Am J Cancer Res 2016;6(2):331-340
www.ajcr.us /ISSN:2156-6976/ajcr0019853

Original Article

The increased excretion of urinary orosomucoid 1 as a useful biomarker for bladder cancer

Fei Li[1*], Zhe Yu[1*], Pengliang Chen[1*], Guangzheng Lin[1], Tieqiu Li[2], Lina Hou[3*], Yuejun Du[1*], Wanlong Tan[1*]

Departments of [1]Urology, [3]Healthy Management, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China; [2]Department of Urology, Mawangdui Hospital of Hunan Province, Hunan Provincial Research Institute of Geriatrics, Changsha 410016, Hunan, P. R. China. [*]Equal contributors.

Received November 16, 2015; Accepted December 3, 2015; Epub January 15, 2016; Published February 1, 2016

Abstract: Improving the early detection rate and prediction of bladder cancer remains a great challenge in management of this disease. To examine the value of urinary orosomucoid 1 (ORM1) for the early detection and surveillance of bladder cancer, two-dimensional differential gel electrophoresis (2-DE) and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF/TOFMS) were applied to identify the differently expressed proteins in urine between bladder cancer and healthy controls. Thirteen different proteins including ORM1 were identified. After verification by western blotting, the ORM1 expressions were quantified in 186 urine samples by enzyme-linked immunosorbent assay (ELISA) correcting for creatinine expression. ELISA quantification showed the urinary ORM1-Cr was found to be higher in bladder cancer patients compared to controls and benign cases (7172.23±3049.67 versus 2243.16±969.01, 2493.48±830.37 ng/ml, respectively, P<0.0001). Furthermore, the pearson correlation analysis indicated that urinary ORM1 had high positive correlation with the pathology classification of bladder cancer. Receiver operating characteristic (ROC) analysis was used to calculate the cut-off value for early diagnosis of bladder cancer, and rendered an optimum cut-off value of 3912.97 ng/mg corresponding to 91.96% sensitivity and 94.34% specificity. Moreover, a cut-off value with 7351.28 ng/mg was utilized to distinguish infiltrating urothelial carcinoma from bladder cancer patients corresponding to 91.89% sensitivity and 90.67% specificity. In conclusion, our findings suggested the elevated urinary ORM1 could be a useful biomarker for bladder cancer. Further research is warranted to elucidate the pathogenic mechanisms of elevated ORM1.

Keywords: Orosomucoid 1, bladder cancer, urine, diagnosis, excretion

Introduction

Bladder cancer, one of the most common cancers, remains a major threat to public health all over the world [1]. Clinically, it is characterized by high recurrent rates and poor prognosis once tumors invade deeper layers. Great progress has been made in diagnostic intensity and treatment, but early diagnosis and effective surveillance of bladder cancer progression remain a substantial challenge. Cystoscopy and bladder biopsy are currently considered as the gold standard for diagnosis of bladder cancer [2-4]. Thereof, the development of a reliable, non-invasive biomarker is of considerable clinical importance, aiming at increasing the early detection rate of bladder cancer and predicting the progression of superficial tumors in time [5].

Human body fluids play a vital role in searching biomarkers for early detection of cancer. Amongst them, urine samples are the most easily obtained compared with serum or plasma specimens in clinical analysis [6]. Furthermore, urine is directly exposed to the bladder epithelium reflecting the risk of bladder cancer at some extent.

ORM1 (Orosomucoid-1, α1-acid glycoprotein), mainly functions as a transport protein in the bloodstream. ORM1 is normally produced by human liver cells, but it can also be produced in endothelial cells and some tumor cells [7]. Briefly, ORM1 plays important roles in modulating the activity of the immune system during the acute-phase reaction [8, 9]. However, the association of urinary ORM1 expression with the disease of bladder cancer is unknown.

In this study, urinary ORM1 levels were identified and significantly elevated in bladder cancer than healthy controls by proteomic methods. Furthermore, after verification of ORM1 expression by western blotting, the ORM1 expressions were quantified by enzyme-linked immunosorbent assay (ELISA) correcting for creatinine expression, aiming to further investigate the association of ORM1 with bladder cancer.

Material and methods

Urinary samples collection and preparation

All subjects in the present study were recruited from the Chinese Han population at Nanfang Hospital (Guangzhou, China) from May 2011 to May 2015. The study protocol was approved by the Nanfang Hospital Ethics Committee. Informed consent was obtained from all donors prior to the study. 112 patients with bladder cancer and 21 cases with benign bladder damage were selected. All patients had their upper tracts cleared via examinations. 53 healthy volunteers had no evidence of disease and were used as control. Pre-cystoscopy voided urine specimens were collected from patients presenting positive findings under suspicion of bladder cancer. The bladder cancer was confirmed by cystoscopy coupled with histopathological information after surgical operations. The diagnosis was conducted by two pathologists in Nanfang hospital of Southern Medical University according to the criteria of World Health Organization classification of tumours [10].

The second voided clean-catch urine samples from subjects were collected in the early morning. Each urine sample (20 ml) was collected into a sterile plastic tube and then immediately centrifuged at 1500×g for 5 min at 4°C to remove cell debris and particulate matter. The supernatant was stored at -80°C for further analysis. All the clinical data of those subjects were collected as well.

