Metabonomic diagnosis of bladder cancer: what are the advantages and considerations?

“Metabonomics demonstrates great potential in characterizing bladder cancer, identification of marker metabolites and shedding insights into the pathogenesis and potential therapeutic targets of the disease.”

Keywords: advantages • bladder cancer • considerations • diagnosis • metabonomics

Bladder cancer (BC) is the fifth most common cancer and is one of the leading causes of death worldwide [1]. The 5-year survival rate for BC is approximately 94% if detected early; thus timely diagnosis and intervention of BC increase patient's survival rate dramatically [2]. However, BC has a high recurrence rate and patients are subjected to life-long surveillance. As a result, the lifetime economic burden per BC patient is higher than other cancer types. Currently, the standard diagnoses of BC are cystoscopy and urinary cytology [3]. While cystoscopy is clinically reliable, it is expensive, invasive and associated with a definite risk of morbidity. On the other hand, noninvasive urinary cytology demonstrates poor sensitivity in detecting low-grade BC (4–31%) [4]. In recent years, several proteinaceous urine-based bladder tumor markers (UBBTMs) have been evaluated for the diagnosis of BC, such as bladder tumor antigen (BTA), NMP22, FDP and ImmunoCyt [5]. However, the specificity and sensitivity of current molecular biomarkers are not adequate to replace cystoscopy [6]. Therefore, there is an impetus to develop sensitive and specific noninvasive biomarkers for the diagnosis and surveillance of BC.

Dysregulated molecular pathways, associated with tumor genes, secrete specific modulated levels of metabolites into biological fluids that may be detectable prior to clinical symptoms, rendering them potential early biomarkers for cancer diagnosis. Metabonomics measures the dynamic multiparametric responses of systems biological metabolome to genetic modifications or

pathophysiological stimuli such as cancers [7]. It allows scientists to survey global dysregulated metabolic pathways and gain holistic insights into cancer etiology and progression. Significant progress has been made using modern metabonomics techniques to characterize BC patients, identify marker metabolites and generate new knowledge in disease biology and potential therapeutic targets [5].

Advantages

The application of metabonomics in the diagnosis of BC is particularly intriguing due to the possibility of using urine for detecting malignancy and determining prognosis. As urine is bathed in close anatomical proximity to the bladder carcinoma, it is expectedly a suitable diagnostic fluid. Advantages of urinary metabonomics profiling of BC include noninvasive sample collection, relative ease of urine analysis, low-cost, circumvention of subjective pathological assessment and urinary metabolic phenotype (metabotype) that is sensitive to environmental, gut microbiota and host genetic factors.

Most current BC metabonomics studies showed that BC patients could be distinctly differentiated from healthy controls with high sensitivity (>90%) and specificity (>90%) [8–14]. A number of potential marker metabolites of BC are derived from metabolic pathways associated with glycolysis, glutathione, tryptophan, purine, pyrimidine, carnitine, citrate, taurine and hippuric acid [5]. Such dysregulated marker metabolites are useful in deciphering the BC biology and have the potential to be translated

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to clinical applications upon further evaluation and validation.

In addition to accurate diagnosis, classification of bladder tumors is important for prognosis of BC. However, reproducibility and consistency in defining superficial bladder tumors is a chronic challenge for pathologists. Notably, Cao *et al.* demonstrated that distinct metabolotypes were associated with low-grade versus high-grade BC as well as pre- versus post-transurethral resected cohorts [15]. Additionally, two BC metabolomics studies exhibited potential in the staging and grading of bladder tumors [8,13]. Based on the direct correlation of metabolic features with pathological status, this technique will provide a mechanistic handle for probing the progression of BC.

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Monitoring treatment and recurrence is critical for BC patients. Recently, Jin *et al.* demonstrated in a large cohort study (138 BC and 121 healthy controls) that BC metabolotype correlates positively with cancer-specific survival time, which in turn underscores the possibility of predicting postdiagnosis survival time of BC patients via metabolomics [14].

As both bladder and kidney are in direct contact with urine, it is possible that marker metabolites associated with BC and kidney cancer are excreted into urine. Intriguingly, it has been reported that metabolomics can discriminate BC and kidney cancer successfully, showing 100% sensitivity and specificity [13].

Considerations

Although metabolomics is a promising approach in the diagnosis and surveillance of BC, several intrinsic limitations still exist. Four key considerations are summarized below.

- The number of patients recruited in most BC metabolomics studies was relatively inadequate. Although external validation was performed in most of the current BC metabolomics studies, the validation cohorts were equally small. Therefore, further validation of potential BC biomarkers should be based on a statistically powerful clinical sample size. To achieve this objective, it becomes pertinent for clinician scientists to conduct multicenter collaborative clinical trials.
- For most current BC metabolomics studies, only healthy controls and BC patients were examined.

BC patients are typically presented with hematuria, a nonspecific symptom that can become a serious confounding variable. Other concomitant conditions such as kidney cancer and urinary tract infection or inflammation may also modulate the metabolotype. Therefore, to better validate the specificity and sensitivity of BC biomarkers, a more diverse patient population is essential and should be carefully enrolled in future clinical trial.

- There is relatively little overlap between the potential biomarkers identified in individual BC metabolomics studies [5]. Metabolotype is highly dynamic and sensitive to environmental factors (e.g., sample storage conditions) [16], host factors (e.g., age, disease, gender, genetic) and external factors (e.g., diet, smoking, chronic medications) [17]. Additionally, the metabolotypes derived from different analytical platforms are variable due to differential chemical space coverage. Therefore, standardization of the metabolomics experiment (e.g., clinical trial design and selection of metabolomics techniques) is critical to ensure good-quality data.
- Global metabolomics are semi-quantitative in measuring biomarkers. However, most studies did not quantitate the potential biomarkers using external sample set, which might be due to the fact that marker metabolite quantitation is difficult and time-consuming, especially if the standard metabolite is not commercially available and has to be chemically synthesized. Despite these challenges, validation of important marker metabolites using quantitative methods remains critical.

Conclusion & future perspective

Early diagnosis and life-long surveillance are clinically important to improve the long-term survival of BC patients. Currently, a noninvasive biomarker with high sensitivity and specificity in detecting bladder tumors is lacking. Metabolomics enables mapping of early biochemical changes in disease and development of predictive biomarkers. Metabolomics demonstrates great potential in characterizing BC, identification of marker metabolites and shedding insights into the pathogenesis and potential therapeutic targets of the disease. In the future, metabolomics may be potentially explored for the holistic clinical management of BC patients from diagnosis to intervention. For instance, rapid evaporation ionization mass spectrometry (REIMS) has been applied for intraoperative analysis of tumor specimens *in vivo* [18]. REIMS intelligent knife (REIMS-iKnife) was shown to be able to differentiate accurately between distinct histological and histopathological tissue types. Such a metabolomics-inspired technology is poten-

tially applicable for the surgical intervention of BC in the future. Notably, near-real-time identification of positive resection margins could help improve the functional outcomes of the bladder, by mitigating surgical trauma and reducing the removal of healthy tissues.

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