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Evaluating the ERG transcription factor as a key driver of prostate cancer

Newcastle University / Centre for Cancer

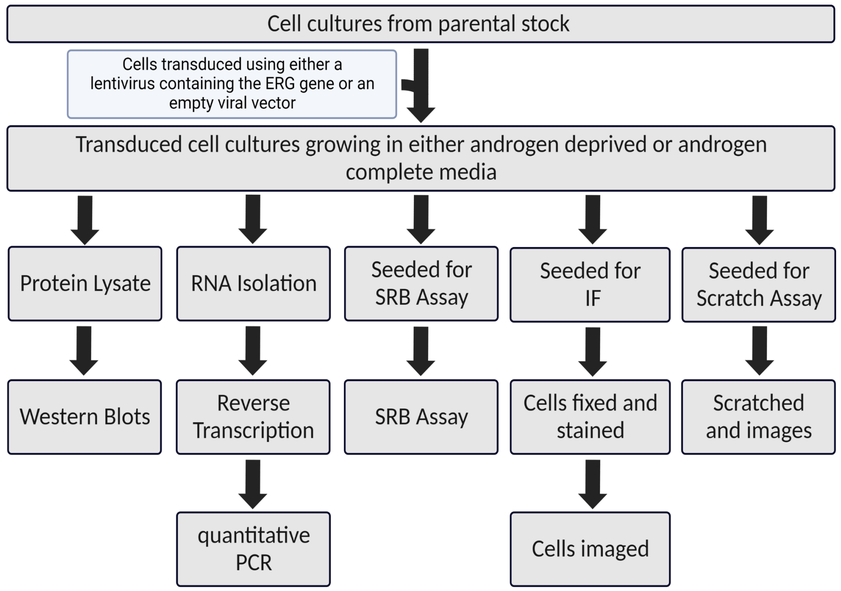
Introduction

- Prostate cancer (PC) is common among men and is one of the leading causes of death in males[1]
- One common PC subgroup involves a fusion between TMPRSS2 and ERG (T-E fusion) and is present in approximately 50% of treatment naïve prostate tumours[2]
- This T-E fusion allows androgen levels to regulate the ERG transcription factor in PC cells leading to increased ERG expression and is associated with an aggressive phenotype.

Aims

- The aims of this project are to examine the effects of over-expressing ERG in PC cell lines.
- This data will be used to determine if ERG is capable of stimulating cell proliferation and activating proteins associated with advanced PC
- These results will provide pilot data to examine the importance of ERG in advanced pre-clinical PC models.

Methods



Results

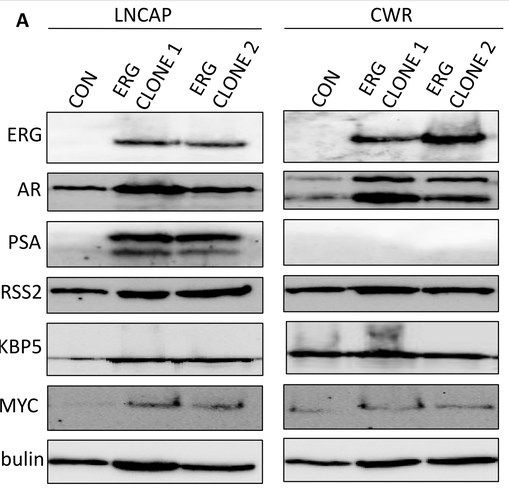


Figure 1a: Western blot data for LNCAP and CWR cells

B

	LNCAP ERG CLONE 1	LNCAP ERG CLONE 2	CWR ERG CLONE 1	CWR ERG CLONE 2	
	1.157	18.025	58.435	480.98	ERG
	2.325	10.185	0.764	1.846	AR
	4.822	12.364	0.451	1.205	PSA
	2.382	3.294	-	-	TMPRSS2
	1.555	76.072	0.506	1.037	FKBP5
	2.850	2.049	0.205	1.115	C-MYC

Figure 1b: qPCR data showing relative fold change in gene expression for LNCAP and CWR cells
Values represent relative changes to control cells transduced with an empty viral vector.

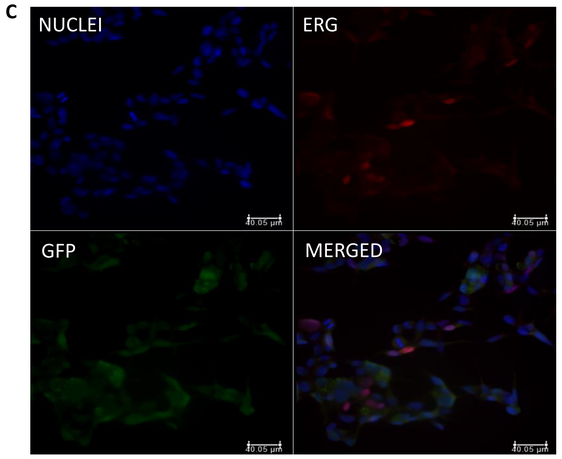


Figure 1c: Immunofluorescence image of LNCAP cells transduced with ERG containing lentivirus. Also show is DAPI, ERG, and green fluorescent protein (GFP)

