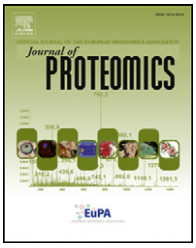
JOURNAL OF PROTEOMICS 85 (2013) 28–43

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot



Identification of potential bladder cancer markers in urine by abundant-protein depletion coupled with quantitative proteomics☆

Chien-Lun Chen[a,b], Tsung-Shih Lin[c], Cheng-Han Tsai[d], Chih-Ching Wu[e], Ting Chung[c], Kun-Yi Chien[d], Maureen Wu[c], Yu-Sun Chang[c,d], Jau-Song Yu[c,d,*], Yi-Ting Chen[c,f,**]

[a]Chang Gung Bioinformatics Center, Department of Urology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan
[b]College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[c]Molecular Medicine Research Center, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[d]Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[e]Graduate Institute of Medical Biotechnology and Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan 333, Taiwan
[f]Department of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan

ARTICLE INFO

Article history:
Received 3 December 2012
Accepted 17 April 2013
Available online 28 April 2013

Keywords:
Urine proteome
Bladder cancer
Biomarkers
Abundant protein depletion

ABSTRACT

In this study, we evaluated the reproducibility of abundant urine protein depletion by hexapeptide-based library beads and an antibody-based affinity column using the iTRAQ technique. The antibody-based affinity-depletion approach, which proved superior, was then applied in conjunction with iTRAQ to discover proteins that were differentially expressed between pooled urine samples from hernia and bladder cancer patients. Several proteins, including seven apolipoproteins, TIM, SAA4, and proEGF were further verified in 111 to 203 individual urine samples from patients with hernia, bladder cancer, or kidney cancer. Six apolipoproteins (APOA1, APOA2, APOB, APOC2, APOC3, and APOE) were able to differentiate bladder cancer from hernia. SAA4 was significantly increased in bladder cancer subgroups, whereas ProEGF was significantly decreased in bladder cancer subgroups. Additionally, the combination of SAA4 and ProEGF exhibited higher diagnostic capacity (AUC = 0.80 and $p < 0.001$) in discriminating bladder cancer from hernia than either marker alone. Using MetaCore software to interpret global changes of the urine proteome caused by bladder cancer, we found that the most notable alterations were in immune-response/alternative complement and blood-coagulation pathways. This study confirmed the clinical significance of the urine proteome in the development of non-invasive biomarkers for the detection of bladder cancer.

Abbreviations: APO, apolipoprotein; LgEs, low grade with early stage; HgEs, high grade with early stage; HgAs, high grade with advanced stage; iTRAQ, isobaric tags for relative and absolute quantitation; LC–MS/MS, liquid chromatography tandem mass spectrometry; proEGF, pro-epidermal growth factor; SAA4, serum amyloid A-4; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TPP, Trans-Proteomic Pipeline; TIM, triosephosphate isomerase.

* *Corresponding author*. Tel.: +886 3 2118800x5171; fax: +886 3 2118891.
** *Corresponding author*. Tel.: +886 3 2118800x3558; fax: +886 3 2118800x3533.
E-mail addresses: yusong@mail.cgu.edu.tw (J.-S. Yu), ytchen@mail.cgu.edu.tw (Y.-T. Chen).

1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.04.024

Biological significance
In this study, we evaluated the reproducibility of abundant urine protein depletion by hexapeptide-based library beads and an antibody-based affinity column using the iTRAQ technique. The antibody-based affinity-depletion approach, which proved superior, was then applied in conjunction with iTRAQ to discover proteins that were differentially expressed between pooled urine samples from hernia and bladder cancer patients. Several proteins, including seven apolipoproteins, TIM, SAA4, and proEGF were further verified in 111 to 203 individual urine samples from patients with hernia, bladder cancer, or kidney cancer. SAA4 was significantly increased in bladder cancer subgroups, whereas ProEGF was significantly decreased in bladder cancer subgroups. Additionally, the combination of SAA4 and ProEGF exhibited higher diagnostic capacity in discriminating bladder cancer from hernia than either marker alone. A marker panel composed by two novel biomarker candidates, SAA4 and proEGF, was first discovered and verified successfully using Western blotting. To the best of our knowledge, the associations of urinary SAA4 and proEGF with bladder tumor and kidney cancer have not been mentioned before. In the present study, we discovered and verified SAA4 and proEGF as potential bladder cancer biomarker for the first time.