2-Dimensional electrophoresis (2-DE) analysis

Equal volume urine specimens from 15 bladder cancer patients and 15 controls were pooled respectively for 2-DE analysis. Of these bladder cancer patients, there were ten cases of non-invasive papillary urothelial carcinoma (five were low grade and five were high) and five cases of infiltrating urothelial carcinoma. The pooled samples were centrifuged using Centricon Plus-20, 10,000 MWCO devices (Millipore, Bedford, MA, USA), and measured by using the Bradford method. 100 μg of isolated urinary protein were separated by 24 cm Immobiline™ DryStrip 3-10 non-linear on an IPGphor apparatus (GE Healthcare) in the first dimension. After equilibration, IPG gel strips were transferred onto a homogeneous SDS-PAGE (12%) using an Ettan DALT twelve system (GE Healthcare) for the second dimension. After fixing for 5 hours, all gels were visualized by a silver staining technique. Protein spots were quantified with ImageMaster 2D Elite software (Amersham Biosciences). All experiments were repeated for three times. Differences in protein spot intensities were analyzed by using quantitative analysis sets after scanning. Protein spots whose intensities either increased or decreased by 2-fold or greater were marked for identification.

Mass spectrometric analysis of proteins

The differentially expressed protein spots were cut from gels, and then identified by matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF/TOF MS) according to previously described procedures [5]. Briefly, Protein spots from gels were digested with trypsin solution, and the peptide mixtures was analyzed by using an ABI Voyager DE-STR mass spectrometer (Applied Biosystems, Foster City, CA, USA). A trypsin-fragment peak was served as internal standard for mass calibration. A list of the corrected mass peaks was the peptide mass fingerprinting (PMF). Monoisotopic peptide masses were assigned and used for database searches with the MASCOT search engine (Matrix Science, London, United Kingdom).

Western blotting analysis

The samples involved four low grade and four high grade non-invasive papillary urothelial carcinoma, eight infiltrating urothelial carcinoma and twelve healthy controls. The extracted proteins from equal 20 ml urine were centrifuged using Centricon Plus-20, 10,000 MWCO devices (Millipore, Bedford, MA, USA) to be concentrated. The prepared urinary proteins were separated by 12% SDS-PAGE respectively and then transferred onto Polyvinylidene Xuoride (PVDF) (Immobilon P, Millipore, Bedford, Massachuse-

Table 1. Urine samples in the study

	Samples for 2-DE		Samples for Western blotting		Samples for ELISA		
	Healthy Control	Bladder Cancer	Healthy Control	Bladder Cancer	Healthy Control	Benign Damage	Bladder Cancer
Number	15	15	12	16	53	21	112
Gender (Male/Female)	7/8	7/8	6/6	8/8	28/25	11/10	69/43
Age (years)	67.64±8.34	69.23±14.11	63.12±7.54	67.89±17.21	65.33±10.57	61.27±9.77	64.85±18.85
Serum creatinine (umol/L)	103.34±29.89	119.35±33.78	95.62±33.85	113.47±36.45	99.54±36.93	101.65±29.84	117.74±49.81
Urine creatinine (mg/dl)	107.57±57.45	115.46±61.68	100.79±45.14	109.58±57.38	97.91±46.03	103.37±40.75	113.29±63.08

Data are expressed as mean ± SD.

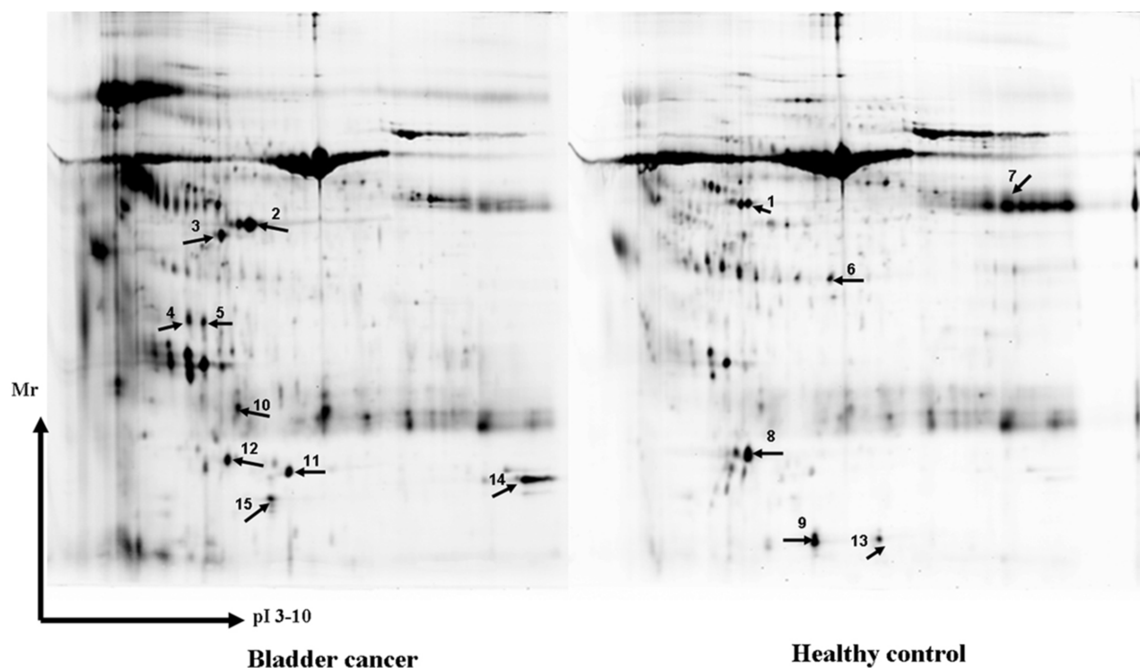

Figure 1. Distribution of all 15 differentially expressed protein spots from urine samples between patients with bladder cancer and healthy controls in two-dimensional difference gel electrophoresis gels.

tts) membranes. The membranes were blocked in TBS solution containing 5% nonfat milk powder and 0.1% Tween-20 for 1 h at 37°C and then incubated overnight at 4°C with the monoclonal antibody against the human ORM1 protein (diluted 1:1000; Abcam, UK). After three times washes in TBS-T for 10-mins each, the membranes were incubated with horseradish peroxidase horseradish (HRP) conjugate of goat anti-rabbit IgG (Bioworld Technology, Louis Park, MN, USA) at a 1:6000 dilution at 37°C for 1 h. The proteins were detected using an enhanced chemiluminescence (ECL, Pierce, Rockford, IL, USA) detection system. Relative intensities were documented and analyzed by densitometry. Triplicate gel images of identical samples were used for analysis. It was quantified by strip densitometry.

