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Diagnostic Utility of Serum Golgi Phosphoprotein 3 in Bladder Cancer Patients

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: This study assessed whether serum Golgi phosphoprotein 3 (GOLPH3) could be used as a biomarker for detecting bladder cancer.

Material/Methods: Enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) assay were performed to measure GOLPH3 expression in serum and tissue samples, respectively, of bladder cancer patients. The associations of serum GOLPH3 expression with clinicopathological factors and the diagnostic accuracy were statistically evaluated using the chi-square test and receiver operating characteristic (ROC) curve analysis.

Results: Compared with the healthy control group, serum GOLPH3 level was distinctly enhanced in bladder cancer patients ($P<0.001$). Moreover, compared to the non-malignant tissues, GOLPH3 showed positive expression in bladder cancer tissues. The abnormal GOLPH3 levels were tightly related to grade ($P=0.018$), tumor stage ($P=0.000$), lymph node status ($P=0.030$), and muscle invasion ($P=0.012$). ROC analysis showed that serum GOLPH3 exhibited a high diagnostic value to distinguish bladder cancer patients from healthy persons. The area under the ROC curve (AUC) was 0.948. The specificity and sensitivity were 92.5% and 83.8%, respectively.

Conclusions: GOLPH3 was highly expressed in bladder cancer patients and could be used as a diagnostic tool.

MeSH Keywords: Diagnosis • trans-Golgi Network • Urinary Bladder Neoplasms

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Background

Bladder cancer is the most common malignancy among males all over the world [1]. The disease displays varied clinical behaviors. Around 90% of bladder cancers are transitional cell carcinoma (TCC), while 5% are squamous cell carcinomas, and 1–2% are adenocarcinomas [2,3]. In 70–80% of cases, the malignancy is low-grade, multifocal, superficial, and papillary, and about 20% of patients have the less common muscle-invasive form [4]. At present, early detection remains one of the most urgent issues in bladder cancer research. Importantly, diagnosis at the early stage of bladder cancer significantly improves the probability of successful patient treatment. Therefore, a non-invasive and sensitive method is required for screening, diagnosis, and monitoring of the disease.

Golgi phosphoprotein 3 (GOLPH3), called GPP34/GMx33/MIDAS in animals, belongs to the trans-Golgi matrix family. GOLPH3 combines with PtdIns (4)P-rich trans-Golgi membranes and MYO18A, imparting tensile force essential for capable tubule and vesicle generation [5–7]. GOLPH3 expression was proved to be able to facilitate cellular transmutation and proliferation by activating serine/threonine kinase mechanistic target of rapamycin (mTOR) [8,9]. Moreover, growing evidence has demonstrated that GOLPH3 as an oncogene has a promising prognostic value in multiple solid tumor types, such as ovarian cancer [10], non-small cell lung cancer [11,12], prostate cancer [13], and colon cancer [14]. Especially, in ovarian cancer, serum GOLPH3 was considered to be a novel valuable indicator in clinical diagnosis [15]. However, there has been little research on the functional role of serum GOLPH3 in bladder cancer patients.

In the present study, we assessed and compared serum GOLPH3 levels in bladder cancer patients and in healthy individuals. The association of serum GOLPH3 with clinicopathological variables was also analyzed statistically, and we assessed the diagnostic capacity of serum GOLPH3 in bladder cancer patients.

Material and Methods

Patients and sample collection

The study was authorized by the Ethics Committee of the Affiliated Hospital of the Academy of Medical Sciences. All participants provided written informed consent.

A total of 117 patients newly diagnosed with bladder cancer were enrolled from the Affiliated Hospital of the Academy of Medical Sciences from October 2015 to May 2017 (power=1.00). The blood samples were collected from included patients after diagnosis. Inclusion criteria were: 1) adults; 2) diagnosed with bladder cancer by histopathological examination; 3) without any anti-cancer treatments such as chemotherapy or radiotherapy; 4) without other malignancies; 5) available clinical records. We excluded patients with diabetes, severe cardiovascular disease, or organ failure, as well as patients with other conditions influencing GOLPH3 expression. Tumor stage was defined based on the tumor-node-metastasis (TNM) staging system of the International Union Against Cancer (1997) and the World Health Organization (WHO). The clinicopathological factors of patients are shown in Table 1. In addition, 107 healthy individuals with the similar age and sex ratio at the same hospital were enrolled as a control group. The healthy individuals had normal medical examination results. None of the healthy individuals had any history of malignancy, bladder disease, or other urological diseases.

