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Clusterin as a Diagnostic and Prognostic Marker for Transitional Cell Carcinoma of the Bladder

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Abstract We investigated the feasibility of profiling and measuring the concentration of clusterin in urine and serum for individuals with transitional cell carcinoma (TCC) of the bladder and comparing it with nontumor controls. In addition, we analyzed the correlation of expression of clusterin in specimens of TCC to various clinicopathologic parameters and prognosis of bladder cancer. Blood and urine samples were used from 68 patients with TCC of the bladder and from 61 patients with benign urological diseases. Enzyme-linked immunosorbent assays (ELISA) were performed for clusterin from serum and urine. Quantitation of clusterin mRNA was carried out in 68 bladder tumor specimens from radical cystectomy or transurethral resection and 26 normal bladder specimens from BPH patients by using RT-PCR method. Correlation for the expression of clusterin mRNA with clinicopathologic parameters was analyzed. Serum and urine clusterin was significantly higher in individuals with bladder cancer than control (p=0.001). Sensitivity and specificity of serum and urine clusterin as a tumor marker for TCC of the bladder was found to be 80%, 91%, 87.1% and 96.7% respectively. Clusterin expression was significantly higher in TCC specimens than normal tissue specimens (P<0.001). Expression of clusterin was significantly higher in patients with invasive TCC of the bladder than that in patients with superficial TCC and control (P<0.001). Overexpression of clusterin mRNA was significantly associated with tumor recurrence and overall survival (p<0.001). The recurrence-free survival time of patients with overexpression of clusterin was significantly shorter than that of patients with weak expression of clusterin (9.8 months vs. 35.2 months). Clusterin may be considered as a potential diagnostic and prognostic biomarker for bladder cancer using urine, serum and/or molecular biology techniques.

Keywords Bladder cancer · Tumor marker · Clusterin · Over-expression · Prognosis

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Introduction

Cancer of the urinary bladder is among the five most common malignancies worldwide[1] and the commonest malignant tumor in Egypt; it accounts for about 20% of all malignant neoplasm of the body [1,2]. Urothelial tumors can be classified into two groups on the basis of histopathology and clinical behavior. At presentation, more than 80% of bladder tumors are non-muscle-invasive papillary tumors (pTa or pT1). The remaining 20% of tumors that show muscle invasion at the time of diagnosis have a much less favorable prognosis [3].

Cystoscopy and urine cytology are the gold standard in the diagnosis and follow up of superficial bladder cancer [4]. Cystoscopy is a naked eye assessment, invasive, uncomfortable and costly. The subjective nature and the low sensitivity of classic urine cytology led to its limited

application and to the development of other replacing testes. Several tumor markers are now available for clinical use; numerous others continue to be evaluated [5]. Unfortunately, all the available tests suffer from high false positive rates, and thus, there is no test available to date that can replace urine cytology [5]. Consequently, the development of noninvasive urinalysis assays using reliable diagnostic markers would be of tremendous benefit to both patients and healthcare providers. Furthermore, the prognosis of patients with advanced bladder cancer is still extremely poor despite recent therapeutic advances [1]. It is, thus, important to investigate the factors that contribute to the acquisition of the resistant phenotype to several therapies, which may provide useful information for the prognosis of patients with bladder cancer.

Clusterin is a multifunctional secretory glycoprotein, which was discovered about 20 years ago from ram rete testis as a protein causing clustering of the erythrocyte [6]. Clusterin is found in all body fluids including plasma, milk, urine and cerebrospinal fluid as well as on the surface of cells lining body cavities [7]. Clusterin can modulate cell-cell and/or cell-matrix interactions, and has a variety of functions including transporting lipoproteins, the inhibition of complement-mediated cell lysis, regulation of survival/apoptosis, tissue remodeling and tumorigenesis [8,9]. Recently, a potential oncogenic role of clusterin in the development and/or progression of several human cancers has been examined. Increased expression of clusterin was detected in prostate, renal cell and lung cancer [10–14]. In this study, we investigated the feasibility of profiling and measuring the concentration of clusterin in urine and serum for individuals with bladder cancer and comparing it with nontumor controls. In addition, we measured the clusterin expression by RT-PCR in specimens of transitional cell carcinoma (TCC) of the bladder obtained from patients undergoing surgical treatment. The results were evaluated with respect to various clinicopathologic parameters and prognosis of bladder cancer.

