Hyperplasia and Carcinomas in Pten-Deficient Mice and Reduced PTEN Protein in Human Bladder Cancer Patients

Hirosi Tsuruta,¹ ² Hiroyuki Kishimoto,² Takehiko Sasaki,³ Yasuo Horie,⁴ Miyuki Natsui,² Yoshiko Shibata,¹ Koichi Hamada,⁵ Nobuyuki Yajima,¹ Koichi Kawahara,¹ Masato Sasaki,¹ Norihiko Tsuchiya,¹ Katsuhiro Enomoto,¹ Tak Wah Mak,⁶ Toru Nakano,⁷ Tomonori Habuchi,¹ and Akira Suzuki²

Departments of ¹Urology, ²Molecular Biology, ³Microbiology, ⁴Gastroenterology, and ⁵Pathology, Akita University School of Medicine, Akita, Japan; ¹The Campbell Family Institute for Breast Cancer Research, and Departments of Immunology and Medical Biophysics, University of Toronto, Toronto, Ontario, Canada; and ⁶Department of Pathology, Medical School and Graduate School of Frontier Biosciences, Osaka University, Suita, Japan

Abstract

PTEN is a tumor suppressor gene mutated in many human cancers. We used the Cre-loxP system to generate an urothelial-specific null mutation of Pten in mice (FabpCreP–Ptenfloxfloxfloxflox (FPtenfloxflox/flox) mice). Histologic examination revealed that all FPtenfloxflox/flox mice exhibited urothelial hyperplasia in which component cells showed enlarged nuclei and increased cell size. With time, 10% of FPtenfloxflox/flox mice spontaneously developed pedicellate papillary transitional cell carcinomas (TCC). This type of tumor also arose in spontaneously developed pedicellate papillary transitional cell carcinomas (TCC). This type of tumor also arose in FPtenfloxflox/flox mice treated with the chemical carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine. FPtenfloxflox/flox urothelial cells were hyperproliferative and showed increased activation of the survival signaling molecules Akt and extracellular signal-regulated kinase. In humans, 53% of primary bladder cancer patients exhibited decreased or absent expression of PTEN protein in either the cytoplasm or nucleus of tumor cells. In early bladder cancers, PTEN expression was repressed in 42% of superficial papillary TCC but in only 8% of cases of carcinoma in situ (CIS). In advanced bladder cancers, PTEN protein was significantly reduced (particularly in the nucleus) in 94% of cases, and this decrease in PTEN correlated with disease stage and grade. Thus, PTEN deficiency may contribute to bladder cancer both by initiating superficial papillary TCC and by promoting the progression of CIS to advanced invasive and metastatic forms. (Cancer Res 2006; 66(17): 8389-96)

Introduction

Bladder cancer is the fifth most common malignancy in the United States, and 95% of these tumors are transitional urothelial cell carcinomas (TCC). Two variants of TCC exist: superficial papillary-type TCC and nonpapillary nodular-type TCC. Superficial papillary TCC, which account for 70% to 80% of all urothelial tumors, present as superficial papillary lesions that are often multifocal and recurrent but only infrequently invade the underlying muscle (1). The 5-year survival rate of this variant (when treated promptly) approaches 90%. However, nonpapillary nodular TCC, which account for 20% to 30% of urothelial malignancies, are invasive at diagnosis and carry a very high risk of further invasion and metastasis. At least 50% of patients with muscle-invasive tumors will die within 2 years of diagnosis (2). Nonpapillary nodular type TCC are believed to develop from carcinoma in situ (CIS), early-stage urothelial tumors of highly malignant potential. Different genetic defects may underlie these bladder cancer variants. Activating mutations of fibroblast growth factor receptor 3 (FGFR3) are frequently (>70%) found in superficial papillary TCC, whereas dysfunction of p53 or Rb is associated with CIS and nonpapillary nodular TCC (3–5).

