Prostatic androgen receptor expression decreases with age and may be regulated by DDX3X in mice

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Introduction

Prostate cancer is the most common non-cutaneous cancer in men in Western countries, estimated to have > 248,000 new cases in the United States in 2021. Another prostate disease, benign prostatic hyperplasia (BPH), affects > 210 million men worldwide and costs about 4 billion dollars in the United States for treatment. Studies suggest that BPH and prostate cancer are closely linked to steroid hormone and hormone receptor changes and aging. However, these detailed cellular and molecular mechanisms remain unclear.

The androgen receptor (AR), which is critical for the maintenance of the normal prostate, is believed to play an important role in the pathology of prostate cancer and BPH. Testosterone (T) and dihydrotestosterone (DHT) are physiological ligands for AR. After entering the cell, T is converted to DHT by 5-α reductase, whereby DHT has a 4-5 fold higher AR binding capacity than T. Once AR is activated, it translocates from the cytoplasm to the nucleus, and initiates the transcription of target genes. While many studies focus on the genetic alterations of AR expression in diseases and normal aging, few pay attention to the translational regulation of AR protein expression. Our previous studies indicated that the RNA helicase, DDX3X, binds to AR mRNA at stress granules and inhibits AR protein translation, which may lead to a failed response to androgen deprivation therapies. DDX3X is a member of Dead-box helicase family, and its functions depends on the localization. Whereas prostatic AR is primarily localized to the nucleus, DDX3X can be found in in both the cytoplasm and nucleus. In the nucleus, DDX3X is involved in gene transcription, RNA splicing, and RNA export; while in the cytoplasm, DDX3X works as translation regulator through interactions with ribosome and eukaryotic initiation factors. Both AR and DDX3X can be found in both stromal and epithelial tissue compartments.

In this study, we evaluated the localization/expression of cytoplasmic DDX3X and nuclear AR in normal aging in the male mouse prostates. Our findings indicate the expression of AR protein decreases with age, which may involve the regulation through a DDX3X mediated mechanism.

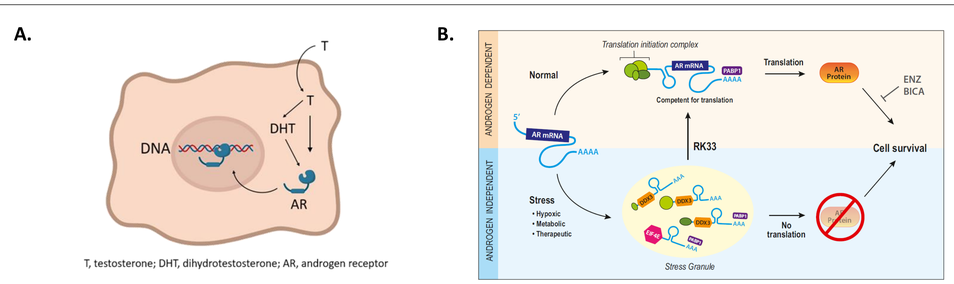

Figure 1. DDX3X inhibits the translation of androgen receptor (AR) by binding to AR mRNA at stress granules.
A. Schematic for cellular AR signaling axis. **B.** Schematic for DDX3X-mediated regulation of AR in castration-resistant prostate cancer (CRPC) [1].

Materials and Methods

- Young (2 months, n = 9) and old (24 months, n = 7) C57Bl6 mice were obtained from Jackson Laboratory.
- Immunofluorescence (IF) staining was performed on young and old mouse anterior prostate tissues.
  - Rabbit anti-androgen receptor antibody (1:250)
  - Rabbit anti-DDX3X antibody (1:200)
  - OPAL 540 fluorophore (1:300)
  - Goat anti-rabbit Alexa Fluor 488 fluorophore (1:1000)
- IF staining was imaged using the Mantra multispectral imaging platform.
- The percentage of AR positivity was quantified with InForm software.