Patients and Methods

Between January 2003 and October 2008, 68 specimens of TCC of the bladder were obtained from patients with primary TCC who underwent surgical treatment in the form of radical cystectomy or transurethral resection. In addition, urine and blood specimens were collected from all TCC patients. Sixty one patients were taken as controls consisted of age-matched nontumor patients admitted to Urology Department at Tanta University during the same period of study (benign prostatic hyperplasia (BPH) "n=26", urolithiasis "n=20" and nonspecific urinary tract infection "n=15"). Specimens of normal urothelial tissue were obtained from 26 volunteers patients with BPH underwent transurethral prostatectomy "TURP". All patients with BPH had normal PSA levels and histologic examination of all prostatic tissues resected proved to be benign in nature. Urine and blood specimens were collected from all control patients. Tissue specimen, urine and blood samples were collected from all patients according to approved informed consent protocols. Ethical approval was obtained from the university ethical review board to perform this study.

Each tissue specimen from TCC patients and controls was snap-frozen and stored at -80°C until assessed. It was routinely confirmed by cryostat sectioning before analysis that carcinoma tissues contained more than 90% cancer cells, and normal bladder tissues from BPH patients did not contain any cancer cells. A single pathologist performed the pathologic examination. The pathologic stage and tumor grade were diagnosed according to the World Health Organization/International Society of Urological Pathology consensus classification [15].

The characteristics of all patients are summarized in Table 1. For patients with TCC of the bladder, the mean age was 54.6 years (range 36–68), men outnumbered women by a ratio of 3.8:1 and the median follow up period was 39 months (range 14–66). In this study, 42 (61.8%) patients were defined as having superficial bladder cancer (18 with pTa and 24 with pT1 TCC) and underwent transurethral resection with or without BCG intravesical instillation. Additional 26 (38.2%) patients were defined as having invasive disease (14 with pT2 and 12 with pT3 TCC) and underwent radical cystectomy. The postoperative examinations for recurrence were performed by routine blood tests and urinary cytology every 3 months, and chest x-ray, intravenous urography, and abdominal and pelvic computed tomography were performed every 6 months. The interval for repeated examinations was appropriately increased in the absence of recurrence 2 years after surgery.

Measurement of Serum and Urine Clusterin by ELISA (Biovender, LLC, USA)

Commercial enzyme-linked immunosorbent assay (ELISA) kit for clusterin was purchased and testing was performed according to the manufactures instructions.

Clusterin Expression by Semiquantitative *RT-PCR*

Total RNAs were isolated from resected benign prostatic hyperplasia and cancer bladder tissue specimens using m-RNA isolation kit following the manufacture instructions (Qiagen, Germany). To investigate relative levels of clusterin mRNA expression, semiquantitative RT-PCR was performed. First strand cDNA was reversely transcribed from 5 μg of the isolated RNAs using SuperScript II kit

Table 1 Serum and urinary levels of clusterin in the cohorts of patients with TCC of the bladder and controls