Mutations of PTEN occur in many human sporadic cancers and in hereditary tumor susceptibility disorders, such as Cowden’s disease (6). PTEN is a multifunctional phosphatase whose major substrate is phosphatidylinositol-3,4,5-trisphosphate (PIP3; ref. 7), a lipid second messenger molecule. PIP3 is generated by the action of phosphatidylinositol 3-kinase (PI3K) that become activated by growth factors or hormones (8). PIP3 in turn activates numerous downstream targets, including the serine/threonine kinase Akt/protein kinase B involved in antiapoptosis, proliferation, and oncogenesis (9). By using its lipid phosphatase activity to dephosphorylate PIP3 at the cell membrane, PTEN negatively regulates the PI3K/Akt pathway and exerts tumor suppressor. PTEN can also dephosphorylate FAK and Shc, activating the extracellular signal-regulated kinase (ERK) pathway (10). Whereas the functions of PTEN at the cell membrane are reasonably well understood, the roles of PTEN and PI3K in the nucleus are less clear. Several lines of evidence point to an additional tumor suppressive role for PTEN in the nucleus. (a) Nucleus-specific expression of PTEN reduces cell proliferation dependent on nuclear PIP3 (11). (b) A nuclear PIP3 receptor is involved in the inhibition of apoptosis (12). (c) PTEN affects the function of nuclear p53 directly and indirectly (13). (d) PTEN binds to and negatively regulates MSP58, a nuclear molecule capable of cell transformation (14). It remains unclear whether PTEN deficiency contributes to the onset or progression of bladder tumors in vivo. In mice heterozygous for a null Pten mutation, bladder cancers are not generally observed (15). In humans, mutation or deletion of PTEN DNA occurs at a low frequency (0-32%) in primary bladder cancers and bladder cancer cell lines (~30%; refs. 16–19), but the status of PTEN protein remains to be definitively investigated. Akt is activated in 55% of primary bladder cancers (20), and PTEN overexpression induces growth suppression and increased sensitivity to doxorubicin in bladder cancer cells in vivo (21). Moreover, inhibitors of PTEN or PI3K reduce the motility and invasiveness of bladder cancer cells.
of these cells (20). However, the role of PTEN in human primary bladder cancers remains to be fully elucidated.

We previously generated conditional mutant mice lacking Pten expression in various tissues and showed that Pten deficiency is usually associated with Akt and ERK activation, enlarged cell size, and tissue hyperplasia with tumor formation (22). Here, we show that mice deficient for Pten in the urothelium \textit{FabpCrePten\textsuperscript{flox/flox}} \textit{(Pten\textsuperscript{flox/flox} mice)} exhibit bladder cell hyperplasia and carcinomas. PTEN may therefore be an important regulator of bladder cancer initiation and/or progression.

Materials and Methods

\textbf{Generation of \textit{FPten}\textsuperscript{flox/flox} mice.} \textit{Pten}\textsuperscript{flox/flox} mice (1290a \texttimes C57BL/6 F6 background), generated as described previously (23), were mated to \textit{FabpCre} transgenic mice (FVB/N \texttimes C57BL/6 F4 background) in which Cre expression is controlled by the fatty acid–binding protein promoter (24). \textit{FabpCre} directs recombination in all cell layers of the transitional epithelium that lines the renal calyces and pelvis, ureters, and bladder by embryonic day 16.5 (24). \textit{Pten}\textsuperscript{flox/\textprime} \textit{mice} were crossed with \textit{FabpCrePten}\textsuperscript{\textprime/\textprime} mice to generate \textit{FPten}\textsuperscript{flox/flox}, \textit{FPten}\textsuperscript{\textprime/\textprime}, and \textit{Pten\textprime/\textprime} offspring that were used in the analyses as homozygous mutant, heterozygous mutant, and wild-type (WT) mice, respectively. \textit{Pten}\textsuperscript{flox/flox} mice were also occasionally used as WT controls because \textit{Pten}\textsuperscript{\textprime/\textprime} and \textit{Pten}\textsuperscript{\textprime/\textprime} mice were indistinguishable in pilot experiments examining histology, bromodeoxyuridine (BedUrd) incorporation, and frequency of N-butyl-N-(4-hydroxybutyl) nitrosamine (BNB)–induced bladder cancer development. The Institutional Review Board of the Akita University School of Medicine approved all animal experiments.

