Seminar article

Metabolomics and bladder cancer

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Abstract

Diagnosis of bladder cancer is primarily made based on clinical presentation and then by direct visualization with cystoscopy. Despite the massive investments recently made to identify urinary-based assays that are able to diagnosis urothelial carcinoma, urine cytology and cystoscopy still remain the gold standard. Recently proof of principle studies have shown that noninvasive urine-based metabolomics, using high pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR), may be able to accurately diagnosis bladder cancer. This review discusses the published studies investigating metabolomics and bladder cancer and the future potential of this developing field. © 2011 Elsevier Inc. All rights reserved.

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Introduction

Bladder cancer is the second most common genitourinary malignancy in the United States with approximately 70,530 new cases diagnosed in 2010 [1]. Diagnosis usually occurs after the presentation of symptoms, predominantly, hematuria. Urine cytology, cystoscopy, and radiologic imaging are currently the primary investigations initially undertaken. They are, however, limited by their poor sensitivity, invasiveness, and cost. Approximately 25% of newly diagnosed patients with bladder cancer present with muscle invasive disease, which often involves expensive and highly morbid treatments with only modest success [2,3]. Patients with superficial disease also have a high risk of progression to muscle invasive disease [4]. Early detection and intervention have been shown to be highly beneficial and can help prevent progression to muscle invasion. Therefore, an accurate diagnostic test for bladder cancer with a high sensitivity, high specificity, and low cost, which is able to identify patients early in the disease process, is desperately needed. In addition, such a test would ideally be able to prognosticate disease status. Furthermore, the recurrent nature of bladder cancer amplifies the importance of finding such a diagnostic tool.

Over the past decade, systems biology has come to the forefront of biomedical research. Recent advances in technology coupled with advances in data analysis have allowed for the study and characterization of genes, transcripts, proteins, and metabolites on a global level [5]. Metabolomics is one of the “omic” sciences that has recently received increased attention as a screening tool in oncology. The premise of metabolomics is founded on detecting changes in cellular metabolic profiles in a diseased state, such as in malignant cells. Tumor cells generally propagate at an increased rate and up-regulate a wide range of metabolic pathways to accommodate their increased division rate. Measuring the metabolic products of these up-regulated and altered pathways potentially allows for the identification and differentiation between tumor and benign tissue. The use of metabolic screening in the detection of bladder cancer is particularly intriguing because of the possibility of using urine samples for detecting malignancy and determining prognosis. Indeed, the direct contact between urothelium and urine would potentially allow urine to be used as a sensitive diagnostic fluid.

Methods of metabolite analysis

Currently, biofluids are analyzed using predominantly 2 analytic platforms: nuclear magnetic resonance (NMR) and mass spectrometry (MS). Each has its own inherent strengths and weaknesses. Separation of the components, specifically with MS, can be performed using either gas chromatography (GC) or HPLC. NMR spectroscopy can

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often stand alone; however, recent developments have coupled the separation advantages of HPLC/GC with NMR detection downstream.

NMR

NMR spectroscopy uses a strong magnetic field to induce molecular resonance with calibration dependent on the addition of a stable isotope. The resonance of a particular substance within the magnetic field is then detected, allowing a NMR spectrum to be determined and specific metabolites to be measured. Perhaps the greatest advantage is the simplicity of sample preparation. In particular, urine samples need only be spun down before analysis. In addition, NMR is able to provide structure analysis and quantitative and qualitative analysis of known compounds. The primary disadvantage of NMR-based urine analysis is the decreased sensitivity compared with MS analysis [6]. Therefore, there is the potential for lower concentration metabolites to be undetected. NMR technology is actively changing, and increasing NMR magnetic field strength may allow this technology to become more sensitive in the future.

HPLC GC-MS

MS analysis is coupled to an upstream separation method, which is usually GC or HPLC. Identification of molecular mass is achieved by ionization of the molecules. Ionization is primarily done by electronic or chemical methods. The ions are then separated by mass using either magnetic or electric fields. GC separates compounds by exploiting the molecular interactions between the carrier gas and column. Therefore, components need to be volatile and able to withstand elevated temperatures. Chemical modification of samples is often needed and may add to the complexity of sample preparation. Advantages of GC over HPLC are the reproducible retention time and available compound libraries. The HPLC-MS platform is a highly sensitive method that allows for the analysis of a large variety of biological molecules. Sample preparation is relatively simple, but the reproducibility is not as clean as GC. Ultra performance liquid chromatography (UPLC) is a more recent advancement of LC. While similar to HPLC, UPLC allows for greater resolution and separation of compounds by decreasing the column size and increasing chromatography pressures. The analytical approaches to metabolomics have been recently reviewed by Wang et al. [5].