Variables	Superficial TCC (n=42)	Invasive TCC (n=26)	Controls (n=)			P value
			BPH (n=26)	Urolithiasis & UTI (n=35)	All controls (n=61)	
Sex:						
Male	33 (78.6%)	21 (80.8%)	26 (100%)	22 (62.9%)	48 (78.7%)	X^2=12.440
Female	9 (21.4%)	5 (19.2%)	0(0.0%)	13 (37.1%)	13 (21.3%)	p=0.014[a]
Age in years:						
Range	36–63	39–68	54–70	27–58	27–70	F=
Mean	52.31	57.08	60.73	43.66	50.93	36.152
SD	7.04	6.29	4.26	5.30	10.91	p<0.001
Serum Level of clusterin (ug/ml):						
Range	410–990	630–1100	90–193	148–198	90–198	F=
Mean	574.05	879.04	168.42	173.06	171.08	351.34[a]
SD	141.77	120.62	21.91	14.24	17.90	p<0.001
Urinary Level of clusterin(ug/ml):						
Range	75–290	100–320	52–99	52–95	52–99	F=
Mean	178.45	245.00	77.15	75.46	76.18	138.0[a]
SD	47.71	55.37	12.24	13.42	12.85	p<0.001

[a] Significant

(Life Technologies, USA) by mixing 5 μg of isolated RNA with random primer, 25 mM $MgCl_2$, 500 mM Kcl, 100 mM tris HCL, 40 U/μl, RNAase inhibitor, 10 mM DNTP mixture and 5 U/μl RT enzyme for a final volume of 20 μl which incubated for 30 min at 42°C. Using reverse-transcribed cDNA, PCR were performed after the following protocol described by Park et al [16]; 2 min denaturation at 96°C, then 36 cycles of a 45 s, denaturation step at 95°C, 1 min annealing at 55ºC, 1 min elongation at 72°C, and a final elongation step for 5 min at 72°C.

Quantitation of clusterin mRNA was carried out using β actin mRNA as an internal standard. This was done using the gel document system computer soft ware (gel-pro-Analyzer). PCR was performed using the following primers for detection of clusterin and β -actin signals, respectively.

Clusterin; (5'ACCTCACGCAAGGCGAAGAC3', (sense) and 5'TCTCACTCCTCCCGGTGCTT3' (anti-sense) and a product with 232 bp was generated. *β actin* Primer sequences used were: β actin: 5'-CCTTCAA CACCCCAGCCA-3' (sense) and: 5'-CCACCAGACA GCACTGTG-3' (antisense) and a product with 529 bp was generated.

Statistical Analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. For quantitative variables, the range, mean and standard deviation were calculated. The difference between means of different studied groups was statistically analyzed using analysis of variance (ANOVA) was calculated and Scheffe test was performed to compare between each two means if F value was significant. Pearson's correlation coefficient (r) was calculated to test the association between two survival and clusterin levels in serum and urine. . For categorical variables, the number and percent distribution was calculated. Chi square was used as a test of significance. For comparison of survival and recurrence free survival in relation to different variables, Mann-Whitney test (Z) was used as the survival was not found to follow the normal distribution. The ROC curve was used to determine the cut of value, sensitivity and specificity of clusterin in urine and serum as a diagnostic test. Regression analysis was used to test the effect of different independent operating variables and detect confounders. Significance was adopted at p<0.05 for interpretation of results of tests of significance [17].

Results

Clusterin Levels in Serum and Urine

We determined clusterin levels in serum and urine of the two groups of patients enrolled in the study: TCC of the bladder (superficial and invasive) and nontumor controls. The means of serum levels for each group, together with urine concentrations, are reported in Table 1. The mean

serum clusterin levels in bladder cancer patients whether superficial (574.05±141.7 μg/ml) or invasive (879.04± 120 μg /ml) were significantly higher than in controls (171.08±17.9 μg/ml) ($p<0.001$). As shown in Table 1, the concentration of clusterin in urine was lower than in serum with some neither difference between normal versus bladder cancer urine. In fact, urinary clusterin was higher in bladder cancer patients (the mean level in superficial TCC was 178.45±47.7 μg/ml and in invasive TCC was 245.0±55.3 μg/ml) than in nontumor controls (76.18± 12.8 μg/ml) ($p<0.001$). The sensitivity and specificity of serum clusterin as a tumor marker for TCC of the bladder was found to be 80% and 91% respectively. Similarly, the sensitivity and specificity of urine clusterin as a tumor marker for TCC of the bladder was found to be 87.1% and 96.7% respectively.

