The Effects of Usp12 on Different Isoforms of Androgen Receptor in Prostate Cancer

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Introduction

Prostate cancer (PCa) affects the prostate gland of males. Its likelihood of occurrence increases with age with more than 40,000 men diagnosed and more than 10,000 deaths per year. [1] PCa cells depend on androgens for growth and survival. Androgens activate the Androgen Receptor (AR), a therapeutic target and a major player in PCa progression (Fig. 1). [2]

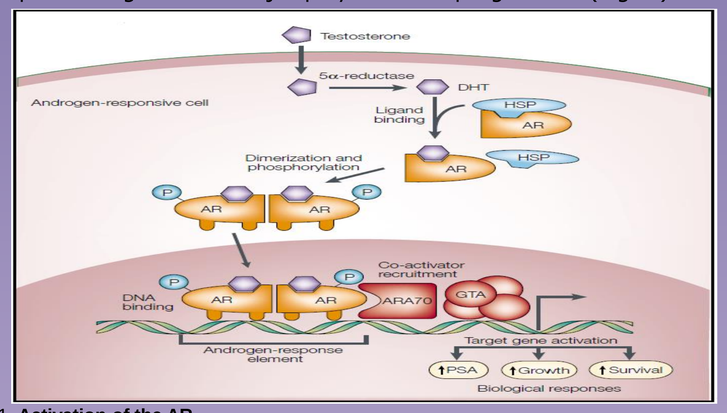

Fig. 1. **Activation of the AR**
Testosterone translocates to AR and is converted to dihydrotestosterone (DHT) by 5α-reductase. DHT is a more active form than testosterone. This event causes dissociation of heat-shock proteins (HSPs) which masks DHT's binding domain. The AR dimerises and can bind to androgen-response elements. AR target genes are activated and leads to activation of PCa. **Adapted from Feldman, B. J., & Feldman, D. (2001) 'The Development of Androgen-Independent Prostate Cancer', Nature Reviews Cancer, 1(1), pp. 34-45.**

In PCa, the AR can be full length AR (flAR) and truncated AR (tAR) isoforms. The AR is divided into four domains: *N*-terminal domain (NTD), DNA binding domain (DBD), Hinge region, and Ligand Binding Domain (LBD). [3]

Alternative splicing of transcribed intronic sequences [2] leads to a loss of the DBD, hence androgens cannot bind. This event brought about different tAR isoforms with varying *C*-terminal lengths.

Ubiquitination, a post-translational modification, is one of the ways of controlling the amount of AR. This process attaches ubiquitin molecules to lysine residues leading to degradation. [4]

Deubiquitination removes ubiquitin molecules stopping degradation using deubiquitinating enzymes (DUB). Ubiquitin Specific Protease 12 (**Usp12**) is a DUB identified in-house small interfering RNA (siRNA) screen that functions as an AR regulator and is involved in DNA damage response.

Aims

The aims of this project were to:

1. Optimise a way of silencing distinct forms of AR.
2. Investigate the effects of Usp12 on different isoforms of AR.

Methods

Transfection of cells using siRNA for AR and Usp12 with scrambled (SCR) siRNA as a control.

Western blotting technique carried out according to manufacturer's instruction using primary and secondary antibodies.

Results

Usp12, present in chromosome 13:

- Has 91% homology to Usp46, another DUB present in chromosome 4, revealing a close relationship.
- Both DUBs deubiquitinate histones 2A, 2B and non-activated notch. [5]

