CYP3A5 promotes aggressive and therapeutically resistant prostate cancer by modulating AR and Wnt Signaling: implications for racially disparate clinical outcomes

Jake McLean[1], Ajay P. Singh[2], Edwin C. Oh [3] and Ranjana Mitra [1]

[1] Roseman University of Health Sciences, Las Vegas, NV, [2] University of South Alabama, Mobile, AL [3] University of Nevada Las Vegas, Las Vegas, NV

Background

Prostate cancer (PC) stands as the second leading cause of cancer-related mortality among American men, with a notably disproportionate impact on African American (AA) individuals. The androgen receptor (AR) signaling pathway plays a key role in the initiation and progression of PC, making it a primary therapeutic target, particularly in advanced disease stages. Aberrant activation of AR is implicated in the development of castration-resistant prostate cancer (CRPC), posing a significant challenge in treatment.

Despite advancements in anti-androgen therapies, including the treatment with novel agents like enzalutamide, metastatic CRPC (mCRPC) remains a formidable clinical hurdle with no definitive cure. Within the context of PC biology, CYP3A5, a monooxygenase expressed in various tissues including the prostate, liver, and intestine, assumes a crucial role in drug metabolism and steroid biosynthesis.

Our prior research has shown the involvement of intratumoral CYP3A5 in AR signaling, facilitating AR nuclear translocation and thereby contributing to disease progression. Moreover, our investigations have highlighted a distinct pattern of CYP3A5 expression, with higher levels observed in AA individuals, primarily associated with the *1 CYP3A5 variant, in contrast to non-Hispanic White Americans (NHWA) who predominantly harbor the *3 variant. This disparity underscores potential ethnic-specific differences in PC pathogenesis and therapeutic responses, warranting further exploration and targeted interventions.

Methods

The methods employed in this study encompassed several key techniques. Genotyping of PC cell lines was achieved through a RT-PCR probe/primer assay. For the quantification of *1 and *3 CYP3A5 RNA transcripts, both qRT-PCR and base scope assays were utilized. Cell fractionation followed by western blotting and immunofluorescence staining were utilized to study AR signaling. Illumina RNA sequencing was conducted using RNA extracted from 13 African American (AA) and 12 Non-Hispanic White American (NHWA) prostatic adenocarcinomas. To explore the involvement of CYP3A5 in enzalutamide resistance, enzalutamide-resistant PC cell lines were developed with a gradual increase in enzalutamide dosage. Changes in AR and CYP3A5 expression between parental and enzalutamide-resistant cell lines were examined using RT-qPCR assay. Furthermore, RNA sequencing and western blot analysis were performed after siRNA treatment to evaluate the impact of CYP3A5 on modulating Wnt signaling.

Results

Analysis of RNA-seq data from patient tumor samples unveiled a significant association between elevated CYP3A5 levels in African American (AA) PC patients and the activation of Wnt-β catenin signaling, mediated by TCF4 overexpression. Notably, all tested AA PC cell lines exhibited the *1 variant and displayed markedly higher CYP3A5 expression levels compared to their non-Hispanic White American (NHWA) counterparts.

The frequency of the *1 variant significantly rises in prostate cancer patients compared to the general healthy population, also indicating a potential association with prostate cancer growth.

Further investigation of enzalutamide-resistant cells revealed distinct patterns: cells of AA origin (MDAPCa2b/EnzR) exhibited heightened CYP3A5 expression, whereas those of NHWA origin (LNCaP/EnzR) did not display such elevation. Interestingly, while MDAPCa2b/EnzR cells showed no change in AR levels, an increase was observed in LNCaP/EnzR cells relative to their parental lines.

To elucidate the functional implications of CYP3A5, a loss-of-function assay was conducted using siRNA-mediated silencing. In MDAPCa2b cells, RNA sequencing post-CYP3A5 inhibition revealed downregulation of key Wnt pathway genes (Wnt5A, Wnt10B, Wnt11, Fzd2, and Dvl3), consistent with previous findings in patient samples indicating upregulated Wnt-β catenin signaling in high CYP3A5 expressing tumors.