Tissue Expression of Clusterin In TCC of the Bladder

All normal bladder tissues examined in this series showed barely detectable or weakly expressed clusterin mRNA, whereas various levels of clusterin expression were noted in TCC specimens. Clusterin mRNA was highly expressed (overexpressed) in invasive TCC of the bladder but was low detectable in superficial TCC. Quantitative evaluation has revealed that the mean level of clusterin expression in invasive TCC was approximately threefold higher than that in superficial TCC and fivefold higher than that in normal tissue ($P<0.002$ and $P<0.001$, respectively).

We analyzed the relationship between the clusterin expression level and several clinicopathologic factors, which revealed that clusterin expression was significantly higher in TCC specimens than normal tissue specimens ($P<0.001$, $\chi 2$ test 30.6) (Table 2). Overexpression of clusterin was detected in 19/26 (73.1%) of patients with invasive TCC of the bladder, which was significantly higher than that in patients with superficial TCC (11/42, 26.2%) ($P<0.001$, $\chi 2$ test 14.3). However, no significant difference was noted in clusterin expression between superficial TCC and normal tissue specimens. Similarly, the clusterin expression level correlated significantly with tumor grade and tumor multiplicity; patients with grade 3 (28/41, 68.3%) showed overexpression of clusterin mRNA than grade 1 or 2 (2/27, 7.4%) ($P<0.001$) (Table 2). But no significant association was found between clusterin expression and other clinico-pathological features, such as patient's gender, age, or tumor size.

In the present study, the overall tumor recurrence was noted in 35 cases (51.5%), 22 of them (62.9%) showed overexpression of clusterin mRNA. Of the 42 patients with superficial TCC, 24 (57.1%) showed intravesical recurrence after endoscopic management, 11 (45.8%) of them showed overexpression of clusterin mRNA. Among the 26 patients with invasive TCC who underwent radical cystectomy, 11

Table 2 Association between clusterin expression and clinico-pathologic factors

Variables	Patients (n)	Clusterin expression						X^2 *P* Value
		Over-expression		Weak expression		Undetectable level		
		n	%	*n*	%	*n*	%	
Group								
BPH	26	0	0.0	19	73.1	7	26.9	30.695
TCC	68	30	44.1	38	55.9	0	0.0	0.001[a]
Stage								
Superficial (≤pT1)	42	11	26.2	31	73.8	0	0.0	14.319
Invasive (≥pT2)	26	19	73.1	7	26.9	0	0.0	0.001[a]
Grade								
G1 or G2	27	2	7.4	25	92.6	0	0.0	24.478
G3	41	28	68.3	13	31.7	0	0.0	0.001[a]
Greatest dimension								
<3	40	14	35.0	26	65.0	0	0.0	3.276
≥3	28	16	57.1	12	42.9	0	0.0	0.070
Multiplicity								
Single	35	10	28.6	25	71.4	0	0.0	7.070
Multiple	33	20	60.6	13	39.4	0	0.0	0.008[a]

[a] Significant

(42.3%) had postoperative recurrence including single organ recurrence in 8 (lung in 3, lymph node in 3, bone in 1 and liver in 1) and multiple organ recurrence in 3. All 11 patients had overexpression of clusterin mRNA. Therefore, the clusterin mRNA expression level significantly correlated with the tumor recurrence rate ($p<0.001$).