Enzyme-linked immunosorbent assay (ELISA) analysis

The expression level of urinary ORM1 protein was measured by a commercially available ELISA kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. The assay has a minimum detectable dose of ORM1 at 0.538 ng/ml, and the linearity was ranged from 3.12 to 200 ng/ml [9]. A standard curve was made with the suppliers' lyophilized human ORM1. The concentrations of ORM1 in urine samples were calculated by adjusting with urinary creatinine (Cr), and expressed as ORM1 -to-Cr ratio (ORM1/Cr nanograms per miligrams of creatinine). Urine creatinine (Cr) levels were measured at the Department of Clinical Laboratory of Nanfang Hospital (Guangzhou, China).

Table 2. Differentially urinary proteins expressed between bladder cancer and healthy control

Master number	Accession No.	Gene Name	Protein molecular weight	Protein PI	Pep. Count[a]	Total ion score	Fold changes[b]	Overall trend[c]
1	Q14624	ITIH4	103521.1	6.51	5	251	-4.96±2.32	down
2	P12830	CDH1	97852	4.58	4	225	7.15±1.29	up
3	P07911	UMOD	72451.4	5.05	9	308	8.85±2.47	up
4	P04746	AMY2A	58354.3	6.6	8	290	5.39±2.31	up
5	P04746	AMY2A	58354.3	6.6	8	307	4.81±2.32	up
6	P01042	KNG1	72995.6	6.34	7	269	-3.74 ±1.39	down
7	P02768	ALB	71317.2	5.92	9	259	-8.31±1.71	down
8	P30740	SERPINB1	42828.7	5.9	5	273	-5.96±2.78	down
9	P01842	IGLC1	11400.6	6.92	3	132	-4.77±1.51	down
10	P02647	APOA1	30758.9	5.56	5	238	4.56±1.79	up
11	P02763	ORM1	23724.8	4.93	3	282	6.81±2.56	up
12	P02763	ORM1	23724.8	4.93	3	298	7.78±3.12	up
13	P01842	IGLC1	11400.6	6.92	3	142	-8.04±3.89	down
14	P02753	RBP4	23337.4	5.76	2	267	5.61±2.81	up
15	P28325	CST5	16080.5	6.71	2	157	2.78±1.23	up

[a]: Calculated by amino acid count. [b]: Calculated based on the spot intensity analysis, represented as mean ± SD. [c]: up: up-regulated in the bladder cancer group. down: down-regulated in the bladder cancer group.

Statistical methods

All data were collected and used for statistical analyses with SPSS software 13.0 (SPSS, Chicago, IL, USA). Values were presented as mean ± standard deviation (SD). Student's t test and one-way ANOVA were used for comparisons between two and three groups respectively. A Spearman analysis was performed to explore the correlations between urinary ORM1-Cr and the clinical features of bladder cancer subjects where appropriate. Univariate (nonparametric rank sum test) and multivariate (logistic regression) analyses were conducted to evaluate the relationship between urinary ORM1 expression and clinical parameters of bladder cancer. Receiving operating curve (ROC) analysis was applied to define the most optimal diagnostic cutoff and the diagnostic performance given by the area under the curve (AUC), evaluating the sensitivity versus its false-positive rate at an optimal cutoff. The results were considered statistically significant at *P*-value <0.05.

Results

Clinical characteristics of subjects

Table 1 has shown the clinical characteristics of all subjects in our study. There were no statistically significant differences in clinical characteristics (e.g. age, sex, serum creatinine and urinary creatinine). Furthermore, there were 112 bladder cancer patients, 21 cases with benign bladder damage and 53 healthy volunteers recruited for ELISA quantitative analysis. Of these 112 subjects with bladder cancer, 75 cases were non-invasive papillary urothelial carcinoma (39 were low grade and 36 were high) and 37 were infiltrating urothelial carcinoma.

Identification of urinary ORM1 by proteomic methods

After 2-DE analysis, we systematically analyzed the differently expressed urinary proteins from patients with bladder cancer and controls. 15 protein spots changed by or over 2-fold were identified by MALDI-FOF/TOF-MS. A total of 12 different proteins were identified (**Figure 1**; **Table 2**). The seven proteins which were significantly up-regulated in bladder cancer included Cadherin-1 (CDH1), Uromodulin (UMOD), Pancreatic alpha-amylase (AMY2A), Apolipoprotein A-I (APOA1), Alpha-1-acid glycoprotein 1 (ORM1) (**Figure 2A**), Retinol-binding protein 4 (RBP4) and Cystatin D (CST5). The other five down-regulated proteins were Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), Kininogen-1 (KNG1), Serum albumin (ALB), Leukocyte elastase inhibitor (SERPINB1) and Ig lambda-1 chain C regions (IGLC1).

