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Genomic Alterations in Liquid Biopsies from Patients with Bladder Cancer

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Abstract

Background: At least half of the patients diagnosed with non–muscle-invasive bladder cancer (NMIBC) experience recurrence and approximately 15% will develop progression to muscle invasive or metastatic disease. Biomarkers for disease surveillance are urgently needed.
Objective: Development of assays for surveillance using genomic variants in cell-free tumour DNA from plasma and urine.
Design, setting, and participants: Retrospective pilot study of 377 samples from 12 patients with recurrent or progressive/metastatic disease. Three next-generation sequencing methods were applied and somatic variants in DNA from tumour, plasma, and urine were subsequently monitored by personalised assays using droplet digital polymerase chain reaction (ddPCR). Samples were collected from 1994 to 2015, with up to 20 yr of follow-up.
Outcome measurements and statistical analysis: Progression to muscle-invasive or metastatic bladder cancer; *t* test for ddPCR data.
Results and limitations: We developed from one to six personalised assays per patient. Patients with progressive disease showed significantly higher levels of tumour DNA in plasma and urine before disease progression, compared with patients with recurrent disease ($p = 0.032$ and 1.3×10^{-6}, respectively). Interestingly, tumour DNA was detected in plasma and urine in patients with noninvasive disease, being no longer detectable in disease-free patients. A significant level of heterogeneity was observed for each patient; this could be due to tumour heterogeneity or assay performance.
Conclusions: Cell-free tumour DNA can be detected in plasma and urine, even in patients with noninvasive disease, with high levels of tumour DNA detectable before progression, especially in urine samples. Personalised assays of genomic variants may be useful for disease monitoring.
Patient summary: Tumour DNA can be detected in blood and urine in early and advanced stages of bladder cancer. Measurement of these highly tumour-specific biomarkers may represent a novel diagnostic tool to indicate the presence of residual disease or to discover aggressive forms of bladder cancer early in the disease course.

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1. Introduction

Bladder cancer (BCa) is the fifth most common cancer in the Western world [1]. About 75% of patients with BCa are diagnosed with non–muscle-invasive BCa (NMIBC) and at least half of the patients have a high recurrence and moderate progression rate (approximately 15%) to muscle-invasive BCa within 5 yr [1,2]. Major clinical challenges are to identify patients at risk for recurrence, progression, and metastasis, and to monitor treatment response. Promising biomarkers have been identified in genomic [3], epigenetic [4], transcriptomic [5,6], and proteomic studies [7]. Furthermore, several studies have focused on delineating diagnostic biomarkers for BCa surveillance [8]. None of the biomarkers have yet reached the clinic because of limited validation and relatively low sensitivity and specificity.

The mutational architecture of bladder tumours has been described by several groups [9–16]. In a recent study, we identified genomic variants in low-stage as well as advanced, localised tumours and found that metachronous tumours shared 25–56% of high-impact mutations and structural rearrangements, thus indicating that genomic changes may be used for BCa surveillance [15]. DNA is continuously released into the blood circulation. In cancer patients, the majority of plasma DNA originates from healthy cells, whereas a minor fraction is tumour DNA [17]. The circulating tumour DNA (ctDNA) contains tumour-specific genomic variants that may be used as unique genetic signatures or biomarkers [18]. Novel techniques identified these genomic variants in DNA from plasma [19]. Genomic variants in cell-free tumour DNA were detected in 75% of patients with advanced cancers in one study [20]. Furthermore, monitoring of large chromosomal rearrangements in plasma from colorectal or breast cancer patients provided evidence that a molecular relapse by ctDNA can be detected earlier than a clinical relapse based on image diagnostics [21].

Here, we established methods for noninvasive disease surveillance in patients with NMIBC, using highly sensitive and tumour-specific personalised plasma- and urine-based assays. Using next-generation sequencing, we identified somatic genomic variants in tumour DNA from six patients with recurrent disease and six with progressive and metastatic disease. Although this is a retrospective study with a relatively low number of patients, we demonstrate that surveillance of BCa patients using plasma- or urine-based personalised assays may be a promising approach for detection of progression and metastatic disease. Importantly, we also showed that ctDNA levels were low, but readily detectable, in plasma from patients without disease progression, whereas they disappeared in disease-free patients.

2. Patients and methods

2.1. Patients and clinical samples

In total, 377 samples comprising blood, tumours, plasma, and urine from six progressive patients (PRO group) and six recurrent patients (REC group) diagnosed with NMIBC were collected between 1994 and 2015 at Aarhus University Hospital, Aarhus, Denmark (Table 1). Patients were followed according to national guidelines and detailed follow-up data were available for all patients. Patients were selected from our biobank based on various criteria (Supplement 1). All patients provided written informed consent, and the study was approved by The National Committee on Health Research Ethics.

2.2. Biospecimen collection and DNA extraction

Fresh tissue samples were stored at −80 °C. Median carcinoma cell percentage was >80% (Table 1). EDTA (Ethylenediaminetetraacetic acid) blood was collected at each visit and centrifuged at 3000 *g* for 10 min. Plasma was centrifuged at 3000 *g* for 5 min. DNA was extracted from an average of 2.2 ml plasma or 3.4 ml urine (Table 2), using the QIAsymphony Circulating NA kit (Qiagen, Hilden, Germany) (Supplement 1).

2.3. Sequencing and variant calling

Three methods were applied to identify genomic variants in tumour and matching germline DNA: whole genome sequencing, whole exome sequencing, and mate-pair sequencing (Supplement 1; Supplementary Fig. 1; Supplementary Table 1a–1c).

2.4. Polymerase chain reaction validation of genomic variants

Genomic variants were evaluated by polymerase chain reaction (PCR) analysing tumour and matched germline DNA (Supplementary Table 1a–1c), using amplicons of 68–300 base pairs (Supplement 1).

2.5. Development of personalised assays

We prioritised genomic variants in common between at least two metachronous tumours (methods 1 and 2), supported by most read pairs or affecting loci harbouring Catalogue of Somatic Mutations in Cancer (COSMIC) cancer genes [22] (method 3). We validated 4–48 genomic variants by PCR, using tumour and matched germline DNA, and the precise breakpoint at base-pair resolution was determined by Sanger sequencing (Supplementary Fig. 1). Breaks were considered tumour specific if absent in matched germline DNA (Supplementary Table 2).

3. Results

We identified tumour-specific genomic variants to develop specific personalised assays for disease surveillance, using liquid biopsy specimens. A schematic overview is shown in Figure 1.

3.1. Defining genetic signatures for individual tumours

We sequenced 22 tumours and matched germline DNA from 12 patients initially diagnosed with NMIBC (Table 1). The PRO group ($n = 6$) included patients with later progression to muscle-invasive or metastatic disease followed for 4–20 yr. The REC group ($n = 6$) included patients with recurrence of NMIBC followed for 7–20 yr, all of whom were alive at the time of analysis (Table 1). We identified varying numbers of tumour-specific

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