We then compared overall survival and recurrence-free rates according to clusterin expression levels (Tables 3 and 4). The recurrence-free and overall survival rates in patients with strong clusterin expression were significantly lower than in those with weak or no expression ($p<0.001$, Figs. 1 and 2). The recurrence-free survival time of patients with overexpression of clusterin was significantly shorter than that of patients with weak expression of clusterin (9.8 months vs. 35.2 months). To evaluate the predictive value of clusterin expression status for bladder TCC recurrence and patient survival multivariate analysis was then performed considering tumor size, multiplicity and pathological grade and stage. Overexpression of clusterin mRNA was significantly associated with tumor recurrence and overall survival (Table 5). Although the overall survival for patients with superficial and invasive TCC was 78.6% and 57.6% respectively, the difference was not statistically significant which may be attributed to the small sample size. Similarly, clusterin levels in serum and urine has been found to be negatively correlated with tumor recurrence and overall survival (Table 6)

Table 3 Factors affecting overall survival in months among studied patients

Variables	Survival in months			*Z*	*P* Value
	Range	Mean	SD		
Age in years:					
≤50	8–66	46.90	22.65	0.023	0.982
>50	9–66	48.00	20.15		
Sex:					
Males	8–66	46.11	21.65	0.720	0.472
Females	9–60	53.64	16.44		
Gene expression:					
Weak	60–66	60.63	1.87	5.446	0.001[a]
Over expression	8–66	31.23	22.24		
Stage					
Superficial (≤pT1)	9–66	50.40	21.12	1.599	0.110
Invasive (≥pT2)	8–66	43.23	21.48		
Grade					
G1 or G2	9–66	58.13	11.22	4.658	0.001[a]
G3	8–66	32.71	22.29		
Greatest dimension					
<3	12–66	52.25	11.60	4.499	0.001[a]
≥3	8–60	33.96	23.40		
Multiplicity					
Single	14–66	58.97	8.84	4.686	0.001[a]
Multiple	8–60	35.67	23.10		

[a] Significant

Discussion

Recent observations indicated an association of clusterin expression with contradictory functions, either cell survival, tumor progression, treatment resistance in vivo, or pro- or antiapoptosis [18,19]. These early apparently ambiguous functions appear to be attributed to the existence of two different but related clusterin protein isoforms, a glycosylated form (secreted or cytoplasmic clusterin of 76–80 kDa) as well as an nonglycosylated form (nuclear clusterin of 49 kDa protein) that are coded by clusterin gene and are derived by alternative posttranslational processes, from the same precursor of 53 kDa protein [20]. These two forms of the clusterin protein can be immunologically distinguished [21]. Nuclear clusterin has been suggested as a cell death protein [21]. Recently, data from in vivo and in vitro studies of clusterin in tumorigenesis have demonstrated that nuclear clusterin was predominantly expressed in normal mucosa of colon and bladder and may act as a pro-apoptosis protein, while cytoplasmic clusterin may function as an anti-apoptosis protein [22].

It is now accepted that the primary function of clusterin in distinct genetic backgrounds of cancer cells is antiapoptotic [18]. This antiapoptotic activity of clusterin may account for the genesis and biologically aggressive behavior of several cancer cells [21]. However, whether increased expression of antiapoptotic clusterin is a common feature of tumorigenesis, thereby protecting cancer cells against apoptotic stimuli that might cause cell death, is still a matter of debate. Moreover, the question whether anti-apoptotic clusterin is the only form of clusterin expressed in cancer, or whether proapoptotic forms of nuclear clusterin are downregulated in distinct tumor entities, has not been definitely answered to date.

Elevated production of clusterin antigen may be detected in body fluids, such as serum and urine. Consequently, it has been speculated that markers derived from clusterin may be used as part of a set of indices for early detection of human cancers [23]. Serum clusterin was identified as a marker of colorectal and anaplastic large-cell lymphoma [24] and it has been recommended that clusterin should be added as a useful marker to antibody panels designed to distinguish systemic anaplastic large-cell lymphoma from classical Hodgkin's lymphoma [24]. Recently, Stejskal and Fiala [25] have examined clusterin concentrations of the urine and serum in 43 patients with bladder cancer by using

Table 4 Factors affecting recurrence free survival in months among studied patients

