Investigating the Mechanism of KDM4B Mediated AR Regulation

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Introduction

As the fourth most common cancer in the UK, prostate cancer accounts for 13% of all new cases. Influenced largely by age it normally occurs in males aged > 50 (Cancer Research UK, 2009).

The androgen receptor (AR) is a nuclear receptor which plays a key role in the transformation and development of the prostate dependant on its interactions with nuclear elements (Gaughan *et al*. 2011).

The demethylase enzyme KDM4B can remove a trimethyl group from histone 3 at lysine 9 in its N terminal tail and levels of KDM4B have been shown to be elevated in both bladder and lung cancers (Toyokwa *et al.* 2011).

KDM4B has been shown to interact and potentially act as a co-activator for the AR.

Aims

The aims of my project were to determine whether;

1. AR methylation is required for the KDM4B demethylase enzyme to interact with the receptor.
2. KDM4B would still be able to act as a cofactor for the receptor if the AR has not been methylated.
3. KDM4B can directly demethylate the AR or if other factors are required.

Methods

Immunoprecipitation, SDS page and Western Blotting

•Immunoprecipitation was performed as described in Guo *et al.* 2012.

•Samples were collected in SDS sample buffer containing 10% β-mercaptoethanol and analysed on 10% SDS PAGE gels.

•Gels were run at 200 V for 1 hour and transferred overnight at 30V. Western blotting was then performed as in GE Healthcare's protocol.

Denaturing Immunoprecipitation

•Same protocol as in immunoprecipitation, only altering the combinations of plasmids used in the transfections.

•Denaturing immunoprecipitation protocol was performed as described in Guo *et al.* 2012.

Reporter Assays

•Cos7 cells were cultured at varying concentrations of KDM4B.

•After six hours full media was changed to either DCC media or DCC media + 10 nM DHT (dihydrotestosterone, steroid used to allow activation of the AR.

•Beta galactosidase (β-gal) and luciferase assays were performed as in Guo *et al.* 2012.

Results

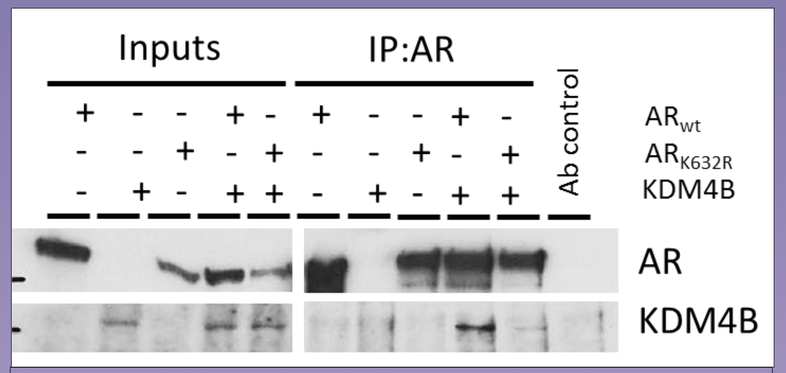
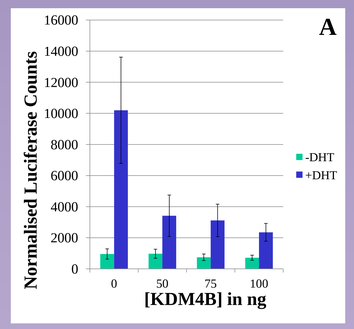


Figure 1: AR methylation may be required for KDM4B-AR interaction.
Co-transfection of HEK293 cells (450,000) with combinations of AR, KDM4B, and mutant AR (mutation at K632R) were performed. AR was immunoprecipitated and pull down confirmed by Western blotting. Hence revealing that AR methylation may be required for interaction between KDM4B and the AR.



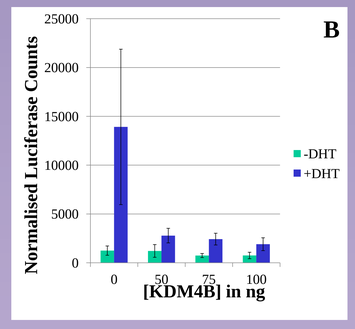


Figure 2: KDM4B can act as a negative cofactor for an unmethylated AR.
Transfection of Cos7 cells (350,000) with pGL2AREIII, pβ-gal and either AR (Figure 2A) or a mutant AR (mutated at K632R) (Figure 2B) with varying concentrations of KDM4B shown above, revealed that AR stimulation did not change in the presence or absence of DHT.

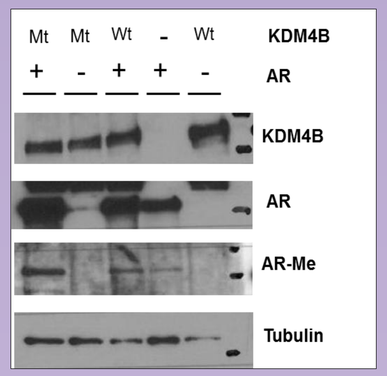


Figure 3: KDM4B may demethylate the AR.
HEK 293 cells were transfected with AR in the presence of wild type KDM4B or the demethylase dead mutant KDM4B. AR was immunoprecipitated and pull down confirmed by Western blotting. An antibody which detects methylated AR was then used to determine the methylation status of the AR in the presence of KDM4B. As there appears to be a greater amount of AR-Me in the presence of the demethylase dead form it may be postulated that KDM4B has the potential to demethylate the AR or at least play a role in the methylation pathway.

1. AR methylation may be required for KDM4B-AR interaction.
Lack of interaction between KDM4B and the mutated AR shown by absence of a band for this co-transfection, suggests that methylation of the AR may need to be present in order for this interaction to occur.

2. KDM4B can/cannot act as a cofactor for an unmethylated AR.
Absence of a change in luciferase values indicates in places KDM4B cannot act as a cofactor for an unmethylated AR.
This is not a definite conclusion, as many of the transfections were inconsistent, while some of the controls failed to work. Further repeats and validation would be needed.

3. KDM4B may/may not directly demethylate the AR.
The difference in the amount of AR-Me present in the demethylase dead KDM4B form and the wild type KDM4B proposes that KDM4B may have the potential to directly demethylate the AR, if not directly, at least playing a role in the pathway of methylation itself.

Conclusions and Further Work

- Repeats and validation of findings would need to be performed to eradicate any discrepancies with controls or inconsistencies in transfections.
- We can conclude however that AR methylation may be required for interaction between KDM4B and the AR.
- Further work would need to be done to determine what initially causes this interaction. Will it be another protein from within the signalling pathway or some other factor that has not been uncovered yet?
- KDM4B cannot act as a cofactor if the AR is demethylated, hence either other factors are involved when the AR is demethylated or it cannot do this at all.
- KDM4B might be able to demethylate the AR at this site but it may not directly do so, it could involve other cofactors or additional steps within the methylation pathway itself.

References

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