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Original article

Plasma thymosin-α1 level as a potential biomarker in urothelial and renal cell carcinoma☆

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Abstract

Objectives: To determine the plasma levels of thymosin-α1 (TA1) and prothymosin-α (PTMA) proteins in renal cell carcinoma (RCC) or urothelial carcinoma (UC) patients, and explore the potential of these 2 molecules as biomarkers.

Materials and methods: Blood samples were taken from 50 consecutive patients with RCC, 97 with UC, and 55 with benign urologic diseases before surgery. Their clinical characteristics were obtained from medical record review. Plasma TA1 and PTMA levels were measured using enzyme-linked immunosorbent assay and their correlation with tumor grade, pathologic stage, and survival were explored.

Results: Plasma TA1 levels were significantly lower in RCC patients than in UC or benign patients, particularly in UC of the renal pelvis patients ($P < 0.0001$). Plasma PTMA levels were also significantly lower in UC patients compared with RCC patients and benign patients ($P < 0.05$). Plasma TA1 levels inversely correlated with pathologic stage both in bladder cancer and RCC patients ($P = 0.03$ and 0.02, respectively). Both plasma TA1 and PTMA did not correlate with tumor grade. Plasma TA1 was a prognostic indicator for progression-free and disease-specific overall survival in bladder cancer patients ($P = 0.008$ and 0.04, respectively).

Conclusions: Plasma TA1 level may be a biomarker for differentiating between UC and RCC. It may also be a prognostic factor for disease progression and disease-specific survival in bladder cancer patients. These findings warrant more studies for validation.

Keywords: Kidney neoplasms; Urothelial neoplasms; Thymosin

1. Introduction

Urothelial carcinoma (UC) denotes tumors derived from uroepithelial cells of the urinary tract. Most are categorized into upper urothelial carcinoma (UUC) and bladder cancer (BC) based on their location. However, a standard biomarker for preoperative differential diagnosis, detection, or follow-up for UC patients remains lacking [1]. Kidney tumors consist of tumors derived from the renal pelvis or parenchyma, and the majority of the latter are renal cell carcinoma (RCC). Although UUC is not common throughout the world [2,3], there is a higher incidence in Taiwan, where UC of the renal pelvis represents approximately 40% of all kidney tumors [4,5]. In clinical practice, it is a bit difficult to differentiate RCC invading centrally into the renal sinus or pelvis from renal pelvis tumors invading into the renal parenchyma using imaging studies alone [6,7]. Thus, much effort is required to explore any useful marker for these issues.

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Prothymosin-α (PTMA) is a small acid protein that plays important roles in several biological functions, including cell apoptosis, cell proliferation, and chromatin decondensation [8]. Previous studies have demonstrated increased and aberrant PTMA expression in human bladder cancers compared with paired normal adjacent bladder tissues using an anti-PTMA antibody against the first 1–31 amino acids (2F11) [9]. PTMA is also overexpressed in UUCs, and its subcellular localization can provide significant prognostic information for subsequent tumor recurrence in the residual urinary tract after nephro-ureterectomy [10]. Moreover, urine PTMA levels may be a novel marker for detecting bladder cancer occurrence or recurrence [11].

Thymosin α1 (TA1) is a polypeptide with immunoregulatory properties first isolated from thymic extracts. TA1 corresponds to the first 28 amino acid residues of PTMA that has been considered the putative precursor of TA1 [12]. Although the real process of TA1 production remains unclear, PTMA is reportedly processed into TA1 by lysosomal asparaginyl endopeptidase [13].

To date, there is no report investigating the clinical relevance of plasma TA1 or PTMA levels in cancer patients. The current study aimed to measure plasma TA1 and PTMA levels in UC and RCC patients and explore whether these 2 proteins have the potential to be tumor biomarkers.

2. Materials and methods

2.1. Study patients and blood samples

Blood samples were collected from 147 consecutive patients, including 97 UC [renal pelvis (n = 29), ureter (n = 15), and urinary bladder (n = 53)] and 50 RCC patients, and from 55 patients with benign diseases. Patients with either newly-diagnosed RCC or UC, or recurrent UC who underwent nephrouretectomy or endoscopic bladder tumor resection were enrolled. Blood samples were collected and stored at −80°C. The presence of RCC or UC was confirmed by pathology reports, and patients were excluded if they had no histologic confirmation of malignancy despite an initial diagnosis based on imaging studies or cystoscopic findings. The Chia-Yi Christian Hospital Ethics Review Board approved the study protocol (IRB number 097019), and each patient provided written informed consent.

To compare with BC or RCC, random, age- and gender-matched control blood samples were taken from the 55 patients with benign diseases treated at the Department of Urology before surgery. These patients had benign urologic diseases, such as urolithiasis, benign prostatic hyperplasia, or inguinal hernia, and underwent endoscopic or surgical intervention. Patients who had a malignancy other than RCC or transitional cell carcinoma (TCC), such as prostate cancer or testicular cancer, were excluded.

Comprehensive patient information collected from medical records was summarized (Table 1). Relevant histologic information, surgical history, and pathologic findings were recorded. Pathologic stage was assigned according to the 2002 TNM staging classification system. The tumor grading system was the Fuhrman nuclear grading system for RCC and the 2004 WHO grading system for UC. All the patients received regular follow-up examinations based on their individual pathologic type.

Table 1
Basic characteristics of the study subjects

Variable	Benign	UUC	BC	RCC
Total no. of patients	55	44	53	50
Male	40	10	39	39
Female	15	34	14	11
Age				
Mean, SD	62.3, 13.6	68.6, 11.4	66.2, 13.9	61.7, 13.5
Median, range	66, 32–86	68, 44–88	68, 33–92	63, 27–83
Follow-up, m (median)	—	17	17	30
Grade*				
I-II		22	36	41
III-IV	—	22	17	9
Pathologic staging				
T0–1	—	20	34	28
T2–4	—	24	19	22
Outcome				
Recurrence	—	7	19	—
Progression	—	4	12	5
Death	—	7	9	9

BC = bladder cancer; UUC = upper urothelial carcinoma; RCC = renal cell carcinoma; SD = standard deviation.

* In RCC, Fuhrman nuclear grading I∼IV was used; in UUC and BC, either low- or high-grade papillary tumors were used according to the 2004 WHO grading.

2.2. TA1 and PTMA enzyme-linked immunosorbent assay (ELISA)

A total of 10 ml venous blood was collected into EDTA-containing vacuette tubes (Greiner Bio-One) preoperatively and centrifuged at 3,000 g (10 min, 4°C). Plasma was aliquoted into 3 fresh Eppendorf tubes and stored at −80°C. Plasma TA1 levels were measured using the human TA1 ELISA kit (APLCO Diagnostics, NH) following the manufacturer's protocol. Briefly, sample or standard solutions were pre-incubated with polyclonal rabbit antibodies directed against synthetic TA1 for 18 hours at 4°C and then added to the TA1-pre-coated microplate. After 90 minutes, the solution was washed out. A peroxidase-conjugated antibody was used for detection and quantification and tetramethylbenzidine (TMB) was the peroxidase substrate. The enzymatic reaction was terminated by an acidic stop solution. A dose response curve of absorbance unit [optical density (OD) at 450 nm vs. concentration] was generated using the values obtained from the standards. The quantity of TA1 present in the samples was determined directly from this curve.

Plasma PTMA levels were measured using an antibody-sandwich ELISA as previously described [11]. Briefly, rab-