Variables	Recurrence free survival in months			*Z*	*P* Value
	Range	Mean	SD		
Age in years:					
≤50	3–50	23.73	18.48	1.065	0.287
>50	3–48	15.90	14.41		
Sex:					
Males	3–50	18.19	17.81	1.076	0.282
Females	3–36	22.88	11.10		
Gene expression:					
Weak	12–50	35.23	11.65	4.345	0.001[a]
Over expression	3–48	9.82	10.56		
Stage					
Superficial (≤pT1)	3–50	23.96	17.65	2.126	0.033[a]
Invasive (≥pT2)	3–24	9.00	6.29		
Grade					
G1 or G2	3–50	30.50	15.22	3.509	0.001[a]
G3	3–48	9.79	10.67		
Greatest dimension					
<3	6–48	23.25	14.37	1.692	0.091
≥3	3–50	17.17	17.44		
Multiplicity					
Single	3–48	29.67	17.52	2.124	0.034[a]
Multiple	3–50	15.65	14.81		

[a] Significant

ELISA test and compared them with 50 patients with benign urological diseases. They found that serum and urine clusterin were significantly higher in individuals with bladder cancer with sensitivity of 73% and 49% respectively and specificity of 55% and 92% respectively. They concluded that urine clusterin could be the possible laboratory marker of bladder cancer. In the present study, we used ELISA to measure the concentration of clusterin