Results

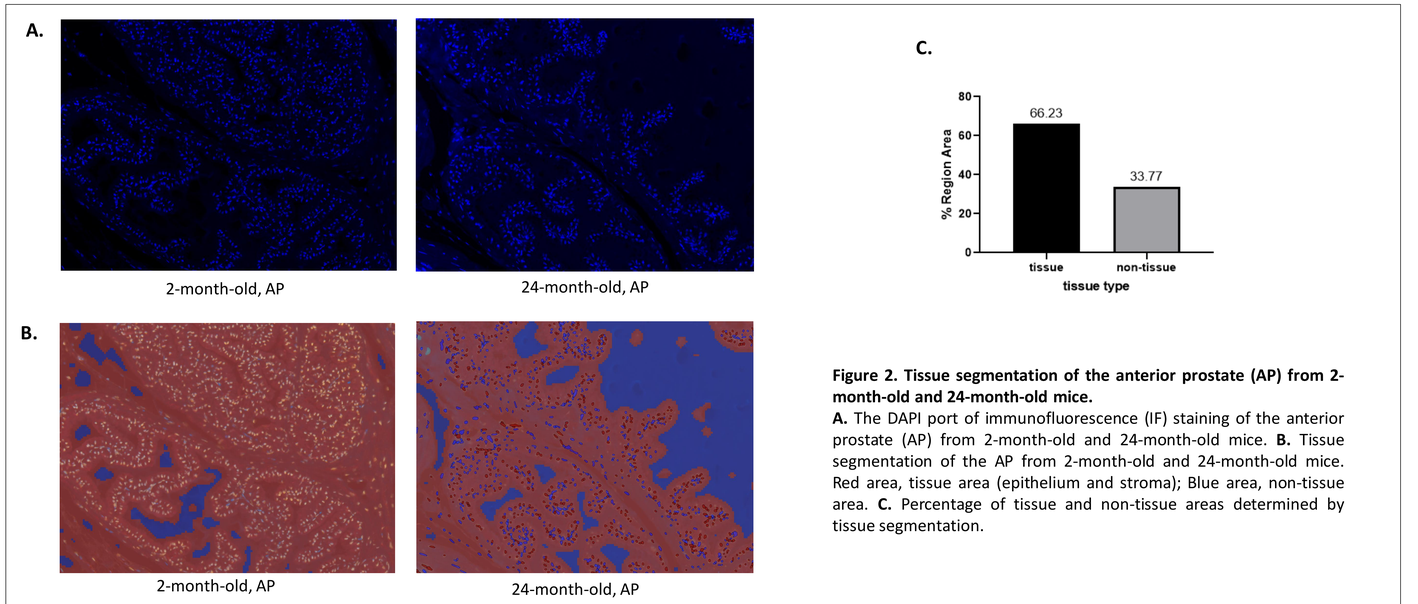

Figure 2. Tissue segmentation of the anterior prostate (AP) from 2-month-old and 24-month-old mice.
A. The DAPI port of immunofluorescence (IF) staining of the anterior prostate (AP) from 2-month-old and 24-month-old mice. **B.** Tissue segmentation of the AP from 2-month-old and 24-month-old mice. Red area, tissue area (epithelium and stroma); Blue area, non-tissue area. **C.** Percentage of tissue and non-tissue areas determined by tissue segmentation.

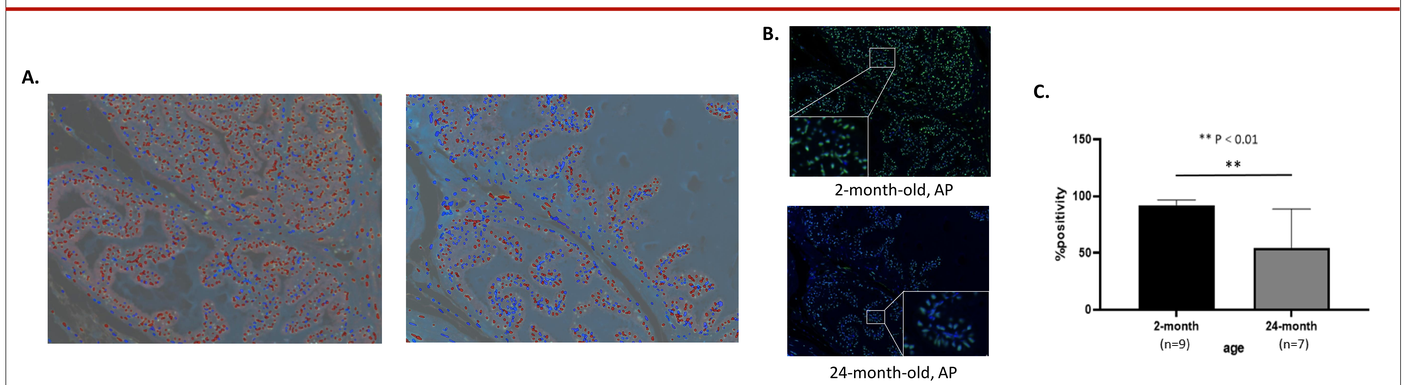

Figure 3. Androgen receptor (AR) protein expression decreases with age in mouse anterior prostate (AP).
A. Cell segmentation of the AP from 2-month-old and 24-month-old mice. Red, AR-positive nuclei; Blue, AR-negative nuclei. **B**. Immunofluorescence (IF) staining of the AP from 2-month-old and 24-month-old mice. Green, AR; Blue, DAPI. **C.** The AP from 2-month-old mice showed significantly higher expression of AR protein than the 24-month-old counterpart.

Results (cont.)

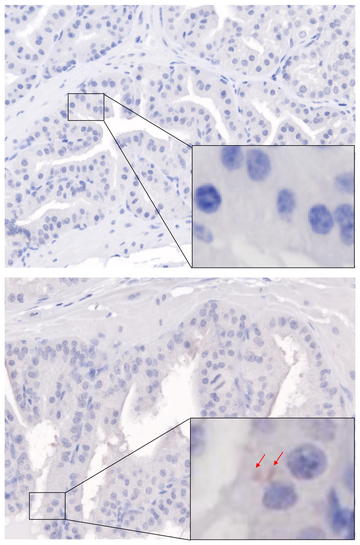

Figure 4. Aging leads to punctate DDX3 expression in mouse anterior prostate.
A. Immunohistochemistry (IHC) staining of the AP from 2-month-old mice. **B.** IHC staining of the AP from 24-month-old mice. Punctate DDX3X indicated by arrows. Brown, DDX3X; Blue, DAPI.

Conclusions

Aging is associated with decreased prostatic AR localization/expression and may involve regulation through the DDX3X mediated mechanism.

Future directions

- Optimize DDX3X IF staining and examine its changes of expression in normal aging.
- Evaluate the expression of DDX3X with treatment of the DDX3X inhibitor RK33 in young and old C57Bl/6 mice.
- Western Blotting (WB) will be carried out to examine the changes of AR and DDX3X expression at protein level.
- Co-localize DDX3X with AR and the stress granule marker PABP1 for investigation of the detailed DDX3X-AR regulation mechanism.

References

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[2]Cancer Statistics, 2021. Siegel R.L. et.al.; *CA CANCER J CLIN*; 2021 (71), 7–33.

[3]Androgen Receptor Dependence. Chaturvedi A. P. and Dehm S. M.; *Adv Exp Med Biol*; 2019 (1210), 333-350.

Acknowledgements

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