ARD1 Acetylation of AR-V7 in Prostate Cancer

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Introduction

- Prostate cancer (PCa) is the second leading cause of cancer-related mortality among men in Western societies, and it affects approximately 1 in every 10 male individuals. Prostate cancer is mediated by the androgen receptor (AR) signaling pathway through its subsequent activation and deregulation. Mutations, amplification, and posttranslational modifications of AR all lead to prostate tumorigenesis.
- The principle treatment for locally advanced and metastatic PCa is androgen-deprivation therapy (ADT). While initially providing remission of the disease for the majority of patients, as shown by a decrease in prostate-specific antigen (PSA), the disease begins to progress after 2-3 years. This advanced state of cancer is known as castrate-resistant prostate cancer (CRPC) and comes with a poor prognosis and high lethality, with a mean survival time of only 16-18 months.
- Despite depleting circulating levels of androgens via ADT, tumor progression is often associated with elevated levels of AR, AR activation, and expression of AR-regulated genes. Several cellular and molecular alterations are related to this post-castration activation of the AR. However, one of the major contributing factors is the expression of AR-splice variants. Recent evidence points to the existence of alternatively spliced forms of AR mRNAs, which encode receptors devoid of the LBD but retain the ability to engage transcriptional machinery and promote the progression of prostate tumorigenesis. The lack of the LBD is responsible for these C-terminal truncated AR variants to be constitutively active while also allowing them to be resistant to current therapeutics such as AR-antagonists which require binding to the LBD for activity. An increased understanding of the mechanisms underlying the pathogenesis of castrate resistance is needed to develop therapeutic approaches for this disease.

Arrest Defective-1 protein (ARD1)

- Arrest defective-1 protein (ARD1) is an acetyltransferase that is overexpressed in prostate cancer.
- ARD1 has been shown to increase the activation of AR leading to a profound increase in tumorigenesis.
- ARD1 acts as a co-activator of AR by acetylating the DNA binding domain (DBD).

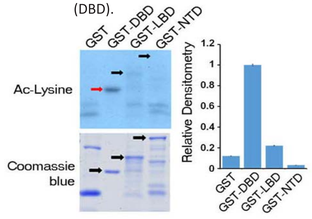


Figure 1. Immunoblots and graphs depicting normalized relative densitometry of individual GST-AR fragments following *in vitro* acetylation assay and Western blot with anti-acetylated lysine antibody.

AR-V7

- AR-splice variant 7 (AR-V7) is the most common AR-splice variant found in CRPC.
- AR activation requires an androgenic ligand, such as testosterone or dihydrotestosterone (DHT), to bind the C-terminal ligand binding domain. Once bound, AR undergoes a conformational change that permits nuclear translocation, DNA binding, and regulation of AR target genes.
- In contrast to AR, AR-V7 lacks the C-terminal ligand binding domain, thus it is purported to have constitutive ligand-independent activity, which leads to prostate tumorigenesis.

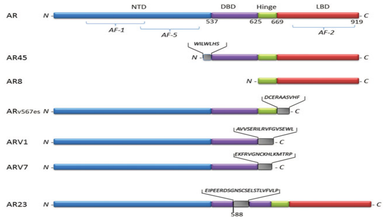


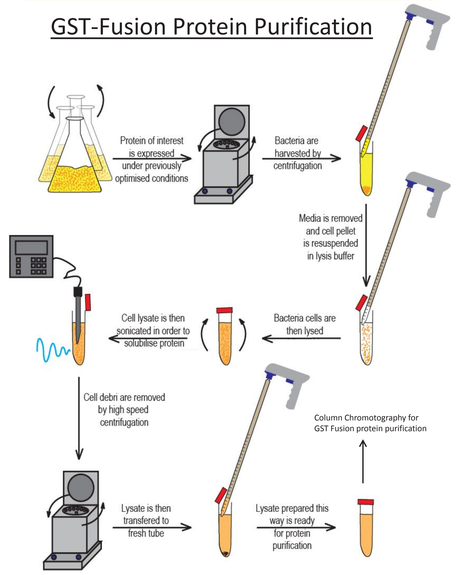
Figure 2. Structural domains of AR and its more important isoforms and splice variants. NTD: N-terminal domain, DBD: DNA binding domain, LBD: ligand binding domain, AF: transcriptional activation function.