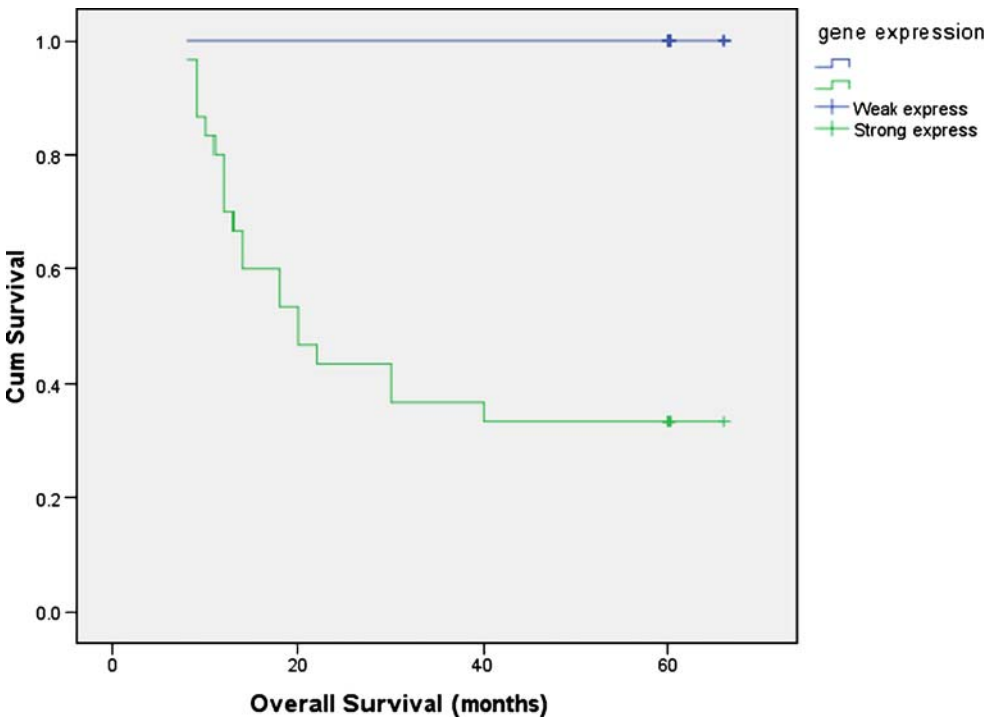

Fig. 1 Overall survival in relation to gene expression

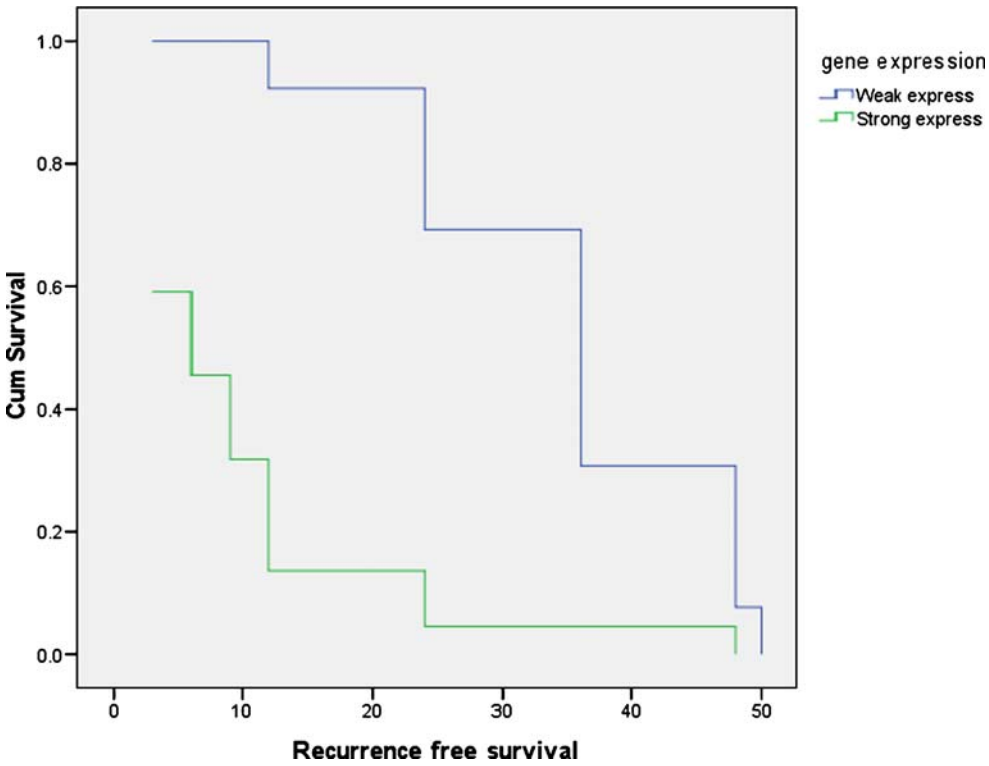

Fig. 2 Recurrence free survival in relation to gene expression

urine and serum. Clusterin was observed to increase from nontumor control to superficial low grade TCC of the bladder to invasive high grade carcinoma in both urine and serum ($p<0.001$). The sensitivity and specificity of serum clusterin as a tumor marker for TCC of the bladder was found to be 80% and 91% respectively. Similarly, the sensitivity and specificity of urine clusterin as a tumor marker for TCC of the bladder was found to be 87.1% and 96.7% respectively.

Recent data indicate that progression towards high-grade and metastatic carcinoma leads to elevated clusterin levels and altered intracellular distribution of nuclear clusterin. Thus, the function of clusterin in tumors may be related to a pattern shift in its isoform production. Overexpression of clusterin was shown in the majority of tumors investigated including prostate cancer [11,26–28], breast carcinoma [12], lung [29], and colon [22] as well as urinary bladder [13,14] cancers. A few reports do, however, suggest decreased clusterin levels in specific cancers, including esophageal squamous cell carcinoma [30], prostate [28], and pancreatic cancer [31]. It is not clear, however, in these studies if cytoplasmic clusterin versus nuclear clusterin expression levels were clearly separable.

