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 5a-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 depend on the localization. Whereas prostatic AR is primarily localized to the nucleus, DDX3X can be found 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 a 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 prostate. Our findings indicate the expression of AR protein decreases with age, which may involve the regulation through a DDX3X-mediated mechanism.

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)
- OPA1 540 fluorochrome (1:300)
- Goat anti-rabbit Alexa Fluor 488 fluorochrome (1:1000)
- IF staining was imaged using the Mantra multispectral imaging platform.
- The percentage of AR positivity was quantified with Inform software.

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Results

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).

Figure 2. Tissue segmentation of the anterior prostate (AP) from 2-month-old and 24-month-old mice.
A. The DAPI part 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.

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 RX33 in young and old C57Bl6 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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