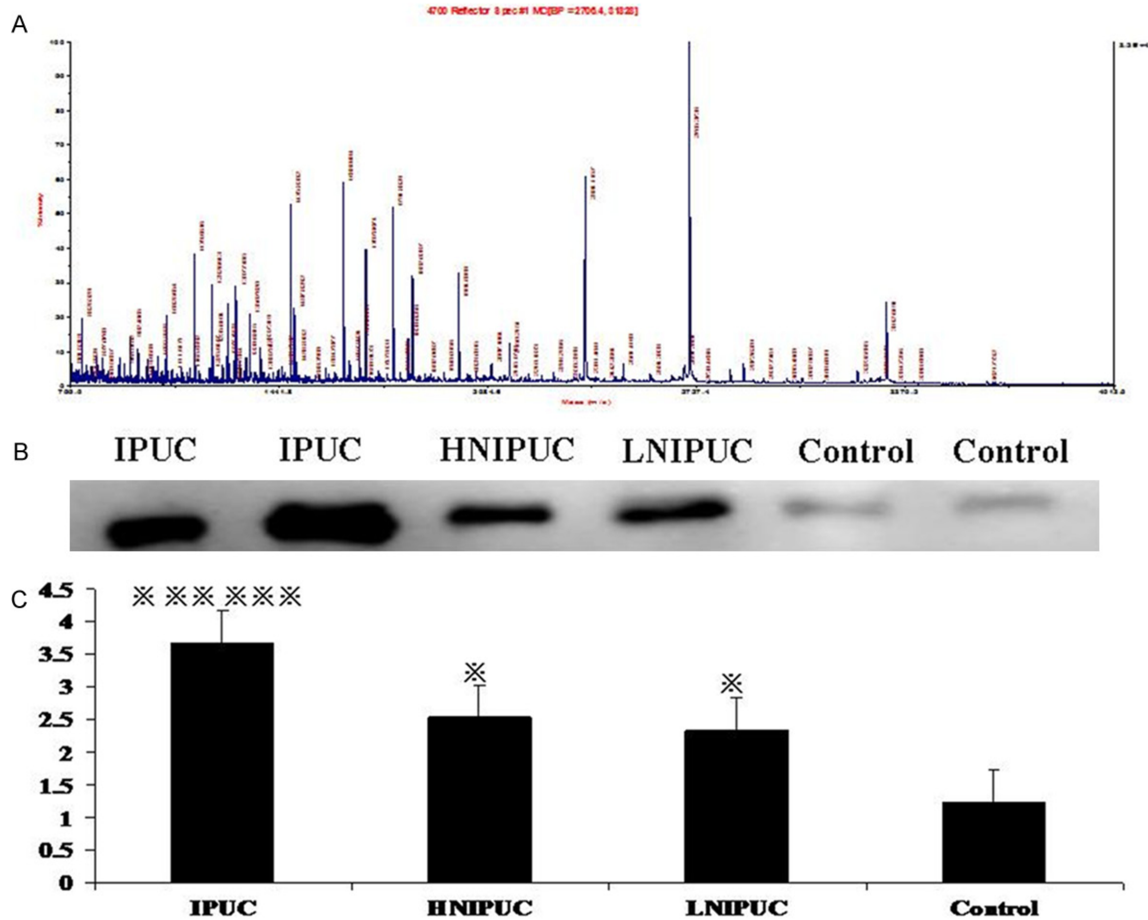

Figure 2. ORM1 expression levels were verified in the urine samples. A. MS of in-gel trypsin digests of the protein and analysis of the depicted peptide spectrum resulted in the identification of ORM1. B. Verification of ORM1 expression in individual urine samples of patients with bladder cancer, bladder benign cases and healthy controls by western blotting. C. The ORM1 expression of western blotting was calculated according to the immunosignals quantified by densitometric scanning. *P<0.05 compared with that of the control group; **P<0.05 compared with that of the LNIPUC group and ***P<0.05 compared with that of the HNIPUC group. LNIPUC: Low grade non-invasive papillary urothelial carcinoma; HNIPUC: High grade non-invasive papillary urothelial carcinoma; IPUC: Invasive papillary urothelial carcinoma.

Analysis of urinary ORM1 by western blotting

To examine the expression of urinary ORM1 in individual samples from patients with bladder cancer and control groups. The results clearly demonstrated that urinary ORM1 was significantly up-regulated in bladder cancer cases that that of the control group. Among the bladder cancer group, the urinary ORM1 expressions in cases of infiltrating urothelial carcinoma were significantly higher than cases of low or high grade non-invasive papillary urothelial carcinoma (**Figure 2B**, **2C**).

Detection of urinary ORM1 expression by ELISA

ELISA was used to quantify urinary ORM1 expression in our analysis After normalization by creatinine level, the urinary ORM1 was markedly elevated in bladder cancer patients than in controls and benign cases (7172.23±3049.67 versus 2243.16±969.01, 2493.48±830.37 ng/ml, respectively, P<0.0001) (**Figure 3A**). After division of the patients into groups by tumour classification, the urinary ORM1 concentrations were 5313.35±1341.39, 5892.76±1943.94 and 10376.49±2677.76 ng/mg for bladder cancer with low grade non-invasive papillary urothelial carcinoma, high grade non-invasive papillary urothelial carcinoma and infiltrating urothelial carcinoma respectively (**Figure 3C**). The relationship between urinary ORM1 levels and the clinical characteristics of bladder cancer patients is presented in **Table 3**. There was no significant association between the urinary ORM1 expression and other clinical fea-