\textbf{Preparation of bladder epithelial cells.} Mouse bladders were minced with scissors and treated with 1,000 units/mL dispase (Godoshusei, Tokyo, Japan) overnight at 4°C. The epithelium was peeled off the bladder wall and trypsinized to produce a single-cell suspension. These cells were suspended in DMEM containing 10% FCS, filtered through a Cell Strainer (Becton Dickinson) and individually analyzed by evaluating forward scatter (FSC). To determine cell ploidy, cells were stained with propidium iodide (PI) and subjected to flow cytometry as described (28). Definitions of pT0 to pT4: pT0, no evidence of tumor; pTa, noninvasive papillary carcinoma; pT1, tumor invasion of subepithelial thickening; pT2, invasion of the muscle layer or deeper.

\textbf{Immunohistochemical analysis.} Immunohistochemical analysis for PTEN was done as described previously (27). Human prostate carcinoma cells were stained in parallel as negative controls, whereas small ureter specimens from healthy renal transplant donors were stained as positive controls. Each tissue section was stained twice using the Cell Signaling Technology anti-PTEN antibody and again with the Cascade Biosciences anti-PTEN antibody. Immunostaining patterns and intensities were scored by two independent, blinded observers.

\textbf{Results}

\textbf{Generation of \textit{FPten}\textsuperscript{flox/flox} mice.} Urothelium-specific Pten-deficient mice were generated by mating \textit{FabpCre} transgenic mice (24) to \textit{Pten}\textsuperscript{\textprime/\textprime} mice (23) in which \textit{Pten} exon 5, which encodes the phosphatase domain, is flanked by loxP sequences (Supplementary Fig. S1A). \textit{FPten}\textsuperscript{flox/flox} mice were born alive and appeared healthy. PCR examination of DNA from bladder epithelial cells of 8-week-old \textit{FPten}\textsuperscript{flox/flox} mice confirmed that efficient Cre-mediated recombination had occurred (Supplementary Fig. S1B). Quantitation of recombination in bladder epithelial cells of \textit{FPten}\textsuperscript{flox/flox} mice was >80%. Western blot analysis of the same cells confirmed a dramatic reduction of Pten protein in the mutant urothelium (Supplementary Fig. S1D).
Development of urothelial hyperplasia and spontaneous superficial papillary TCC in the absence of Pten. Histologic examination of 8-week-old FPTenfllox/flox mice revealed urothelial hyperplasia. In contrast to the urothelia of WT bladder and ureter, which are composed of only three to four cell layers (basal, intermediate, and superficial), FPTenfllox/flox urothelia were significantly thicker and showed five to seven cell layers (Fig. 1A). In addition, absolute numbers of bladder epithelial cells were increased 1.6-fold over WT levels at 8 weeks and 2.6-fold at 48 weeks (Fig. 1B). Intriguingly, although polarity was normal, the size of individual FPTenfllox/flox bladder epithelial cells was greater than that of FPTen+/+ bladder epithelial cells as determined by flow cytometric evaluation of FSC (Fig. 1C, top). Because this enlargement of individual cells was observed in both diploid and tetraploid fractions of the total bladder epithelial cell population, the cell size enhancement was not due to polyploidy (Fig. 1C, bottom). Thus, Pten deficiency induces a thickening of the urothelial layer that is due to increases in cell number and cell size.

We next analyzed the spontaneous onset of TCC in FPTenfllox/flox mice by sacrificing individuals at about every 10 weeks from 30 weeks to 80 weeks (Fig. 1). Of these tumors, three of four were observed in bladders from 50 WT and 15 FPTenfllox/+ mice. No urothelial hyperplasia or TCC were observed in bladders of four experiments. Data are number of cells with a given FSC value and are one trial representative of four experiments. Bottom, DNA content (ploidy; N) of the cells (top) was determined by PI staining.