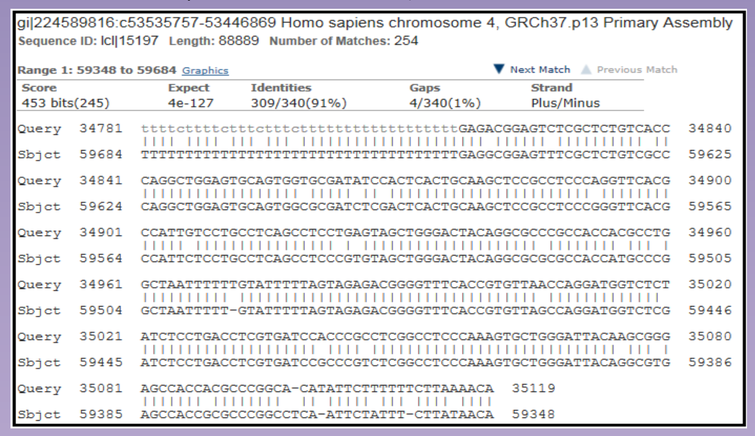

Fig. 2. **Sequence alignment of Usp12 and Usp46**
Analysis carried out using BLAST and showed a 91% sequence homology when Query (Usp12) and Subject (Usp46) were aligned.

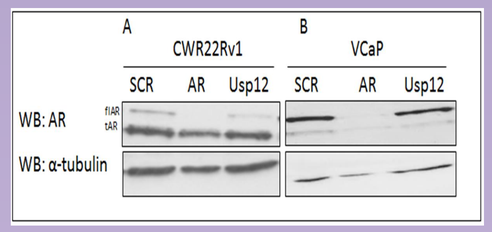

Fig. 3. **The effect of AR and Usp12 silencing 72 hours post-transfection**.
CWR22Rv1 and VCaP cells were treated with the indicated siRNA and cultured in full media. Cells were lysed at 72 hours followed by Western blotting for AR and α-tubulin.

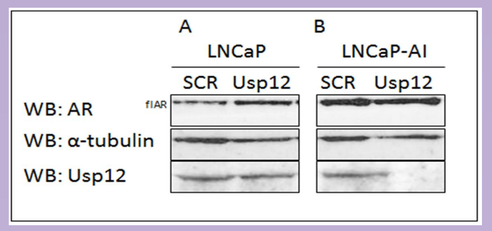

Fig. 4. **The effect of Usp12 silencing 96 hours post-transfection**.
LNCaP and LNCaP-AI were treated with siRNA as indicated and were cultured in full media. After 96 hours, cells were lysed followed by Western blotting for AR, α-tubulin, and Usp12

1. **Usp12 silencing in CWR22Rv1 reduced flAR protein and had no effect on tAR protein (Fig. 3A).**
   CWR22Rv1 contains a duplicated exon 3 encoding a zinc finger of DBD and is sensitive to androgen stimulation. After treatment with AR siRNA; flAR, and not tAR was silenced. Usp12 siRNA treatment caused a decrease in flAR compared to SCR. Hence, Usp12 siRNA treatment may have a reducing effect on flAR and no effect on tAR protein levels.

2. **Usp12 silencing in VCaPs decreased flAR and tAR protein (Fig. 3B).**
   VCaP had an amplified AR and was sensitive to androgen stimulation. After treatment with AR siRNA, flAR and not tAR may have been silenced. Usp12 siRNA decreased both flAR and tAR protein level compared to SCR.

3. **No conclusion about Usp12 in LNCaP cells (Fig. 4A).**
   LNCaP cells are responsive to androgen stimulation and have no tAR. No conclusion could be made about the effect of Usp12 on flAR because silencing Usp12 was unsuccessful.

4. **No effect on flAR protein when Usp12 was silenced in LNCaP-AI (Fig. 4B).**
   LNCAP-AI cells were derived from parental LNCaP but androgen independent. Usp12 silencing may be successful and from this conclusion, it may have no effect on flAR when compared to SCR treated control.

Conclusion

- We successfully silenced AR in the cell lines used.
- Usp12 plays a role in AR isoforms of CWR22Rv1 and VCaP.
- In LNCaP and LNCaP-AI, Usp12 may play no role in flAR.
- Repeats need to be carried out to confirm the results above as there was not enough time to repeat the experiment.

These findings may aid in understanding if Usp12 can be a target for PCa treatment in different patients.

References

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