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Differential PTEN Protein Expression Profiles in Superficial versus Invasive Bladder Cancers

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Key Words
PTEN · Tumor-suppressor gene · Transitional cell carcinoma · Bladder

Abstract
Introduction: The prognostic significance of PTEN protein loss in bladder cancer is not well established. The objective of this study was to investigate the PTEN expression profile in superficial noninvasive papillary transitional cell carcinoma (TCC) versus invasive TCC and compared the results with pathological and clinical parameters. ***Materials and Methods:*** Bladder tumor samples were obtained from 29 patients who underwent surgery for superficial (n = 11) and invasive (n = 18) bladder cancers at the Akdeniz University Hospital. The patient profile including sex, age, histological grade and the stage, presence of carcinoma in situ, cystoscopy findings (tumor size, location, multiplicity) were obtained by examining the patients' medical records. No patient received anticancer agents prior to the operation. Western blotting was performed using bladder carcinoma samples in order to determine the level of PTEN protein expression for each patient. ***Results:*** Only 4 (13.7%) patients with bladder carcinoma manifested a decrease in the level of PTEN expression. Regarding the correlation between tumor stage and the PTEN expression, with the exception of patient 23 all patients who displayed a reduction in PTEN expression had muscle-invasive TCC. ***Conclusion:*** Future studies with a clinical follow-up will be needed to determine if those superficial tumors with decreased PTEN expression are going to progress to a later stage. Based on our results PTEN by itself does not seem to be a good candidate as an independent marker to predict the behavior of bladder cancers.

Introduction

Transitional cell carcinoma (TCC) of the bladder is the fifth most common solid malignancy in the United States, and is diagnosed in approximately 54,000 patients and results in 12,000 deaths annually [1]. Bladder cancer is characterized by a diverse biological behavior. 70–80% of these tumors present as superficial lesions that recur in 30–90% of the patients [2]. 15–20% of these recurrences eventually become invasive/metastatic [3]. Patients with invasive carcinomas are usually treated by radical cystectomy. Nonetheless, metastatic disease appears after surgery in approximately 50% of the cases [4]. While several biological and molecular parameters have been consid-

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ered as potential prognostic markers for bladder cancer, so far only the tumor grading and staging have been the most important prognostic variables [5]. There is, however, significant intra- and inter-observer variation in the reporting of tumor grade and stage, therefore, more reliable and objective indicators of prognosis are required [6]. Therefore, current investigations have been targeting to identify molecular markers for prognosis and metastatic potential of bladder cancer.

The PTEN gene (MMAC1-mutated in multiple advanced cancers) is a novel candidate tumor suppressor located on chromosomal band 10q23.3 [7]. PTEN encodes a dual-specificity phosphatase which dephosphorylates focal adhesion kinase resulting in the inhibition of cell migration, spreading and focal adhesion formation. PTEN plays an important role in the modulation of the 1-phosphatidylinositol 3-kinase pathway, which is involved in cell proliferation and survival [8]. A few studies have investigated the role of PTEN in the tumorigenesis of bladder cancer [9–11]. However, the prognostic significance of PTEN protein loss in bladder cancer is not well established. The objective of this study was to investigate PTEN expression in superficial noninvasive papillary TCC versus invasive TCC and comparing these results with pathological and clinical parameters.

Materials and Methods

Clinical Assessment of Patients with Bladder Cancer

Bladder tumor samples were obtained from 29 patients who underwent surgery for superficial and invasive bladder cancers at the Akdeniz University Hospital. Of these 29 cases, 11 cases had superficial TCC, and 18 had invasive TCC. Tumor grade 1:2:3 ratio was 1:9:19. The 25 male and 4 female patients had a mean age of 65 (range 40–83) years. The mean follow-up for patients was 7.2 (range 1–19) months. All patients underwent transurethral resection of the primary tumor. Blocks were selected by viewing the original pathologic slides. The patient characteristics including sex, age at the time of surgery, histological grade and stage, presence of carcinoma in situ, and cystoscopy features (tumor size, location, multiplicity) were obtained by examining the medical records. No patient received anticancer therapy prior to the operation. Staging was performed according to the 1997 TNM classification, while grading was based on the World Health Organization (WHO) classification [12, 13]. Recurrence was defined as any evidence of tumor in a retained bladder at least 3 months after treatment. Disease progression was defined as the development of invasive carcinoma (stage pT1 or higher) when the initial diagnosis was pTis, pTa, or the development of muscle-invasive carcinoma (stage pT2 or higher) when the initial diagnosis was pT1. Cystoscopy was performed every 3 months for 2 years after transurethral resection, then every 6 months from 3 to 4 years and annually after 4 years.