Western blot analysis following CYP3A5 inhibition in both LNCaP and MDAPCa2b cells demonstrated upregulation of Axin and downregulation of LRP6 and Dvl-3, indicative of CYP3A5's regulation of the canonical Wnt-β catenin pathway. Moreover, MDAPCa2b cells exhibited downregulation of Wnt 5A and Wnt 11A/B, molecules known to influence the Wnt non-canonical pathway.

Collectively, these findings suggest that the *1 CYP3A5 variant in African Americans likely contributes to the aggressive behavior and therapeutic resistance observed in PC, serving as a molecular determinant underlying disparate clinical outcomes.

Relevance to VA

- Almost 90% of the VA population are males and 50% of this population is over 65 years of age which is very close to the average age of prostate cancer patients at first diagnosis (67 years).
- Almost 12% of the VA male population is African American (AA), AAs present with more aggressive therapeutic resistant prostate cancer. NCI recommends prostate cancer screening at age 50 for normal individuals and at age 45 for persons of AA origin. The overexpression of CYP3A5 in AA population due to presence of *1 variant suggests more active AR signaling.

Results

Differential expression of CYP3A5 in NHWA and AA population

Frequency of wild type variant (*1)		
1	Non-Hispanic White Americans	5%
2	African Americans	73%
3	Japanese	29%
4	Chinese	27%
5	Korean	30%

Table 1: Frequency of wild-type variant (*1). The wild type (*1) CYP3A5 is predominantly expressed in African Americans (AAs).

Cell lines	Origin	CYP3A5
LNCaP	NHWA	*3/*3
C4-2	NHWA	*3/*3
22RV1	NHWA	*3/*3
PC-3	NHWA	*3/*3
DU145	NHWA	*3/*3
MDAPCa2b	AA	*1/*3
RC77T/E	AA	*1/*3

Table 2: CYP3A5 SNP genotyping of PC cell lines. All tested NHWA PC cells carry the *3 variant whereas all the AA PC cell lines carry one (*1) variant.

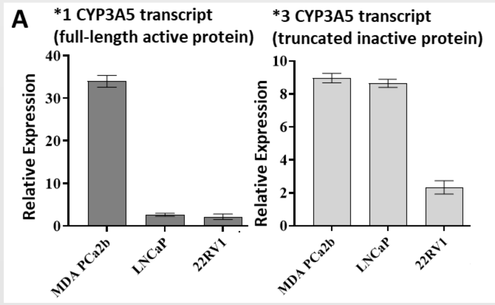
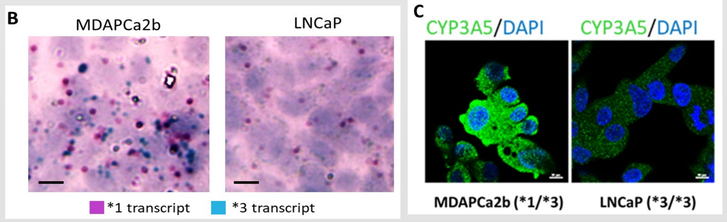


Figure 1. Quantification of CYP3A5 mRNA splice variants. (A) Quantification of splice variants using TaqMan probe assay. qRT-PCR was performed to quantify the CYP3A5 splice variants in MDA PCa2b, LNCaP, and 22RV1 PC cell lines. Separate TaqMan-based primer and probe pairs were designed to detect only the specific variants. TaqMan GAPDH probe/primer set was used as an internal control. **(B) Base scope assay to quantify splice variants.** Custom base scope probes were designed to identify the CYP3A5 *1 (full-length protein-pink) and mutant *3-(truncated protein-cyan) mRNA splice variants. (bar-10μm). **(C) CYP3A5 protein expression in PC cell lines.** Immunofluorescence staining showing CYP3A5 expression in LNCaP-(NHWA) and MDAPCa2b-(AA) PC cells. Confocal image showing a z-stack passing through the nucleus. The size bar is 10μm