Figure 1. Urothelial hyperplasia in FPTenfllox/flox mice. A, H&E staining of mouse bladder sections. Left, normal bladder and ureter epithelial cells from an 8-week-old WT (+/+) mouse; right, urothelial hyperplasia of the bladder and ureter from an 8-week-old FPTenfllox/+ (fllox/flox) mouse. Bar, 10 μm. B, increased cell number. Total bladder epithelial cells were counted in WT and FPTenfllox/flox mice of the indicated ages. Representative of three trials using four mice per group. Columns, mean; bars, SE. *, P < 0.05, statistical differences determined using Student’s t test. C, enlarged cell size. Top, single-cell suspensions of bladder epithelial cells from 32-week-old WT and FPTenfllox/flox mice were subjected to flow cytometry and FSC was determined as a measure of cell size. Data are number of cells with a given FSC value and are one trial representative of four experiments. Bottom, DNA content (ploidy; N) of the cells (top) was determined by PI staining.

Increased susceptibility of FPTenfllox/+ mice to BBN-induced carcinogenesis. To examine induced urothelial carcinogenesis, FPTen+/+, FPTenfllox/+ and FPTenfllox/fllox mice of 8-10 weeks of age were orally given BBN, a known initiator of urothelial carcinomas. Individual mice were sacrificed at every 8 weeks until 24 weeks and examined for urothelial tumors. Surprisingly, TCC, including CIS and dysplasia (Fig. 3A), were observed in 50% and 25%, respectively, of FPTenfllox/+ mice and in 33% and 11%, respectively, of FPTenfllox/fllox mice as soon as 16 weeks after BBN administration (Fig. 3B). Some TCC were associated with squamous differentiation (Fig. 3A). In contrast, CIS and dysplasia were observed in only 8% and 8%, respectively, of WT mice at 16 weeks. No significant differences were observed among the genotypes in water intake, urinary excretion, food intake, or body weight (data not shown), indicating that all animals experienced identical carcinogen exposure and that BBN was no more toxic to the mutants than to WT mice.

Detailed histologic examination revealed that all BBN-induced tumors in WT mice were nonpapillary TCC (Fig. 3A, top left and C, left, a-c), malignancies that develop from CIS. Pedicellate papillary carcinomas were not observed in WT mice (Fig. 3C, left, a-c). In contrast, 50% of tumors in FPTenfllox/fllox mice were pedicellate papillary carcinomas with evident outer (against the luminal side) projections (Fig. 3A, bottom left, and C, left, d-f). When the outer projection and inner invasion scores were plotted (Fig. 3C, right), FPTenfllox/fllox mice showed a significant increase in outer projections due to the frequent onset of pedicellate papillary carcinomas. To determine whether loss of heterozygosity (LOH) of the WT Pten allele was the mechanism of tumor onset in FPTenfllox/+ mice, we used PCR to monitor the presence of Pten exon 5. LOH was not observed in any FPTenfllox/+ tumor (Fig. 3D). Thus, BBN accelerates the onset of urothelial malignancies in Pten-deficient mice, particularly papillomavirus carcinomas, and this acceleration is not due to LOH of the WT Pten allele.

Increased proliferation associated with urothelial hyperplasia. Tissue hyperplasia can arise from either an increase in cellular proliferation or a decrease in apoptosis. TUNEL staining of WT bladders revealed very few apoptotic cells, and there was no apparent further reduction in FPTenfllox/fllox bladders (data not shown). To determine if cell division was increased in FPTenfllox/fllox bladders, urothelial cell proliferation was evaluated by BrdUrd incorporation. High levels of BrdUrd incorporation were observed in the bladder epithelial cells of both 10-week-old FPTen+/+ and FPTenfllox/fllox mice (Fig. 4A, left). However, whereas 0.67% of WT urothelial cells were BrdUrd+, >5.67% of FPTenfllox/fllox urothelial cells had incorporated BrdUrd (Fig. 4A, right). Most BrdUrd+ FPTenfllox/fllox cells were located in the basal layer, although some intermediate and superficial layer cells were also labeled. Thus, increased proliferation is the primary mechanism by which Pten deficiency induces hyperplasia in the mouse bladder.

