Early upregulation of AR and steroidogenesis enzyme expressions after 3 months of androgen-deprivation therapy.

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Abstract

Background: Androgen-Deprivation Therapy (ADT) is a standard treatment for advanced prostate cancer (PCa). However, there is a high recurrence or progression rate during ADT. Until now, there is no evidence on when the progression starts. This study would like to evaluate the early response of intraprostatic androgen receptor (AR) and steroidogenic enzyme gene expressions in ADT.

Methods: Prostate tissue samples were taken from PCa patients with urinary retention, who had ADT (ADT-PCa; n=10), and further grouped into ≤12 months (n=4) and ADT >12 months (n=6). ADT-PCa group were then compared with BPH (n=12) and primary (no treatment) PCa tissues (n=16). AR and steroidogenic enzyme genes were extracted from Formalin Fixed Paraffin embedded (FFPE) tissues and analysed using rtPCR. Protein expressions were evaluated by immunohistochemistry of specific antibodies.

Results: AR gene expression was found higher in ADT-PCa group compared to BPH and primary PCa. Both ADT ≤12 and > 12 months subgroups had significantly higher relative gene expression of AR (p 0.01 and 0.03) compared to primary PCa. AR protein expression in ADT-PCa group showed an increase trend in ADT ≤12 months subgroup and a significantly elevated expression AR protein in ADT >12 months subgroup compared with PCa (100%; p <0.01). Half (50%) of ADT ≤12 months patients were found to have upregulation of AR, and one undergone upregulation from only 3 months of ADT. A trend of elevating relative gene expression of SRD5A3 were also found within the groups given ADT.

Conclusion: There are upregulation of AR and steroidogenic enzymes in ADT-PCa patients, as early as 3 months without showing PSA elevation. Steroidogenic enzyme, especially SRD5A3 expression was also showing upregulation before PSA rises.

Introduction

Along the years, there have been notable improvements in the development of Prostate Cancer (PCa) treatment, resulting in better prognosis. (1). However, 40% of early PCa patients are still at risk of progressing into more advanced stage (1), needing Androgen Deprivation Therapy (ADT) as the current standard palliative treatment (2). Although ADT may result in prolonged overall survival (3), better response to metastasis (4), and decreased PSA level (5), the effects are only temporary. The disease will progress further after 2-3 years of treatment (6). This late stage of PCa is called castration resistant prostate cancer (CRPC), as PCa cells are evolved and capable of both hormone-dependent and hormone-independent pathways for cell proliferations and survival (7).

The finding of CRPC has instigated more focus on the disease. One important mechanism is androgen receptor (AR) signalling pathway, contributing a key role in PCa progression (8-10). The known mechanisms of resistance include AR overexpression, amplification, hypersensitivity, mutations, AR variants (ARVs), androgen-independent AR activation, also intratumoral and alternative androgen production. (8-10). Furthermore, findings suggested that the increased level of intraprostatic AR would lead to a disease progression (10). Another downstream steroidogenic enzyme within the AR signalling pathway, 5α-reductase and AKR1C3, also became an agent of interest (7-12). These enzymes are responsible in converting

androgens into dihydrotestosterone (DHT), which needed by PCa cells. (11). Currently, there are three identified isozymes of SRD5A, encoded by 3 different genes; SRD5A1, SRD5A2, and SRD5A3 (11). AKR1C3, SRD5A1 and SRDA2 have been extensively studied (7-12), and marked level changes were reported along the course of PCa development and progression (7-12). The SRD5A1 and SRD5A3 were associated with androgen response elements (ARE) that promotes transcriptions of target genes, leading to cell homeostasis, angiogenesis, differentiation, and apoptosis (13). It is also believed that the two isozymes play important roles in the production of androgens in prostate cells (11). On the other hand, the role of SRD5A3 is still obscure, though associations with DHT production and AR activation have been suggested (14). Upon these findings, AR, AKR1C3, and 5α-reductase are proven indicative of PCa progression, as difference of levels were documented between malignant and non-malignant cells. The changes following ADT treatment however, has never been reported, though it has been associated with further disease progression and recurrences.

Currently, no study has ever reported the start of disease progression after ADT. This study hypothesized that progression of the disease would likely to occur within a short time interval after commencement of ADT. Therefore, this study would like to evaluate the early effect of ADT by investigating the changes of AR and steroidogenic enzymes within the intraprostatic cells before as represented in BPH and primary PCa cases and after ADT treatment, as an indicator of the disease progression.

