MEDICAL UNIVERSITY OF VIENNA

Characterization of the androgen receptor in androgen-sensitive and castration-resistant human prostate cancer cell lines

Julia Raitanen[1,2,3], Katarína Benčurová[1,2], Patrik Schwarzl[1], Gerda Egger[1,4], Marcus Hacker[2], Thomas L. Mindt[1,3], Markus Mitterhauser[1,2], Theresa Balber[1,2]

[1]Ludwig Boltzmann Institute Applied Diagnostics, Vienna, Austria
[2]Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Vienna, Austria
[3]Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria
[4]Department of Pathology, Medical University of Vienna, Vienna, Austria

Objective

The androgen receptor (AR) and its signaling axis in the progression of prostate cancer is the key to our understanding of castration resistance. As depicted in Figure 1, the proposed signaling pathway of the AR starts with testosterone (T), being transported to the cell by sex hormone binding globulin (SHBG), where it either directly binds to the AR or is reduced to dihydrotestosterone (DHT; higher affinity to AR). Ligand binding leads to dissociation of heat shock proteins (HSP), conformational change, subsequent phosphorylation (P), homodimerization and finally binding to androgen responsive elements (ARE), promoting gene transcription. Androgen-sensitive prostate cancer is often treated with androgen deprivation therapy (ADT), using antiandrogens and luteinizing hormone-releasing hormone (LHRH) analogs e.g., or 5α-reductase inhibitors (5ARI). However, ADT and cancer progression often lead to androgen-independent prostate cancer.

This *in vitro* study was designed to investigate AR expression, localization and functionality in androgen-sensitive and castration-resistant human prostate cancer cell lines, using (i) 16β-[^{18}F]fluoro-5α-dihydrotestosterone ([^{18}F]FDHT, Figure 2), a radiotracer for positron emission tomography targeting the AR [1], as well as (ii) specific antibodies for immunofluorescence (IF) and Western blot (WB).

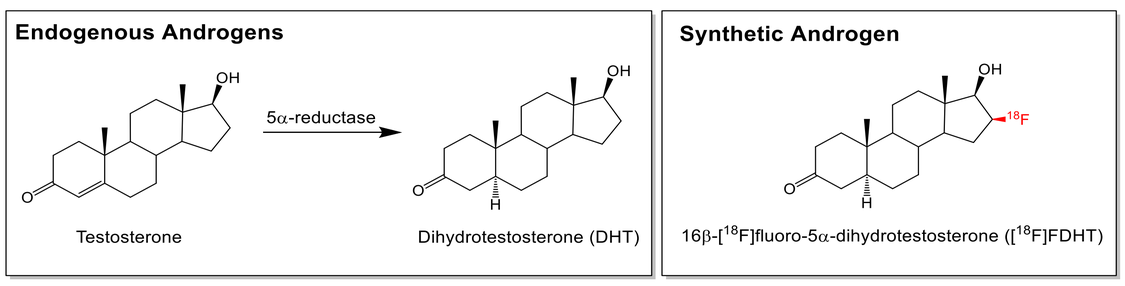

Figure 2: Endogenous androgens in contrast to synthetic androgen [^{18}F]FDHT.