Blood samples from all participants were collected using tubes without anticoagulant. These samples were centrifuged at 3500 g for 5 min at room temperature. Serum was transferred into RNA-free EP tubes for every 500-µl aliquot and maintained at –80°C until further analysis. In addition, the malignant and adjacent non-cancerous tissues were also collected from the bladder cancer patients through radical cystectomy and transurethral resection.

Enzyme-linked immunosorbent assay (ELISA) for serum GOLPH3

GOLPH3 levels in serum samples were determined with a commercially available ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the protocol of the manufacturer (Bio Vendor Laboratory Medicine, Inc., Brno, Czechoslovakia and EXBIO, Prague, Czechoslovakia). Calibration curves were made using purified standards. Curve fitting was completed through either linear or four-parameter logistic regression based on the directions of the manufacturer.

Immunohistochemistry (IHC) assay for GOLPH3 protein in bladder cancer tissues

The protein expression of GOLPH3 in bladder cancer tissues was estimated through IHC analysis, and the experimental procedures were performed according to the standards. In brief, the tissue sections were deparaffinized by xylene and rehydrated using ethanol. After being washed with PBS buffer 3 times, the tissue sections were heated at 100°C for 30 min in buffer (pH=6.0) to retrieve the antigens. Next, the tissue sections were incubated with the primary anti-GOLPH3 antibody (Abcam, 1: 150) overnight at 4°C. Subsequently, the tissues were incubated with horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody (1: 200, Abcam, USA) for 30 min at room temperature. Finally, the tissues were stained using 3,3'-diaminobenzidine (DAB) solution, followed by counter-staining with

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Table 1. The relationship of serum *GOLPH3* expression with clinicopathological features.

Features	No. of cases (n=117)	*GOLPH3* expression		χ^2	P value
		Low (n=58)	High (n=59)		
Age (years)					
<60	54	26	28	0.081	0.775
≥60	63	32	31		
Sex					
Male	82	40	42	0.069	0.793
Female	35	18	17		
Tumor size (cm)					
<3	72	34	38	0.414	0.520
≥3	45	24	21		
Grade					
Low	68	40	28	5.558	0.018
High	49	18	31		
TNM stage					
1–2	81	51	30	18.882	0.000
3–4	36	7	29		
Lymph node metastasis					
Negative	91	50	41	4.728	0.030
Positive	26	8	18		
Muscle invasion					
No	63	38	25	6.304	0.012
Yes	54	20	34		

hematoxylin. The staining range was scored as follows: 0 points, <5%; 1 point, 5–25%; 2 points 26–50%; 3 points, 51–75%; and 4 points, >75%. The staining intensity was defined as follows: 0 points, nonstaining; 1 point, light yellow; 2 points, brownish yellow; and 3 points, brown. The final scores were the product of staining range and staining intensity. Low expression was defined as ≤3 points and high expression was defined as >3 points.

Statistical analysis

Data synthesis was conducted using the SPSS, version 18.0 (SPSS, Inc., Chicago, IL, USA). Graphs were plotted with Origin 9.0. All continuous data are listed as mean ± standard deviation (SD). We used the 2-tailed *t* test to compare differences between cases and controls. The chi-square test was used to analyze the potential association of serum GOLPH3 expression with clinicopathological variables. Receiver operating characteristic (ROC) curve method was used to assess the diagnostic significance of serum GOLPH3 in bladder cancer patients. The statistical power of the study sample size was analyzed by GPower 3.1 software. A 2-sided *P* value <0.05 was defined as statistical significance.

Results

Serum GOLPH3 expression in bladder cancer patients

We examined the expression level of GOLPH3 in the serum specimens of bladder cancer patients and in healthy controls. The serum GOLPH3 in healthy controls was 114.43±84.82 pg/ml compared with 394.25±149.46 pg/ml in serum samples of bladder cancer patients. The statistical analysis indicated that the serum GOLPH3 level was higher in bladder cancer patients than in healthy persons (*P*<0.001, Figure 1).

