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Full Length Article

Quantitative analysis of plasma cell-free DNA and its DNA integrity in patients with metastatic prostate cancer using ALU sequence

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KEYWORDS

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Abstract *Background:* Prostate cancer (PC) is the most common cancer affecting men, it accounts for 29% of all male cancer and 11% of all male cancer related death. DNA is normally released from an apoptotic source which generates small fragments of cell-free DNA, whereas cancer patients have cell-free circulating DNA that originated from necrosis, autophagy, or mitotic catastrophe, which produce large fragments.

Aim of work: Differentiate the cell free DNA levels (cfDNA) and its integrity in prostate cancer patients and control group composed of benign prostate hyperplasia (BPH) and healthy persons.

Methodology: cf-DNA levels were quantified by real-time PCR amplification in prostate cancer patients (n = 50), (BPH) benign prostate hyperplasia (n = 25) and healthy controls (n = 30) using two sets of ALU gene (product size of 115 bp and 247-bp) and its integrity was calculated as a ratio of qPCR results of 247 bp ALU over 115 bp ALU.

Results: Highly significant levels of cf-DNA and its integrity in PC patients compared to BPH. Twenty-eight (56%) patients with prostate cancer had bone metastasis. ALU115 qpcr is superior to the other markers in discriminating metastatic patients with a sensitivity of 96.4% and a specificity of 86.4% and (AUC = 0.981)

Conclusion: ALU115 qpcr could be used as a valuable biomarker helping in identifying high risk patients, indicating early spread of tumor cells as a potential seed for future metastases.

Introduction

Prostate Cancer is the sixth most common cancer in the world. Prostate cancer (PC) is the sixth most common type of cancer in the world. It is the highest cancer in elderly males (> 70 years of age) with substantial impact on morbidity and

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mortality rates. It accounts for 29% of all male cancer and 11% of male cancer related death [1].

Prostate cancer patients' faces multistep challenges during the course of the disease. At diagnosis using prostate specific antigen (PSA) test with its low specificity result in a lack of cancer detection in a significant proportion of patients [2]. In contrast, men receiving radical treatment for presumed locally confined prostate cancer often develop disease relapse post treatment, and in the majority of these patients the disease was more extensive than it appeared pre-treatment [3]. Early occult dissemination of PC cells from the primary tumor through the bloodstream to secondary organs is a critical step in tumor progression.

Dissemination of PC cell from primary tumor may occur due to the overexpression of MUC1-C oncoprotein, which enhances the epithelial-mesenchymal transition (EMT) and therefore promoting metastasis in epithelium-derived carcinoma. [4] Similarly, MYC oncogene is also responsible for the development and progression of prostate cancer [5] and changes in promoter hypermethylation are driven by DNA methyltransferase (DNMTs) [6]. But detection of these markers requires sophisticated methods and has been studied on cell lines and cultures.

Currently, repeated measurements of PSA blood serum levels are done after the primary treatment of PC (e.g., surgery or radiotherapy). However, the increase in PSA concentrations (also called biochemical recurrence) cannot distinguish between a local or metastatic relapse. In addition, even if overt metastases are subsequently confirmed by current imaging technologies (e.g., bone scans), the patient has become already incurable [2]. Thus, a biomarker which can be used as a valuable tool in identifying high risk patients, indicating early spread of tumor cells as a potential seed for future metastases is highly needed.

In healthy individuals, the main source of free circulating DNA is apoptotic cells which release uniformly truncated DNA fragments (~185–200 base pair) in length, in contrast to DNA released from malignant cells via necrosis or mitotic catastrophe, autophagy and mitochondrial catastrophe, which varies in size because of random and incomplete digestion of genomic DNA, which leads to raised levels of circulating cell-free DNA (cfDNA) with longer fragments in serum or plasma. [7]. The use of plasma/serum cell-free DNA has attracted the interest of clinicians in oncology [8], prenatal diagnosis [9], and hematology [10]. Patients with cancer have a higher concentration of circulating cell-free DNA as compared to healthy people [8], since cfDNA in cancer patients often bears similar genetic and epigenetic features to the related tumor DNA, there is evidence that some of the cfDNA originates from tumoral tissue. It is possible that circulating cell-free DNA may serve as a non-invasive, rapid and sensitive biomarker for molecular diagnosis, prognosis and monitoring treatment response in various types of cancers [11,12]. The use of cfDNA and DNA integrity has been studied in several studies including Prostate Cancer [13–15].

Most of the plasma DNA of normal individuals belongs to the Arthrobacter luteus (ALU) repeat family. The ALU sequences are about 300 base pairs long and are therefore classified as short interspersed elements (SINEs) typically 300 nucleotides in length, that account for more than 10% of the human genome, among the class of repetitive DNA elements. [16]. The ALU is the most abundant repeated sequence in the human genome, with a copy number of 1.4×10^6 per genome. ALU elements multiply within the genome in a retroposition process through RNA polymerase III-derived transcripts from evolution. [17]. Several studies used ALU amplicons and Cell-free circulating DNA integrity which is the ratio of repeated sequences of ALU (247 and 115 bp) [15,18]. The ALU 115 primers amplify small fragments (truncated by apoptosis) and the ALU 247 primer amplifies longer DNA fragments. Therefore, using direct q-PCR of ALU repeats with properly designed primer sets can detect as little as 0.01 pg of DNA (equivalent to about 1/300 of the genome in a single cell) with high linearity. Moreover, using plasma directly as template set can dramatically increase the sensitivity of size-dependent DNA measurement by eliminating artifacts associated with DNA purification [18].

The present communication aimed to differentiate the cell free DNA levels (cfDNA) and its integrity in prostate cancer patients and control group composed of benign prostate hyperplasia (BPH) and healthy persons. And study the relationships between the results and the clinicopathological findings to evaluate the prognostic value of these markers in the detection of included cases.

Patients and methods

This is a cross sectional study that included 105 cases gathered during the time period from February 2014 to June 2015. A group of 50 patients were newly diagnosed Prostate Cancer. They were recruited from the oncology outpatient clinic at National Cancer Institute – Cairo University. The stage of prostate cancer was classified using the TNM staging system, according to the American Joint Committee on Cancer (AJCC).

Eligibility criteria for patient selection in our study were as follows: (1) valid informed consent form, (2) availability of blood samples before prostate biopsy and (3) availability of complete clinical and serum PSA data for each patient. Patients with other malignancies were excluded, in addition to control group composed of 25 patients with BPH who were recruited from the Urology Department at Kasr El-Aini Hospital, Cairo University. And 30 apparently healthy men based on clinical and laboratory examinations with no history of malignant prostate disease, with PSA values less than 4 ng/ml, and no symptoms of BPH at all.

Ethics statement

Informed written consent was taken from all participants prior to enrollment in this study according to Human Ethics Committee approval. The study protocol was reviewed and approved by the Ethical Committee of NCI., Cairo University (IRB No. 00004025) and (FWA No 00007284).

Blood collection and DNA isolation

Peripheral venous blood of 3 ml was collected into EDTA containing tubes and processed within 2 h after venipuncture. To ensure cell-free plasma collection and to prevent cellular contamination, all EDTA-blood samples were centrifuged in 2 steps (3000 rpm for 10 min and then 12,000 rpm for 10 min). The cell-free plasmas were stored at −20 °C until extraction.

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