# Assessing the Contribution of KMT5A Phosphorylation to AR Signalling In Prostate Cancer

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## INTRODUCTION

As the most common cancer among men in the UK, prostate cancer accounts for a quarter of cases of cancer diagnosed in men (Cancer Research, 2014). The androgen receptor is a nuclear receptor which plays a key role in the normal development and transformation of the prostate dependant on its interactions with nuclear elements (Heinlein and Chang, 2013). KMT5A, a mono-methyltransferase which mono-methylates lysine 20 on histone 4 (H4K20Me1), has been shown to potentially act as an AR regulator(Coffey *et al*, unpublished data). Furthermore, KMT5A acts differently in models of CRPC and early PC. This could be due to how KMT5A is regulated itself between these two models which then has an impact on AR signalling. Therefore, the aim of this study was to investigate whether KMT5A phosphorylation (pKMT5A) at serine 29 affects androgen receptor signalling in prostate cancer.

## METHODS

### B-gal/Luciferase Assay

Transfected 293T cell line with flag-AR and each of the flag-KMT5A constructs in full media. Cells were then starved by changing full media to steroid depleted media (DCC) for 24 hours, followed by dihydrotestosterone (DHT) stimulation at 10 nM for 24 hours, to determine the activity of AR.

### Denaturing Immunoprecipitation

To investigate the level of endogenous pKMT5A in models of CRPC (LNCaP-AI) to androgen dependent PC (LNCaP), by pulling down serine-phosphorylated proteins and blotting for KMT5A.

### Native Immunoprecipitation

Performed in 293T cell line transfected with flag-AR and each KMT5A constructs, flag WT KMT5A, flag-S29A (phospho-mutant) and flag-S29D (phospho-mimetic) KMT5A looking at the interaction of AR and KMT5A constructs.

### SDS PAGE Western Blotting

Samples were collected in SDS sample buffer with 10% β-mercaptoethanol and resolved on 12% PAGE gels, transferred to nitrocellulose membrane, blocked, incubated in primary and secondary antibodies and then developed.

## REFERENCES

1.Cancer research (2014) 'Cancer statistics 2014.' Available [On-line] at cancerresearchuk.org.
2.Heinlein.A and Chang.C (2013) 'Androgen Receptor in Prostate Cancer.' Endocrine Reviews: 25(2): 276-308.

## RESULTS

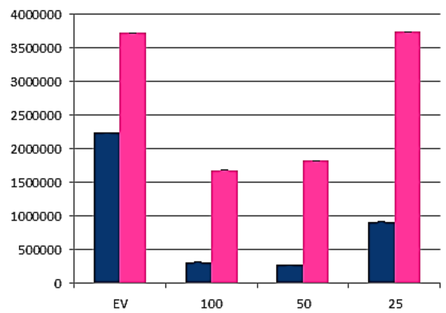



**Figure1** . WT flag tagged KMT5A in different concentration and a positive control EV was co-expressed with AR in 293T cell line in DCC and DHT treatment to determine the activity of AR using B-gal/Luciferase Assay.

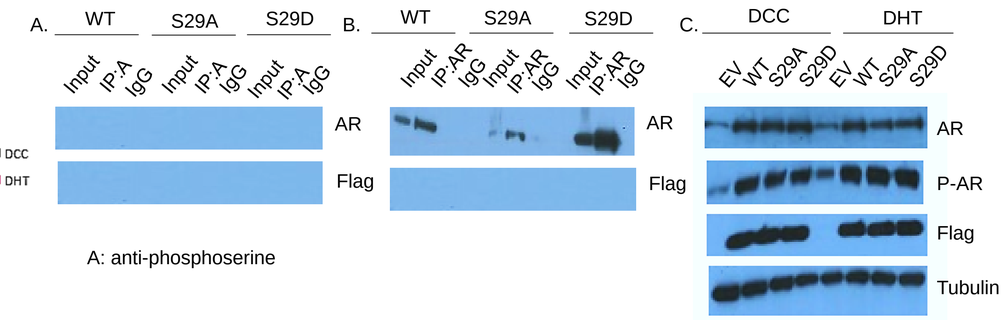
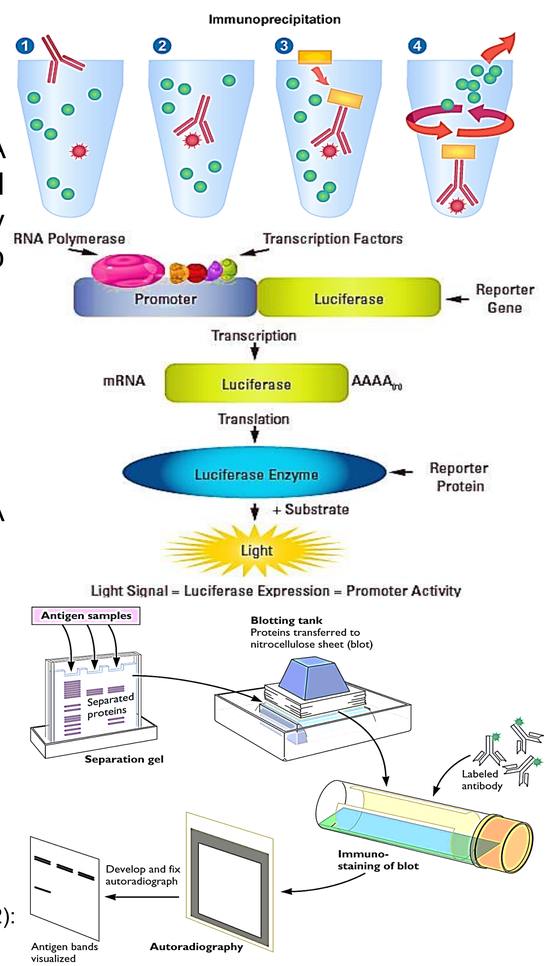



**Figure2** . (A) Pull down serine-phosphorylated proteins using anti-phosphoserine antibody in denature I.P and blot for KMT5A. (B) Pull down AR with AR antibody in native I.P and blot for KMT5A. (C) KMT5A constructs treated with DCC and DHT was blot for AR,P-AR, KMT5A and α-tubulin.




## DISCUSSION

We expected that the increased stability of pKMT5A would activate AR activity. However, from the results attained, we can suggest that the higher the concentration of WT-KMT5A, the lower the AR activity achieved when treated with DHT. We also expected to see a higher level of pKMT5A in LNCaP-AI, but due to unsuccessful denaturing IP experiment, this requires further work. Fortunately, when investigating the interaction of pKMT5A and KMT5A-AR, the native IP was successful as AR was pulled down but no KMT5A was detected in the Western blots. Hence, repeats and validation of experiments are required. On the other hand, due to inconsistent results produced, determining pKMT5A and its role in regulating AR phosphorylation also requires further repeats and validation of the experiment to improve the results.

## CONCLUSION AND FUTURE WORK

Due to inconsistent results attained and time constraints, no conclusive conclusions can be drawn. However, if the pKMT5A is the dominant form of KMT5A in LNCaP-AI cells representative of CRPC, it may impact AR signalling differently to LNCaP cells which represent androgen-dependent PC. The increased stability of pKMT5A is expected to activate AR activity. Consequently, this leads to resistance of current therapeutics directed against the androgen-AR. However, it is still unknown how KMT5A acts differently. Therefore, repeats and validation of experiments and results would need to be performed to further investigate KMT5A-AR studies in LNCaP and LNCaPAI.

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