Positive GOLPH3 expression in bladder cancer patients

The protein expression profile of GOLPH3 in bladder cancer tissues was estimated through IHC. Representative IHC

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images are shown in Figure 2. Among the bladder cancer tissues, 83.8% (98/117) showed positive staining results, while only 17.1% (20/117) of non-malignant tissues exhibited positive staining. The levels of GOLPH3 protein were significantly higher in bladder cancer tissues than in non-cancerous specimens ($P<0.001$).

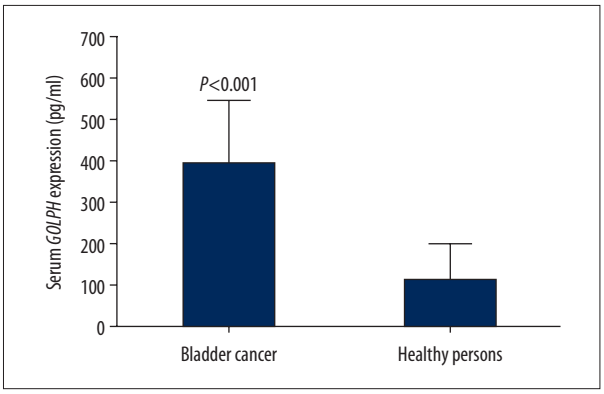

Figure 1. Serum GOLPH3 expression in bladder cancer. Compared with healthy persons, GOLPH3 expression was obviously increased in bladder cancer patients ($P<0.001$).

Association between serum GOLPH3 level and clinicopathological parameters

In order to explore whether serum GOLPH3 expression was associated with the clinicopathological parameters, we divided all patients into 2 group according to the median value of serum GOLPH3 (405.70). Patients who expressed levels of GOLPH3 above 405.70 pg/ml were assigned to the high GOLPH3 expression group, while the rest were assigned to the low GOLPH3 expression group. Close associations were observed between serum GOLPH3 expression and grade ($P=0.018$), tumor stage ($P=0.000$), lymph node status ($P=0.030$), and muscle invasion ($P=0.012$), but not with age, sex, or tumor size (all $P>0.05$, Table 1).

In addition, we also compared GOLPH3 expression between muscle-invasive and non-muscle-invasive patients. ELISA results demonstrated that serum levels of GOLPH3 were significantly higher in muscle-invasive patients than in non-muscle-invasive

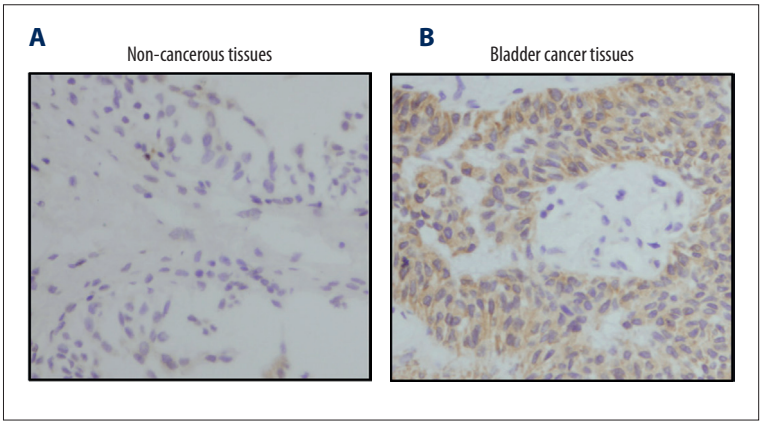

Figure 2. Representative IHC images for GOLPH3 protein expression in non-cancerous tissues (**A**) and bladder cancer tissues (**B**) (400×). GOLPH3 protein showed positive expression in bladder cancer tissues, while its expression in non-malignant tissues was negative.