Western Blot Analysis

After the confirmation of tumor presence, tumor tissues were homogenized in Triton X-100 buffer with 500 m*M* HEPES (pH 7.0), 150 m*M* NaCl, 10% glycerol, 1 m*M* EDTA, and 1.2% Triton X-100. SDS-PAGE, blotting and developing procedures of the Western blot assay were performed according to the protocol published previously [14]. A polyclonal antibody raised against PTEN (Santa Cruz, Cat No. SC-9145) was utilized to detect PTEN expression in bladder tissue. As an internal control, GAPDH expression was detected using GAPDH antibody (Biodesign, Cat No. H86504) to normalize PTEN expression in each blot. Densitometric analysis was performed using Scion Image software (NIH). Control samples were surgically obtained from the pathologically normal bladder epithelium of the same patient. The average of this densitometric analysis was used to compare and find the percent reduction in PTEN expression in patients with bladder tumor.

Results

Bladder tissue was obtained surgically from 11 patients with superficial and 18 patients with advanced bladder cancer. Five of the superficial bladder tumor patients were staged as pTa and 6 as pT1. Ten of the advanced bladder tumor patients were staged as pT2a, 4 as pT2b, 3 as pT3a and 1 as pT3b. In addition, 1 patient had lymph node metastasis (pT3bN+) and 2 patients had bone metastases and were assigned as pT3aM+.

Bladder samples were analyzed by Western blotting to determine the level of PTEN expression as described in the Materials and Methods. Tumor samples and their PTEN expression status are listed in table 1. Only 4 (13.7%) cases (patients 7, 21, 22 and 23) manifested a decrease in the level of PTEN expression (fig. 1). As far as the tumor stage and PTEN expression are concerned, 3 of 4 patients with a reduction in the level of PTEN expression had muscle-invasive TCC. Although muscle-invasive TCC was not found in case 23, the patient still showed an about 85% reduction in the level of PTEN expression.

Discussion

The prognosis of patients with bladder cancer is strongly dependent on whether the lesion is superficial or invasive upon initial presentation. In addition, a significant number of patients presenting with superficial disease have invasive tumor during follow-up. Understanding how superficial bladder cancer progresses to invasive forms of the disease is of paramount importance for early diagnosis and successful treatment. The molecular

mechanism underlying bladder cancer progression is still being debated. Cytogenetic analyses of bladder tumors indicated a spectrum of molecular changes, yielding important clues about tumor initiation and progression. Loss of heterozygosity (LOH) at 10q is a common event in the later stages of bladder cancer and is found predominantly in muscle-invasive tumors [15–17].

PTEN (MMAC1/TEP1), a tumor-suppressor gene that maps to chromosome 10q23, is a dual-specific lipid phosphatase that antagonizes the phosphatidylinositol (PI) 3-kinase pathway by dephosphorylating its end product, the lipid second-messenger PI 3,4,5-triphosphate. Mutation and deletions in PTEN have been found in numerous cancers, including glioblastomas, prostate and endometrial carcinomas [18–20]. Recent studies indicate that

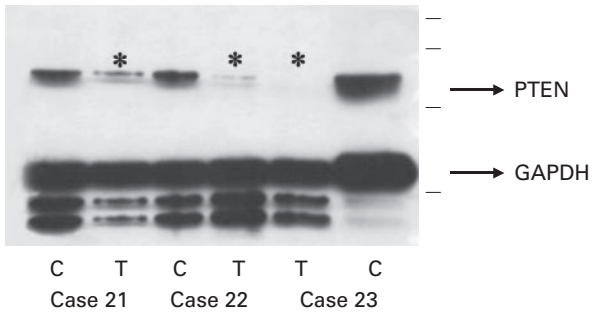

Fig. 1. Western blot analysis of PTEN protein expression in human bladder tumors. C = Control; T = tumor.

Table 1. Clinical and pathological characteristics of bladder cancer specimens in connection with their PTEN expression profiles

