DAX-1 Regulation of Metastatic Gene Expression in Prostate Cancer Cells

Brandon Reyes, Roxxana Beltran, Dr. Christina Tzagarakis-Foster

UNIVERSITY OF SAN FRANCISCO
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Background:

DAX-1 is an orphan nuclear receptor which has been implicated in **D**osage-sensitive sex reversal, **A**drenal hypoplasia congenita on the **X** chromosome gene **1** as well as its role in various cancers. This nuclear receptor has no known ligand, lacks typical domains that allow direct binding to DNA, and instead have alanine and glycine reach repeats with motifs typically found in coactivators and corepressors.

Prostate cancers have been traditionally diagnosed with bone metastases at which point the cancer has advanced rather significantly. Metastasis occurs when the cancer cells are able to detach and assume a mesenchymal state. These cells can also undergo a process called epithelial mesenchymal transition to traverse cell linings into different tissues. This can occur with a loss of proteins such as E-cadherin, a protein responsible for cell adhesion. Its expression is decreased by TWIST and SLUG proteins. RUNX2 on the other hand plays a crucial role in the formation of osteoblastic metastases.

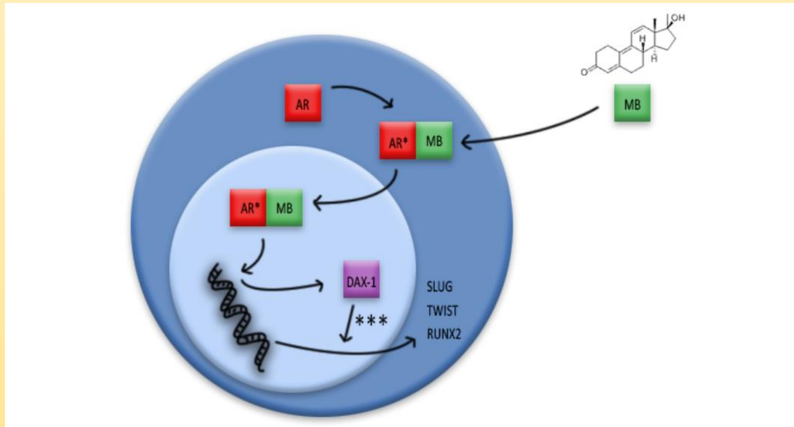


Figure 1 Basic putative mechanism of metribolone (MB) treatments with androgen receptor (AR) and the effects with DAX-1. DAX-1 is suspected to influence the following proteins involved in metastasis: SLUG, TWIST, and RUNX2; labeled by three asterisks.

Introduction:

Metastatic marker interactions with DAX-1 are of interest given prostate cancers progressions. DAX-1 has been shown to be expressed in LnCap cells but not in LCR1 cells as shown in **Figure 2**. The LCR1 cell line is a CRISPR knockout of DAX-1 so that it is not expressed in the cells. The figure shows the results of the CRISPR knockout to be significantly successful and the presence of androgen receptor in both cell lines.

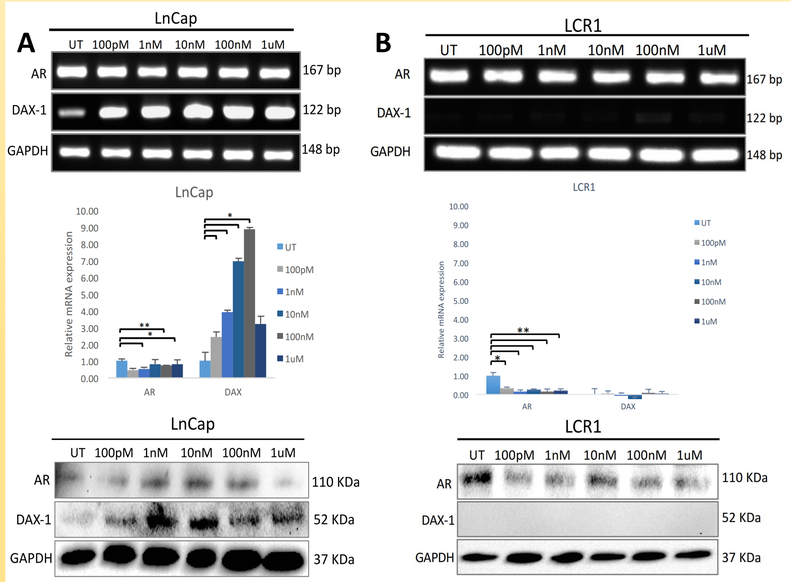
Due to the fact that LCR1 doesn't express DAX-1 innately, the effects caused by metribolone observed will be identify key roles DAX-1 is involved in. From previous research in the lab some influenced targets have been identified in proliferation, apoptosis, and metastasis to hone in particular factors. Identifying these targets individually characterize the influence DAX-1 has on metastatic processes.

