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Molecular Status of BRAF Mutation in Prostate Adenocarcinoma: The Analysis of 100 Cases in North-East of IRAN

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ABSTRACT

Background and Objective: *BRAF* mutations were studied in various populations for prostate carcinoma (PC); however, mutations in BRAF gene are unusual compared to *KRAS*. Oncogenic activating of BRAF mutations were studied lately in almost 0%-10% of prostate cancer cases.

Methods: In this retrospective study, we gathered 100 formalin-fixed paraffin-embedded samples of prostate adenocarcinoma. A hundred archived samples of adjacent benign prostatic hyperplasia were chosen as normal control. This study was done in pathology laboratory of Qaem Hospital during 2013-2015.

Results: Total number of 200 PC and normal cases was investigated for BRAF V600E mutation. The BRAF V600E mutation was found in only 4 patients but it was not detected in normal cases. There were no significant differences between patient and control groups for this mutation (P>0.99). The frequency of BRAF V600E mutation was not significant in different age groups (P>0.285); the most frequency was related to the age range of 71-80. No significant difference was observed between tumor grade and BRAF mutation (P=0.21).

Conclusion: According to our findings, BRAF gene mutations did not play essential role in PC. Therefore, anti-BRAF (V600E) could not be considered as a proper target for therapy.

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Introduction

Prostate cancer (PC) is considered as the sixth most prevalent cancer in the world and the third most significant one in men. It is increasing globally, with powerful heterogeneity among various areas (1, 2).

PC is the second leading cause of malignancy worldwide. In 2016, approximately 189,090 new cases and 26.120 deaths occurred in the United States due to PC. It is more prevalent in the US and Europe than Southeast Asia. In China, the incidence is 1.6 per 100,000, while it is 9.119 per 100,000 in the United States (3). Although the outbreak is low in East Asia, it is accompanied with high levels of granularity and tumor volume (4). It is rare at age below 40 but increases with age. For example, benign prostatic hyperplasia is more prevalent in men over 60 years of age.

Race is one of the most important risk factors in this regard. African-American men include 50-70% of cases and they are more likely from Caucasians (5-7). PC is the third most visceral cancer in Iran and the seventh cause of death due to cancer (8). However, PC incidence is low in Iran that might be due to lack of proper registration system (7, 8). The lowest reported PC in Iran was related to Ardabil with the rate of 0.4% per 100,000 (8).

The clinical configuration of PC has considerably changed over the past few years; it is a reason of death in 10 % of men in Western countries (2). Although,

PC incidence and mortality has been ascending (9), it is still one of the main reasons of morbidity and mortality in Iran (10, 11). Although, primary stage of this disease can be cured by androgen ablation therapy impressively, almost 20% of prostate tumors will relapse and finally progress to an androgen resistance state. Therefore, a better comprehending of molecular pathways of cancer expansion is needed to progress to a new therapeutic approach (1). The difference is not only relevant to the domain of diagnosis of latent PC but also to the genetic or environmental factors (1).

Studies have demonstrated that genetic variations are consequential for prostate carcinogenesis. Nonetheless, few oncogenes or tumor suppressor genes have been relevant to PC, and the substantial molecular mechanisms regarding its progression are poorly understood (12).

The constitutive activation of RAS pathway has been distinguished in many cancers including PC (13). *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 (14). *BRAF* mutations are reported frequently in malignant melanomas and thyroid papillary carcinomas; it has lower rate of incidence in other types of human cancer (15, 16). BRAF mutations were studied in PC in various populations; however, they are unusual in comparison with KRAS mutations (15). Although BRAF mutations have not been detected by various studies in white patients, a new publication reported 10.2% BRAF mutation rate in PC in Korean patients (12). Accordingly, practical ethnic diversity in BRAF mutations in PC cannot be divested. Oncogenic activation of BRAF mutations was studied lately in almost 0%-10% of PC cases (12, 16). The V600E BRAF mutant protein is fundamentally activated and perhaps the reason of occurrence in oncogenesis. Significantly, small-molecule inhibitors of BRAF are noticed in the treatment of cancers with activated BRAF (17). 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 (18). The identity of mutant BRAF proteins is important in a subset of PC for prognostic and therapeutic point of view (17).

This study aimed to characterize the prevalence of *BRAF* codon 600 mutations in tumoral tissue specimens from the patients with prostatic adenocarcinoma and benign prostatic hyperplasia.

Materials and Methods

Patients and materials

In this retrospective study, 100 formalin-fixed paraffin-embedded (FFPE) samples of prostate denocarcinoma, and 100 samples of adjacent benign prostatic hyperplasia were examined. They were archived in pathology laboratory of Qaem Hospital during 2013-2016. Data were extracted from existing document files in Qaem center. According to the data extracted from the present evidence files in this center, no separate sampling was done from the patients in this study, and the archived samples were used for the experiment; thus, a consent form was not required

FFPE blocks were chosen and examined by two pathologists (Jafarian MJ and Ayatollahi H). Each tumor was graded in order to conform to the Gleason system (11). The areas including minimum 80% of tumor tissues on the slides were chosen for analysis. Tumoral samples included 19 cases having Gleason score <6 and 81 cases having Gleason score > 6.

The exclusion criteria were: 1) the incompleteness and uncertainty of the information required in the medical records of patients, 2) paraffin blocks with high levels of tissue, 3) paraffin blocks with high necrosis in tissue, and 4) patients without confirmed diagnosis and no PCR result.

Using the computer system and the contents of the hospital file the following information of patients were gathered: age, type of tumor, grade, and telephone number. Each patient was carefully and individually examined. After diagnosis and tumor grade confirmation the incisions under 10-micron were tak-

en from the paraffin blocks under sterile conditions.