Case No.	Sex	Age year	Tumor number	Tumor size, cm	Treatment	Histology	Grade and stage	Time to recurrence months	Follow-up months	PTEN expression %
1	E	77	1	2	TUR	TCC	II, pT2a	–	6	100
2	E	83	1	3	TUR	TCC	I, pTa	–	7	100
3	K	67	4	1	TUR	TCC	II, pTa	–	6	100
4	E	73	2	4	TUR	TCC	III, pT2a	–	8	100
5	E	67	1	1.5	TUR	TCC	II, pT1	–	5	100
6	K	75	3	2	TUR	TCC	II, pT2a	–	9	100
7	E	73	4	2	TUR	TCC	III, pT2a	–	7	64
8	E	58	5	2	TUR	TCC	II, pTa	–	3	100
9	K	79	3	6	TUR	TCC	III, pT1	–	9	100
10	E	73	4	5	TUR	TCC	III, pT3a/M+	2	6	100
11	E	73	3	4	Cystectomy	TCC	III, pT1	–	3	100
12	E	72	1	3	TUR	TCC	II, pTa	–	2	100
13	E	46	1	3	Cystectomy	TCC	III, pT2a	–	1	100
14	E	70	2	2	TUR	TCC	III, pT2a	–	2	100
15	E	55	3	3	TUR	TCC	III, pT1	–	2	100
16	E	61	2	7	Cystectomy	TCC	III, pT2b	–	2	100
17	E	58	1	3	Cystectomy	TCC	III, pT2b	–	7	100
18	E	53	4	2	Cystectomy	TCC	III, pT2a	6	8	100
19	K	68	5	3.5	TUR	TCC	III, pT3a	2	12	100
20	E	54	1	4	Cystectomy	TCC	II, pT1	–	12	100
21	E	86	2	1	TUR	TCC	III, pT2a	24	18	58.8
22	E	64	5	2	TUR	TCC	III, pT2a	–	18	21.9
23	E	52	1	3	TUR	TCC	II, pTa	–	19	5
24	E	61	1	1	TUR	TCC	III, pT1	–	18	100
25	E	64	4	3	Cystectomy	TCC	III, pT2b	–	6	100
26	E	58	1	3	Cystectomy	TCC	III, pT2b	–	7	100
27	E	73	3	5	Cystectomy	TCC	III, pT3a/M+	–	3	100
28	E	66	2	5	Cystectomy	TCC	III, pT3b/N+	–	3	100
29	E	40	4	1	TUR	TCC	II, pTa	3	4	100

TUR = Transurethral resection; TCC = transitional cell carcinoma.

PTEN is mutated or deleted in a significant proportion (14–23%) of invasive bladder cancer with LOH at 10q [16, 21]. In addition, primary human bladder tumors of all stages overexpress PI 3-kinase and have significantly higher PI 3-kinase activity (5- to 20-fold) than adjacent normal epithelium [22]. These finding indicate that the PI 3-kinase pathway is aberrantly activated in bladder tumors and may be involved in bladder tumorigenesis and/or progression. The PI 3-kinase pathway regulates key processes that are relevant for tumor growth and progression, including cell-cycle progression, survival, motility, invasion and angiogenesis [23, 24]. The serine/threonine kinase Akt is a major downstream target of PI 3-kinase and is important in each of these processes [25]. Akt is also essential for the growth of PTEN-null embryonic stem cells as aggressive teratomas in mice [26]. Kagan et al. [27] recently demonstrated the involvement of chromosome 10q23.3 in advanced invasive bladder TCC. In 29% of the informative cases, allelic losses were found clustered within a 2.5-cM region on this band [27]. Another recent study on the LOH in primary bladder cancers has reported frequent (23%) LOH around the PTEN locus [16]. Inactivation of PTEN by homozygous deletions was detected in 6% of the specimens with LOH. Inactivating mutations were detected in 2 of 25 (8%) specimens screened for mutations in the PTEN coding region [16]. The investigators argued that the frequency of the second inactivating event, in bladder tumors with LOH of 10q23.3, is too low to conclude that PTEN is the inactivated tumor-suppressor gene within this region [16]. Furthermore, Aveyard et al. [17] confirmed the finding of LOH at 10q which is associated with muscle-invasive bladder tumors, where analysis of PTEN identified homozygous deletion in 2/63 tumors with no detectable mutations. Since this group found only deletions of PTEN in both bladder carcinoma cell lines and tumors, they suggested that homozygous deletions may be a more common mechanism for PTEN inactivation. Although Wang et al. [21] also reported homozygous deletion of PTEN in both bladder cell lines and tumors, they found that 6/35 (17%) tumors harbored PTEN mutations. Liu et al. [9] searched for evidence that PTEN is a tumor-suppressor gene in bladder TCC. They found that PTEN was inactivated by homozygous deletions and mutations in 3 of 11 (27%) bladder cancer lines. However, none of the 33 bladder TCC specimens examined had a mutation or deletion in the coding region. These results suggest that PTEN is not the primary target for inactivation in bladder TCC and that another gene, in close proximity to the PTEN locus, within this region of frequent allelic loses, may be the target for inactivation [9]. In the present study, we also found that only 4 (13.7%) cases manifested the decrease in the level of PTEN expression. All reductions in the level of PTEN expression except 1 (case 23) were identified in muscle-invasive TCC and, interestingly, case 23 (grade II, pTa) still showed a drastic reduction in the level of PTEN expression (85%). While, future studies with clinical follow-up will be required to determine if those superficial tumors with decreased PTEN expression are going to progress to a later stage, according to our findings PTEN does not seem to be the relevant molecule solely responsible for the progression of bladder carcinomas.

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