Activation of Akt and ERK in Pten-deficient urothelial cells. Pten regulates the Akt pathway via PI3 dephosphorylation (7, 15) and the Ras/ERK pathway via FAK and Shc dephosphorylation (10). Our previous demonstrations that both Akt and ERK...
are constitutively activated in Pten-deficient cells showing abnormal proliferation or apoptosis (23, 29, 31) prompted us to analyze the phosphorylation of Akt and ERK in bladder epithelial cells from 8-week-old FPten+/+ and FPtenflx/flx mice. Phosphorylation levels of both molecules (pAkt and pERK) were significantly elevated in the latter (Fig. 4B). However, the onset of bladder cancers in mice also involves p53, Rb (5), and PATCHED (28). Significantly, Pten directly affects p53 expression...
However, there were no obvious differences between WT and FPtenflox/flox urothelial cells in the expression of any of these molecules (Fig. 4B). Thus, in bladder cancer epithelial cells, the primary mechanism driving increased cell proliferation and consequently urothelial hyperplasia seems to be the loss of Pten-mediated regulation of Akt and ERK activation. Importantly, whereas Akt activation was consistently higher in BBN-induced tumors obtained from FPtenflox/flox mice compared with those from FPtenflox/flox mice, ERK activation was observed in 50% of tumors from both genotypes (Fig. 4C). Thus, the accelerated onset of TCC in FPtenflox/flox mice is most likely due to the activation of Akt rather than ERK.

**Frequent reduction of PTEN protein expression in human bladder carcinomas.** Mutation or deletion of PTEN DNA occurs at only a low frequency in human primary bladder cancers and in bladder cancer cell lines (16–19). A single study of PTEN protein expression in bladder cancers has been reported, in which 29 bladder cancer samples were analyzed by Western blotting. Only 13.7% patients manifested a decrease in PTEN protein expression (33). However, this study did not differentiate between cancerous and normal cells or between nuclear and cytoplasmic PTEN protein expression. To determine PTEN protein expression in the cytoplasm and nucleus of tumor cells only, we did immunostaining on samples from 68 patients with primary bladder cancers. Noncancerous bladder epithelial cells within a given tissue section (Fig. 5A) and normal urothelia in the ureters of three healthy donors (Fig. 5B, a) served as positive controls. Anti-PTEN staining of bladder epithelium was variable even in healthy donor ureter samples, but nuclear PTEN expression was consistently weakest in the basal layer. The weakest staining in normal ureter epithelial cells, or in noncancerous epithelial cells within the same tissue section, was scored as +2 (normal). PTEN expression in the cytoplasm and nuclei of bladder cancer cells ranged from absent (0) to below normal (+1) to normal (+2; Fig. 5B, b–d). Levels of PTEN protein expression in the nuclei of cancer cells were lower than in the cytoplasm (Supplementary Table S1). Whereas 47% of patients showed normal PTEN staining, the remaining 53% showed below normal or absent PTEN expression in either the cytoplasm or the nucleus (Fig. 5C). Of these, 6% showed loss of PTEN protein in both the cytoplasm and the nucleus. With respect to TCC variant type, PTEN expression in either the cytoplasm or the nucleus was reduced in 42% of superficial papillary TCC (pT1) but in only 8% of CIS (pT1u; Fig. 5D, top left). There was a statistically significant correlation between reduced PTEN protein and TNM stage or tumor grade (Fig. 5D; Supplementary Table S2). Importantly, PTEN protein was reduced or absent in 94% of advanced bladder cancer patients (pT2 or greater), particularly in the nucleus (Fig. 5D). PTEN protein did not vary significantly according to sex, age, or tumor number (Supplementary Table S2).

**Discussion**

Pten deficiency exerts a potent tumorigenic effect on urothelium in mice. Urothelium-specific Pten deletion in mice resulted in urothelial hyperplasia due to hyperproliferation and the onset of superficial papillary urothelial cancers in 10% of the mutant animals. In humans, the reduction or loss of PTEN protein expression was observed in 42% of superficial papillary bladder cancers (pT1) and in 94% of advanced bladder cancers (pT2 or greater). Our urothelium-specific Pten-deficient mice may furnish a useful model for human bladder cancer in which to analyze mechanisms underlying the onset of urothelial cancers and to explore drugs for the treatment of these malignancies.

