Research Article

Bladder Cancer New Biomarkers in Liquid Biopsies

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Abstract

Bladder cancer is one of the most common neoplasia in men in the developed countries. Diagnosis and surveillance are made by bladder examination through cystoscopy making this one of the most expensive on cost/patient. After tumor removal, clinical staging is important for prognosis and treatment decision as non-muscle invasive (Ta and T1) and invasive (T2+) are treated in a completely different way. Today no noninvasive method has enough sensitivity to substitute cystoscopy or histological examination for tumor clinical staging. Our aim is to identify and quantify in urine, proteins that can detect and classify bladder tumors. A biomarker study was conducted using urine samples from: individuals with bladder cancer; individuals with other genitourinary disorders and individuals without urological diseases. Several proteins were found to successfully help in the discrimination of the bladder cancer stages Ta, T1 and T2+. Two biomarker-panels were developed, one capable of detecting bladder cancer presence and other able to distinguish Ta, T1 and T2+. Our results show a significant difference between urinary proteome in patients with different bladder cancer stages. This may allow through liquid biopsies predict patient's cancer stage. A validation study is on progress to attest this biomarker panel's accuracy.

Keywords: Bladder cancer; Biomarkers; Liquid biopsies; Diagnosis

Introduction

Bladder Cancer (BC) is the seventh most commonly diagnosed cancer in the male population worldwide, while it drops to eleventh when both genders are considered [1]. The worldwide age-standardized incidence rate (per 100,000 person/years) is 9.0 for men and 2.2 for women. In the European Union the age-standardized incidence rate is 19.1 for men and 4.0 for women. Worldwide, the BC age-standardized mortality rate (per 100,000 person/years) was 3.2 for men *vs.* 0.9 for women in 2012 [1]. Bladder cancer incidence and mortality rates vary across countries due to differences in risk factors, detection and diagnostic practices, and availability of treatments. The variations are, however, partly caused by the different methodologies used and the quality of data collection [2]. The incidence and mortality of BC has decreased in some registries, possibly reflecting the decreased impact of causative agents [3].

Approximately 75% of patients with BC present with a disease confined to the mucosa (stage Ta, CIS) or submucosa (stage T1); in younger patients (<40) this percentage is even higher [4]. Patients with TaT1 and CIS have a high prevalence due to long-term survival in many cases and lower risk of cancer-specific mortality compared to T2 to T4 tumours [1,2].

The diagnosis of bladder tumors is made through cystoscopy. In patients with bladder injury, previously seen through outpatient cystoscopy, they should be referred for a new cystoscopy, under general anesthesia, with complete transurethral resection (TURB) of the tumor. In addition to resecting the tumor, TURB aims to obtain a sample of the muscle wall, so that the tumor is properly staged. When CIS is found, random biopsies of the remaining bladder walls should be obtained. In the case of invasive injury, add investigation by Computed Tomography (CT) of the abdomen and pelvis (or nuclear Magnetic Resonance Imaging (MRI) if renal dysfunction) and laboratory tests to assess liver and kidney functions.

Additional tests should always include urinary cytology, which can be performed with cystoscopy, in addition to tests to evaluate the upper urinary tract, such as urography (by MRI or CT) or Ultrasound (US) of kidneys and urinary tract or, in limiting the use of contrast, CT without contrast with retrograde ureteropielography. Bone scintigraphy is indicated in cases of elevated alkaline phosphatase or symptoms. Consider Positron Emission Computed Tomography (PET-CT) in selected cases of patients with musculoskeletal disease.

The examination of voided urine or bladder-washing specimens for exfoliated cancer cells has high sensitivity in G3 and high-grade tumors (84%), but low sensitivity in G1/LG tumors (16%). The sensitivity in CIS detection is 28-100% (LE: 1b). Cytology is useful, particularly as an adjunct to cystoscopy, in patients with HG/G3 tumors. Positive voided urinary cytology can indicate an urothelial carcinoma anywhere in the urinary tract; negative cytology, however, does not exclude its presence [5]. Driven by the low sensitivity of urine cytology, numerous urinary tests have been developed [6]. None of these markers have been accepted for diagnosis or follow-up in routine practice or clinical guidelines. Promising novel urinary biomarkers, assessing multiple targets, have been tested in prospective multicenter studies, with a very high negative predictive value [7,8].

Objectives

Diagnosis and surveillance are made by bladder examination through cystoscopy making this one of the most expensive on cost per patient. After tumor removal, clinical staging is fundamental for prognosis and for treatment decision as non-muscle invasive (Ta

and T1) and invasive (T2+) are treated with a completely different procedure. Nowadays no noninvasive method has enough sensitivity to substitute cystoscopy or histological examination for tumor clinical staging. Our aim is to identify and quantify in urine, proteins that can detect and classify bladder tumors.