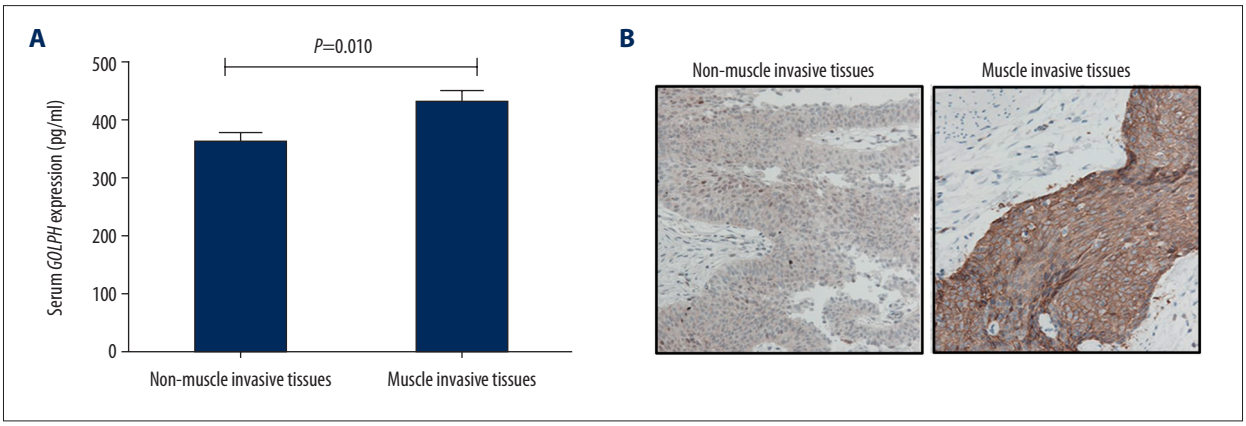

Figure 3. Comparison of GOLPH3 levels between non-muscle-invasive and muscle-invasive serum (**A**) and tissue (**B**) samples. ELISA demonstrated that serum levels of GOLPH3 were significantly higher in muscle-invasive cases than in non-muscle-invasive cases ($P=0.010$) (**A**). The representative IHC images for GOLPH3 protein in non-muscle-invasive (negative staining) and muscle-invasive tissues (positive staining) (**B**) (200×).

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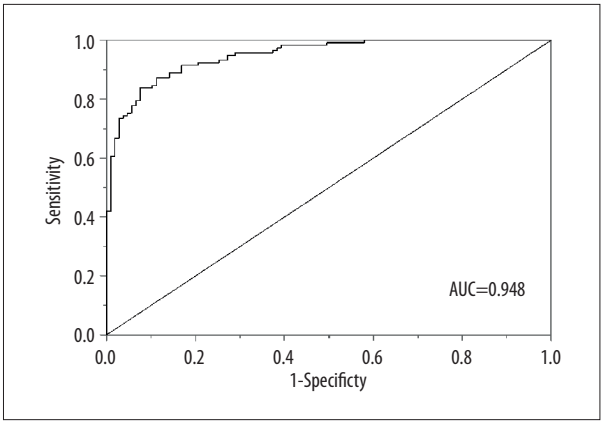

Figure 4. Receiver operating characteristics (ROC) curve analysis for serum GOLPH3 to determine the optimal cutoff value that distinguished bladder cancer patients from health persons. The area under the curve (AUC) was 0.948 and the optimal cutoff value was 235.97 pg/ml. The specificity and sensitivity were 92.5% and 83.8%, respectively.

patients (*P*=0.010) (Figure 3A). IHC was performed to investigate GOLPH3 expression in bladder cancer tissues. As displayed in Figure 3B, GOLPH3 showed positive staining results in muscle-invasive tissues and negative results in non-muscle invasive tissues. The positive rate was up to 92.6% (50/54) in muscle-invasive tissues, but was only 76.2% (48/63) non-muscle invasive specimens with positive GOLPH3 staining (*P*=0.016).

Diagnostic performance of serum GOLPH3 in bladder cancer

An ROC curve was drawn to determine a best cutoff point for distinguishing bladder cancer patients from healthy controls. The result showed that the area under the ROC curve (AUC) of serum GOLPH3 reached 0.948 (95%CI: 0.922–0.974). At an optimal cutoff value of 235.97 pg/ml, the specificity and sensitivity were 92.5% and 83.8%, respectively (Figure 4).

