The Egyptian Journal of Medical Human Genetics (2016) 17, 223–228

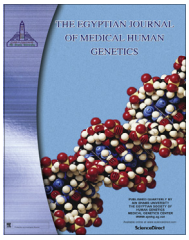
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Partial association of restriction polymorphism of the ligand binding domain of human androgen receptor in prostate cancer

Mohamed Hessien [a,*], **Ahmed El Barbary** [a], **Saad el Gendy** [b], **Amira Nabil** [a]

[a] *Department of Chemistry, Section of Biochemistry, Faculty of Science, Tanta University, Egypt*
[b] *National Cancer Institute, Cairo University, Egypt*

Received 2 September 2015; accepted 22 September 2015
Available online 2 November 2015

KEYWORDS

Androgen receptor;
Ligand binding domain;
Prostate cancer;
Benign prostatic hyperplasia;
Polymorphism

Abstract *Background:* Human androgen receptor (AR) functions as a steroid-hormone activated transcription factor. The receptor binds to its ligand (testosterone or dihydrotestosterone) and is translocated to the nucleus to stimulate the transcription of androgen responsive genes. Mutations in the ligand binding domain (LBD) impair the receptor activity and play a crucial role in the development and progression of prostate cancer (PCa).

Materials and methods: This work was designated to investigate the restriction integrity of the LBD and its association with benign prostatic hyperplasia (BPH) and prostate cancer. Exons of this domain (exons: 4–8) were amplified from prostate tissue of BPH and PCa patients and the restriction polymorphism was investigated by *SmlI*, *HphI* and *Tsp45I* restriction enzymes in both BPH and PCa groups.

Results: Data revealed the integrity of exons 4–6 in both BPH and PCa patients. Exons 7 and 8, however have kept their constitutional pattern only in BPH patients. *Hph1* site showed an abnormal restriction pattern in 40% and 26.7% of PCa patients. Also, *Tsp45I* demonstrated restriction polymorphism in 20% and 13% of PCa patients.

Conclusion: Our results indicate that the loss of the restriction integrity in the C-terminal part (exons: 7 and 8) of the LBD is associated with the progression of benign prostatic hyperplasia to prostate cancer.

1. Introduction

Prostate cancer is the sixth leading cause of cancer-related deaths among men [1]. Prostate cancer cells usually acquire a large number of genetic alterations including point mutations, deletions, amplifications and translocations. Also, a wide range of epigenetic modifications was reported, such as

* Corresponding author at: Department of Clinical Biochemistry, College of Medicine, King Khalid University, Saudi Arabia.
E-mail address: Mohamed.hessien@fulbrightmail.org (M. Hessien).
Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.09.004

Table 1 Mean ages and prostate specific antigen (PSA) in benign prostatic hyperplasia and prostate cancer patients.

	Groups			
	Benign prostatic hyperplasia		Prostate cancer	
n	15		15	
	Age (yr)	PSA (ng/ml)	Age (yr)	PSA (ng/ml)
Mean	58.9	7.1	61.9	1.9[a]
SEM	2.59	1.7	2.9	0.4
Range				
Minimum	48.0	1.40	47.0	0.5
Maximum	82.0	21.2	75.0	1.8
Normality test (*KS*)	0.135	0.21	0.218	0.312
P value	> 0.1	> 0.1	> 0.1	> 0.1

[a] Significant difference between the indicated group vs BPH.

changes in the acetylation and/or methylation patterns in addition to chromatin remodeling [2,3]. This type of cancer develops through a gradual progression through which the benign prostatic epithelial cells progress to prostatic intraepithelial neoplasia (PIN), invasive adenocarcinoma, distant metastatic disease and androgen refractory metastatic disease [4]. This transformation is characterized by several diagnostic nuclear morphological features, such as nuclear and nucleolar enlargement and alterations in chromatin structure [5].

The dependence of prostate cancer on androgen stimulation was described few decades ago [6]. Androgens (testosterone or dihydrotestosterone) bind to the androgen receptors (AR) and the liganded receptor is translocated to the nucleus, binds the androgen responsive elements (ARE), affects the transcription of androgen-regulated genes (e.g. prostate-specific antigen, PSA) and ultimately stimulates the proliferation and inhibits apoptosis of prostate cancer cells.

Many studies have reported that androgen receptor mutations and polymorphism are deeply involved in prostate cancer [7] and [8]. This receptor is encoded by AR gene, located on the X chromosome, at Xq11-12 [9], which consists of 6 functional domains "labeled A through F" [10]. The ligand binding domain (LBD) (domain E) represents the attachment site of androgens and has activation function-2 (AF2), which is responsible for agonist induced activity. Also, the LBD functions as a nuclear export signal [11]. Mutations in LBD were found to restrict the binding of testosterone or DHT to the receptor and subsequently impair AR mediated transactivation. The literature has reported unlimited number of genetic abnormalities in different domains of the AR gene. Most of these genetic abnormalities were detected in prostate cancer (PCa) patients. Also, the majority (79%) of mutations, identified in the LBD in PCa patients, were clustered to three discrete regions that influence the receptor activity [12].

A few studies have investigated the genetic integrity of AR gene during the progression toward cancer, especially in patients with androgen insensitivity syndrome (AIS) and/or patients with prostate cancer. Hence, this work was designated to investigate the genetic integrity of LBD in patients with benign prostatic hyperplasia (BPH) and those who developed prostate cancer (PCa) compared to the constitutional restriction pattern.

2. Subjects and methods

2.1. Patient population and grouping

The study included 30 male patients. All subjects were selected from inpatient of the catheterization units of El-Hussein hospital, Al-Azhar University, and Shebin El-Kome hospital and from El-Mataria Institute. Samples were obtained after surgery under informed consent, following the regulations and approval of the ethics committee and in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Patient's medical history and PSA levels were recorded with special attention to any associated medical problems. The inclusion criteria were benign prostatic hyperplasia (BPH) or prostate cancer (PCa). Patients were divided into two groups. Group I ($n = 15$) included patients with BPH, their ages ranged from 48 to 82 years (average ± SEM: 58.9 ± 2.59 years), whereas group II included 15 patients diagnosed with PCa, their ages ranged from 47 to 75 years (average ± slandered deviation: 61.8 ± 2.9 years) (Table 1).

2.2. Extraction of genomic DNA and exons amplification

Genomic DNA was extracted from tissues of BPH and PCa tumors using QlAmp DNA (GENELUTE) following the manufacturer's instruction. The DNA yield was measured at 260 nm and its integrity was tested by running onto 1.5% agarose gel. Exons 4–8 of the LBD were amplified using segment specific primer pairs (Table 2). In each amplification, 100 ng/μl was amplified in a PCR mix containing 1X (5 μl of 10X buffer) (Stratagene), 0.2 mM each dNTP (2 μl dNTPs mix), 100 pmol/μl (2 μl) of each exon specific primers (Integrated DNA Technologies, USA) and 2.5 U (0.5 μl) *Taq* DNA polymerase (GENE KRAFT, Germany). The reaction mixture was brought to 50 μl with molecular biology grade water.

Reactions of exons 4/5 and 6 were subjected to a thermal cycling program consisting of initial denaturation at 94 °C for 5 min, followed by 35 cycles of annealing at 55 °C for 30 s, primer extension at 72 °C for 40 s and denaturation at 94 °C for 30 s followed by a single extension at 72 °C for 5 min. For