Methods

Samples

Formalin Fixed Paraffin embedded (FFPE) prostate tissue were taken from the Department of Pathology Anatomy, Cipto Mangunkusumo General Hospital (RSCM). The study was done on three groups of patients: 1) Benign Prostatic Hyperplasia (BPH) group (n=12), 2) primary PCa group (n=16, sampled in year 2009-2015), 3) PCa after ADT group (ADT-PCa) (n=10). Sample retrieval method for BPH and primary PCa tissues are provided in Table 1, while ADT-PCa tissues were taken primarily from TUR-P. Further subgrouping of the ADT-PCa group was done into ADT ≤12 months and ADT >12 months based on duration of ADT. All use of specimens was in compliance with the guidelines of the Local Review Board and Ethics Committee 520/UN2.F1/ETIK/2015, and prostatic tissues were obtained with patient consent at the time of procedure.

RNA extraction from FFPE tissue

Paraffinization was done by prior isolation of the RNA using High Pure RNA Paraffin Kit (Roche, Germany). The sample used was FFPE prostate tissues which were cut with a thickness of 5-10 µM from each sample of respective group (BPH, primary PCa, and ADT-PCa). DNA extraction was done from the FFPE samples. Total RNA extracted was 50 µl and was stored in a -80° freezer.

Quantitative Real-time PCR

Quantification of gene expression (qPCR) was done on AR, AKR1C3, SRD5A1, SRD5A2, SRD5A3, using QuantiTect SYBR Green RT-PCR kit. 18 S gene was used as a housekeeping gene for internal control for its

invariant expression across tissues and cells. qCPR reaction mix (2x QuantiTect SYBR Green RT-PCR Mix, Forward Primer, Reverse Primer, free water nuclease) and 2 uL cDNA sample was used as a template. Specific primer used in this study can be seen in **Table 1**. The measurement was done using Mx3000P (agilent) tool with amplification program. Cycle threshold (Ct) was obtained by using MxPro (agilent) software. Relative gene expression was measured by using Livak method or also known as delta delta threshold cycle (ΔΔCt).

Immunohistochemistry

Immunohistochemistry examination was done from FFPE preparation. Each sample was cut into 4 μm tissue put and warmed (in 58℃), and was deparaffinized using xylol I, II, III. The specimens were then rehydrated in abs, 96%, and 80%, then undergone Heat induced Epitope retrieval (HIER) in pH6 through pressure boiler 125℃ and cooled in 90℃. Dual endogenous enzyme block was done using H_2O_2 0,3% and ethanol 95%. Staining using specific antibodies were done for expression of AR and AKR1C3 and the steroidogenic enzymes (SRD5A1, SRD5A2, SRD5A3) using antibodies obtained from Sigma Aldrich (St Louis, Missouri) (15). Mouse monoclonal Anti-AR (WH0000367M1) (15,16), mouse monoclonal Anti-AKR1C3 (A6229) (15,17), rabbit polyclonal anti-SRD5A1 (HPA051402) (15), rabbit polyclonal anti-SRD5A2 (SAB2105567) (15), and rabbit polyclonal anti-SRD5A3 (HPA027006) (15,18). All of the antibodies were validated and referenced (Human Protein Atlas project and peer-reviewed) and widely used in immunohistochemistry research field as referenced by Sigma Aldrich. Further description can be seen in **Supplementary Table 1**.

Incubation was done in labelled polymer HRP followed by chromogenization in DAB (substrate DAB: chromogen DAB was 1:20) using DAKO autostainer KIT. The specimens were then counterstained using Hematoxylin. The specimens were then dehydrated (abs, 80%, 96%), cleared (Xylol I,II,III), and mounted on slides with coverglass.

The examination was performed visually by three examiners, blinded for the clinical groups of the patients, and then confirmed by experienced pathologist. Microscopic semiquantitative examination was done on 10 random fields per specimen containing minimum 500 cells using ImageJ (Research Service Branch, NIH.gov) in 400 times magnification. The expression of the protein will be positive if cells were stained brownish in the cytoplasm or the nuclei with a granular pattern.