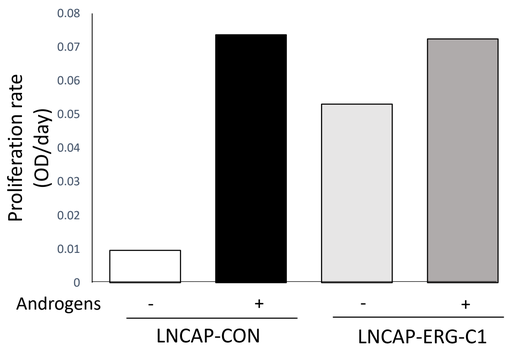


Figure 2: Proliferation data (SRB) for LNCAP cells transduced with a control or ERG containing lentivirus. Cell lines grown in the absence (-) or presence (+) of 1nM synthetic androgen

	ERG CLONE 1	ERG CLONE 2
FZD4	0.002	0.002
BRD2	2.528	1.681
PRKD1	2.839	3.858

Table 1: qPCR data showing relative fold change for gene expression in LNCAP cells. Values represent relative changes to control cells transduced with an empty viral vector.

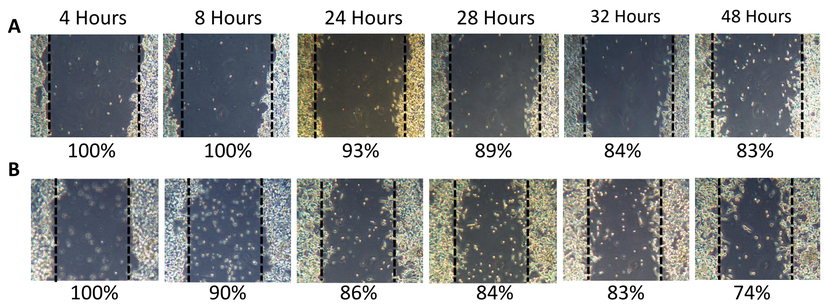


Figure 3: Scratch assay for LNCAP cells transduced with a control lentivirus (A) or an ERG lentivirus (B). Percentages indicate the relative width of the scratch at the indicated time point. Marked is the location of the initial scratch. All cells grown in full media

Discussion

- Figure 1a Indicates the successful of both cell lines with ERG. Expression of ERG **enhances** the expression of several AR regulated genes including PSA and AR itself.
- Figure 1b shows the relative levels of the indicated genes. Importantly, this data **correlates** with the western blot data (figure 1a)
- Figure 1c shows evidence or the successful ERG lentivirus transduction and expression of ERG protein.
- Figure 2 (proliferation data) demonstrates LNCAP control cells show increased proliferation in response to androgens. ERG over expression stimulate proliferation in the absence of androgens to similar levels as control cells with androgen.
- Table 1 shows the effects of ERG overexpression on ERG regulated genes. FZD4 is strongly **down regulated** whilst BRD2 and PRKD1 are upregulated. Similar results were demonstrated in CWR cells (data not shown)
- The scratch assay (figure 3) shows the migration of cells over time and demonstrates little impact of ERG overexpression on cell motility.

Conclusion

- The data suggests that ERG overexpression **impacts** the expression levels of several genes at both the RNA and protein level.
- These findings are supported by the current knowledge that ERG is capable of **impacting** the expression of androgen regulated genes.
- Proliferation data also demonstrates that ERG overexpression allows for the **maintenance** of cell proliferation in the absence of androgens to a comparable level observed when androgens are present.
- This data might suggest that ERG functions to **maintain** the levels of AR-regulated genes in the absence of androgens potentially through ERG's postulated ability to **activate** AR[3]
- Although ERG is associated with cell motility and invasion[1], only a small increase in motility was observed in ERG overexpressing cells. This result may be a consequence of experimental growth conditions
- The data in table 1 shows the **almost complete absence** of FZD4 gene expression in ERG overexpressing cells. This result conflicts with published data and requires further investigation[4]. The results for BRD2 and PRKD1 **fit established** trends for these genes
- Future experiments could explore the consequences of reducing the high levels of ERG expression in VCAP cells which contain an endogenous T:E fusion.

Acknowledgements

- I would like to thank Craig Robson for being my supervisor and allowing me the opportunity to take part in this research. I would also like to thank all the other staff and students working in the Paul O'Gorman building for their help and support

References

(1) Ashrafizadeh M, Paskeh MDA, Mirzaei S, Gholami MH, Zarrabi A, Hashemi F, et al. Targeting autophagy in prostate cancer: preclinical and clinical evidence for therapeutic response. J Exp Clin Cancer Res. 2022;41(1):022-02293.
(2) Adamo P, Ladomery MR. The oncogene ERG: a key factor in prostate cancer.
(3) Wasmuth EV, Hoover EA, Antar A, Klinge S, Chen Y, Sawyers CL. Modulation of androgen receptor DNA binding activity through direct interaction with the ETS transcription factor ERG. Proceedings of the National Academy of Sciences. 2020;117(15):8584-92.
(4) Birdsey Graeme M, Shah Aarti V, Dufton N, Reynolds Louise E, Osuna Almagro L, Yang Y, et al. The Endothelial Transcription Factor ERG Promotes Vascular Stability and Growth through Wnt/β-Catenin Signaling. Developmental Cell. 2015;32(1):82-96.