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Urologic Oncology: Seminars and Original Investigations ▮ (2018) ▮▮▮–▮▮▮

UROLOGIC ONCOLOGY

Original article

Improving the genetic signature of prostate cancer, the somatic mutations

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Received 13 July 2017; received in revised form 30 December 2017; accepted 12 March 2018

Abstract

Background: Somatic mutations have been related to the highest incidence of metastatic disease and different treatment responses. The molecular cause of prostate cancer (PC) is still unclear; however, its progression involves alterations in oncogenes and tumor suppressor genes as well as somatic mutations such as the ones in *PIK3CA* gene. A high percentage of PC is considered sporadic, which means that the damage to the genes occurs by chance after birth (mainly somatic mutations will drive the cancer event). However, little is known about somatic mutations in PC development.

Materials and methods: We evaluated prostate biopsies in the main somatic mutations genes (*PIK3CA*, *TP53*, *EGFR*, *KIT*, *KRAS*, *PTEN*, and *BRAF*) among individuals with PSA values > 4 ng/ml ($n = 125$), including affected and unaffected PC subjects.

Results: Mutations in *KIT* gene are related to aggressive PC: TNM stages II to III, Gleason score ≥ 7 and D'Amico risk ($P = 0.037$, 0.040, and 0.017). However, there are no statistical significant results when more than 3 somatic mutations are presented in the same individual. In relation to environmental factors (smoking, diet, alcohol intake, or workplace exposure) there are no significant differences in the effect of environmental exposure and the somatic mutation presence. The most prevalent mutations among patients with PC are c.1621A > C (rs3822214) in *KIT*, c.38G > C (rs112445441) in *KRAS* and c.733G > A (rs28934575) in *TP53* genes. *KRAS*, *KIT*, and *TP53* genes are the most prevalent ones in patients with PC.

Conclusions: Somatic alterations predisposing to chromosomal rearrangements in PC remain largely undefined. We show that *KIT*, *KRAS*, and *TP53* genes have a higher presence among patients with PC and that mutations in *KIT* gene are related to an aggressive PC. However, we did not find any environmental effect in somatic mutations among PC individuals.

Keywords: Hormonoresistant; *KIT* gene; Prostate cancer; Somatic mutation; Treatment

1. Introduction

In prostate cancer (PC), structural genomic rearrangements, including translocations (e.g., *TMPRSS2-ERG*) and copy number aberrations (e.g., 8q gain, 10q23/*PTEN* loss) are key mechanisms driving tumorigenesis [1]. Somatic events are associated with structural genomic rearrangements but in PC remain largely undefined [2]. Although somatic mutations occur with a low to an intermediate frequency among cancer patients (2–20%), their role in cancer is clearly highlighted, mainly in cancer progression and treatment management [3,4]. Recent large-scale sequencing efforts such as The Cancer Genome Atlas (TCGA) have revealed a complex landscape of somatic mutations in various cancer types [5]. For example, in colorectal cancer, somatic *BRAF* V600E mutation is associated with poor outcomes irrespective of the received

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https://doi.org/10.1016/j.urolonc.2018.03.012
1078-1439/

treatment and regarded as poor prognosis markers [6]. Current data about whole-exome sequencing and whole-genome sequencing have provided a window into the biology, that drives oncogenesis and PC tumors progression, by enabling unbiased exploration of somatic mutations in prostate tumors that span the spectrum of aggressiveness disease [7]. These findings suggest that the genome-wide interplay between somatic single nucleotide variants, indels, and structural variants is important for understanding the repertoire of genomic aberrations that contribute to PC. In spite of these findings, considerable work remains to understand the relationship between somatic genomic alterations and tumor aggressiveness [8].

