Urine *MACC1* Plays an Indicative Role in Diagnosing Bladder Cancer

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Abstract

Background: Present study aimed to estimate the diagnostic efficacy of urine Metastasis-associated in colon cancer 1 (*MACC1*) for bladder cancer patients.

Methods: Urine *MACC1* expression was measured in bladder cancer patients and controls via quantitative real-time polymerase chain reaction (qRT-PCR) assay. Chi-square test was used to assess the correlation of urine *MACC1* levels with clinicopathological features of the patients. The diagnostic performance of urine *MACC1* was evaluated by receiver operating characteristics (ROC) curve.

Results: Urine *MACC1* was obviously elevated in bladder cancer patients compared with controls ($P<0.001$). What's more, abnormal urine *MACC1* expression was significantly correlated with differentiation ($P=0.013$), tumor stage ($P=0.023$), lymph node metastasis ($P=0.033$) and distant metastasis ($P=0.026$). Furthermore, according to ROC analysis, urine *MACC1* could distinguish bladder cancer patients from controls with an AUC (the areas under the ROC curve) of 0.909. The cut-off value of urine *MACC1* for bladder cancer diagnosis was 1.275, with 79.8% specificity and 87.3% sensitivity.

Conclusion: Urine *MACC1* may be act as a biomarkers for diagnosis of bladder cancer.

Background

Bladder cancer is one of the most frequently diagnosed malignancies in the urinary system around the world [1]. About 70–80% cases are confirmed as non-muscle-invasive (Ta and T1 tumors) bladder cancer (NMIBC), and the rest belongs to be muscle-invasive (T2-T4 tumors) bladder cancer (MIBC) [2]. Although the NMIBC is not life-threatening, with a five year-survival more than 90% [3]. However, approximate 50–70% of NMIBC will relapse, and 10–15% of them progress to MIBC within five-year of diagnosis [4]. Therefore, early diagnosis and postoperative monitoring are crucial for treatment and clinical outcomes of bladder cancer patients. At present, cystoscopy is the golden standard for early screening and monitoring of bladder cancer. However, the high cost and invasive procedures may limit its wide application [5]. Therefore, non-invasive biomarkers are in urgent need for patients with bladder cancer. The genetic materials, such as protein, DNA and RNA, derived from bladder tissues could be detected by easy methods in urine specimens that are considered as promising candidates for early detection and monitoring of bladder cancer [6, 7].

Metastasis-associated in colon cancer 1 (*MACC1*) initially detected in colon cancer [8] is confirmed a new cancer-related gene, located on human chromosome 7 (7p21.1). *MACC1* has the capacity to induce cell proliferation, migration and invasion [9, 10]. Moreover, numerous reports have proved that *MACC1* was highly expressed in human tumors, such as nasopharyngeal carcinoma, glioma, gastric cancer and so on [11–13]. In bladder cancer, Xu et al. reported that knockdown of *MACC1* using RNA interference could induce inhibition on proliferation and invasion of bladder cancer cells in vitro [14]. However, the expression patterns of urine *MACC1*, as well as its diagnostic efficacy for bladder cancer remained poorly known.

In the present study, we measured the expression status of *MACC1* in urine samples collected from bladder cancer patients and non-malignant controls. Moreover, the relationship between urine *MACC1* level and clinicopathological factors was also analyzed. In addition, the diagnostic value of urine *MACC1* was evaluated in bladder cancer patient.

Methods

Patients and sample collection

The study was examined and approved by the Ethical Committee of Huaihe Hospital of Henan University. All participants signed written informed consents in the study.

118 patients who were newly diagnosed with bladder cancer were recruited from Huaihe Hospital of Henan University. Diagnosis of bladder cancer was based on pathological examinations. None of the patients had received any anti-tumor treatments. The demographic data and medical history of the patients were recorded in Table 1. Tumor classification was established according to Union for International Cancer Control (UICC) 2010 TNM system. At the same time, 104 age- and gender-matched subjects, including 92 healthy volunteers and 12 subjects with urolithiasis, were collected from the same hospital as controls.