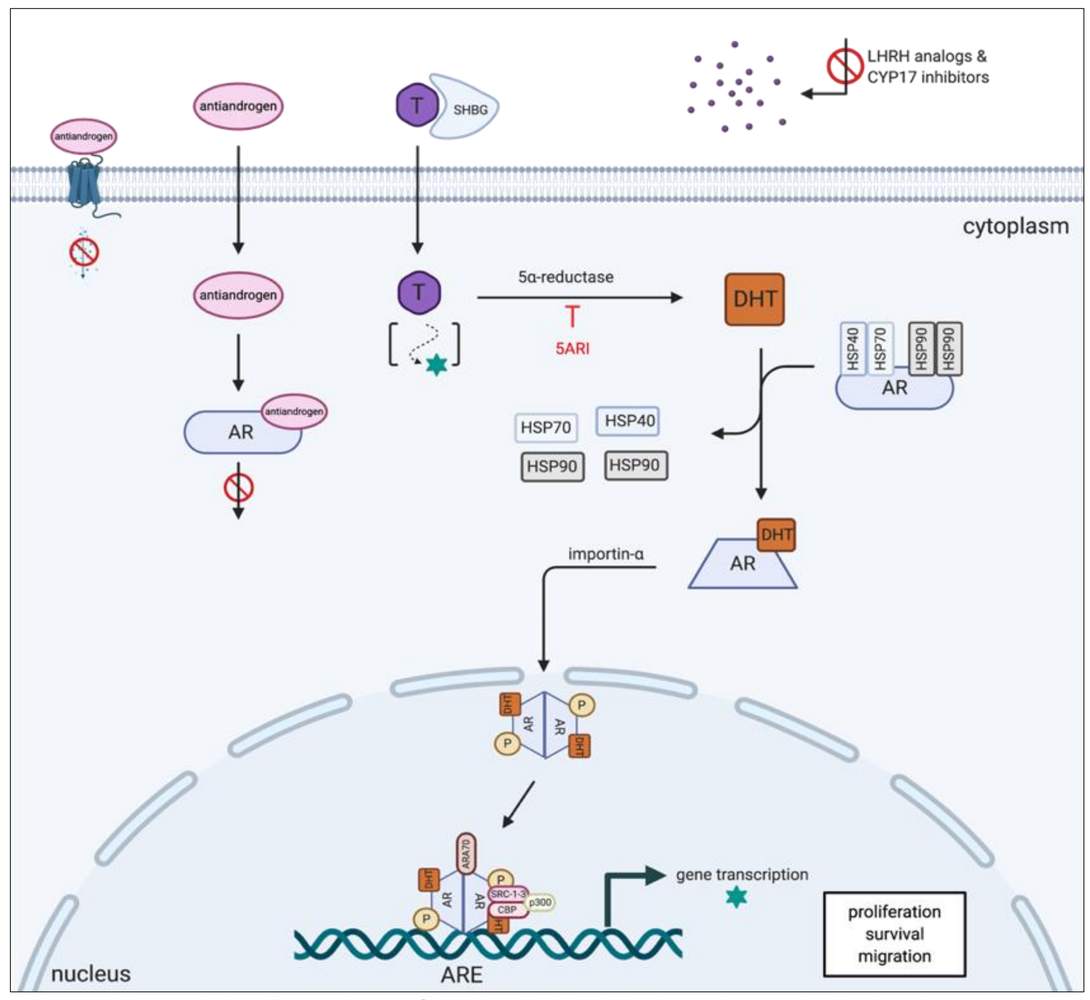

Figure 1: Proposed signaling pathway of the AR & its inhibition by androgen deprivation therapy.

Methods and Materials

Cellular [^{18}F]FDHT uptake was investigated in androgen-sensitive (LNCaP [2]) and androgen-independent (PC-3 [3]) human prostate cancer cell lines by collecting membrane-bound, internalized and nuclear fractions. WB analyses as well as IF were employed to determine target expression and receptor localization in support of obtained data.

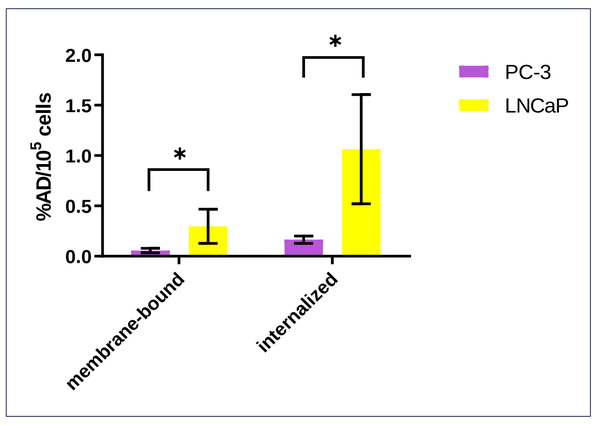

Figure 5: Specific [^{18}F]FDHT uptake (membrane-bound & internalized fractions). Percentage of applied dose per 10^5 cells (%AD/10^5 cells), n=4 in triplicates, unpaired t-test: *p<0.05

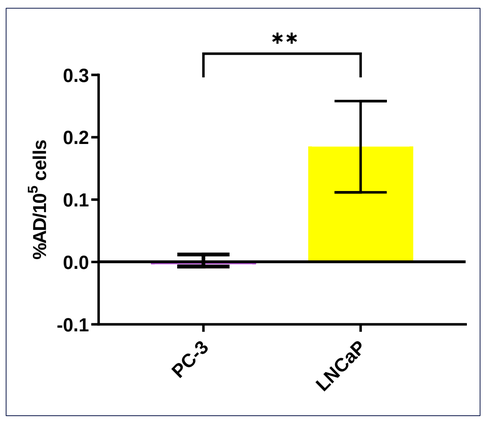

Figure 6: Specific nuclear [^{18}F]FDHT uptake. Percentage of applied dose per 10^5 cells (%AD/10^5 cells), n=4 in triplicates, unpaired t-test: **p<0.01

Results

Significantly higher specific [^{18}F]FDHT membrane binding and nuclear uptake was found in LNCaP cells compared to PC-3 cells (Figure 5 and 6). WB analyses (Figure 7 and 8) and IF (Figure 9) confirmed the presence of ARs of different isoforms (full-length: AR-B and truncated: AR-A) in both investigated cell lines. LNCaP cells demonstrated cytoplasmic and pronounced nuclear AR expression, while in PC-3 cells only the AR variant was detected.