Spontaneous bladder cancers in FPtenflox/flox mice developed late in life (>40 weeks of age) and at low frequency. Although this late onset could be due to the extremely low proliferative rate of urothelium compared with skin and intestinal epithelia (34), it could also imply that the onset of superficial papillary bladder cancers requires secondary genetic or epigenetic events in addition to Pten deficiency. Because Pten deficiency increases susceptibility to carcinogens, and humans are continuously exposed to these agents in the environment, loss of Pten
function may allow a carcinogen to cause additional gene alterations. Indeed, the same carcinogens cause different bladder tumors in rats and mice. Rats develop papillary bladder tumors regardless of the carcinogen used, and these cancers become invasive only if a large dose of carcinogen is given for a prolonged period (35). In mice, the same carcinogens cause primarily urothelial dysplasia, CIS, and nonpapillary tumors that easily become invasive (36). This species difference may be related to the fact carcinogen-induced bladder tumors show a higher frequency of H-Ras mutation and a lower frequency of p53 mutation in rats than in mice (36–38). In our study, the majority of BBN-induced bladder cancers were nonpapillary in the WT but papillary in the Pten-deficient mutants. The reduced Pten present in BBN-treated mutant mice may have led to Akt and ERK hyperactivation. This hyperactivation might then accelerate the growth of a tumor initiated by a BBN-induced p53 mutation and influence CIS and nonpapillary TCC to become papillary. It should be noted that, compared with our observations, previous studies using the BBN-induced tumor model reported higher frequencies of bladder cancers at earlier times in WT mice (28, 39). These differences may be due to the relatively low dose of BBN given in our study, the more advanced age of our mice, or the variations in genetic background.

The occurrence of bladder cancers in FpTENfl/fl mice has recently been reported (40). Consistent with our findings, Yoo et al. found that bladder epithelial cells in FpTENfl/fl mice were larger than those of FpTEN+/- mice and that the frequency of spontaneous bladder cancers was significantly lower than the incidence of other cancers in these mutant mice. Yoo et al. speculated that mechanisms other than constitutive Akt activation might be important for the onset of bladder cancers because these workers did not observe the activation of either Akt or its downstream signaling molecules in FpTENfl/fl bladder epithelial cells. However, our results clearly show that Akt is strongly activated both in spontaneous bladder cancers and in BBN-induced tumors of FpTENfl/fl mice. We therefore believe that Akt activation is crucial for the onset of murine bladder cancers. Another discrepancy arises with regard to increased bladder epithelial cell proliferation, which Yoo et al.

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Figure 5. Reduction or absence of PTEN expression in human primary bladder cancers. A and B, representative urothelial sections immunostained for PTEN protein expression. A, left, region of normal bladder epithelium (score +2); right, adjacent region showing the epithelium of a primary bladder cancer lacking PTEN expression (score 0) in both the cytoplasm and the nucleus. Bar, 10 μm (top) and 100 μm (bottom). B, a, normal PTEN protein expression in urinary epithelial cells from a healthy donor (score +2). b, PTEN expression is absent in the nucleus (score 0) but normal (score +2) in the cytoplasm. c, PTEN is reduced (score +1) in both the nucleus and the cytoplasm. d, PTEN is absent (score 0) from both the nucleus and the cytoplasm. Bar, 5 μm. C, percentages of bladder cancer patients showing reduction or loss of PTEN expression in the cytoplasm and/or nucleus. In total, PTEN expression is either reduced or lost in 53% of bladder cancer patients. D, relationship between the level of PTEN expression in the cytoplasm and/or nucleus (as defined for C) with TNM stage (left) or tumor grade (right). Note that PTEN staining in either the cytoplasm or the nucleus is decreased in 42% of pTa patients but in only 8% of pTis patients. Nuclear PTEN expression is absent in the majority of advanced bladder cancers (pT2 or greater).
Moreover, the total number of bladder epithelial cells was much higher in our study. Furthermore, the intracellular distribution of PTEN cannot be determined by this method. In our study, we used immunostaining to evaluate PTEN expression in the cytoplasm and nucleus of individual cells.