Table 1
The relationship between circulating *MACC1* and clinicopathological variables of bladder cancer cases

Variables	No. of case n = 118	MACC1 expression		χ^2	Pvalue
		Low (n = 54)	High (n = 64)		
Age (years)					
< 53	58	25	33	0.325	0.569
>=53	60	29	31		
Gender					
Male	92	45	47	1.670	0.196
Female	26	9	17		
Tumor size (cm)					
< 3.75	67	32	35	0.249	0.617
>=3.75	51	22	29		
Differentiation					
High-moderate	51	30	21	6.173	0.013
Low	67	24	43		
Tumor stage					
Ta-T1	48	28	20	5.151	0.023
T2-T4	70	26	44		
Lymph node metastasis					
Negative	53	30	23	4.556	0.033
Positive	65	24	41		
Distant metastasis					
Absent	42	25	17	4.975	0.026
Present	76	29	47		

Urine samples were collected from each participant in the morning, and then centrifuged at 600 × gravity for 5 minutes at 4°C. Supernatant was decanted, aliquoted into Eppendorf tubes and maintained at -80°C until use.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from urine samples using TRIzol reagent (Invitrogen, Grand Island, NY, USA) based on the protocols of the manufacturer. The concentration and purity of RNA were measured at 260 and 280 nm with BioPhotometer plus (Eppendorf AG, Hamburger, Germany).

Then the first chain of cDNA was generated by reverse transcription utilizing the HiFi-MMLV cDNA Kit (Beijing CoWin Biotech Co. Ltd., Beijing, China) according to the instruction of the manufacturer. QRT-PCR was carried out using a SYBR green I Master Mix kit (Invitrogen). The primers sequence was synthesized according to the previous report [14]. β -actin was employed as reference control. The expression of *MACC1* gene was normalized to the internal control, and calculated using $2^{-\Delta\Delta Ct}$ method. Each test was repeated at least three times.

Statistical analysis

All statistical analyses were carried out using SPSS version 18.0 (SPSS, Chicago, USA), and graphs were plotted by GraphPad Prism 5.0 (GraphPad Software). The statistical differences of *MACC1* expression between bladder cancer patients and controls were assessed via independent Student *t* test. Chi-square test was applied to analyze the association of urine *MACC1* with clinicopathological features of bladder cancer patients. The diagnostic capacity of urine *MACC1* for bladder cancer was evaluated by receiver operating characteristics (ROC) curve. The specificity and sensitivity, as well as the area under the curve (AUC) value were calculated to estimate the diagnostic performance of urine *MACC1* for bladder cancer. *P* value less than 0.05 was regarded as statistical significance.

Results

Baseline characteristics of the bladder cancer patients

A total of 118 bladder cancer patients including 92 males and 26 females were included in our study, and their average age was 58.76 ± 10.56 years. 51 patients had tumor size more than 3.75 cm. The pathological examinations suggested that 67 patients exhibited low differentiation, and 51 patients exhibited high and moderate differentiation. According to TNM staging system, 48 patients were diagnosed with stages Ta-T1, and 65 patients diagnosed with stages T2-T4. Lymph node metastasis was observed in 65 patients, while distant metastasis was observed in 76 patients. The clinical information of the included patients were summarized in Table 1.

Urine *MACC1* level in bladder cancer patients

We analyzed the expression levels of *MACC1* mRNA in urine samples of 118 bladder cancer patients and 104 controls. According to qRT-PCR assay, urine *MACC1* levels were dramatically up-regulated in bladder cancer patients, compared to the controls ($P < 0.001$, Fig. 1).

Association of urine *MACC1* with clinicopathological parameters

To further analyze the relationship between urine *MACC1* levels and clinicopathological parameters, we divided all patients into two groups based on the median value of urine *MACC1* expression: low *MACC1* expression group (n = 54) and high *MACC1* expression group (n = 64). As indicated in Table 1, the elevated urine *MACC1* expression was distinctly correlated with bladder cancer patients' differentiation ($P = 0.013$), tumor stage ($P = 0.023$), lymph node metastasis ($P = 0.033$), and distant metastasis ($P = 0.026$). Nevertheless, urine *MACC1* levels had no association with other clinicopathological factors, such as age, gender, or tumor size (all $P > 0.05$).

Diagnostic performance of urine *MACC1* in bladder cancer

ROC curve analysis illustrated that urine *MACC1* could distinguish bladder cancer patients from healthy controls with an AUC (the areas under the ROC curve) of 0.909 (95%CI: 0.872-946) (Fig. 2). At a cutoff value of 1.275, the optimal specificity and sensitivity values were 79.8%, and 87.3%, respectively (Fig. 2).

Discussion

Bladder cancer is a heterogeneous disease with unpredictable clinical course, representing a leading cancer-related deaths [15]. At present, there are several major risk factors identified in bladder cancer, such as cigarette smoking, chronic infection by *Schistosoma haematobium*, and exposure to the carcinogens [16, 17]. Early diagnosis and close monitoring are key for clinical outcomes of patients with bladder cancer. Unfortunately, there are no recommended biomarkers for bladder cancer in routine clinical application until now [18]. In current study, we confirmed that the expression profiles of urine *MACC1* were significantly different between bladder cancer patients and non-malignant individuals. Urine *MACC1* might be a potential diagnostic biomarker for bladder cancer.