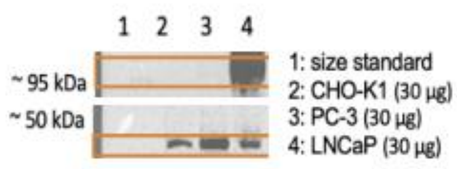

Figure 7: Cropped image of WB 1. Anti-AR directed against N-terminus of AR shows AR-B expression at ~95 kDa; Anti-beta-actin shows beta-actin at ~42 kDa.

Figure 8: Cropped image of WB 2. Anti-AR directed against amino acids 299-315 of AR shows AR-B expression at ~95 kDa (orange) & potential AR-A expression at ~87 kDa (red); Anti-beta-actin shows beta-actin at ~42 kDa.

Conclusion

[^{18}F]FDHT uptake in all fractions was significantly higher in androgen-sensitive LNCaP than in androgen-independent PC-3 cells suggesting androgen-dependence as the driving force for higher expression of androgen-binding membrane proteins. This data further suggests that androgen-sensitive cells quickly translocate the tracer-receptor complex to the nucleus to initiate gene transcription and consequent tumor growth. However, the AR isoform expressed by castration-resistant PC-3 cells is not capable of binding relevant amounts of [^{18}F]FDHT, pointing at impaired AR functionality. This *in vitro* data contributes to a deeper understanding of [^{18}F]FDHT PET in a clinical context.

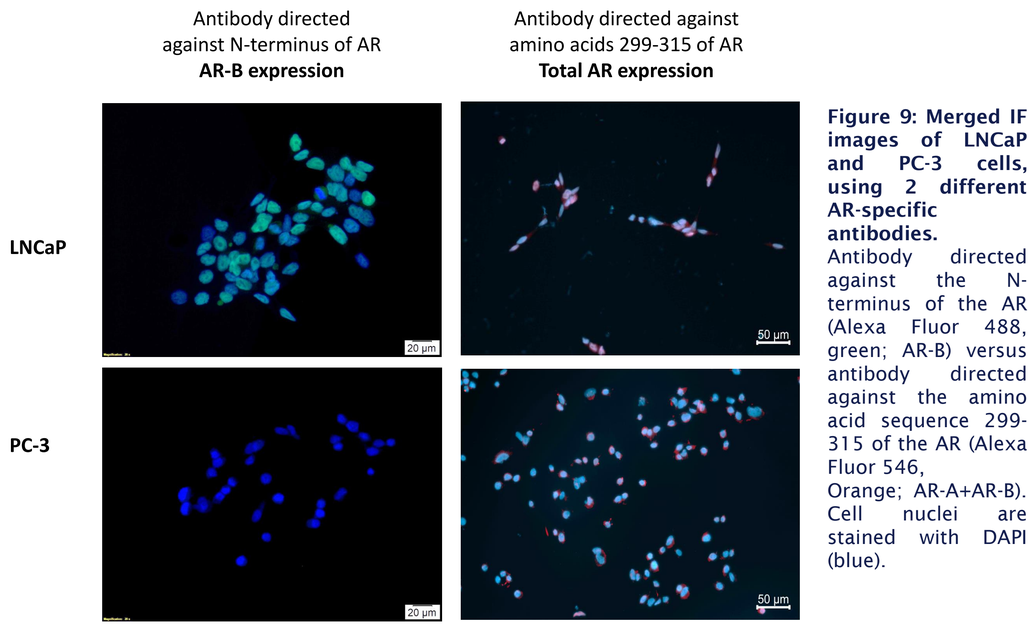

Figure 9: Merged IF images of LNCaP and PC-3 cells, using 2 different AR-specific antibodies. Antibody directed against the N-terminus of the AR (Alexa Fluor 488, green; AR-B) versus antibody directed against the amino acid sequence 299-315 of the AR (Alexa Fluor 546, Orange; AR-A+AR-B). Cell nuclei are stained with DAPI (blue).

References

[1] A. Liu, et al. "Synthesis of High Affinity Fluorine-Substituted Ligands for the Androgen Receptor. Potential Agents for Imaging Prostatic Cancer by Positron Emission Tomography" J. Med. Chem., vol. 35, no. 11, pp. 2113–2129, 1992
[2] T. M. Chu, et al. "Lncap model of human prostatic carcinoma" Cancer Res., vol. 43, no. 4, pp. 1809–1818, 1983
[3] M. E. Kaighn, et al. "Establishment and characterization of a human prostatic carcinoma cell line (PC-3)" Invest Urol., vol. 17, no. 1, pp. 16–23, 1979