PTEN expression in human bladder cancers. Point mutations or LOH of PTEN DNA are observed in ~6% and 5%, respectively, of human primary bladder cancers and in 13% and 14%, respectively, of bladder cancer cell lines (16–19). These low frequencies suggest that mutation of the PTEN gene itself does not play a major role in bladder carcinogenesis. However, the function of an intact gene can be lost through promoter hypermethylation, alternative splicing of pre-mRNA, or post-translational modifications. The actual frequency of PTEN abnormalities in bladder cancers thus may have been underestimated. As well, PTEN protein exists in the nucleus, although it lacks a traditional nuclear localization signal (41). Differentiated and resting cells have shown preferential nuclear localization of PTEN (27, 42), and nucleus-specific expression of PTEN can suppress cell growth (27, 42), and nucleus-specific expression of PTEN may also acquire a growth advantage and progress to invasive and metastatic nonpapillary nodular type TCC.

Reduced or absent PTEN in either the cytoplasm or the nucleus was found in 36 of the 68 (53%) bladder cancer samples examined. About 6% of cases showed loss of PTEN in both the cytoplasm and the nucleus, consistent with previously reported LOH percentages reported for bladder cancer patients (16–19). These values may in fact be underestimates, because the weakest staining of PTEN in control ureter or noncancerous bladder epithelial cells was scored as normal, and the most intense PTEN staining in a cancerous tissue was taken as its score.

Proposed mechanism for the onset of urothelial cancers. We found that 42% of primary superficial papillary bladder cancers (pT1) showed reduced PTEN, whereas 92% of CIS (pTis) samples showed normal PTEN. This result suggests that PTEN loss initiates a fraction of superficial papillary cancers (Fig. 6, top pathway). In mouse models, the overexpression of genes encoding growth factor–related signaling molecules, such as H-Ras, FGFR3, and epidermal growth factor (EGF) receptor (EGFR), causes urothelial hyperplasia (44–46) that can progress to superficial papillary carcinomas when additional (unknown) genes are presumably altered. In humans, activating mutations of FGFR3 have been found in >70% of superficial papillary cancers (3). Significantly, engagement of FGFR3 or EGFR stimulates Akt and ERK signaling (47). However, the gene whose alteration pushes hyperplasia into a carcinoma remains unknown. PTEN deficiency augments the activation of Akt and ERK pathways triggered by various growth factors, including FGF and EGF (48). Furthermore, urine usually contains high concentrations of growth factors, particularly EGF (49). Thus, PTEN-deficient urothelial cells may have an enhanced susceptibility to external signaling that results in hyperproliferation, which in turn increases the chance of additional genetic alterations that could tip the balance toward malignancy.

PTEN inactivation may also play a role in the progression of CIS to nonpapillary nodular invasive TCC (Fig. 6, bottom pathway). We found that PTEN expression was down-regulated in the cytoplasm or nucleus of many advanced nonpapillary bladder tumor cells. Most advanced nonpapillary TCC are believed to be derived from CIS (2) that were initiated by p53 or Rb mutation (5). Subsequent inactivation of PTEN may confer on these cells a growth advantage or resistance to apoptosis that leads to invasiveness or metastasis. EGFR may also be involved in this step, because EGFR overexpression occurs in 40% to 60% of bladder cancers and EGFR expression is highest in late-stage urothelial cancers (48, 50). Both PTEN and EGFR regulate the Akt and ERK pathways, and this activation, coupled with impaired p53 and Rb functions, may spur the development of advanced nonpapillary cancers.

Our finding that PTEN deficiency may contribute to the malignancy of both superficial papillary and invasive nonpapillary bladder cancers may make this regulator an attractive target for new therapeutics designed to treat these tumors.

Acknowledgments


Grant support: Ministry of Education, Science, Sports and Culture, Japan, Kowa Life Science Foundation, and Suzuken Memorial Foundation.

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We thank Dr. Tetsuo Noda (Tohoku University) and Drs. Junko Sasaki and Shunsuke Takasuga (Akita University) for helpful discussions and technical expertise and Dr. Jeffrey I. Gordon (Washington University School of Medicine) for providing the FabpCre transgenic mice.
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