Assessing SGK1 Inhibition as a Therapeutic Intervention in Castrate Resistant Prostate Cancer

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1. Introduction

Prostate cancer (PC) is the most common urological disease in men. In 30% of resistance cases, PC is resistant to PI3K signalling pathway inhibition.

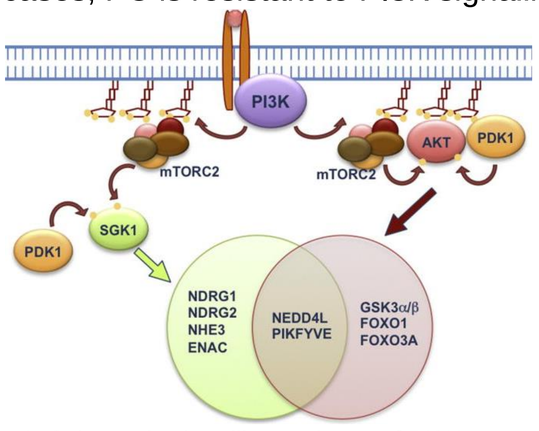

Fig 1. Schematic signalling pathway of PI3K, activating effectors PDK1 and SGK1, and the proteins specifically associated with each kinase or coregulated by both.

Fig 2. Current Therapeutic strategies of inhibiting the PI3K pathway in prostate cancer

PI3K activation recruits PDK1 kinase and leads to the activation of mTORC2. mTORC2 and PDK1 together leads to cancer progression. However, there is now growing evidence that SGK1, another PI3K signalling effector, also conveys PI3K inhibition resistance. The aim of the project is to validate the underlying effects of SGK1 inhibition on PC cell lines and ultimately demonstrating its implications in stopping growth of PC.

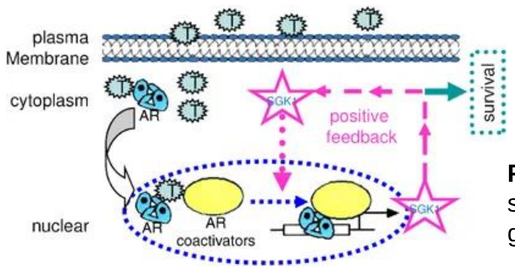

Fig 3. Proposed mechanism for SGK-1 expression in AR-dependent survival and for the positive feedback in AR-dependent survival and gene expression

2. Methods

- Cells were plated at a density of 250,000 cells per ml in steroid depleted media and kept in an incubator at 37℃, 48 hours prior to drug treatments.
- Prostate cancer cells lines VcaP and LNCAP were treated with different combinations of EMD (SGK1 inhibitor) as shown in **Fig 4**;

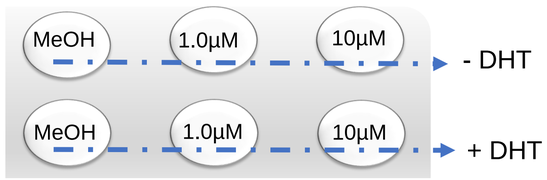

Fig 4. Layout of the drug treatments at different EMD concentrations

- 24h post-treatment, whole cellular proteins extracts were used for Western blots.
- Western Blots were analysed using a BioRad Chemidoc.

3. Results

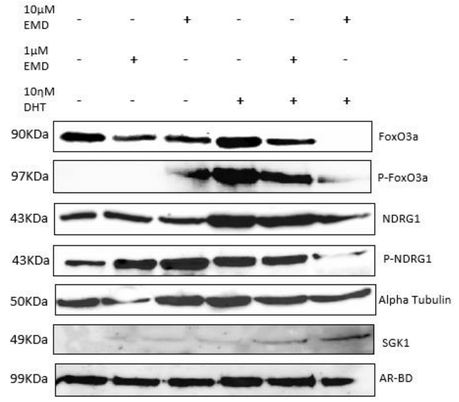

Fig 5. Western blots of VCaP cell lines treated with EMD showing the levels of different proteins

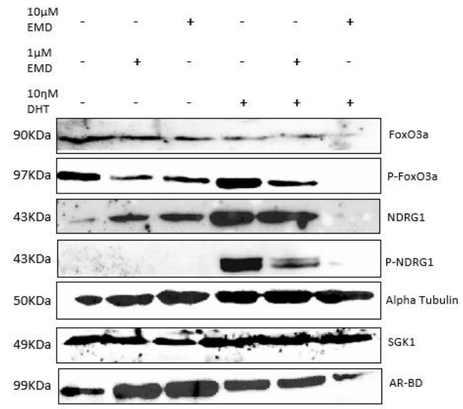

Fig 6. Western blots of LNCAP cell lines treated with EMD showing the levels of different proteins

- SGK1 protein levels were increased in the presence of DHT, supporting previous studies that show SGK1 expression is under regulation of the AR. However, this was not affected by SGK1 inhibition with EMD.
- Increased P-FoxO3a protein was detected in the presence of DHT and was consequently reduced with increasing EMD concentrations. This supports the theory that SGK1 remains active after androgen stimulation thus phosphorylating FoxO3a into P-FoxO3a. However at higher EMD concentration no P-FoxO3a is detected as the SGK1 inhibitor outcompetes the AR stimulation.
- A similar trend happened with P-NDRG1 except, P-NDRG1 is un-detected in the absence of DHT in LNCAP cells, demonstrating differing protein expression across castration-resistant PC cell lines.
- FoxO3a protein expression was decreased in both the non DHT and DHT arms at the 10 μM EMD concentration.

5. Conclusion

10 μM EMD concentrations significantly reduced phosphorylation of two key SGK1 target proteins confirming its ability as an inhibitor in two different CRPC cell lines. Further investigation needs to be achieved to see how this inhibition will ultimately affect proliferative potential.

References

Fig 1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5658788/ **Fig 3**. https://www.nature.com/articles/4402227.pdf?origin=ppub
Fig 2. https://www.sciencedirect.com/science/article/pii/S1535610816302641