Discussion

Bladder cancer is a global public health problem and is the most expensive cancer to treat. Early detection of bladder cancer is the most promising approach to extend patient survival and improve patient quality of life. Bladder cancer diagnosis hinges on invasive examination of the bladder (cystoscopy) and biopsy of a bladder tumor [16,17]. Unfortunately, several limitations exist for the detection methods, such as invasion, low sensitivity, and high financial cost. Therefore, a reliable, objective, rapid, and precise predictor for diagnosis of bladder cancer is urgently needed. Bladder cancer is a complex disease which is regulated by interactions between environmental and genetic factors. Genetic factors play crucial roles in progression of bladder cancer. For example, the study carried out by Gong et al. reported that Rab11 as an oncogene could enhance the malignant progression of bladder cancer through activating the NF-κB signaling pathway [18]. Given their key roles in tumorigenesis, genetic factors are considered promising candidate biomarkers for cancer. Li et al. suggested that the expression of B7 homolog 1 (B7H1) is closely correlated with disease progression and clinical outcomes of bladder cancer, and it might be a potential prognostic biomarker [19]. Cancer Susceptibility Candidate 2a (CASC2a), a long non-coding RNA, was confirmed to be significantly correlated with disease progression of bladder cancer, and might be useful as an indicator for early recurrence [20]. In the present study, we explored whether cancer-related genes could provide an effective approach for early screening and prognosis evaluation for patients with bladder cancer.

GOLPH3, located on human chromosome 5p13, is a phosphorylated protein, which has a role in anterograde and retrograde Golgi trafficking [8,21]. A large body of evidence proves that GOLPH3 is upregulated in several human tumors, such as esophageal squamous cell carcinoma [2]2, prostate cancer [23], renal cell carcinoma [24], and pancreatic cancer [25], indicating that elevated GOLPH3 level has an effect on onset and progression for various tumor types. Interestingly, several investigators reported that circulating GOLPH3 level is also upregulated in tumors, revealing that circulating GOLPH3 as a noninvasive and easy indicator for the detection of cancers. Fan et al. demonstrated that the difference in the levels of serum GOLPH3 in ovarian cancer patients was statistically significant compared with that in the benign group and the control group, suggesting serum GOLPH3 as a valuable diagnostic biomarker of the disease [15]. Based on ELISA results, Hu et al. reported that, compared to healthy individuals, serum concentrations of GOLPH3 were remarkably higher in gastric cancer patients [26]. However, to the best of our knowledge, few studies have assessed the relationship of serum GOLPH3 level with the detection of bladder cancer.

Through our research, we determined GOLPH3 levels in bladder cancer patients and healthy persons. It was revealed that serum GOLPH3 was highly expressed in bladder cancer patients compared with healthy persons, and GOLPH3 showed positive expression in bladder cancer tissues in comparison with non-malignant tissues. GOLPH3 might act as an oncogene in the disease. The result was in line with the previous study reported by Zhang et al., who proved that GOLPH3 was obviously upregulated in bladder cancer tissues and cells, and was a promising prognostic indicator and therapeutic target for bladder cancer [27]. The study also showed the association of serum GOLPH3 expression with clinicopathological variables, indicating that abnormal GOLPH3 level was significantly

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correlated with grade, tumor stage, and lymph node status. It was demonstrated that GOLPH3 is involved in the development and metastasis of bladder cancer, and the authors revealed that in most human urothelial bladder cancer tissues, GOLPH3 expression levels are higher than in matched normal bladder tissues [28]. More importantly, according to serum GOLPH3 levels, we plotted the ROC curve, showing that serum GOLPH3 had a meaningful diagnostic significance in patients with bladder cancer.

The present study has several limitations. First, the sample size was relatively small, which might reduce the strength of our results. Second, all subjects were from China, so the results may not be generally applicable to populations of other ethnic origins. As a consequence, despite the unlikely effect of ethnicity, use of serum GOLPH3 expression as a diagnostic biomarker must be confirmed in diverse ethnic populations. Third, although serum GOLPH3 is a noninvasive biomarker for early detection of bladder cancer, the criterion standard for bladder cancer should also be based on cystoscopy or urine cytology. Serum GOLPH3 might be used as an adjunct tool to screen individuals with high risk of bladder cancer. In addition, we found that the expression of GOLPH3 was closely association with clinical parameters of bladder cancer patients. Serum GOLPH3 might be useful as an indicator to monitor treatment effects. Further multi-center investigations are required to address the above issues.

Conclusions

GOLPH3 was higher in serum samples of bladder cancer patients compared to healthy controls. It might be a potential index for bladder cancer diagnosis.

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