H-Score Calculation to measure Protein expression

The positive epithelial cells were then quantified using semiquantitative scoring of 0, +1, +2, and +3 using expression scores for each cell. The score +1 is defined as low positive, +2 is moderate positive, and score +3 is high positive. Quantification of protein expressions was done by measuring H-score. H-score was measured using a formula:

See formula 1 in the supplementary files.

BPH group mean H-Score was measured as a cut-off to determine which samples are upregulated or downregulated of protein expressions, and further analysed for statistical comparison. The percentage of

upregulated samples were presented.

Statistical Analysis

Statistical analysis was performed using Graphpad Prism 7 (La Jolla, CA). The Mann Whitney U test was used to determine the comparison between gene expressions among the groups, comparing ADT with BPH and ADT with PCa. P<0.05 was defined as statistically significant. Fisher exact test was used to compare the specified groups in protein expression analysis and association with clinic-pathologic parameters.

Results

Sample characteristic

In 2007-2015, we were able to evaluate 10 patients who still had retention after ADT treatment (ADT-PCa group), within the mean age of 67,27±9,69. The group were then subdivided into two, group that had administration of ADT for under 12 months (4 samples) with 5 (3-9) months median time of administration and above 12 month (6 samples) with 30 (15-70) months median time of ADT administration. Initial PSA levels were 93.3 (58.14-784.60) in ADT under 12 months, and 176.9 (104.23-200.40) in ADT under 12 months group. Gleason score of patients were mostly high (8-10), T-staging and prostate volume were comparable between the two groups. The ADT type given were similar and comparable between the two, where 50% of patients had orchyde and 50 % LHRH agonist and anti-androgen in group ADT under 12 months, while in above 12 months group they were 67% and 33% respectively. We also included comparison groups BPH patients (12 samples), primary PCa (16 samples). Detailed characteristics of the patients are described in **Table 2**. There was statistically significant difference of PSA level between the groups, which the highest PSA were found in ADT >12 months group.

Gene relative expression

AR gene expression was found higher in ADT-PCa group compared to BPH (Median difference of 1.1; p-value 0.05) and primary PCa (Median difference of 1,83; p <0.01). The ADT ≤12 months patients having the highest AR gene relative expression (median 5.14). AKR1C3 showed an increased trend for ADT-PCa subgroups in comparison with BPH and primary PCa (**Figure 1**).

There were no significant differences of SRD5A1 and SRD5A2 gene expressions between the groups. However, a decreased trend of SRD5A2 gene expressions were reported (Figure 1). On the contrary, an overall increased trend of SRD5A3 expressions were reported between BPH and ADT-PCa subgroups, with ADT ≤12 months subgroup having significantly highest SRD5A3 relative expression (Median difference of 9.39 in ADT ≤12 months vs Primary PCa, p 0.13; Median difference of 11.3 in ADT ≤12 months vs BPH, **p 0.02**) (**Figure 1**).

Protein expression by Immunohistochemistry

AR and AKR1C3 expressions were found in both the nucleus and cytoplasm, meanwhile the protein expressions of SRD5A1, SRD5A2, and SRD5A3 were found predominantly in cytoplasm (Figure 2).

Upregulation of protein expressions were described in **Table 3**. AR protein expression in ADT-PCa group were 80% upregulated compared with Primary PCa group (p <0.01), showing an increase trend in ADT ≤12 months subgroup compared with PCa group (p 0.2) and a significantly higher expression AR protein in ADT >12 months subgroup (100; p <0.01). **(Table 3).** No upregulation in AKR1C3 were found in both ADT-PCa subgroups.

Association between protein expression in prostatic tissue of ADT-PCa patients with the clinico-pathological parameters

A further analysis towards the ADT-PCa patients was done by observing the upregulated protein expressions and its association with the clinical status of the patients (**data was not shown**). This study focused on the analysis the subgroups of ADT-PCa Patients, ADT ≤12 months (table 4), to see the initial changes within the intraprostatic tissue due to ADT. Half of the patients with ADT ≤12 months were found to have upregulation of the AR, while all of the ADT >12 months patients have undergone the upregulation of AR. Furthermore, one patient among ADT ≤12 months subgroup that had undergone upregulation were found to have been given ADT only for 3 months of duration. However, no upregulation of AKR1C3 expression in ADT ≤12 months subgroup was noted. No notable finding was found in SRD5A1, SRD5A2, and SRD5A3 protein expressions. There was no notable significant difference between the upregulated and non-upregulated ADT ≤12 months subgroup in clinical and pathological parameters.