Materials and Methods

A biomarker study was conducted using urine samples from individuals with bladder cancer (stages Ta - 6 patients, T1 - 6 patients and T2+ - 6 patients) [1]; individuals with other genitourinary disorders (BPH; lithiasis) - 6 patients [2]; individuals without urological diseases - 6 patients [3]. Using Filter Aided Sample Preparation, urinary proteins were purified and digested. Liquid Chromatography coupled to Mass Spectrometry was used for the identification and quantification of digested urinary peptides. The authors confirm that written patient consent and permission to publish have been obtained.

Results

Several proteins were found to successfully help in the discrimination of the bladder cancer stages Ta, T1 and T2+. Two biomarker-panels were developed, one capable of detecting bladder cancer presence and other able to distinguish Ta, T1 and T2+. In Figures 1-3 are showed the difference between healthy patients, patients with genitourinary disorders and bladder cancer patients. In Figures 4 and 5 are showed the difference between Ta, T1 and T2+ stages. In Figure 6 proteins presented are the ones that were significantly up regulated in T2+ in comparison to Ta and to T1.
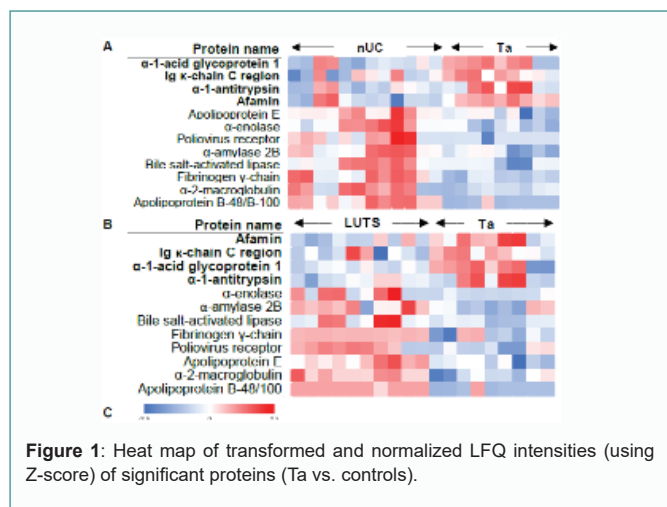

Figure 1: Heat map of transformed and normalized LFQ intensities (using Z-score) of significant proteins (Ta vs. controls).
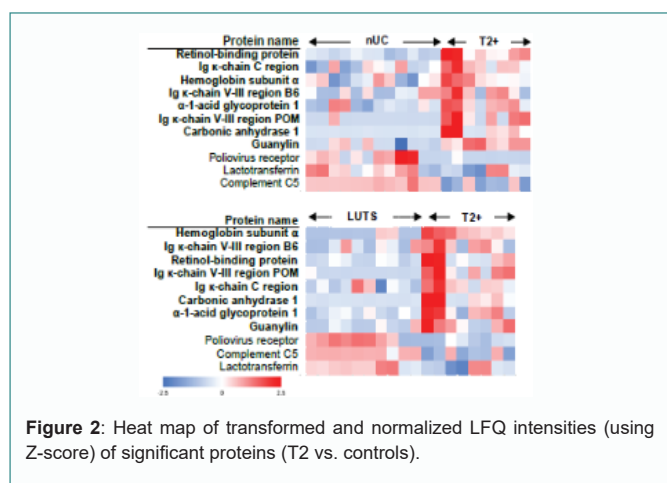

Figure 2: Heat map of transformed and normalized LFQ intensities (using Z-score) of significant proteins (T2 vs. controls).
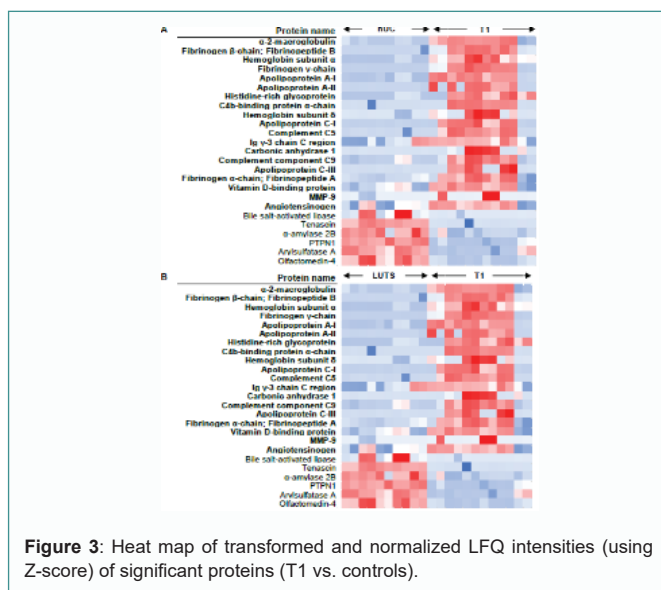