MACC1 encodes a protein containing four domains: ZU5, SH3 and two C-terminal death domains [19]. Growing evidences have demonstrated that the over-expression of *MACC1* may contribute to malignant tumor progression through enhancing cell proliferation, migration and invasion [8, 20–22]. The expression pattern of *MACC1* in human fluids may have the possible to serve as a biomarker for early diagnosis and prognosis evaluation in several types of human cancers. For instance, Ashkorab *et al.* reported that *MACC1* transcripts were obviously increased in plasma samples of colon adenoma patients compared with normal patients. Moreover, plasma *MACC1* could distinguish the colon adenoma patients from healthy individuals [23]. Tan *et al.* showed that serum *MACC1* was highly expressed in breast cancer patients, and it might be a reliable biomarker for early diagnosis and prognosis evaluation in breast cancer patients [24]. A study carried out by Wang *et al.*, reported that plasma *MACC1* expression exhibited significantly increased in non-small cell lung cancer patients, and might be a promising non-invasive factor for diagnosis and prognosis of the disease [25]. Taken together, the expression of *MACC1* in human fluids shows specific to cancer, which may be employed as biomarker for diagnosis and

monitoring of cancer. However, the clinical significance of urine *MACC1* in bladder cancer was rarely reported.

In this study, urine *MACC1* mRNA expression was examined in bladder cancer patients and controls using qRT-PCR method. The expression of *MACC1* was significantly increased in urine samples of bladder cancer patients in comparison to controls. The result was in line with the previous study reported by Xu et al., revealing that *MACC1* mRNA levels were up-regulated in human urothelial carcinoma [14]. In addition, we estimated the association of urine *MACC1* expression with clinicopathological parameters of bladder cancer patients. The abnormal *MACC1* expression in urine samples was remarkably associated with tumor differentiation, tumor stage, lymph node metastasis, and distant metastasis. The bladder cancer patients with high expression of *MACC1* were more likely to undergo malignant disease progression. The in vitro experiments carried out by Xu et al. suggested that *MACC1* could regulate the biological behaviors of human bladder urothelial carcinoma cell line T24 [14]. Over-expression of *MACC1* might contribute to progression of bladder cancer through enhancing the malignant biological behaviors of cancer cells.

Given the different levels of urine *MACC1* in bladder cancer cases and healthy individuals, we hypothesized that urine *MACC1* might be a potential diagnostic biomarker for bladder cancer. ROC curves were plotted to estimate the diagnostic performance of urine *MACC1* in bladder cancer. Urine *MACC1* could distinguish bladder cancer patients from the non-malignant controls with high sensitivity and specificity. Urine *MACC1* might act as a valid and reliable biomarker for early screening of patients suffering from bladder cancer.

This study had certain limitations. Firstly, the number of patients recruited in the study was relatively small, and further studies with a large scale number of patients are required to confirm our findings. Secondly, the patients corrected in our study all came from one hospital that might cause bias to our results. In addition, the molecular mechanisms underlying the oncogenic function of *MACC1* remained poorly known. Further in vitro and in vivo experiments are required to address the issues. Therefore, multicenteric studies should be considered to verify the value of *MACC1* as a diagnostic biomarker for bladder cancer.

Conclusions

In conclusion, urine *MACC1* level is up-regulated in bladder cancer patients and closely associated with tumor metastasis and progression. Urine *MACC1* may be a valuable biomarker for early screening of bladder cancer.

List Of Abbreviations

Metastasis-associated in colon cancer 1 (*MACC1*)

Quantitative real-time polymerase chain reaction (qRT-PCR)

Receiver operating characteristics (ROC)

Non-muscle-invasive (Ta and T1 tumors) bladder cancer (NMIBC)

Muscle-invasive (T2-T4 tumors) bladder cancer (MIBC)

Union for International Cancer Control (UICC)

Area under the curve (AUC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Huaihe Hospital of Henan University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors' contributions

H.Z., X.L. design of the work; W.T. the acquisition, analysis, L.G. interpretation of data; Z.Y. the creation of new software used in the work; X.B. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

Figure 1

Expression of urine MACC1 determined by qRT-PCR assay in bladder cancer patients and controls. Urine MACC1 levels were significantly increased in bladder cancer patients compared with controls (***: $P < 0.001$).

Figure 1

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Figure 2

Receiver operating characteristics (ROC) curve analysis for the diagnostic accuracy of urinary MACC1 in bladder cancer. The AUC (areas under the ROC curve) value was 0.909 (95%CI: 0.872-0.946). The specificity and sensitivity were 79.8% and 87.3%, respectively. All the data revealed that urine MACC1 could distinguish bladder cancer patients from the controls, and the cut-off value was 1.275.

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