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RESEARCH ARTICLE

Investigating the Genetic Change in BRAF Gene Causing Manifestation of Prostate Cancer in a Sample of Iraqi Patients

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Abstract

This study included 100 patients suffering prostate cancer, 15 persons with family history of cancer served as positive control, and 30 healthy men served as negative control. Prostate cancer tissues were collected from surgical room and subjected to DNA extraction for further processing. Four primers were designed to investigate lethal changes at BRAF location related to this disease. Six pathogenic mutations were found among patients included in this study. These were rs1131692058, rs180177042, rs397507484, rs121913364, rs121913377, rs113488022, and rs1131692058. These represented ingle nucleotide polymorphism except for rs1131692058 which was found a deletion mutation. More data showed that positive control were less specific to associate with BRAF primer suggesting they bear variations susceptible for lethal change that may developed to cancer.

Introduction

Prostate cancer is the most common noncutaneous malignancy among men. Although prostate cancer is often a slow-growing malignancy, it remains the third leading cause of cancer deaths in men [1].

Most patients are asymptomatic at diagnosis; prior to the availability of prostate-specific antigen (PSA) testing, the most common presenting symptoms were urinary retention, back pain, bone pain, and hematuria.3 Risk factors for prostate cancer include sub-Saharan African ancestry, family history, certain genetic mutations [2], and older age (Eggener et al., 2015).

Mutations of the *BRAF* gene were first identified and implicated in human cancers by [3]. *BRAF*, which has been implicated in human cancer, is one of three highly conserved serine-threonine protein kinase genes (*ARAF*, *BRAF*, and *CRAF*) in the RAS-RAF-MEK–ERK cascade [4]. Mutations in the *BRAF* gene have been reported in 7%-15% of all human cancers, with melanoma having one of the highest incidences (40%-70%). The most common locus of mutation is at position V600, causing constitutive hyper activation, proliferation, survival, and oncogenic transformation.

The constitutive activation of RAS pathway has been distinguished in many cancers including prostate cancer (PC) [5]. *BRAF* is a component of the RAF family of serine/threonine kinases; it has hot-spot mutations at codon 600 in the kinase domain, which is considered for more than 90% of *BRAF* mutations in human cancers. BRAF gene mutations mostly occur in 30 positions in the kinase zone and most of the mutations occur in two regions, which include the second loop G and its active site [6].

BRAF mutations were studied in PC in various populations; however, they are unusual in comparison with KRAS mutations [7]. BRAF mutation is one of the causes of resistance to treatment in patients with cancer. Therefore, using BRAF inhibitors is an important target for anticancer drugs development. The identity of mutant BRAF proteins is important in a subset of PC for prognostic and therapeutic point of view [8]. However this study aimed to identify the mutations in BRAF gene associated with prostate cancer in Iraqi men.

Materials and Methods

Sample collection: a total number of 100 tissue samples were collected from patients suffering prostate cancer after surgery, while blood samples were drawn from 15 positive control subjects who have a family history of cancer, and 30 healthy men.

Extraction of DNA

DNA was extracted from tissue samples and blood using blood DNA Maxi extraction kit type FABGK-300 from Favorgen/Taiwan according to company instructions.

PCR Amplification

Four primers design to amplify BRAF gene were used for PCR amplification. Primers sequences are as follow:

Primer name	Sequence 5'-3'	Amplicon size / bp
BR-1-F	TGCATTTGGGATTGTTCTGTATGA	370
BR-1-R	AAACGCACCATATCCCCCTG	
BR-2-F	TGCATTTGGGATTGTTCTGTATG	301
BR-2-R	TGTTTGGAAACCAGCCCGAT	
BR-3-F	AGACGGGACTCGAGTGATGA	617
BR-3-R	TCATACAGAACAATCCCAAATGC	
BR-4-F	GCATTTGGGATTGTTCTGTATGA	370
BR-4-R	GAAACGCACCATATCCCCCT	

PCR amplification was conducted under the following conditions: initial denaturation at 94^{0}C for 5 min, followed by 35 cycles of denaturation at 94^{0}C for 30 sec, annealing at 55^{0}C for 45 sec, and extension at 72^{0}C for 30 sec

Statistical Analysis

The Fisher exact test and Q score was carried out to measure the communications between BRAF mutation and the histopathological particularities of tumors. P value less than 0.05 was considered statistically significant. The SPSS software (version 16.0, Chicago, IL, USA) was applied for statistical analysis.