Amongst the group given ADT £ 12 months, there was one sample that had only AR increased protein expression, one sample that had only SRDA1, 2, and 3 increased protein expressions, one sample that had AR and SRDA 1 and 2 increased protein expressions, and one that had no up-regulation of proteins expressions. Elaborate H-scores of the protein expressions are available in the **Supplementary table 2**.

Discussion

Though castration by ADT has been the standard frontline therapy for advanced stage of PCa, disease progression is predicted to occur, into a state of castrate-resistant prostate cancer (CRPC). To better understand the changes towards the progressive state, elaborate study onto the mechanisms of resistance is important. The upregulation of AR signalling pathway intraprostatic tissue is one of the resistance mechanisms in CRPC. This study found upregulation of AR and steroidogenic enzymes in ADT treated PCa patients. Furthermore, this study, in accordance to other study, have found that CRPC can be developed in less than 12 months after commencement of ADT. (19) However, until now, there were no study evaluate how early the resistant mechanism in the prostate starts to overcome ADT.

This study delves into the early response to ADT, by evaluating intraprostatic AR and steroidogenic enzymes changes using prostate tissue from patients who still complained a urinary retention during ADT. It revealed a notable unique finding in the subgroup of patients who only had ADT under 12 months. There were two patients that had a high intratumoral AR gene and protein expression after 3 months of ADT. It can be speculated that the resistance mechanism to ADT (10) through upregulation of AR might start as early as 3 months. To best of our knowledge, this was the first study that showed an early AR upregulation in human

PCa tissue during ADT. This early resistant mechanism should warn clinicians to detect this process when starting ADT. (20).

Other interesting result that is AR was the only gene which upregulated in early state (3 months). PCa cell might start to overcome low serum androgen level due to ADT by increasing AR expression first (10-13). It suggests that the early mechanism to overcome low serum androgen level is increasing an AR expression (10-13). Many in-vitro studies have shown upregulation of AR expression illustrating adaptations of the prostatic cells to increase sensitivity to low androgen level after treatment of ADT (21,22,23). However these phenomenon can only be seen in orchiectomy as an ADT patient. It might show that an abrupt decreasing serum testosterone level induces the upregulation of AR. Furthermore, here are many known mechanisms of AR changes, i.e. amplification, mutation, that have also been reported in ADT >12 months condition (8-10). However, this study only examined the protein expression and did not further evaluate the other AR changes i.e. AR amplification, AR mutation.

It has been known that PCa cell growth is promoted by androgen, especially DHT (12). This study found that the 5α-reductase isoenzymes, which regulate the conversion of T into DHT (11,12), were increased in ADT treated PCa patients. Similar to other studies, SRD5A1 (14,24) and SRD5A3 (14,25) were upregulated in ADT-PCa patients compared with the ADT naïve PCa Patients, and SRD5A2 to be downregulated (14,24,26). However, up until now there has been very limited information whether the isozyme is involved in the process of androgen biosynthesis. Interestingly, this study found that SRD5A were the only steroidogenic enzyme which upregulated in ADT ≤12 months patients. This SRD5A upregulation were also related to AR upregulation. This speculates that after or simultaneously PCa cell upregulate AR expression, PCa cells upregulate SRD5A expression which is the closest enzyme is responsible the conversion of T to DHT (14). Thus, developing a new strategy or compound which targeted SRD5A can reduce the risk of early resistance.

Among the PCa-ADT patients with duration under 12 months, there were three patients showing upregulation of gene with increased protein expression. Three from four showed upregulation of AR, consisting of one patient with AR and SRD5A1, 2, and 3; one patient with upregulation of AR and SRD5A1 and 2; and one patient only had AR upregulation. These finding is novel to our research, as no one has ever investigated further below the cut-off of 12 months. This study would like to suggest that a possible upregulation of AR and the steroidogenesis enzyme (namely, SRD5A1, SRD5A2, and SRD5A3) as a compensation mechanism to the low testosterone level due to ADT (14). Our study showed, that patients that develop upregulated SRD5A1, 2, or 3 showed increased expression of protein later than those of AR, ranged from 7-9 months of ADT duration for SRDA1,2 and 3 compared with AR that occurred ranged from 3-7 months of ADT duration. This suggests that AR increased activity as a compensatory mechanism comes first, then followed by SRD5A1, 2, and 3. However, further studies with more patients is needed in order to conclude the compensatory response.