DNA extraction

For each FFPE sample, tumoral area was labeled on the slide by the pathologist; then, tissue samples with 0.9 mm in diameter were separated from the selected area in each paraffin block and placed in 1.5 ml microtubes. In the next step the dried tissue samples were de-waxed by xylene and threaded by ethanol. YTA DNA Tissue Kit (Yekta-Tajhiz-Azma Co, Tehran, IRAN) was used for DNA extraction, according to the YTA protocol. The quality and concentration of the DNAs were determined by Nano Drop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

PCR amplification and direct sequencing

The PCR reaction was performed for BRAF V600E mutation in 25 µl final volume comprising nearly 100 ng of genomic DNA, 12 µl DW,10 µl Master mix (Amplicon, Denmark), 100 nmol/L of each primer; Forward 5′,-TGCTTGCTCTGATAGGAAAATG-,3′ and Reverse 5′,-AGCCTCAATTCTTACCATCCA-,3′. PCR amplification was carried out by denaturation at 94°C for 5 min following 35 cycle of94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, and the last extension at 72°C for 30 sec in the Veriti 96 wells thermo cycler (Applied Biosystems, USA). The PCR products were electrophoresed on 2% agarose gel for 40 min. All samples were sequenced by direct sequencing method using an ABI automatic sequencer and the results were analyzed by CLC sequence viewer.

Statistical Analysis

The Fisher exact test and Q score was carried out to measure the communications between BRAF V600E 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 200 cases (100 PC and 100 benign prostatic hyperplasia cases) were gathered 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).

BRAF V600E Status

The numbers of 200 PC and normal cases were investigated for BRAF V600E mutation. BRAF V600E

Table 1. Clinical characteristics of 100 patients with prostate cancer

Histopathology factors	BRAF V600E mutation:		All patients: N (%)	BRAF V600E mutation:%	*P-value*
	Positive:	Negative:			
Age: (Year)					
40-50	0	3	3 (3%)	0%	*P*= 0.285
51-60	0	9	9 (9%)	0%	
61-70	0	31	31 (31%)	0%	
71-80	3	45	48 (48)	75%	
81-90	1	7	8 (8%)	25%	
91-100	0	1	1 (1)	0%	
Cumulative Gleason score:					
<6	0	19	19	0%%	*P*= 0.21
7-10	4	77	91	5.19%	

mutation was detected in 4 patients but not in normal cases. There were no significant differences between patient and control groups for this mutation (P>0.99). According to the Table 1, the frequency of BRAF V600E mutation was not significant in different age groups (P>0.285) but the most frequency belonged to the age range of 71-80. No significant difference was observed between tumor grade and BRAF gene mutation (P=0.21)

Discussion

The molecular variations contained in the pathogenesis of prostate adenocarcinoma are less known. Accordingly, the vital steps that mark the shift from the primary phases of PC progression to more critical stages of this disease are not fully recognized (20). The main parameters which manage the treatment and prognosis are in the pathological phase as yet. Recognizing the molecular pathways of cancer growth and progression such as RAS, RAF and MAP kinase pathway and RAF kinase inhibitors such as Sorafinib can be helpful in PC treatment (21). Due to the progression in novel molecular targeted treatments, like anti-EGFR molecules, novel therapeutic markers disseveration is in need (15).

Currently, molecular tests are part of Personalized Medical Oncology. They are used for identifying cancer promoters for target therapy. Choosing the appropriate test for somatic mutations is based on the type of mononization, clinical and laboratory conditions (22).

This investigation was the first study which assessed BRAF in prostate cancer patients in North-East of Iran. Sadjadi et al. have reported a low rate of this mutation in Iran in recent years; while Salmaninejad et al. (8, 15) did not report any mutation. However, our results showed that the mutation rate is higher in our region which is close to the other countries.

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.

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 (23, 24). 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 (23).

In this study, we determined BRAF V600E mutation in 4% of PC patients. Liu et al. in USA (12) and Burger et al. in Germany (25) studied BRAF mutation and they could not find any mutation in PC patients under study. This outcome was inconsistent with our results. BRAF mutations were detected in 10.2% of Korean PC patients; it was correlated with higher Gleason scores and clinical stages (12).

Salmaninejad et al. evaluated 35 PC patients in Iran by sequencing method and reported no BRAF mutation, which was different from our results (15). Jonathan et al. in 2012 reported no BRAF mutation in prostate cancer patients evaluated by FISH method in China either (26). Cho et al. detected BRAF mutation (10.2%) in 206 PC patients by RFLP-PCR; it was more prevalent than our studied population (27).

In our study, PC cases were divided in two groups, of which one group owned <6 (19 patients) and the other had >6 Gleason score (81 patients). Cho et al. analyzed 206 prostate cancer patients in 3 graded groups included <6 Gleason score (27 cases), intermediate Gleason score (132 cases) and high Gleason score (47 cases) (27). Liu et al. studied 93 prostate cancer patients in 3 grades including low grade (24 cases), intermediate grade (50 cases) and high grade (19 cases) (23). Salmaninejad et al. analyzed 35 PC cases with low grade (7 cases) and high grade (13 cases) (15).

In our study, we did not detect BRAF gene mutation in <6 Gleason score, conforming to the other studies. Cho et al. and Salmaninejad et al. found BRAF mutation in intermediate and high grades; they did not report it in low grade carcinoma (15, 27).

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. Unfortunately, only 4 V600E mutations were seen in 100 cases; therefore, the sample size of 100 cases might be too small to detect BRAF. 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 (V600E) 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 (V600E) 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 Iran (8). 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 preventative 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.

Conclusion

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 PC.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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