Hypothesis

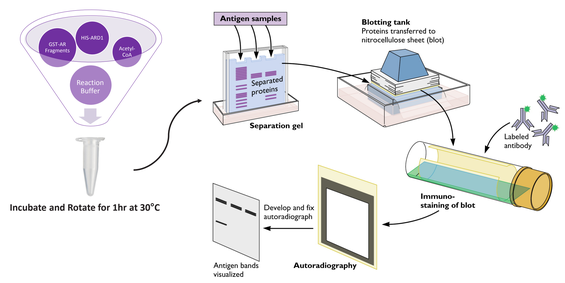
- Given that AR-V7 retains the DBD region of AR, we hypothesize that ARD1 overexpression may act as a co-activator of AR-V7 by acetylating the DBD domain. This allows for increased nuclear translocation, target gene expression, and ultimately CRPC.

Methods

GST-Fusion Protein Purification



In Vitro Acetylation



Results

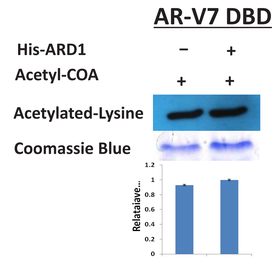


Figure 5. Immunoblots and graph of normalized relative densitometry of GST-AR-V7 DBD following *in vitro* acetylation assay and Western blot with anti-acetylated lysine antibody.

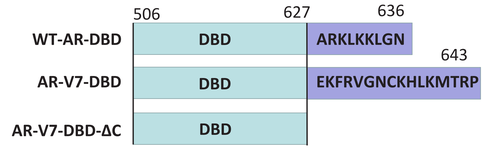


Figure 6. Comparison of the amino acid sequence of WT-AR-DBD, AR-V7-DBD, and AR-V7-DBD- ΔC.

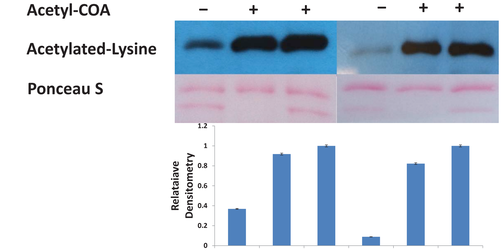


Figure 7. Immunoblots and graph of normalized relative densitometry of GST-AR-V7 DBD and GST-AR-V7 DBD-ΔC following *in vitro* acetylation assay and Western blot with anti-acetylated lysine antibody.

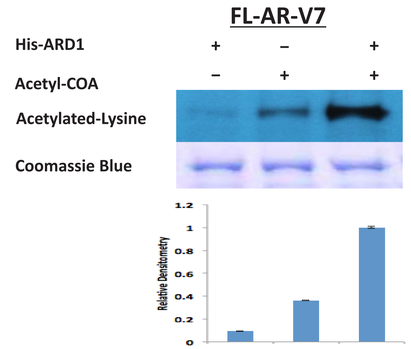


Figure 8. Immunoblots and graph of normalized relative densitometry of GST-FL-AR-V7 following *in vitro* acetylation assay and Western blot with anti-acetylated lysine antibody.

Conclusion

- *In vitro* acetylation of AR-V7 DBD demonstrates auto-acetylation.
- Further investigation reveals that, without the 16 amino acid C-terminal region, auto-acetylation still occurs.
- The absence of auto-acetylation in WT-AR-DBD must be due to the presence of the 9 C-terminal amino acids inhibiting auto-acetylation.
- *In vitro* acetylation of FL-AR-V7 resulted in minimal auto-acetylation and a profound increase in acetylation with ARD1 present.
- ARD1-dependent acetylation of FL-AR-V7 could be responsible for the constitutively active nature of the receptor, leading to increased tumorigenesis in CRPC.
- Blocking the interaction of ARD1 with AR-V7 or inhibiting ARD1-mediated acetylation could be a potential target for CRPC drug therapy.

Future Directions

Determine the acetylation site in AR-V7 by *in vitro* and *in vivo* assays.

Investigate the pathological impacts of AR-V7 acetylation in PCa tumorigenesis *in vitro* and *in vivo*.

Develop Ab specific for AR-V7-Ac to determine if an increase in AR-V7 acetylation correlates with CRPC progression.