Figure 2

Relevant receptor genes and products given the metribolone treatments in LnCap and LCR1 cell lines. PCR (top), qPCR (middle), and western blot (bottom) analyses performed for the various protein targets. GAPDH serves as a control for comparison given the treatments.

A. LnCap cell work: PCR analysis reveals presence of all three targets and the upregulation of DAX-1 with treatments. qPCR analysis shows the changes in AR and DAX-1 to be significant in the metribolone treated samples. Western blot shows presence of target proteins with the upregulation of DAX-1 taking effect.

B. LCR1 cell work :PCR analysis here instead shows a distinct lack of DAX-1 thanks to the CRISPR knockout. qPCR analysis shows the significant decrease in AR and lack of DAX-1 across the cell line. Western blot shows presence of target proteins with the entire lack of DAX-1 protein.



Acknowledgements

Farhad Vesuna, Paul van Diest, Ji Hshiung Chen, Venu Raman. Twist is a transcriptional repressor of E-cadherin gene expression in breast cancer. Biochemical and Biophysical Research Communications. Volume 367, Issue 2, 2008.
Karen M. Hajra, David Y-S. Chen and Eric R. Fearon. The SLUG Zinc-Finger Protein Represses E-Cadherin in Breast Cancer. Cancer Res March 15 2002 (62) (6) 1613-1618

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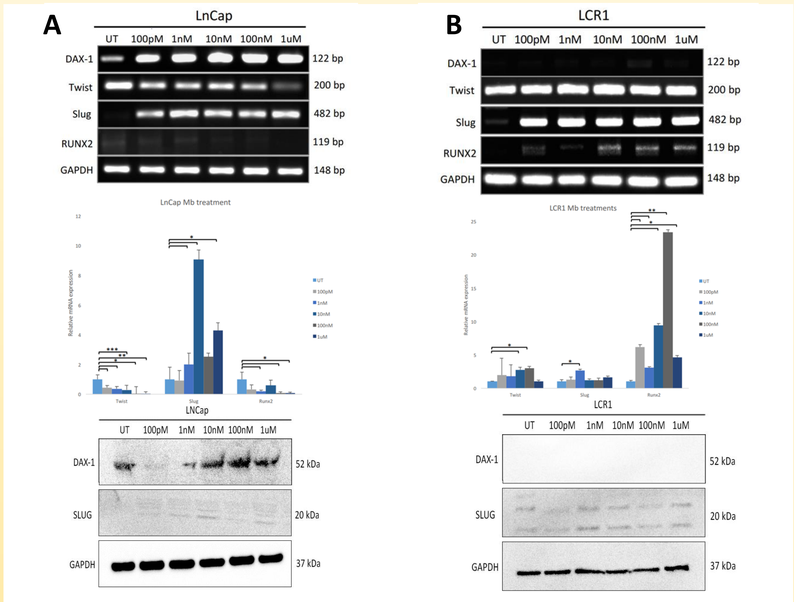

Figure 3

Metastatic genes and products given the metribolone treatments in both cell lines. PCR (top), qPCR (middle), and western blot (bottom) analyses performed for the various protein targets. GAPDH serves as a control for comparison given the treatments.

A. LnCap cell work: PCR show effects of metribolone on TWIST and SLUG and no effect on RUNX2. qPCR identifies significant changes in all three but mainly in TWIST expression. Western blot analysis shows an increase in DAX-1 protein production with the increasing metribolone treatments and SLUG expression.

B. LCR1 cell work: PCR show increase of RUNX2 in higher doses and no effect on TWIST and SLUG. qPCR identifies significant changes in all three but mainly in RUNX2 expression again. Western blot analysis shows an increase in DAX-1 protein production with the increasing metribolone treatments and no SLUG expression change in the absence of DAX-1.

Conclusion

DAX-1 shows a significant influence over the metastasis related proteins and is therefore a potential target for therapies given its role in repressing the metastatic changes. We see that in LnCap cells, the metribolone is able to upregulate DAX-1. This can be associated with the relevant changes in the protein targets TWIST, SLUG, and RUNX2. These changes can be attributed to DAX-1 working in conjunction with activated androgen receptors due to the fact that LCR1 cells seem to be unresponsive to the metribolone treatments. A key difference is found in DAX-1 expression which seems to decrease TWIST and SLUG expression but RUNX2 seems to require some other factor given its expression at high metribolone dosages. Further western blot analysis is required to complete this work.