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Review article

The role of cell-free circulating DNA in the diagnosis and prognosis of prostate cancer☆

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Abstract

The presence of small amounts of circulating DNA in plasma was demonstrated 60 years ago. Since then, cell-free DNA has been tested for quantity, fragmentation pattern, and tumor-specific sequences in patients with various malignancies. Recent studies have shown that cell-free DNA levels are distinctly increased in most patients with prostate cancer (PCA) and that the DNA fragmentation pattern is different from healthy individuals and patients with benign prostate disease. The origin of this circulating DNA remains largely unknown, but it is established that a small fraction of the DNA is derived from the tumor itself, and genetic (allelic imbalances) and epigenetic (DNA methylation) alterations are regularly detected in patients with PCA. The detection of increased DNA levels and tumor-specific DNA sequences may provide diagnostic and prognostic information. The recent findings in the emerging field of cell-free DNA will be discussed.

Keywords: Cell-free DNA; Prostate cancer; Methylation; Allelic imbalance; Biomarker

1. Introduction

Prostate cancer (PCA) is the most common malignancy in men in industrialized countries. It was estimated that 198,280 men will be diagnosed with PCA and 27,360 men will die from PCA in 2009 in the USA [1]. Since the introduction of the prostate-specific antigen (PSA) test, the incidence of PCA approximately doubled in the USA [1], while the mortality decreased only modestly. It is therefore assumed that PSA screening leads to the detecting of clinically insignificant PCA. The recently published first results from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial [2] and the European Randomized Study of Screening for Prostate Cancer (ERSPC) trial [3] also demonstrate the problem of PCA overdiagnosis: after a median follow-up of >7 years, the PCA incidence was distinctly higher in the screening arms while PCA-specific mortality was similar [2], and only slightly increased in the control arm [3]. Thus, there is a clear need for a biomarker that allows identifying the clinically significant PCA.

The existence of circulating cell-free DNA in human plasma was reported in 1948 [4]. It was recognized that patients with cancer have higher levels of cell-free DNA compared with healthy individuals [5] as well as patients with different nonmalignant diseases [6]. Especially the widespread availability of the quantitative real-time PCR increased the number of studies on cell-free DNA in various malignancies. It seems that most patients with solid tumors have increased DNA levels, which allow the discrimination from patients with nonmalignant disease and healthy individuals (e.g., lung [7], colon [8], cervical [9], ovarian [10], breast [11], testis [12], bladder [13], and prostate [14] cancer). Cell-free DNA levels may therefore serve as a noninvasive universal cancer biomarker.

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The reason for increased cell-free DNA levels in cancer patients' blood remains largely unknown. It was discovered in the 1980s that the tumor itself contributes to the DNA [15], but most of the DNA is derived from healthy cells [16,17]. The analysis of DNA fragmentation patterns revealed that cancer patients' DNA shows an apoptotic as well as a necrotic pattern [16,18], and the pattern seems to be different in various cancer entities: DNA integrity was increased in patients with colon [8], testicular [12], head and neck [19], and breast [20] cancer patients, indicating predominantly necrotic breakdown. On the other hand, in patients with PCA [21] and bladder cancer [13], mainly short, presumably apoptotic DNA fragments were circulating. Interestingly, the amount of tumor-specific DNA sequences are enriched in the fraction of short DNA fragments [22]. It was shown that the clearance of cell-free DNA from the bloodstream occurs rapidly: the half-life time of fetal DNA in the blood of mothers after delivery was approximately 16 minutes [23]. Cell-free DNA seems to be eliminated by renal [24] and hepatic [25] mechanisms, however, cell-free DNA is also sensitive to plasma nucleases (e.g., DNase 1). It is unknown whether a different clearance time is also contributing to the higher levels of cell-free DNA in cancer patients.

2. Cell-free DNA detection methods

The cell-free DNA is extracted, using standard commercially available DNA kits, from serum or plasma. Interestingly, DNA levels are approximately 6 times higher in serum than plasma. It is unknown what is causative for higher serum DNA levels; a loss of DNA in plasma during purification procedures or a relevant contamination with leukocyte DNA was excluded [26]. However, delayed processing (i.e., >6 hours) of blood samples caused a significant increase in serum DNA concentrations [27] and DNA integrity [28], thereby indicating the contamination by leukocyte DNA during storage. Furthermore, diverse DNA isolation kits are different in the DNA isolation efficacy [29]. Thus, the analysis of cell-free DNA requires a standardized processing procedure.

Different methods have been used to determine the concentration of cell-free DNA. A simple spectrophotometry was reported [18,14], however the concentration of cell-free DNA is within the range of nanograms, and thus a polymerase chain reaction (PCR)-based quantification method [30] seems to be more accurate and sensitive. Quantitative real-time PCR also allows characterizing the DNA fragmentation pattern [10]. Apoptosis-induced cleavage of DNA results in DNA fragments of approximately 180 bp. Thus, quantification of a small and a long PCR product allows indirect drawbacks about the underlying cell-death entity.

At least a fraction of cell-free DNA is derived from tumor cells, and thus the detection of tumor-specific DNA alterations is feasible, but more difficult, because of a high background of normal DNA. The detection of epigenetic alterations requires either chemical (bisulphite treatment alters cytosine to uracil in case of unmethylated DNA and methylated and unmethylated DNA are discriminated using specific primers in the subsequent performed PCR) or enzymatic treatment (cleavage of unmethylated DNA using methylation-sensitive restriction enzymes, methylated DNA remains intact and is detected with a PCR). For the detection of allelic imbalances, the DNA of patients' leukocytes and the cell-free DNA are amplified with fluorogenic PCR primers, and the PCR products are analyzed using a capillary-electrophoresis. Leukocyte and cell-free DNA peaks in the capillary-electrophoresis are compared in order to detect allelic imbalances.

3. Cell-free DNA levels

Ten studies with more than 650 PCA patients and 350 control subjects have examined the levels of cell-free DNA in patients with PCA [14,21,30–37]. Although the comparison of the different studies is difficult because of the use of diverse DNA isolation and detection methods, most studies demonstrate a diagnostic role of cell-free DNA (see Table 1 for details). The first study, published in 2004, failed to demonstrate significant differences between patients with

Table 1
Diagnostic information of cell-free DNA levels

Study	*n* (PCA)	*n* (control)	Sensitivity	Specificity	AUC	Source	Detection method
Jung 2004 [14]	91*	93	n.s.	n.s.	n.s.	Plasma	Fluorometric assay
Allen 2004 [31]	27†	10	85%	73%	n.r.	Plasma	PCR
Papadopoulou 2004 [32]	12	18	58%	94%	0.840	Plasma	Dipstick
Papadopoulou 2004 [32]	12	13	58%	92%	0.708	Plasma	PCR
Boddy 2005 [33]	78	99	n.s.	n.s.	n.s.	Plasma	PCR
Chun 2006 [34]	142	19	n.r.	n.r.	n.r.	Plasma	Spectrophotometry
Ellinger 2008 [21]	168	42	88%	64%	0.824	Serum	PCR
Altimari 2008 [35]	64	45	80%	82%	0.881	Plasma	PCR
Cherepanova 2008 [36]	5	59	n.r.	n.r.	n.r.	Plasma	Fluorometric assay
Schwarzenbach 2009 [37]	69	10	n.r.	n.r.	n.r.	Plasma	Spectrophotometry

AUC = area under curve; n.s. = not significant; n.r. = not reported.
* Mixed cohort of patients with metastatic and localized disease.
† Including 12 patients with high grade PIN.