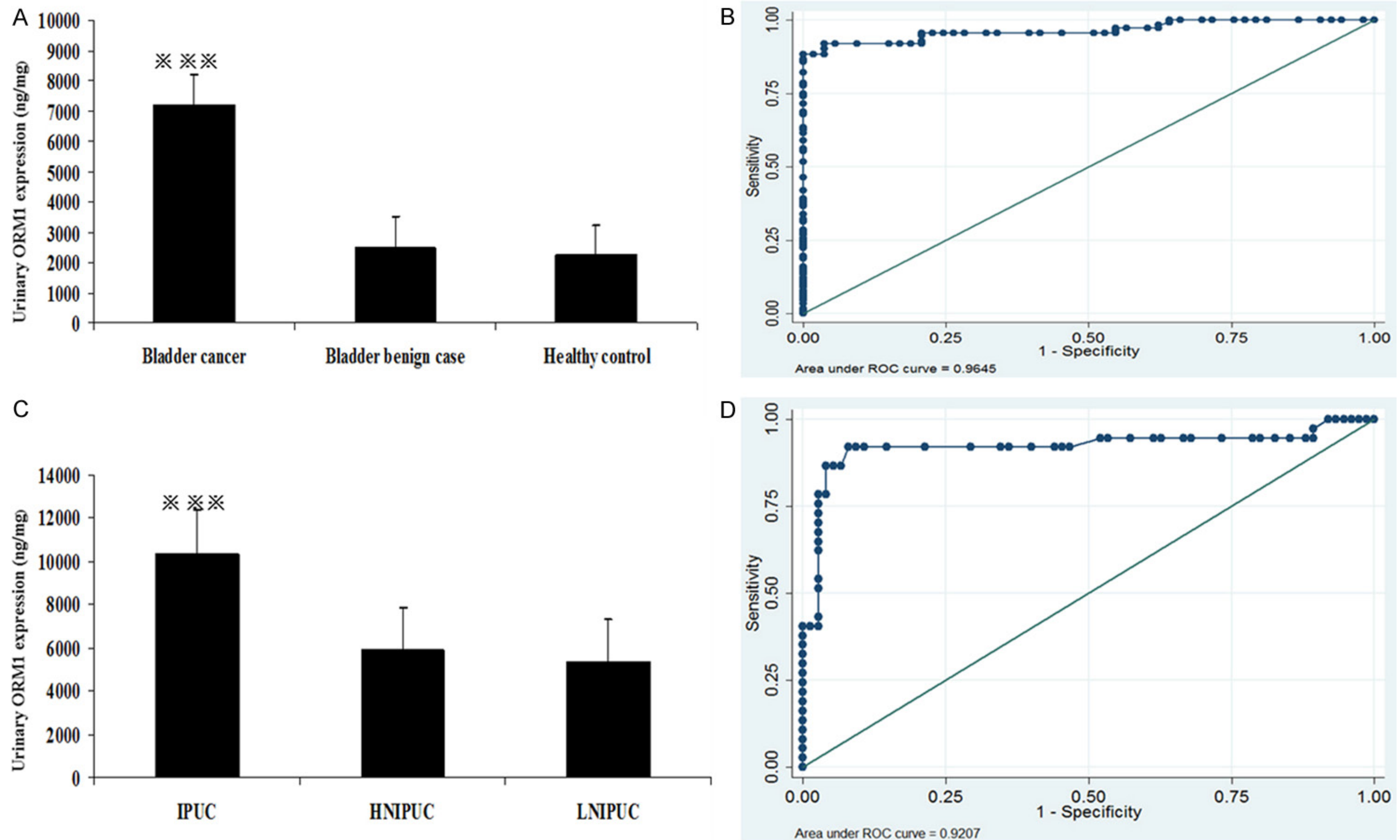

Figure 3. Urinary ORM1 as a useful biomarker for Bladder cancer. A. ELISA was used to quantify urinary ORM1 levels in 112 bladder cancer patients, 21 cases with benign bladder damage and 53 healthy volunteers. *P<0.05 compared with that of the control group; **P<0.05 compared with that of the bladder benign group. B. ROC curve of urinary ORM1 as a marker for detecting bladder cancer was based on a series of 185 urine samples. The optimal cut off was 3912.97 ng/mg, and the area under the ROC curve (AUC) was 0.965 (95% confidence interval (CI) 0.923-0.987). C. The expression level of ORM1 in different pathology classification of bladder cancer. *P<0.05 compared with that of the LNIPUC group and **P<0.05 compared with that of the HNIPUC group. HNIPUC: High grade non-invasive papillary urothelial carcinoma; IPUC: Invasive papillary urothelial carcinoma. D. ROC curve of urinary ORM1 as a marker for distinguish infiltrating urothelial carcinoma from bladder cancer patients. The optimal cut off was 7351.28 ng/mg, and the area under the ROC curve (AUC) was 0.921 (95% confidence interval (CI) 0.853-0.963).

Table 3. Associations of urinary ORM1/Cr level with bladder cancer

Clinical feature	Number	Urinary GC levels (ng/mg)	P
Age ≥65 years	52	6988.25±3107.29	0.256
Age <65 years	60	7401.97±4451.57	
Male	97	7257.12±4297.54	0.548
Female	15	6806.27±3317.95	
With diabetes	34	6884.79±4187.56	0.741
Without diabetes	78	7647.29±3533.47	
With hypertension	45	7579.46±3557.75	0.642
Without hypertension	67	6952.42±3972.41	
With hematuria	61	7502.19±3788.85	0.756
Without hematuria	51	7009.85±4387.69	
With urinary inflammation	40	6652.58±4477.84	<0.0001*
Without urinary inflammation	72	7290.58±4156.75	
New cases	66	7467.87±3719.97	0.256
Recurrences	46	6647.45±3859.74	
No metastasis	91	7002.36±2998.69	0.113
Metastasis	21	8045.45±4841.74	
Low grade non-invasive papillary urothelial carcinoma	39	5313.35±1341.39	<0.0001*
High grade non-invasive papillary urothelial carcinoma	36	5892.76±1943.94	
Infiltrating urothelial carcinoma	37	10376.49±2677.76	

Data are expressed as mean ± SD. *Significant *P<0.05.