Results

Clinicopathologic Findings

Clinicopathologic findings of 100 cases were collected from medical records and files. The average age of patients and prostatic hyperplasia cases were 70.83 ± 8.9 (range 40-100) and 68.95 ± 8.5 (range 40-90), respectively. No statistically significant difference was detected within groups (patients and control) and age (P=0.13). The most frequent of age range were 71-80 and 61-70 in patients and control cases, respectively (Table 1).

Table 1: Clinical characteristics of 100 patients with prostate cancer

Histopathology factors Age: (Year)	BRAF mutation: Positive:	BRAF mutation: Negative:	All patients: N (%)	BRAF V600E mutation:%	P-value
40-50	3	2	5 (5%)	0%	P= 0.285
51-60	4	5	9 (9%)	0%	
61-70	10	21	31 (31%)	0%	
71-80	3	45	48 (48)	75%	
81-90	2	7	9 (9%)	25%	
Cumulative Gleason score:					
<6	0	19	19	0%%	P= 0.21
7-10	4	77	91	5.19%	

Molecular Analysis and BRAF Status

DNA from all subjects included in this study was isolated and subjected to PCR amplification using specific primers designed for this purpose. Resulting amplicons were sent for sequencing by Macrogen Company, Korea. Amplification results are shown in Figure 1.

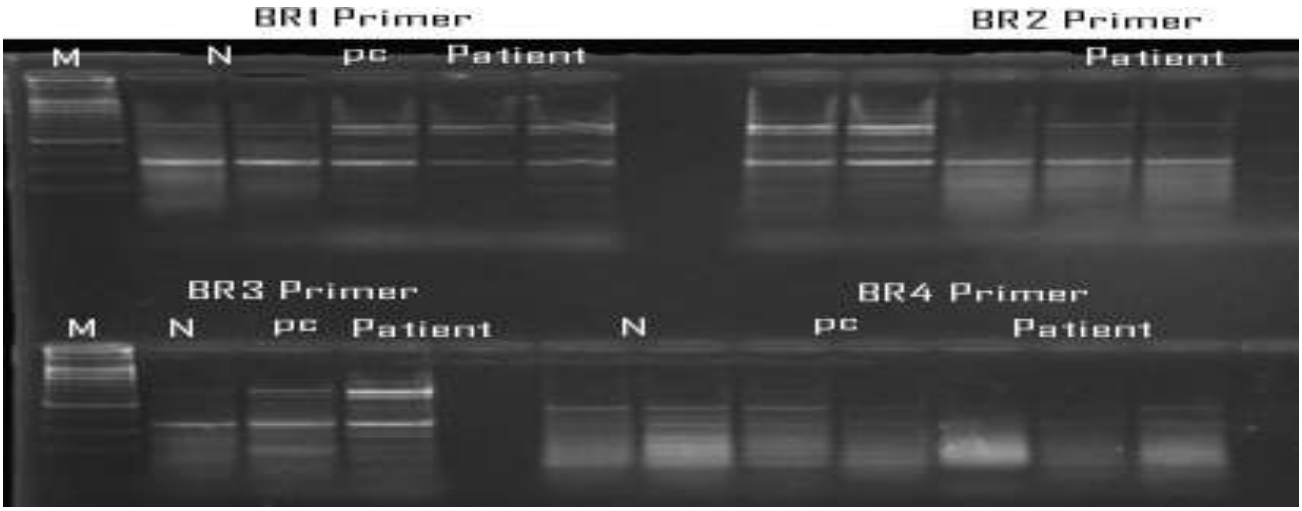

Figure 1: Amplification of BRAF specific region using primers designed for this study. M is 100 bp markers DNA, N is normal and healthy subjects, pc is positive control subjects, whereas patients are subjects with prostate cancer

Detection of Mutations

Six pathogenic mutations were found associated with PC. These were identified after obtaining DNA sequence of amplified regions of BRAF gene and listed in Table 2.

