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Polymorphism of 3 β -Hydroxysteroid Dehydrogenase-1 in patients with prostate cancer in middle Euphrates of Iraq

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Abstract--Androgens play critical roles in prostate carcinogenesis as well as prostate cancer progression. Androgen-deprivation therapy (ADT), which reduces testosterone production and inhibits androgen action in prostate cancer cells, has been the criterion standard therapy for metastatic prostate cancer (1). Although initially most prostate cancers respond well to ADT, most patients eventually progress to castration-resistant prostate cancer (CRPC), which is mainly thought to be because androgen receptor reactivation is induced by several mechanisms. One of those mechanisms have been identified to be intratumoral androgen synthesis mostly from adrenal precursor steroids and at least in part due to de novo synthesis from cholesterol which is supported by increased expression of several genes encoding steroidogenic enzymes including HSD3B, HSD17B, and SRD5A in CRPC. Among them, HSD3B1 gene encodes 3 β -hydroxysteroid dehydrogenase-1, which is mainly expressed in peripheral tissues including the prostate (another isoform, 3 β -hydroxysteroid dehydrogenase-2 was mainly expressed in adrenal gland and gonad in human) and is a rate-limiting enzyme required for all pathways of dihydrotestosterone synthesis. Genetic polymorphism in HSD3B1 encoding 3 β -hydroxysteroid dehydrogenase-1 has been shown to be associated with oncological outcome for prostate cancer. This study to investigate the significance of missense polymorphism in HSD3B1 gene (rs1047303) among men with prostate cancer.

Keyword---polymorphism, 3β -Hydroxysteroid Dehydrogenase-1, patients, prostate cancer

Introduction

The prostate is both an accessory gland of the male reproductive system and a muscle-driven mechanical switch between urination and ejaculation. The prostate gland is of the male reproductive system. In adults, it is about the size of a walnut (1) and it is an average weight of about 11 grams, usually ranging between 7 and 16 grams (2). Prostate cancer is the second most common non-cutaneous cancer in men and a leading cause of death, with an estimated 174,650 new cases and 31,620 deaths in the United States in 2019 (3). Prostate cancer cells, like normal prostate cells, require androgens to grow and survive. Growth of prostate cancer depends on the ratio of the rate of cell proliferation to the rate of cell death (4). In prostate cancer, the rate of proliferation is higher than that of death, resulting in continuous net growth. Androgens play critical roles in prostate carcinogenesis as well as prostate cancer progression. Since 1941, androgen-deprivation therapy (ADT), which reduces testosterone production and inhibits androgen action in prostate cancer cells, has been the criterion standard therapy for metastatic prostate cancer.

Although initially most prostate cancers respond well to ADT, most patients eventually progress to castration-resistant prostate cancer (CRPC), which is mainly thought to be because androgen receptor reactivation is induced by several mechanisms (5). One of those mechanisms have been identified to be intratumoral androgen synthesis mostly from adrenal precursor steroids and at least in part due to de novo synthesis from cholesterol (6,7,8) which is supported by increased expression of several genes encoding steroidogenic enzymes such as HSD3B (9). HSD3B1 encodes 3β -hydroxysteroid dehydrogenase-1, which is mainly expressed in peripheral tissues including the prostate, breast, skin, and placenta (10). Dihydrotestosterone synthesis in prostate cancer from adrenal DHEA/DHEA-sulfate requires enzymatic conversion in tumor tissues. 3β -hydroxysteroid dehydrogenase-1 is an absolutely necessary enzyme for such dihydrotestosterone synthesis and is encoded by the gene HSD3B1 which comes in 2 functional inherited forms described in 2013. The adrenal-permissive HSD3B1(1245C) allele allows for rapid dihydrotestosterone synthesis. The adrenal-restrictive HSD3B1(1245A) allele limits androgen synthesis (11).