In the present study, we found that the mean expression level of clusterin mRNA in invasive TCC specimens was significantly higher than that in superficial TCC or normal tissue specimens. Based on the semi-quantitative analysis of clusterin mRNA levels, we found that the clusterin expression level correlated significantly with pathologic stage i.e., overexpression of clusterin was more frequently detected in higher stages (pT2 and pT3) when compared to

Table 5 Results of multiple regression analysis for variables affecting the overall and recurrence free survival

Variables	Overall survival		Recurrence free survival	
	t	p	t	p
Gene expression	4.331	0.001[a]	3.086	0.004[a]
Grade	0.593	0.556	0.159	0.875
Multiplicity	1.512	0.136	1.888	0.069
Size	3.327	0.001[a]	–	–
Stage	–	–	0.260	0.797

[a] Significant

Table 6 Association of survival in relation to serum and urine clusterin levels

Survival	Serum clusterin		Urine clusterin	
	r	p	r	p
Total survival	-0.488	0.001[a]	-0.399	0.001[a]
Recurrence free survival	-0.452	0.006[a]	-0.366	0.031[a]

[a] Significant

that in lower stages (pTa and pT1). Moreover, we found that the clusterin expression level correlated significantly with tumor grade and multiplicity, but not with tumor size. In addition, 42.3% of patients with invasive TCC showed postoperative recurrence, all of them showed overexpression of clusterin mRNA. These findings provide evidence that the increased expression of clusterin is involved in TCC tumorigenesis and progression and acts as an independent predictor of postoperative recurrence in patients with superficial and invasive TCC who underwent surgery.

In the current study, we further examined the usefulness of overexpression of clusterin mRNA as a prognostic predictor and revealed that the overall survival rate for patients with high clusterin expression was significantly lower than that for patients with low clusterin expression. This is in agreement with many studies which have also documented that increased expression of clusterin was involved in the development and progression of several types of carcinomas, including breast, prostate and kidney carcinomas [10–14]. Taken together, clusterin expression may be an important prognostic factor of aggressive nature of several human cancers, including TCC of the bladder.

Miyake et al [13] have reported a close relationship between the expression level of clusterin in TCC of the bladder by Northern blot analysis and pathologic stage and tumor grade when examining human bladder carcinoma specimens. Moreover, they found that the strong expression of clusterin mRNA was an independent predictor of tumor recurrence and associated with a significantly lower overall survival rate indicating that strong clusterin expression could be used as a novel predictor of prognosis of patients with TCC of the bladder. The results in the present study are in agreement with that reported by Miyake et al. The same investigators in another study also demonstrated in vitro that human KoTCC-1 bladder carcinoma cells showed a dose-dependent increase of clusterin mRNA expression induced by Cisplatin treatment [32]. Similarly, Kruger et al [14] found that clusterin immunoreactivity, tumor stage, and nodal status were significantly associated with disease-related survival in cases of muscle invasive bladder cancer treated by radical cystectomy. They also found that bladder carcinoma specimens with high p53 immunoreactivity displayed clusterin expression in a significantly greater proportion of cases (32%) than those with low p53 immunoreactivity. Although clusterin immunoreactivity showed only a trend towards an independent prognostic relevance in Kruger et al study, they conclude that it may be used, in addition to conventional and other immunohistochemical prognostic factors, as a supplementary tool to provide more prognostic information in patients undergoing cystectomy for muscle-invasive bladder cancer [14].

These findings suggest that additional useful information about the appropriate adjuvant therapy and prognosis in patients with invasive TCC could be acquired by measuring the expression level of clusterin; that is, aggressive adjuvant therapies should be performed in patients with strong clusterin expression, and patients with weak clusterin expression might not require intensive adjuvant therapies. Many reports by Miyake et al have demonstrated that clusterin could be an optimal therapeutic target for advanced prostate cancer using antisense oligodeoxynucleotide technology [33–35].

In conclusion, a large body of evidence now supports important functions of clusterin protein expression in the pathogenesis and progression of bladder cancer. Clusterin may be considered as a potential diagnostic and prognostic biomarker for bladder cancer using urine, serum and/or molecular biology techniques. Although recent findings are very promising, future studies will have to meet the challenge to clarify specifically the roles of clusterin as potential targets for therapeutic modalities for effective cancer therapy.

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