Many studies have shown that there is a shifting to an adrenal androgen usage for maintaining DHT level by upregulated of AKR1C3 expression (10-14). This study found that AKR1C3 can only be found in patients treated with ADT above 12 months duration, which is in accordance to other study (27). The further question is why the AKR1C3 is not upregulated in early state, based on steroidogenic pathway, AKR1C3 is an

upstream enzyme that converts adrenal androgen to downstream androgen which needed as a source of DHT (12-14). To support the previous statement, we hypothesize that PCa cell will increase AKR1C3 expression after the AR and SRD5A upregulation. However, it has been shown that there are many variations of AKR1C3, SRD5A and AR expression in each ADT >12 months patients. It might due to the dynamic process in steroidogenesis. The AR or steroidogenesis enzymes were regulated based on 'real time' condition which is needed by PCa cells.

The main limitation of our study is the low sample size included. However, this is the first study which tried to evaluate the AR signalling pathway changes during ADT in human prostate tissues. This study also revealed an important finding in which PCa cell may adapt to low androgen level, caused by ADT, before rising its PSA level. This finding is not the first significant study with tantalizing information limited by low sample size. A study done by Alsinnawi M, et al has contributed a significant prognostic information where high expression of SLCO gene may result in worse Disease-free survival (DFS) with only 11 samples included in the study (28). Although small in number, the result of the mentioned study were in concordance with the result of Terakawa T, et al. team, examining the similar outcome, only with more number of patients included (n=494) (29). Similar studies had similarly low sample size yet give significance in practice. Tiwari et al showed AR and its transcriptional co-repressor REST modulate SPINK1 expression, and plausible role of SPINK1 in the progression of Neuroendocrine prostate cancer with only few samples (30). Another study by Cheung et al, using only 11 samples per group founded that the Actin alpha cardiac muscle 1 (ACTC1) gene expression would play a role in compensating ADT administration for PCa as a response to ADT-induced muscle loss (31).

Beside the lacking of sample numbers, other limitation of our study were limited samples available due to some non-utilizable old specimens unsuitable for RNA extraction and protein expression evaluation. Another limitation of this study, is using the median expression of each gene in BPH tissues as cut off in defining upregulation in other samples. This was the only available method, as currently no official cut off to define upregulation of AR and steroidogenic gene in immunohistochemistry staining that has been validated.

In conclusion, there are upregulation of AR and steroidogenic enzymes in PCa patients which were treated with ADT. The early AR and SRD5A upregulation can be found in 3 month in ADT patient. This indicates that the early evaluation of AR and SRD5A expression in intraprostatic tissue should be done. Further strategic treatment should be targeting AR and SRD5A enzyme to overcome early resistance to ADT.

Declarations

Disclosure

Consent for publication

All individual data being included in the study have been approved by the patients by signing a written consent for the whole research process and publication.

Availability of Data ana Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

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Authors Contribution

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Conflict of Interest

The authors declare that they have no conflict of interest

Ethical Clearance

This research involves human participants. All procedures performed in studies involving human participants/samples were in compliance with the guidelines of the Local Review Board and Ethics Committee 520/UN2.F1/ETIK/2015, and data were obtained with patient consent at the time of procedure/data collection.

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Tables

Table 1. Primers for rtPCR

Gene	Forward	Reverse
AR	CAT TGA GCC AGG TGT AGT GT	CCA GTT CAT TGA GGC TAG AGA G
AKR1C3	TGC AGG TTT TTG AGT TCC AGT	TGG CTA GCA AAA CTA TCA CGT T
SRD5A1	ACG GGC ATC GGT GCT TAA T	CCA ACA GTG GCA TAG GCT TTC
SRD5A2	GGAGTCCTTCAAGGCTACTATCT	CACCCAAGCTAAACCGTATGT
SRD5A3	GTC ATC TGC CCA TCA GTA TAA	GAA TGA CCA CTC CTG CTT TAT
18 S	AAA CGG CTA CCA CAT CCA AG	CCT CCA ATG GAT CCT CGT TA