Methods

Blood samples were obtained from 90 patients at the 54–88 ages diagnosed with histopathological confirmed prostate cancer (CaP). The CaP patients were recruited from Department of Urology and archived in Department of Clinical and Molecular Pathology of the Imam Hussein (peace be upon him) Teaching Hospital in the Holy Karbala Governorate and Merjan Medical City in Babylon Governorate/ Iraq. Ninety candidates of this group have neither symptoms nor signs of essential PCa by doing PSA serum level with per rectal examination of prostate and they are healthy otherwise. Data was collected by ways of a personal interview for every individual to obtain information about their smoking status

and about their age, ethnicity and family history. Almost 10 ml venous blood was drawn from each candidate when visit the oncology center for treatment. Two ml of this sample were collected in EDTA tube for DNA extraction and PCR. The remaining was transferred into a clean plain tube, and left at room temperature for nearly thirty minutes for clotting, then centrifuged. Polymorphism Detection for HSD3B1 (rs1047303) used the primers that have been used to detect this polymorphism

Forward primer	5'-GTCAAATAGCGTATTCACCTTCTCTTAT-3'
reverse primer	5'-GAGGGTGGAGCTTGATGACATCT-3'

After performing the PCR assay as in figure, 4 samples were selected for each gene (3 samples from patients versus 1 control sample), those samples with their primers were sent to MacroGen Corporation in Korea (MacroGen Inc. Geumchen, Seoul, South Korea) specialized in analyzing the sequencing products of those samples. The PCR products of the HSD3B1 (rs1047303) polymorphism gene were dependent on the lengths (bp 215), the result of the sequences was compared with those of the DNA sequences of the previously mentioned and globally registered genes, from which the reference was extracted. <https://www.ncbi.nlm.nih.gov> PCR sequencing products were analyzed, purified, lined up and analyzed along with NCBI samples using BioEdit 6 GenBank (DNASTAR reference database) software, Madison. Sequence Alignment Editor Software Version 7.1 www.ncbi.nlm.nih.gov (<https://www.ncbi.nlm.nih.gov/>) National Center for Biotechnology Information.

Result

Genomic DNA was extracted from patient whole blood samples. HSD3B1 (rs1047303) genotyping was performed by sequencing technology by sent the samples to MacroGen Inc. Geumchen, Seoul, South Korea company. Briefly, pathologic complete response amplification was performed using special PCR Master Mix kit. The primers, annealing temperature, and cycle numbers were as follows: 5'-GTCAAATAGCGTATTCACCTTCTCTTAT-3' and 5'-GAGGGTGGAGCTTGATGACATCT-3', annealing temperature: 65°C, 35 cycles, respectively. The pathologic complete response products were purified and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) on a Genetic Analyzer 3130XL (Applied Biosystems). Sequence data were visualized using Sequence Scanner Software version 1.0 (Applied Bio edit) as shown in figure 1

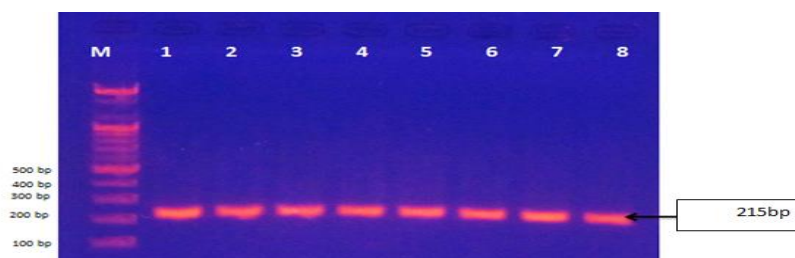


Figure 1. Electrophoresis for PCR production to the HSD3B1 gene on 70 volts for 1 hr. 2% agarose

The result of gene sequencing shows the change A allele with C allele at site of SNP as show in figure 2