Figure 3: Heat map of transformed and normalized LFQ intensities (using Z-score) of significant proteins (T1 vs. controls).
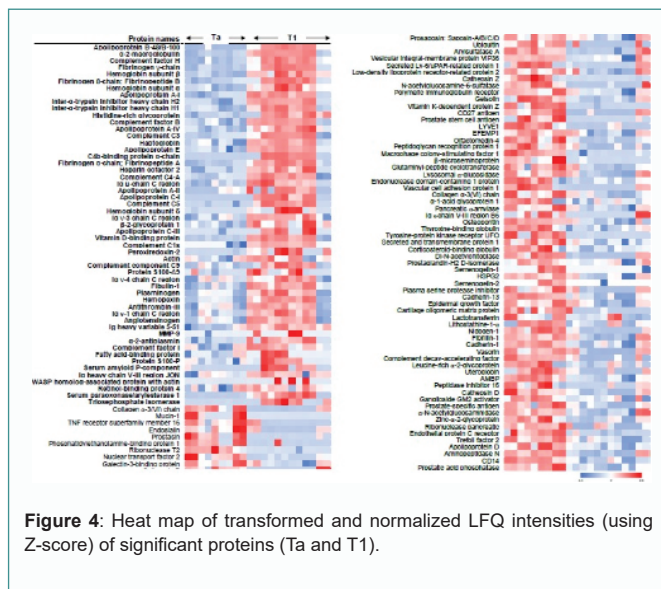

Figure 4: Heat map of transformed and normalized LFQ intensities (using Z-score) of significant proteins (Ta and T1).
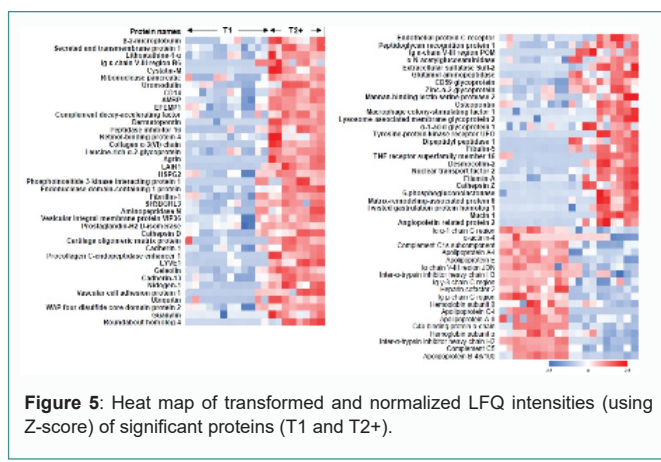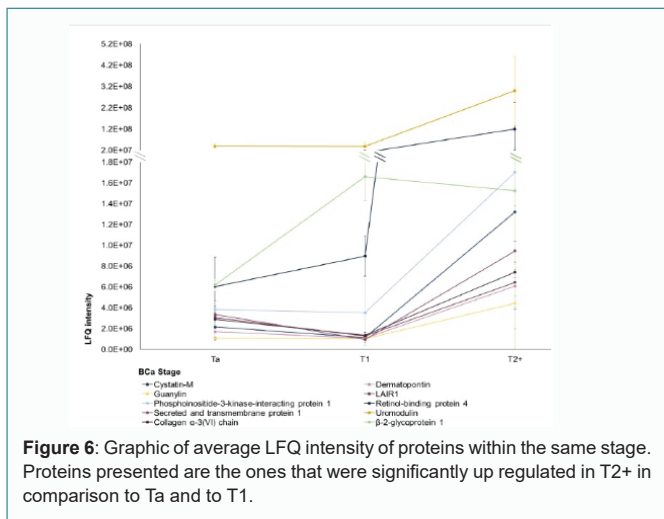

Figure 5: Heat map of transformed and normalized LFQ intensities (using Z-score) of significant proteins (T1 and T2+).

Conclusion

Our results show a significant difference between urinary proteome in patients with different bladder cancer stages. This may allow through liquid biopsies predict patient's cancer stage.

Figure 6: Graphic of average LFQ intensity of proteins within the same stage. Proteins presented are the ones that were significantly up regulated in T2+ in comparison to Ta and to T1.

A validation study is on progress to attest this biomarker panel's accuracy.

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