Metabolomics and bladder cancer

A metabolomics focused approach to assist in the diagnosis of bladder cancer is promising. Numerous studies have used the metabolic differences between malignant and benign tissues to diagnose various other cancers [7–10]. Preliminary studies using metabolic profiling and pattern recognition have been used to successfully diagnose patients with breast and prostate cancer [8,10–12]. NMR studies have analyzed breast tissue profiles and consistently found elevated tCho, lower glycerophosphocholine, and glucose levels compared with benign tissue [13–15]. Bathe et al. demonstrated that metabolic profiling from surgical specimens of breast cancer tissue could predict a malignant phenotype with sensitivity and specificity between 83% and 100% [16]. Prostate cancer, likewise, seems to correspond with elevated tCho, phosphocholine, lactate, and alanine [17]. Moreover, citrate levels detected with 1H-NMR from prostatic fluid have been shown to outperform PSA in prostate cancer detection, independent of age [18,19].

Urinary markers of bladder cancer have been extensively investigated. Despite recent investment into this field of research, none of these novel markers is superior to cystoscopy [20,21]. Several studies have compared the metabolic profiles of urine in patients with bladder cancer to controls. Issaq et al. compared urine metabolic profiles of 48 healthy individuals and 41 patients with urothelial carcinoma [22]. Samples were frozen, stored, thawed, centrifuged, and directly analyzed using HPLC coupled to a hybrid triple-quadrupole time of flight mass spectrometer. The profiles were then compared using two statistical methodologies: principal component analysis (PCA) and orthogonal partial least square-discriminate analysis (OPLS-DA). When the data were analyzed with the OPLS-DA methodology, all patients could be discriminated into cancer and control groups with sensitivity and specificity of 100%. The PCA analytical technique, while less accurate, correctly predicted 40 of 41 and 46 of 48 of the patients and controls, respectively (sensitivity, 98% and specificity, 96%) [22]. Another similar study examined urine samples of 24 patients with superficial or T1 bladder cancer to 51 control patients using gas chromatography and time of flight mass spectrometry (GC-TOFMS). Sample preparation, given the separation properties of GC, was significantly more complex than the HPLC study mentioned above. Using OPLS-DA, this study was also able to discriminate the two groups with sensitivity and specificity of 100% [23].

NMR spectroscopy has been effectively used to aid in the diagnosis of breast, prostate, and parasitic infections with promising results [24–26]. Srivastava et al. compared 400 MHz. H1 NMR spectra analysis of urine samples taken from patients with noninvasive bladder cancer to controls [27]. The patients were limited to predominantly pTa and pT1 stages. Interestingly, they identified increased taurine levels and decreased hippurate and citrate levels in the urine of patients with bladder cancer. While only 33 patients were analyzed, this study shows that metabolomics using NMR in minimally prepared urine samples is possible. It remains to be determined if this technique can be modified to detect muscle invasive bladder tumors using serum and/or urine.
Table 1
Summary of studies using metabolomic profiling in the detection of bladder cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Metabolomic profiling method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issaq et al.</td>
<td>HPLC-MS</td>
<td>Correct identification of 48 of 48 controls and 41 of 41 bladder cancer patients using OPLS-DA.</td>
</tr>
<tr>
<td>Pasikanti et al.</td>
<td>GC-MS</td>
<td>Using multivariate principle component analysis and OPLS-DA, bladder cancer patients were differentiated from controls with 100% sensitivity.</td>
</tr>
<tr>
<td>Srivastava et al.</td>
<td>1H NMR spectroscopy</td>
<td>Significant variations in urine concentrations of hippurate, citrate, and taurine in bladder cancer patients compared with controls.</td>
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</tbody>
</table>

External validation of these techniques and larger studies are needed to ascertain if the limitations of these analytical techniques can be overcome. The initial cost of HPLC/GC and NMR spectroscopy is large, and dedicated personnel are required. Nevertheless, these techniques are used in analytical labs across the country. Sample preparation for GC and some HPLC techniques can be laborious and time-consuming and, despite automated sequence, individual sample runs can last more than 60 min. Finally, biological variations from diet, common genetic polymorphisms, and medications have not been evaluated. Sample storage and stability also require further investigation.

Newer technologies such as UPLC and 900 MHz. NMR spectroscopy are currently available but have not been used to analyze the metabolome of bladder cancer patients. Further, combining the separation properties of GC/HPLC-MS with tandem NMR may allow more accurate differentiation of metabolomes. Alternatively, we may have to rely on more traditional “pet scans” given that Willis et al. have shown that dogs are capable of identifying urothelial cancers from urine samples using olfactory senses [28,29]. These preliminary studies, summarized in Table 1, collectively support the notion that metabolomics may become an important diagnostic tool.

Summary

The use of metabolomics as a diagnostic tool in bladder cancer is in its infancy but remains extremely promising. Technological advances to date have allowed proof of principle studies with NMR and MS. Improvements in technology are likely to further advance this field. Using metabolomics as a screening tool for bladder cancer certainly warrants further instigation. Monitoring treatment and recurrence has not been investigated and should be pursued.

References