Table 2. Basic characteristic of patients

	BPH n=12	Prostate Cancer			P-Value[b]
		Primary PCa n=16	ADT-PCa n=10		
			Duration ≤12 Months n=4	Duration >12 Months n=6	
Age	64.2 ± 8.6	63.4 ± 7.0	58.75 ±3.4	72±9.3	0.05
Sample retrieval method					
Biopsy	5 (71%)	5 (31%)	-	-	-
TUR-P	2 (29%)	5 (31%)	4 (100%)	6 (100%)	
Radical Prostatectomy	-	6 (38%)	-	-	
Gleason Score					0.76
≤ 7	-	4 (25%)	-	1 (17%)	
8 - 10	-	12 (75%)	4 (100%)	5 (83%)	
T Staging[a]					0.91
1	-	6 (37%)	-	-	
2	-	4 (25%)	2 (50%)	3 (50%)	
3	-	3 (19%)	-	1 (17%)	
4	-	3 (19%)	2 (50%)	2 (33%)	
Lymphatic Involvements	-	1 (7%)	NE	NE	-
Initial PSA[a]	9.5 (1.4-36.7)	49.3 (4.6-600)	93.32 (58.14-784.60)	176.9 (104.23-200.40)	**0.01***
Prostate volume[a]	51.6 (28-73.6)	43.6 (13.4-80.5)	30.5 (28.6-36.68)	39.7 (27.6-94.2)	0.39
ADT Type					0.76
Orchyde	-	-	2 (50%)	4 (67%)	
LHRH agonist + antiandrogen	-	-	2 (50%)	2 (33%)	
ADT Treatment Duration	-	-	5 (3-9)	30 (15-70)	-

[a] Initial PSA at diagno and prostate volume are presented in median (min-max)

[b] Sample retrieval method, T staging, and Lymphatic involvements are presented as percentages

[c] Statistical significance was measured by comparing Primary PCa with ADT-PCa using Pearson chi square for Gleason score, T stages, and ADT Type; ANOVA for age; Kurskall Wallis test for initial PSA level and Prostate Volume.

[d] ADT treatment duration is presented in months

N.E : not evaluated

* P-value <0.05 is significant

Table 3. Expression of target proteins in the epithelial cells of the prostate tissue

Expressions of proteins	H-score cut-off[a]	Upregulated cancer tissues							
		Primary PCa (n=7)		ADT-PCa (n=10)					
				≤ 12 months (n=4)			>12 months (n=6)		
		n	%	n	%	Compared to primary PCa[b]	n	%	Compared to primary PCa[b]
AR	170	1	14	2	50	0.2	6	100	**<0.01***
AKR1C3	117	2	29	**0**	**0**	0.23	1	17	0.61
SRD5A1	123	5	71	2	50	0.47	4	67	0.85
SRD5A2	254	3	43	2	50	0.81	3	50	0.79
SRD5A3	123	7	100	1	25	**<0.01***	3	50	**0.03***

[a] H-Score cut-off was measured from mean H-score of BPH group (n = 6) as mentioned in the method, to determine upregulation of protein expressions in malignant tissues (Primary PCa and ADT ≤ 12 months and >12 months)

[b] Statistical significance was measured for p-value by comparing the H score of ADT ≤ 12 months and >12 months with Primary PCa using Pearson Chi Square

* P-value <0.05 is significant

Table 4. Prostate cancer patients treated with ADT characteristics and clinical and pathological parameters

Characteristics	AR			SRD5A1			SRD5A2			SRD5A3		
	ADT < 12 Months		P-Value	ADT < 12 Months		P-Value	ADT < 12 Months		P-Value	ADT < 12 Months		P-Value
	High (n=2)	No (n=2)		High (n=2)	No (n=2)		High (n=2)	No (n=2)		High (n=1)	No (n=3)	
Age	59.5 ± 0.7	58 ± 5.6	0.75	56.5 ± 3.5	61 ± 1.4	1	56.5 ± 3.5	61 ± 1.4	1	54	60.3 ± 1.5	0.07
T Stages			0.05			0.32			0.32			0.25
1	--	-		-	-		-	-		-	-	
2	100%	-		50%	50%		50%	50%%		-	67%	
3	-	-		-	-		-	-		-	-	
4	-	100%		50%	50%		50%	50%		100%	33%	
Gleason score[a]			0.25			0.25			0.25			0.50
≤ 7	50%	-		-	50%		-	50%		-	33%	
08-10	50%	100%		100%	50%		100%	50%		100%	67%	
PSA level[a]	160.6 (17.9-303.3)	20.2 (10.1-30.4)	0.19	156.7 (10.1-303.3)	22.3 (17.9-30.4)	1	156.7 (10.1-303.3)	22.3 (17.9-30.4)	1	10.1	30.4 (17.9-303.3)	0.5
ADT Type			-			-			-			-
Orchyde	2	-		1	1		1	1		-	1	
LHRH agonist ± antiandrogen	-	2		1	1		1	1		1	2	
ADT Treatment Duration	5 (3-7)	6 (3-9)	-	8 (7-9)	3	-	8 (7-9)	3	-	9	3 (3-7)	-