Table 4. The multivariate analysis of urinary ORM1/Cr level and clinical features

Feature	*p*-value (uni)	*p*-value (multi)	OR	95% CI
Age	0.723	0.297	1.121	0.487-1.521
Sex	0.412	0.215	1.215	0.321-1.715
Diabetes	0.442	0.311	1.745	0.714-1.962
Hypertension	0.741	0.485	2.312	0.505-3.547
Hematuria	0.951	0.412	1.451	0.325-2.032
Urinary inflammation	<0.001	0.024	1.841	1.123-2.565
Recurrences	0.685	0.314	0.954	0.856-1.285
Metastasis	0.315	0.278	3.089	0.523-3.542
Pathology classification	<0.001	<0.001	2.432	1.253-3.589

tures of bladder cancer such as age, gender, diabetes, hypertension, hematuria, recurrence and metastasis, except for classification of pathology and urinary inflammation (**Table 3**).

Correlation and multivariate logistical regression analyses

The Spearman rank correlation was 0.712, which indicated that the levels of urinary ORM1 had a strong positive correlation with the pathologic stage of bladder cancer (P<0.0001). Furthermore, the multivariate analysis suggested that the urinary ORM1 content was associated with the urinary inflammation (OR: 1.841, 95% CI: 1.123-2.565, P=0.024) and the classification of tumor pathology in bladder cancer (OR: 2.432, 95% CI: 1.253-3.589, P<0.001) (**Table 4**).

Urinary ORM1 as a useful biomarker for bladder cancer

After quantitative measurement of ORM1 in 186 urine samples by ELISA, ROC curves were applied to assess the potential utility of urinary ORM1 in early diagnosis and surveillance of bladder cancer, ROC analyses rendered an optimum cut-off value of 3912.97 ng/mg corresponding to 91.96% sensitivity and 94.34% specificity. The area under the ROC curve (AUC) of ORM1-Cr for diagnosis of bladder cancer was 0.965 (95% confidence interval (CI) 0.923-0.987) (**Figure 3B**). Furthermore, we tested whether ORM1-Cr could be utilized to distinguish infiltrating urothelial carcinoma from bladder cancer patients. ROC analyses indicated a cut-off value with 7351.28 ng/mg corresponding to 91.89% sensitivity and 90.67% specificity. The AUC was 0.921 (95% CI 0.853-0.963) (**Figure 3D**).

Discussion

The incidence and prevalence of bladder cancer remain increasing globally. The early detection and long term prognosis of this disease are still challenge, because cystoscopy is an invasive procedure for patients, and the commonly used cytology has low sensitivity and high variability [11-13]. However, easily applicable screening markers for the early detection of the clinical course of bladder cancer are currently lacking. Therefore, to search for a better noninvasive method for reliable detection of new or recurrent bladder cancer is needed. Proteomic method is a useful tool to find novel biomarker or key proteins in disease of cancer.

In this present study, we have identified thirteen differently expressed proteins in urine from the patients of bladder cancer and normal control by the means of 2-DE analysis and MALDI-FOF/TOF-MS. Of these identified proteins, the association of urinary ORM1 quantitation with bladder cancer has not been examined before, though ORM is found to be significantly increased in urine samples of patients with bladder cancer [14]. Of the protein ORM1 expression was confirmed by Western blot analysis. 186 urine samples were examined by ELISA to correlate urinary ORM1 levels with clinical significance. Our findings have shown that urinary ORM1 could be a potential tumor marker in early diagnosis and surveillance of bladder cancer.

Orosomucoid (ORM) is a polymorphic acute-phase reactant with immunosuppressive properties [7, 15]. Previous investigations have suggested that ORM and other acute-phase reactants may act as blocking factors protecting tumor cells against immunological attack, thereby contributing to the 'immune escape' of the tumor [15, 16]. Many previous studies have demonstrated that serum level of ORM is elevated in inflammatory and lymphoproliferative disorders and cancer, such as breast, lung and ovary cancer [17, 18]. Only recently have some studies begun to look at changes in ORM levels in the urine of patients with certain diseases. Christiansen *et al.* [19] discovered that the ORM expressions in urine were significantly increased in patients with type 2 diabetes, even in normal buminuric patients [20]. The finding was in line with the enhanced urinary ORM concentrations in pregnant women with pre-gestational type 1 diabetes to predict pre-eclampsia [21].

Different ORM types have different roles in the progression of cancers [17]. ORM1 (Orosomucoid-1, α1-acid glycoprotein 1) mainly functions as a transport protein in the bloodstream, which is normally produced by human liver cells, and can also be produced in endothelial cells and some tumor cells as well [7, 8, 22]. Some studies have demonstrated ORM1 has a regulatory dampening effect on the inflammatory cascade, thereby protecting against tissue damage from excessive inflammation [23, 24]. The exaggerated excretions of urinary ORM were also detected in hypertensive subjects and in chronic heart failure patients [22]. However, the association of urinary ORM1 levels with bladder cancer is unclear.