CYP3A5 regulates AR nuclear localization and activation

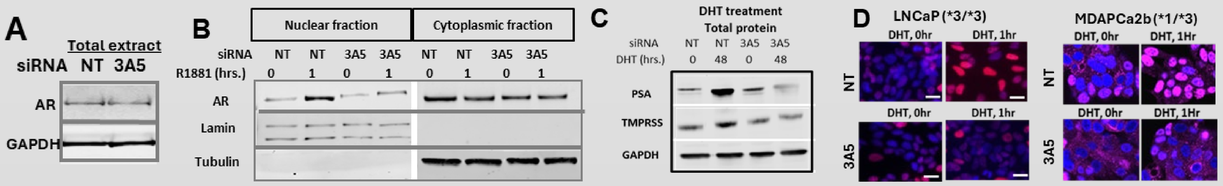


Figure 2: CYP3A5 inhibition blocks AR nuclear translocation and downstream signaling. (A &B) Effect of CYP3A5siRNA (3A5) on total AR, and AR present in the cytoplasmic and nuclear fractions in LNCaP cells. NT-non target siRNA control. **(C)** TMPRSS2 and PSA expression is reduced in CYP3A5 siRNA treated cells after DHT induction. (D) Immunofluorescence imaging after CYP3A5 inhibition on and DHT induction (Dihydrotestosterone), AR (red/pink), DAPI-blue. The white bar is 50μm.

Wnt pathway genes are upregulated in patients with high CYP3A5 expression (*1)

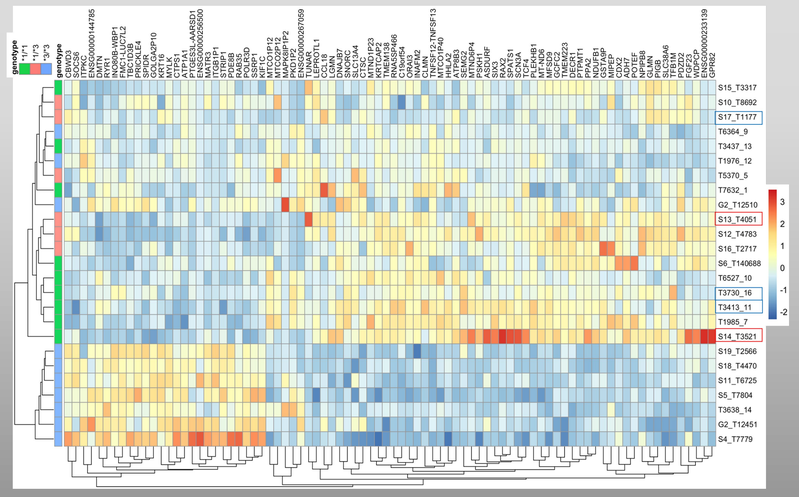


Figure 3: Heat map comparison of differentially expressed genes: RNA sequencing was performed using PC samples from AA and non-Hispanic White American (NHWA) patients. CYP3A5 genotyping separated them into two groups: a) carrying wt-CYP3A5 (*1/*1 and *1/*3; N=14); and b) mutant CYP3A5 (*3/*3; N=11) variants. The samples' ancestry was confirmed using markers. Genes that displayed ≥log2-fold difference in expression were identified and plotted in a hierarchical cluster heat map. Red shows higher expression and blue shows lower expression.

Symbol	Log2 Fold change	P adj
SEMG2	4.65	0.00489
ZNRF3-IT1	4.52	0.04001
CCL18	4.5	0.04001
U3	3.26	0.04001
MT-ND6	2.43	0.04001
SCARNA9	2.15	0.02686
NRCAM	2.05	0.04586
LINC00853	2.01	0.04001
TCF4	1.21	0.04843
SLC38A6	1.19	0.04939
HHLA3	0.94	0.04586
DECR1	0.72	0.0238
THTPA	-2.02	0.03348
NEFH	-3.24	0.04586

Table 3: Differentially expressed genes in PC patients carrying wild type CYP3A5 (*1). List of genes differentially expressed between the two groups (*1/*1+*1/*3 vs *3/*3) after RNA sequencing (p-adj <0.05). The listed overexpressed genes are known to be highly expressed in PC and promote aggressive disease. Wnt signaling is shown to be upregulated in the *1 CYP3A5 patient population.

Does CYP3A5 overexpression increase PC aggressiveness?

Frequency of wild-type variant (*)			
Normal healthy population		Prostate cancer patient population	
Non-Hispanic White Americans	African Americans	Non-Hispanic White Americans	African Americans
5%	73%	18%	100%

Table 4: Frequency of *1 variant shows a significant increase in prostate cancer patients as compared to the healthy population. The natural occurrence of *1 variant is lower in the general population compared to prostate cancer patients pointing towards its possible role in promoting prostate cancer growth. For this analysis 60 patient samples were genotyped for CYP3A5 variant (*1 or *3). The ancestry of the patient samples were confirmed using ancestry markers.

