throughout the course of the experiment. Cells were imaged at *t*=00hrs and *t*=72hrs using the InCell Analyzer 2200 (*GE Healthcare Life Sciences*). A dead cell percentage was then calculated.
- Final timepoint staining with Hoechst 33342 (*Invitrogen*; *Ex/Em 350/461*), Phalloidin 488 (*Invitrogen*) and Alpha Tubulin ab18251 (*Abcam*) was also carried out
- Happy Cell© (HC) 3D media was used to develop iSnai1 spheroids in a Corning Mini Bioreactor which were then transplanted into 96 well plates (HC media) for experiments.
- Dox was added to and maintained in these spheroids for 5 days to induce Snai1 overexpression. Dox was then removed and replaced with normal culture media for 21 days.



Results

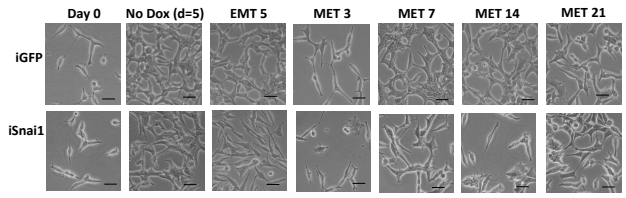


Figure 1: Morphological changes observed during treatment with doxycycline hyclate over a period of 5 days and subsequent phenotypic changes upon removal of dox (reseeded) at days 3, 7, 14 and 21 in 2D monolayer. Scale bar represents 50µm

Results

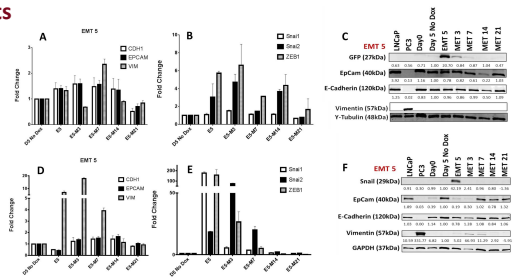


Figure 2: qRT-PCR and western blotting results for the iGFP and iSnai1 models grown in 2D. All samples normalised to housekeeping gene RPL32). Figures A, B and C show the results for iGFP control model and figures D, E and F show the results for iSnai1 model. Mean ± SEM shown.

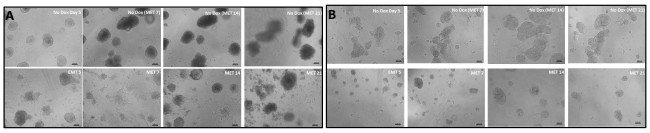


Figure 3: 3D spheroids in Happy Cell© media showing morphological changes upon treatment with doxycycline hyclate (dox) at for 5 days and subsequent phenotypic changes upon removal of dox for days 7, 14 and 21. Panel A shows spheroids grown in HC-RPMI 5% FBS media and Panel B shows spheroids grown in HC-RPMI 5% CSS media. Scale Bar represents 100µm.

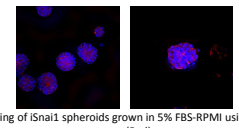


Figure 4: Immuno Fluorescence staining of iSnai1 spheroids grown in 5% FBS-RPMI using Hoechst 33342 (33342) and Phalloidin 488 (Red).

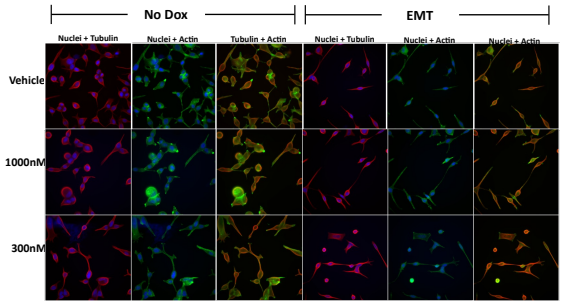


Figure 5: Endpoint staining of no dox and EMT cells treated with doxorubicin at 1000nM and 300nM for 72 hours.

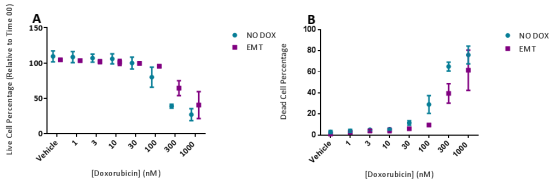


Figure 6: A) Live cell percentage at different concentrations of Doxorubicin at *t*=72 hrs calculated relative to live cells at *t*=00hrs **B)** Dead cell percentage at different concentrations of Doxorubicin at *t*=72 hrs. Mean ± SD.

Conclusion

- LNCaP inducible iSnai1 EMT model growing in 2D was validated using qRT-PCR and western blotting.
- When grown in 3D, iSnai1 LNCaP spheroids lost their smooth surface and cells appeared to move away from the spheroids, upon addition of dox. A reversion in this effect was observed upon removal of dox.
- An increase in the dead cell percentage was observed at higher concentrations of 100nM, 300nM and 1000nM in both EMT and no dox control groups. However, the dead cell percentage in the EMT group was relatively lower compared to the no dox cells indicating that cells undergoing an EMT are more resistant to doxorubicin.

References & Acknowledgments

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