In this analysis, our finding showed that the expression of ORM1 was significantly increased in the bladder cancer group compared with healthy controls in individuals through the method of western blotting. Following quantitative measurements of 186 urine samples by ELISA analysis, it was identified that the levels of urinary ORM1 were significantly higher in the urine samples from patients with bladder cancer than in the urine from controls and benign cases. Furthermore, the Spearman analysis indicated that urinary ORM1 had a strong positive correlation with the tumor pathology classification of bladder cancer, meaning that the elevated urinary ORM1 expression was associated with invasive nature of bladder cancer, Furthermore, the multivariate analysis indicated that the urinary ORM1 expression correlated with urinary inflammation ($P=0.024$) and pathology classification of bladder cancer ($P<0.001$). In addition, ROC curves suggested at an optimum cut-off value of 3912.97 ng/mg with 91.96% sensitivity and 94.34% specificity respectively is appropriate for diagnosis of bladder cancer patients, and a cut-off value of 7351.28 ng/mg with 91.89% sensitivity and 90.67% specificity could be used to detect the infiltrating urothelial carcinoma.

However, the mechanisms underlying the elevated ORM1 expression in urine from bladder cancer patients are unclear. One possible explanation could be that the increased ORM1 may be associated with the inflammatory activation in bladder cancer patients. Inflammation

has been proved to play a pivotal role in the development of bladder cancer through both animal and human research [14, 25-27]. Another possibility may be regarding the ORM1 produced by vascular endothelial cells, especially angiogenically activated blood vessels [14]. One characteristic phenomenon of angiogenically activated blood vessels is the abnormal vascular leakage or permeability in the development of cancer disease [28, 29]. This would explain the highest increase in mean protein amount of ORM in bladder cancer with infiltrating urothelial carcinoma than cases of low or high grade non-invasive papillary urothelial carcinoma. Therefore, further studies are needed to confirm the reasons leading to the significantly elevated urinary ORM1 in bladder cancer patients.

There are several limitations in the current study that needed to be considered when contemplating the potential value of urinary ORM1 in early detection and surveillance of bladder cancer. Firstly, one of the most important points that needed to be addressed is about the about the specificity of this biomarker. Though our findings were measured in 112 bladder cancer patients, 21 cases with benign bladder damage and 53 healthy controls, the control groups were limited probably resulting in an overestimation of the specificity of ORM1. Furthermore, we did not detect an association between urinary ORM1 levels and other clinical characteristics of bladder cancer such as age, gender, diabetes, hypertension, hematuria, recurrence and metastasis, but we could not exclude other known factors that may affect the urinary ORM1 expression. Thereby, further validation of the urinary marker in bladder cancer is necessary to be considered through larger-scale studies with adequate control groups and adjusted factors.

Conclusions

To sum up, our findings have shown that the ORM1 expression levels in urine are significantly increased in patients with bladder cancer compared to controls. Furthermore, the expression level of urinary ORM1 is positively associated with the pathological classification of bladder cancer. Thus, our study indicates that the urinary ORM1 levels may be a potential biomarker for the early diagnosis and surveillance of bladder cancer. Further research is warranted to elucidate the pathogenic mechanisms of elevated ORM1 and its putative mechanistic role in the pathogenesis of bladder cancer.

Acknowledgements

This work was supported by Dean's research fund of Nanfang Hospital, the Southern Medical University (2013C022) (F.L.), the Natural Science Foundation of Guangdong Province of China (2014A030310424) (F.L.), Southern medical university scientific research plan (20-1311) (F.L.), Guangdong Provincial science and technology innovation project of education (2013KJCX0039) (W.L.T.), Guangdong Provincial Science and technology projects (2013-B022000067) (W.L.T.), the National Science Foundation of Guangdong Province of China (2015A030313289) (W.L.T.) and the National Natural Science Foundation of China (No. 81-272844) (W.L.T.).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Wanlong Tan and Yuejun Du, Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China. Tel: +86-20-616-41762; Fax: +86-20-616-41762; E-mail: tanwanlong@gmail.com (WLT); Tel: +86-20-627-87210; Fax: +86-20-627-87210; E-mail: duyuejunsmu@126.com (YJD); Dr. Lina Hou, Department of Healthy Management, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, P. R. China. Tel: +86-20-616-76532; Fax: +86-20-616-76532; E-mail: lnhou2010@163.com

References

[1] Li F, An SL, Zhou Y, Liang ZK, Jiao ZJ, Jing YM, Wan P, Shi XJ, Tan WL. Milk and dairy consumption and risk of bladder cancer: A meta-analysis. Urology 2011; 78: 1298-1305.

[2] Feldman AS, Banyard J, Wu CL, McDougal WS, Zetter BR. Cystatin B as a tissue and urinary biomarker of bladder cancer recurrence and disease progression. Clin Cancer Res 2009; 15: 1024-1031.

[3] Mitropoulos D, Kiroudi-Voulgari A, Nikolopoulos P, Manousakas T, Zervas A. Accuracy of cystoscopy in predicting histologic features of bladder lesions. J Endourol 2005; 19: 861-864.