+ is upregulated protein expression group; - is downregulated protein expression group

[a] Variable Age are presented in mean ± SD; PSA level and prostate volume are presented in median (min-max)

[b] Statistical significance was measured by comparing the clinical parameters of upregulated and non-upregulated ADT-PCa samples using Independent T test for Age; Pearson chi square test for Gleason score; Mann-Whitney test for T-stages and PSA level

* P-value <0.05 is significant

Figures

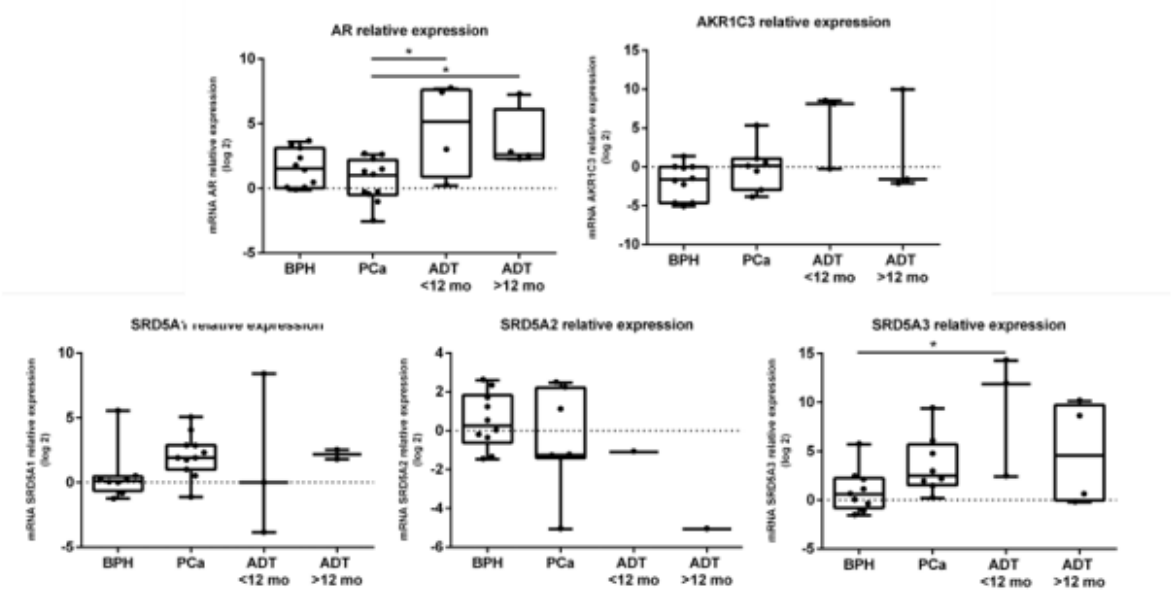

Fig. 1. Gene Relative expressions analysis. Gene Relative expressions analysis was done by quantification of real-time one-step RT-PCR using Mx3000P (agilent) tool on tissue samples of BPH group (n=12), Primary PCa group (n=13), ADT ≤12-months Group (n=4) and ADT above 12 months Group (n=6) for five different genes: AR(a), and several intraprostatic steroidogenic enzymes; AKR1C3 (b), SRD5A1 (c), SRD5A2 (d), SRD5A3 (e) in the prostate tissues. Statistical analysis was performed by using Mann-Whitney Test, comparing each group to another in a paired manner. * $p < 0.05$ and ** $p < 0.01$. Standard errors of the means are indicated by bars. (a-e) All experiments were performed at least two times.