Expression of AR and CYP3A5 in Enz-resistant PC lines

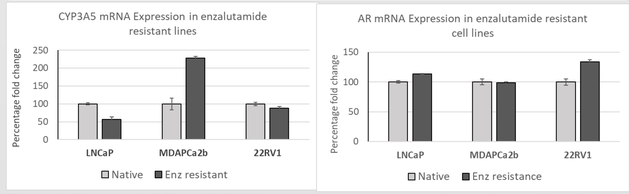


Figure 4: Changes in AR and CYP3A5 expression in enzalutamide resistant PC cells. We used qRT-PCR to analyze RNA from both regular and enzalutamide-resistant prostate cancer cells. We created the enzalutamide-resistant cells by gradually increasing the enzalutamide dose until they became resistant, then continued to maintain them to that dose. The final enzalutamide concentrations varied for each cell line: LNCaP (8μM), MDAPCa2b (10μM), and 22RV1 (12μM). We kept corresponding control cells in DMSO (vehicle control).

Effect of CYP3A5 siRNA on Wnt pathway genes

Symbol	Fold change	P adj
WNT5A	-2.30	0.018
WNT11	-3.09	0.0422
WNT10B	-2.97	1.58E-04
FZD2	-2.12	0.0053
FRAT2	-2.25	2.72E-07
DVL3	-1.483	6.51E-05
APCDD	-1.379	0.0037
CTNNBIP1	1.386	0.0266
GBP1	3.27	5.72E-08
GBP2	2.146	2.78E-05
LRP4	2.10	0.0052
WNT7B	1.377	0.012

Table 5: RNA sequencing study showing modulation of Wnt pathway genes after CYP3A5 siRNA treatment in MDAPCa2b cells. Wnt pathway genes differentially expressed between the two groups: Non-target siRNA vs CYP3A5 siRNA treated cells.

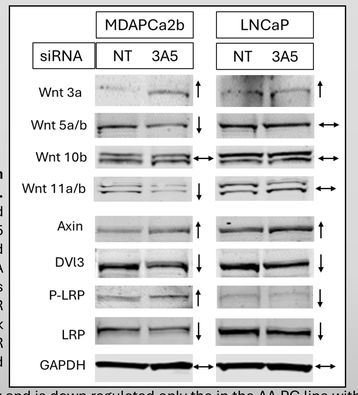


Figure 5: Effect of CYP3A5 siRNA on Wnt signaling pathway genes. Western blot analysis was performed using non-target (NT) and CYP3A5 siRNA treated LNCaP (*3/*3) and MDAPCa2b (*1/*3) PC cells. Wnt 3A is upregulated in both cell lines which is often observed when AR signaling is blocked due to cross talk between Wnt-canonical and AR pathways. Wnt 5a/b, Wnt 10a/b and Wnt 11a/b are mostly associated with the Wnt-non canonical pathway and is down regulated only the in the AA PC line with CYP3A5 inhibition.

Hypothesis and Future Directions

We hypothesize that the wild-type CYP3A5 variant (*1), more prevalent in (AA) prostate cancer patients, promotes aggressive disease and therapeutic resistance through a novel CYP3A5/AR/TCF4/β-catenin signaling axis. Future studies will investigate the functional role of CYP3A5 in regulating TCF4 signaling in prostate cancer using patient samples and *in vitro* models. We will:

- Analyze protein expression and localization of CYP3A5, AR, nuclear β-catenin, nuclear TCF-4, nuclear JNK, and nuclear NFAT in PC tumor samples from NHWA and AA patients.
- Employ *in vitro* models to elucidate the mechanism by which CYP3A5 *1 variant regulates TCF4 signaling and its downstream effects on AR function and therapeutic resistance.
- We will also explore how socioeconomic factors that contribute to stress affect hormonal and inflammatory profiles (cortisol, leptin, resistin, IL6, etc.), and lead to aggressive PC. We will further investigate potential links to CYP3A5 overexpression via PXR/CAR pathways known to be triggered by elevated hormonal and cytokine levels.