1. Introduction

Bladder cancer is one of the most common urinary tract carcinomas. According to the most recent estimates of the American Cancer Society, there were 69,250 new cases of bladder cancer in the United States and 14,990 deaths from bladder cancer annually in 2011 [1]. Major risk factors for the development of bladder cancer include cigarette smoking and exposure to specific carcinogens [2].

Prominent among the major clinical problems associated with bladder cancer is the high recurrence rate of superficial tumors; 50–70% of newly diagnosed tumors recur within five years and progression to invasive disease will occur in 10–20% of patients. Therefore, patients with bladder tumors must undergo life-long surveillance [3]. This requirement for lifetime surveillance, taken together with the cost to treat recurrent bladder cancer tumors and complications associated with treatment, imposes a significant economic burden. Screening of high-risk populations may be beneficial in the detection of early-stage tumors before they become invasive or in surveillance to detect recurrence [4]. Thus, the most important clinical challenges are early detection of bladder tumors and discovery of non-invasive stage/grade discriminators or predictors of tumor progression. Integration of multiple markers has the potential to further improve bladder cancer diagnosis and progression monitoring.

Urine, derived from plasma by filtration through the renal glomerulus, has become one of the most widely used clinical samples for biomarker discovery. It is estimated that approximately 70% of urinary proteins are derived from the kidney and urinary tract [5–7]. Many abundant plasma proteins are also present at high concentrations in urine specimens [8–10], making it more difficult to detect potential biomarkers present at low concentrations. Antibody-based affinity-depletion approaches are able to simultaneously remove several of the most abundant proteins from various kinds of body fluids in a single step [11–13]. Simply removing 2 to 20 of the most abundant proteins using immune-depletion approaches has been shown to allow more protein spots to be visualized by two-dimensional electrophoresis or a larger number of proteins to be identified using liquid chromatography (LC)-based proteomic strategies [14,15].

An alternative strategy for enriching low-abundance proteins uses a combinatorial hexapeptide ligand library immobilized on a solid-phase matrix [16–18]. The amount of each specific protein captured by the library is limited by the number of the ligands with suitable affinity interaction. Saturation of affinity ligands limits the amounts of abundant proteins captured, preventing retention of an excess of highly abundant proteins. Therefore, the concentrations of low-abundant proteins are enriched relative to the original composition of the specimen, decreasing the dynamic range of the concentration difference in a biological sample. However, the interaction of a complicated peptide mixture with a random peptide library is an intricate process that can be affected by competition among multiple association/dissociation mechanisms, kinetics of binding affinity, and stoichiometry. When this approach is applied to the discovery of biomarkers in biological fluids, poor reproducibility of the depletion step may cause problems in candidate selection in subsequent proteomic studies. Although hexapeptide-based library beads and columns containing immobilized antibodies are widely used techniques for depletion of abundant proteins in body fluids [16,17], detailed characterizations and comparisons of the two approaches have been limited [17,19], particularly for specimens of urine, a biofluid with low protein levels but a high salt concentration.

In this study, we used the iTRAQ (isobaric tags for relative and absolute quantitation) technique to evaluate the reproducibility of abundant-protein depletion using hexapeptide-based library beads and an antibody-based affinity column—the Agilent human 14 multiple affinity removal system (MARS) column. The antibody-based affinity-depletion approach was then applied in conjunction with iTRAQ to discover proteins differentially expressed between pooled urine samples from age-matched hernia and three subgroups of bladder cancer patients. Several proteins present at markedly different levels were further verified in individual samples using Western blot analyses and a multiplexed Bio-plex assay. The verified proteins were found to be potential markers for early detection and diagnosis of bladder cancer.