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Telomerase activity in urine sediments as a tool for noninvasive detection of bladder cancer

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Abstract

Telomerase is extensively investigated as potential diagnostic and prognostic marker in human tumors. In this study, we determined telomerase activity in histological specimens and voided urine of 52 human bladder cancers. Using the PCR-ELISA method telomerase activity was found in 21 (88%) of the 24 tumor tissues and in the corresponding sediments from voided urine of patients with superficial bladder carcinoma (Ta/T1). In case of muscle-invasive tumors (T2–T4), telomerase activity was found in 27 (96%) of the 28 tumor tissues and in 26 (93%) of the 28 urine sediments. Enzyme activity was not detected in 13 control urine sediments. Telomerase activity was not significantly associated with clinicopathological parameters supporting the diagnostic rather than prognostic value of this marker in bladder cancer. The present study demonstrates that telomerase activity detection in voided urine has high potential for noninvasive diagnosis of superficial bladder tumors.

Keywords: Telomerase activity; Bladder cancer; Urine sediments; TRAP assay

1. Introduction

Telomeres are distal structures at the ends of all eukaryotic chromosomes composed of hundreds to thousands of tandem hexanuclotide $(TTAGGG)_n$ repeats and a plethora of associated proteins [1]. They stabilize chromosome ends by preventing end-to-end fusion of chromosomes, nuclear degradation and interchromosomal recombination [2]. In most normal cells, telomeres shorten with each cell division as a result of inability of DNA polymerase to replicate chromosome ends [3]. Too short telomeres trigger DNA damage/apoptotic signal and cellular senescence [4]. Telomere length shortening can be reversed by telomerase, an enzyme that extends the lifespan of the cells [5]. Telomerase is a specialized ribonucleoprotein polymerase composed of RNA subunit, hTR (human telomerase RNA), and a catalytic protein component hTERT (human telomerase reverse transciptase), which can elongate telomeric DNA using its own RNA subunit as a template [6]. Telomerase activity is generally found in stem cells, hematopoietic progenitor cells, activated lymphocytes, fetal and cancer cells [7–11]. As telomerase activity is observed

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in a variety of malignant tumors, it is considered as a sensitive marker that enables quick and highly specific identification of malignant transformation [12–15]. Recently, detection of telomerase activity in voided urine of patients with bladder cancer seems to be a useful tool in cancer diagnosis, prognosis and monitoring residual disease [16].

Bladder cancer accounts for over 10,000 deaths in Central and Eastern Europe and 2000 deaths per year in Poland [17]. Because superficial bladder cancer progress to invasive disease in 15–30% it is crucial to diagnose these patients at the earliest stage as possible and predict these who will progress and thus will require more aggressive therapy. Results gained by conventional bladder diagnostical tools based on cystoscopy, cytology, histology of the tumor and its clinical behavior used to predict a specific outcome of bladder cancer treatment are far from perfect and are well demonstrated in literature especially concerning superficial bladder tumors [18]. In this regard, it has been shown that telomerase is readily detected in human bladder cancer tissues [19,20]. However, clinical utility of telomerase activity measurement in exfoliated urinary cells is controversial since it was observed in some studies [20] but not in others [21]. In our study, we address the question of whether PCR-based telomerase activity detection in voided urine may be used as a sensitive marker of bladder cancer?

2. Materials and methods

2.1. Patients

Bladder cancer specimens and voided urine were obtained from 52 patients who underwent transurethral resection (TUR) or cystectomy at the Department of Urology at the Medical University of Gdansk. Investigations reported here were approved by the Medical University of Gdansk Ethics Committee. The specimens were staged according to the fifth edition of the TNM classification [22] and each tumor was reviewed for histological grading according to WHO (1973). In addition, 13 urine specimens were obtained as normal control; two of these specimens were obtained from patients undergoing cystoscopy from reasons unrelated to bladder cancer and 11 from healthy volunteers. Approximately 50 ml of the first morning voided urine sample was collected prior to surgery, immediately cooled in ice and centrifuged at $1600\times g$ for 7 min at 4 °C. The treatment of these samples immediately after collection reduces the denaturing effects of RNases and proteases that are present in the urine. Urinary sediments were washed twice with cold phosphate buffered saline and after last centrifugation the cell pellet was suspended in 200 μl of ice-cold lysis buffer (CHAPS) provided with a commercial kit. After 30 min of incubation on ice, the lysates were centrifuged at $16{,}000\times g$ 4 °C, and the supernatant was rapidly frozen in liquid nitrogen and stored at −80 °C until the assay. Frozen tissue samples were powdered in liquid nitrogen, followed by homogenization in 200 μl ice-cold lysis buffer (CHAPS) and incubated for 30 min at 4 °C. The lysates were centrifuged at $16{,}000\times g$ for 20 min at 4 °C, frozen and stored as described above until use. Tissues collected included tumor tissue that was confirmed by subsequent histopathological examination at the Pathology Department of the Medical University of Gdansk. The patients' mean age was 62 years (ranging from 32 to 79). The clinicopathological characteristic is presented in Table 1.

2.2. Telomerase activity assay

Telomerase activity in tissue and urine sediment samples was measured using TeloTTAGGG

Table 1
Clinicopathological characteristics of 52 bladder cancer patients

Variable	No. Pts. (%)
Female	11 (21)
Male	41 (79)
Age	
≥65	21 (40)
<65	31 (60)
Grade	
Low grade	20 (38)
Moderate/high grade	32 (62)
Pathological stage	
Pta	3 (6)
pT1	21 (40)
pT2–pT4	28 (54)
Lymph node status	
Positive	8 (15)
Negative	44 (85)