[4] Yang MH, Chu PY, Chen SC, Chung TW, Chen WC, Tan LB, Kan WC, Wang HY, Su SB, Tyan YC. Characterization of ADAM28 as a biomarker of bladder transitional cell carcinomas by urinary

proteome analysis. Biochem Biophys Res Commun 2011; 411: 714-720.
[5] Li F, Chen DN, He CW, Zhou Y, Olkkonen VM, He N, Chen W, Wan P, Chen SS, Tan WL. Identification of urinary Gc-globulin as a novel biomarker for bladder cancer by two-dimensional fluorescent differential gel electrophoresis (2D-DIGE). J Proteomics 2012; 77: 225-236.
[6] Van Tilborg AA, Bangma CH, Zwarthoff EC. Bladder cancer biomarkers and their role in surveillance and screening. Int J Urol 2009; 16: 23-30.
[7] Sorensson J, Matejka GL, Ohlson M, Haraldsson B. Human endothelial cells produce orosomucoid, an important component of the capillary barrier. Am J Physiol 1999; 276: 530-534.
[8] Fournier T, Medjoubi-N N, Porquet D. Alpha-1-acid glycoprotein. Biochim Biophys Acta 2000; 1482: 157-171.
[9] Hou LN, Li F, Zeng QC, Su L, Chen PA, Xu ZH, Zhu DJ, Liu CH, Xu DL. Excretion of urinary orosomucoid 1 protein is elevated in patients with chronic heart failure. PLoS One 2014; 9: e107550.
[10] Eble JN, Sauter G, Epstein JI, Sesterhenn IA. Pathology and genetics of tumours of the urinary system and male genital organs. IARC Press; 2004. pp. 359.
[11] Konety B, Lotan Y. Urothelial bladder cancer: Biomarkers for detection and screening. BJU Int 2008; 102: 1234-1241.
[12] Quek ML, Sanderson K, Daneshmand S, Stein JP. New molecular markers for bladder cancer detection. Curr Opin Urol 2004; 14: 259-264.
[13] Jou YJ, Lin CD, Lai CH, Chen CH, Kao JY, Chen SY, Tsai MH, Huang SH, Lin CW. Proteomic identification of salivary transferrin as a biomarker for early detection of oral cancer. Anal Chim Acta 2010; 681: 41-48.
[14] Irmak S, Tilki D, Heukeshoven J, Oliveira-Ferrer L, Friedrich M, Huland H, Ergun S. Stage-dependent increase of orosomucoid and zinc-alpha2-glycoprotein in urinary bladder cancer. Proteomics 2005; 5: 4296-4304.
[15] Logdberg L, Wester L, Immunocalins. A lipocalin subfamily that modulates immune and inflammatory responses. Biochim Biophys Acta 2002; 1482: 284-297.
[16] Andersson LC, Gahmberg CG. Surface glycoproteins of resting and activated human T lymphocytes. Mol Cell Biochem 1979; 27: 117-131.
[17] Duche JC, Urien S, Simon N, Malaurie E, Monnet I, Barre J. Expression of the genetic variants of human alpha-1-acid glycoprotein in cancer. Clin Biochem 2000; 33: 197-202.
[18] Yin WH, Chen JW, Jen HL, Chiang MC, Huang WP, Feng AN, Young MS, Lin SJ. Independent prognostic value of elevated high-sensitivity C-reactive protein in chronic heart failure. Am Heart J 2004; 147: 931-938.
[19] Christiansen MS, Hommel E, Magid E, Feldt-Rasmussen B. Orosomucoid in urine is a powerful predictor of cardiovascular mortality in normoalbuminuric patients with type 2 diabetes at five years of follow-up. Diabetologia 2005; 48: 386-393.
[20] Christiansen MS, Hommel E, Magid E, Feldt-Rasmussen B. Orosomucoid in urine predicts cardiovascular and over-all mortality in patients with Type II diabetes. Diabetologia 2002; 45: 115-120.
[21] Christiansen MS, Hesse D, Ekbom P, Hesse U, Damm P, Hommel E, Feldt-Rasmussen B, Mathiesen E. Increased urinary orosomucoid excretion predicts preeclampsia in pregnant women with pregestational type 1 diabetes. Diabetes Res Clin Pract 2010; 89: 16-21.
[22] Lisowska-Myjak B, Krych A, Kolodziejczyk A, Pachecka J, Gaciong Z. Urinary proteins, N-acetyl-beta-D-glucosaminidase activity and estimated glomerular filtration rate in hypertensive patients with normoalbuminuria and microalbuminuria. Nephrology (Carlton) 2011; 16: 403-409.
[23] Gahmberg CG, Andersson LC. Leukocyte surface origin of human alpha1-acid glycoprotein (orosomucoid). J Exp Med 1978; 148: 507-521.
[24] Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple risk factor intervention trial. Am J Epidemiol 1996; 144: 537-547.
[25] Davis M, Bunin DI, Samuelsson SJ, Altera KP, Kinders RJ, Lawrence SM, Ji J, Ames MM, Buhrow SA, Parman T. Characterization of batracylin-induced renal and bladder toxicity in rats. Toxicol Pathol 2014; 43: 519-529.
[26] Lee ST, Lu MH, Chien LH, Wu TF, Huang LC, Liao GI. Suppression of urinary bladder urothelial carcinoma cell by the ethanol extract of pomegranate fruit through cell cycle arrest and apoptosis. BMC Complement Altern Med 2013; 13: 364.
[27] Said N, Theodorescu D. RhoGDI2 suppresses bladder cancer metastasis via reduction of inflammation in the tumor microenvironment. Oncoimmunology 2012; 1: 1175-1177.
[28] Shu X, Wu W, Mosteller RD, Broek D. Sphingosine kinase mediates vascular endothelial growth factor-induced activation of ras and mitogen-activated protein kinases. Mol Cell Biol 2002; 22: 7758-7768.
[29] Roudnicky F, Poyet C, Wild P, Krampitz S, Negrini F, Huggenberger R, Rogler A, Stohr R, Hartmann A, Detmar M. Endocan is upregulated on tumor vessels in invasive bladder cancer where it mediates VEGF-A-induced angiogenesis. Cancer Res 2013; 73: 1097-1106.