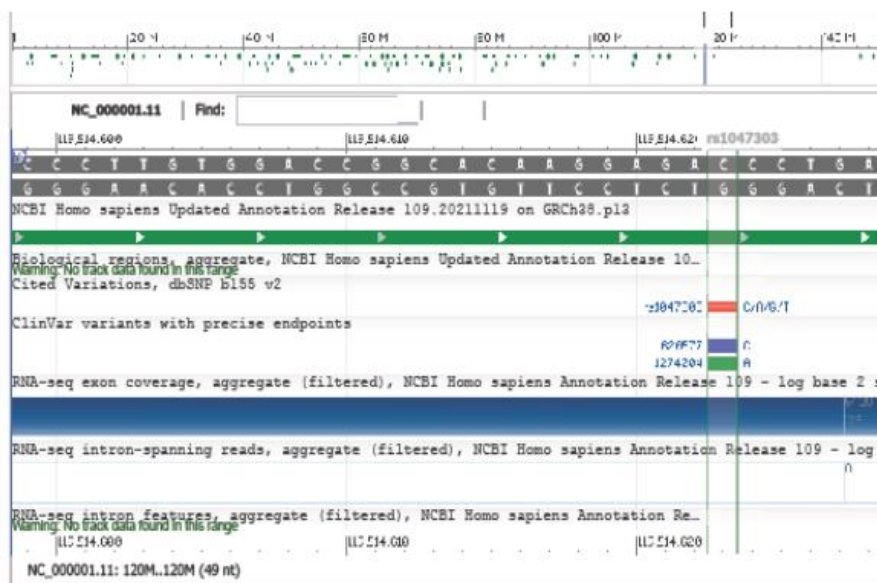


Figure 2. show site of A _ C snp for HDS3B1 gene

There was significant difference in expression of SNP between patients group and control group. where it was expressed more often in patients than in control. P value 0.015 which is significant at p value <0.05 as show in below table 1

Table 1
Allele Frequency of HDS3B1 gene

	C dominant N% SD	A dominant N% SD	Marginal Totals	Row
Patients	87 (54.96) (0.3)	3 (6.04) [2.7]	90	
Control	72 (36.04) [0.45]	18 (4.75) [4.12]	90	
Marginal Column Totals	159	21	180	

Discussion and Conclusion

There are several enzymes that convert adrenal androgens to testosterone and/or DHT have increased expression in castration-resistant disease (12). One such enzyme is 3b-hydroxysteroid dehydrogenase isoenzyme-1 (3b-HSD1, encoded by HSD3B1), which catalyzes the rate-limiting step of potent androgen synthesis from adrenal precursor steroids in peripheral tissues (e.g., prostate, breast, skin, placenta) (13, 14). Here, the study discusses the impact of an A→C missense-encoding single nucleotide polymorphism (SNP) in HSD3B1 nucleotide position

1245 (clustered refSNP ID 1047303) to augment potent androgen synthesis from adrenal precursors. The study discusses the impact of an A→C missense-encoding single nucleotide polymorphism (SNP) in HSD3B1 nucleotide position 1245 (clustered refSNP ID 1047303) to augment potent androgen synthesis from adrenal precursors. We and others researchers have referred to the HSD3B1(1245C) allele as the variant allele because it is minor allele. However, HSD3B1(1245C) is also the allele in the HSD3B1 reference sequence (15, 16)

Shiota *et al.* (17) again study the relationship between the adrenal-permissive genotype and worse post ADT clinical outcomes in 104 Japanese men treated with AD for metastatic disease. Men possessing one or two copies of the adrenal-permissive allele had a higher HR of progression compared with homozygous wild-type men. Similarly, in a cohort of 44 Spanish men with PCa treated with ADT, Gil *et al.* (18) discover the adrenal-permissive genotype to be associated with inferior PFS compared with the adrenal-restrictive one. This study done on Iraqi men with CaP to show There was significant difference in expression of SNP between patients group and control group. where it was expressed more often in patients than in control. P value 0.015 which is significant at p value <0.05 where A allele changes to C allele.

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