In PC, somatic mutations rate is in the medium to lower range (0.31 mutations/Mb) in comparison with other tumors like lung squamous cell carcinoma (8.4 mutations/Mb) or malignant melanoma (30 mutations/Mb); even though the rate is also moderate between localized and advanced PC [9]. When talking about somatic mutations in PC there are some candidate genes such as *AR*, *TP53*, *KLF6*, *EPHB2*, *CHEK2*, *ZFHX3* (formerly known as *ATBF1*), *NCOA2*, *PTEN*, *MYC*, *PIK3CA*, *FOXA1*, *KIT*, and various histone-modifying genes [9]. Despite its high incidence, one of the PC main challenges is related to its high heterogeneity, which makes risk stratification and selecting treatments strategies difficult, because tumors classified in the same risk group exhibit different clinical behavior [10]. The inclusion of expression patterns, molecular and genetics biomarkers in PC could create a specific profile classification to assess risk and treatment options [11]. For instance, a recent study has showed an effective prognostic prediction model in relation with several atypical somatic mutations signatures. This model combines the genetic signatures with NICE (National Institute for Health and Care Excellence) factors and improve the prognosis prediction of genetic features or NICE features when they are used alone [10].

The role of environmental factors (like tobacco smoke [including second hand smoke], diet, radiation, and occupational exposures) in cancer development has long been evident from epidemiological studies, and with fundamental implications for primary prevention. There is a clear detailed database of cancer risk molecular effects, and it is well established that environmental factors exert a relevant influence on mutations in all cancers [12]. There are described mutations' signatures related to cancer such as UV damage producing high levels of mutations at Py-Pysequences; tobacco and tobacco smoke exposure with an increase rate of transversional mutations associated with adducts in lung cancer [12]. Moreover in other hormonal cancers, such as breast cancer, the exposure to multiple endogenous and exogenous environmental factors (such as alcohol, smoking, and obesity) are established as risk factors, affecting estrogens metabolism [13].

The term gene and environmental interaction is relevant in cancer cases, and for that reason we make a focus point of the study on the environmental factors effect on somatic mutations and PC risk. Studying the environmental influence includes everything that surrounds us both internally and externally, such as patients with PC and that mutations in *KIT* gene toxicants, hormones, diet, psychosocial behavior, and lifestyle [13].

By September 2013, 125 men with PC clinical symptoms and who had a prostatic biopsy were collected for this study. The main objective of the present work is to obtain a good stratification of patients with high-risk PC giving details in initial steps of the progression of the tumor by the analysis of clinical and environmental exposure data.

2. Materials and methods

2.1. Patient and samples

Men enrolled in this study were selected by urologists of the *Virgen de las Nieves* University Hospital, Granada, Spain. The inclusion criteria were subjects with total PSA/free PSA under 20%, and PSA values above 4 ng/ml. All individuals underwent a systematic 20-core ultrasound guided biopsy to limit the false negative rate. Men with histological confirmed prostatic adenocarcinoma comprised the patient group and negative biopsy individuals were considered as controls. Moreover, patients with positive biopsy were analyzed for T stage, serum PSA, Gleason score and were categorized according to D'Amico risk classification (low, intermediate, and high risk). After primary therapy, PSA was monitored every 3 or 6 months in patients, during 43 months to evaluate the existence of biochemical recurrence (Table 1).

Tissue samples were obtained with 20-core ultrasound guided biopsy as parallel and close cylinders. The anatomopathological analysis classified each biopsy cylinder according to cancer presence/absence. We have analyzed the parallel cylinder to the one previously analyzed by the pathologist. With this methodology we tried to take cells throughout the whole sample, which assures having a deep analysis spectrum and making sure to cover the same sample that the pathologist has analyzed.

Despite the fact that a total of 300 samples were registered, we selected the most representative (samples with no missing data of clinical follow-up, TNM stage Gleason score, and environmental exposure data) 125 tissue samples for somatic mutation analysis. Several samples were discard because of samples' quality, so the total final samples that fulfill all the criteria were reduced to $n = 119$ (PC [$n = 60$] and no patients with PC [$n = 59$]). Moreover, we have quantified the samples with Qubit 4 Fluorometer and NanoDrop 2000c (Thermofisher Scientific, MA). These systems provide quantification and purity assessments for DNA; those samples that were under 1.8 in the 260/280 rate in fluorometry measurements; or those with a difference value over 3 between the fluorescence and