Figure 1

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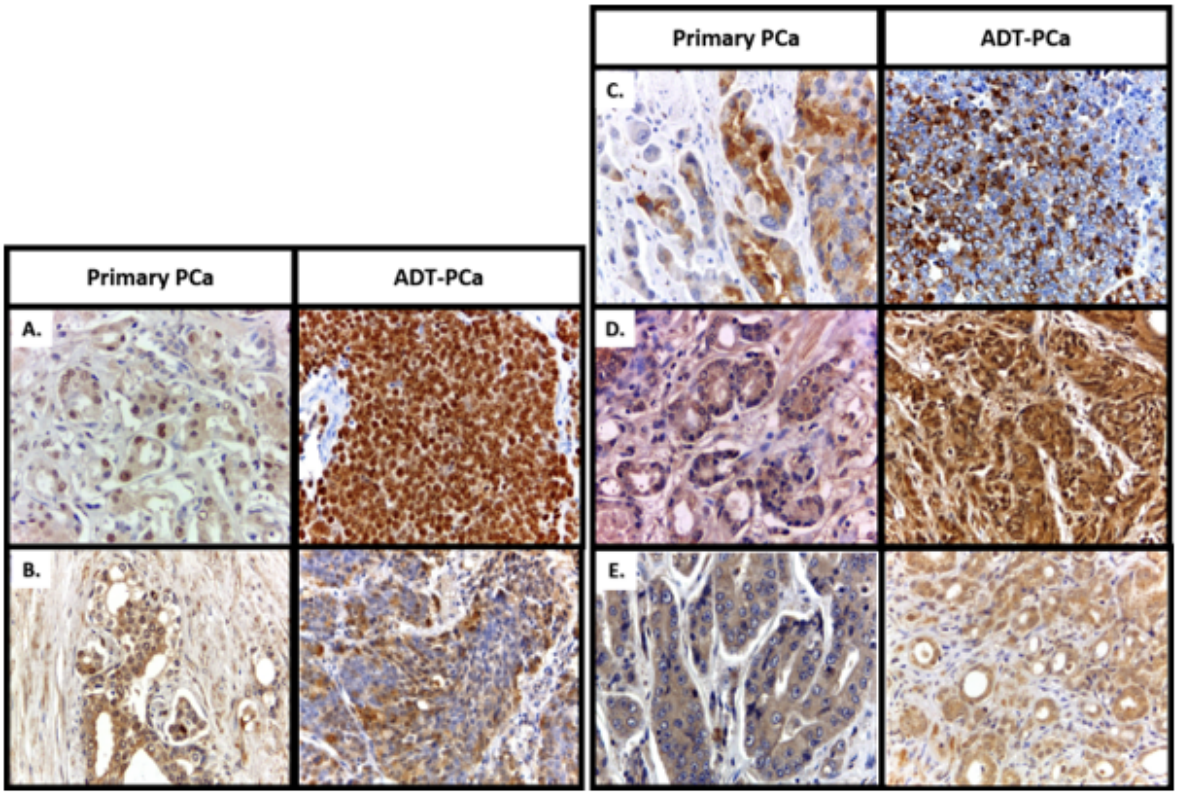

Fig. 2. Immunohistochemistry of prostate cancer tissue stained for AR, AKR1C3, SRD5A1, SRD5A2, and SRD5A3. Comparison between the protein expressions of AR and intraprostatic steroidogenic enzymes in the prostatic cell of Primary PCa and ADT-PCa groups: A) AR, B) AKR1C3, C) SRD5A1, D) SRD5A2, and E) SRD5A3. Images were taken from sliced FFPE samples of patients taken between 2009-2014 in RSCM. Images were captured in 400.000 times magnification

Figure 2

Immunohistochemistry of prostate cancer tissue stained for AR, AKR1C3, SRD5A1, SRD5A2, and SRD5A3. Comparison between the protein expressions of AR and intraprostatic steroidogenic enzymes in the prostatic cell of Primary PCa and ADT-PCa groups: A) AR, B) AKR1C3, C) SRD5A1, D) SRD5A2, and E) SRD5A3. Images were taken from sliced FFPE samples of patients taken between 2009-2014 in RSCM. Images were captured in 400.000 times magnification

Supplementary Files

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- Supplementarytables.docx
- formula1.PNG