Table 2: SNP linked to Gene (gene ID: 107985664) Via Contig Annotation

rs1131692058	**140,734,769 - 140,734,776**	**indel**	**BRAF and 1 more**	**splice acceptor variant, nc transcript variant, intron variant**	**Pathogenic**
rs180177042	140,749,365	single nucleotide variant	BRAF and 2 more	missense variant, nc transcript variant	Pathogenic
rs397507484	140,753,333	single nucleotide variant	BRAF and 3 more	missense variant, nc transcript variant, intron variant	Pathogenic
rs121913364	140,753,334	single nucleotide variant	BRAF and 2 more	missense variant, nc transcript variant, intron variant	Pathogenic
rs121913377	140,753,335 - 140,753,337		BRAF and 2 more	missense variant, nc transcript variant, intron variant	Pathogenic
rs113488022	140,753,336	single nucleotide variant	BRAF and 3 more	missense variant, nc transcript variant, intron variant	Pathogenic

Discussion

The molecular variations contained in the pathogenesis of prostate cancer are less known. Thus, the steps that mark the shift from the primary phases of PC progression to more critical stages of this disease are not fully recognized [9]. The main aspects which manage the treatment and prognosis are in the pathological phase as yet. Due to the progression in novel molecular targeted treatments, like anti-EGFR molecules, novel therapeutic markers disseveration is in need [7].

Recent reports proposed that approximately 10% of PCs may have BRAF mutations. Different frequencies of cited mutation have been reported in different populations which may be related to various ethnic backgrounds [10]. The presence of BRAF mutations indicated that there could be easily recognizable patients who might be assigned as a joint clinical path or even helpful for targeted treatment [11]. According to the present and other studies, the frequency of mutations in BRAF gene was low in PC. In this regard, PC cannot be an appropriate target for anti-BRAF (V600E) treatments. Therefore, we need to seek for other molecular abnormalities such as RAS / RAF / MAP Kinase.

According to the evaluated papers and current study, the correlation between high Gleason score and BRAF mutation was concluded. Actually, a major limitation of this study was the small population size.

Thus, we suggest using a larger sample size for more sophisticated studies and eliminating the bias caused by the low sample size. The mutation detection method for BRAF was direct PCR in our study. For better and more accurate examination, more novel methods such as ARMS-PCR are recommended. According to this study, the role of BRAF gene mutation in the development of PC was less colorful. It is advisable to check other targets of the RAS / RAF / MEK / MAPK route, such as EGFR and IGFR that activate the RAS / RF / MEK pathways in PC. As a result, our study evaluated one of the PC risk factors and proposed potential risk factors which are particular to the Iranian population.

Our study is a novel research in the cited subject in Iraq. It seems that high morbidity and mortality of PC in our country may generate enhanced burden of disease in Iran in the future decades. Unless eventative proceedings, which can be extended, we suggest more care and follow up for these patients. The effects of more precise techniques and other involved genes can be evaluated for this cancer therapy. According to this study which proved the presence of BRAF mutation in few cases of prostate cancer, the use of anticancer drugs that affect tyrosine kinase pathway may be useful. Nevertheless, subjects with family history of cancer may be candidate for PC or other types of cancer to appear. The specificity of primers used in this study were less with positive control than normal subjects as calculated by us suggesting presence of nucleotide variation within BRAF gene which may develop to pathogenic mutation at a particular stage of development.

Conclusions

According to our findings, the role of BRAF gene mutations in PC is low, thus, anti-BRAF therapy could not be a good treatment strategy. It is needed to follow other molecular abnormalities such as RAS / RAF / MAP kinase pathways to evaluate the progress of PCa.

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