Fez1/Lzts1-deficient mice are more susceptible to N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) carcinogenesis

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Introduction

Although bladder cancer is the fifth most common cancer in USA (1), the molecular mechanisms that determine neoplastic transformation in the bladder urothelium are still unknown. No specific or exclusive cytogenetic aberration has been identified for urothelial carcinoma (UC), but numerous non-random deletions, gain of chromosomes, polyploidization and formation of isochromosomes have been linked to it (2).

In 1999, we identified the Fez1/Lzts1 (LZTS1) tumor suppressor gene on chromosome 8p22 (3). Subsequently, we demonstrated that the LZTS1 expression is often altered in human cancers with diverse histogenetic backgrounds, including those derived from prostate, breast, esophagous and stomach (3–5). Furthermore, LZTS1 expression was often altered in human cancers with diverse histogenetic backgrounds, including those derived from prostate, breast, esophagous and stomach (3–5).

To further investigate the role of LZTS1 in the development of bladder cancer, we utilized heterozygous and nullizygous Lzts1 mice in a chemically induced carcinogenesis model. Fifty-eight mice consisting of 25 Lzts1+/+, 17 Lzts1+/− and 16 Lzts1−/− were treated with N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN). Results showed that there was a significant increase in neoplastic lesions in the Lzts1−/− (82.3%) and Lzts1−/− (93.8%) versus Lzts1+/+ (8.0%) mice after BBN treatment. No difference in cancer incidence between Lzts1+/− and Lzts1−/− was observed. Collectively, these findings indicate that loss of one or both LZTS1 alleles hampers the normal defenses of urothelial cells against carcinogens, favoring bladder cancer development. Therefore, LZTS1 may become an excellent target for gene therapy in advanced bladder carcinoma.

Abbreviations: BBN, N-butyl-N-(4-hydroxybutyl) nitrosamine; PCR, polymerase chain reaction; UC, urothelial carcinoma.
follows: (i) simple hyperplasia and mild dysplasia; (ii) moderate and severe dysplasia; (iii) in situ carcinoma and (iv) invasive carcinoma. For each case, only the most severe lesions were considered in the statistical analysis. Severe dysplasia and carcinoma in situ were grouped together. The slides were randomized so that the three pathologists (R.B., A.V., and R.V.I.) evaluating the slides did not know the nature of the treatment received by each animal. The association between Lzts1 genotype, presence of neoplastic lesions and bladder tumors was evaluated via the $\chi^2$ test. Differences were considered statistically significant at $P < 0.05$. All the statistical evaluations were performed with STATA software (Stata Corporation, College Station, TX).

Results

As we reported previously (9), our data confirmed the higher susceptibility of Lzts1-deficient mice to develop tumors under the effect of a carcinogen using heterozygous and nullizygous Lzts1-deficient mice in a chemically induced carcinogenesis model. In the Lzts1$^{+/+}$ group (Table I), simple hyperplasia and mild dysplasia were detected in 20 mice (80.0%), three mice (12.0%) showed moderate to severe dysplasia and only two mice (8.0%) presented neoplastic lesions (one with in situ carcinoma and the other with invasive carcinoma). On the contrary, 14 of 17 (82.3%) Lzts1$^{+/−}$ and 15 of 16 (93.8%) Lzts1$^{−/−}$ mice harbored invasive carcinomas. Mild to moderate dysplasia was diagnosed in the remaining Lzts1$^{+/−}$ (3/17) and Lzts1$^{−/−}$ (1/16) mice. Neoplastic lesions in the urothelium adjacent to advanced tumors were frequently observed. In most cases, as described previously (13, 14), we observed squamous differentiation of neoplastic lesions. Specifically, 11 of 14 tumors in the Lzts1$^{+/−}$ and 12 of 15 tumors in the Lzts1$^{−/−}$ mice showed squamous features. Furthermore, cancer infiltrated the deeper muscle in 10 of 14 Lzts1$^{+/−}$ mice and 11 of 15 Lzts1$^{−/−}$ mice, respectively. The remaining tumors invaded the superficial muscle in both Lzts1$^{+/−}$ and Lzts1$^{−/−}$ mice. No significant histopathological differences were observed between wild-type and knock-out mice. When we used three outcome categories (Table I), the global $P$ comparing all three groups was 1.3E-13, underlying the differences of outcomes across the genotypes. The pair-wise probabilities were as follows: wild-type versus heterozygous, $P = 1.4E-9$; wild-type versus homozygous$^{−/−}$, $P = 2.5E-14$ and heterozygous versus homozygous$^{−/−}$, $P = NS$.

Table I. Summary of the urothelial lesions observed in the three different genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal/hyperplasia, mild dysplasia, $n$ (%)</th>
<th>Moderate dysplasia/severe dysplasia/in situ, $n$ (%)</th>
<th>Carcinoma, $n$ (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lzts1$^{+/+}$</td>
<td>20 (80.0)</td>
<td>3 (12.0)</td>
<td>2 (8.0)</td>
<td>25</td>
</tr>
<tr>
<td>Lzts1$^{+/−}$</td>
<td>1 (5.9)</td>
<td>2 (11.8)</td>
<td>14 (82.3)</td>
<td>17</td>
</tr>
<tr>
<td>Lzts1$^{−/−}$</td>
<td>0</td>
<td>1 (6.3)</td>
<td>15 (93.8)</td>
<td>16</td>
</tr>
</tbody>
</table>

Discussion

The short arm of chromosome 8 is frequently deleted in human cancers and its deletion is commonly associated with a more aggressive malignant phenotype (15, 16). In previous studies, we have identified the LZTS1 gene on chromosome 8p22 (3), a region which showed loss of heterozygosity in invasive UC tumors (12, 17–19), and shown that the LZTS1 protein is lost or decreased in most of the UC-derived cell lines and primary human urothelial bladder cancers analyzed (10). Moreover, there was a statistical association between decrease or absence of LZTS1 and high-grade bladder tumors. By restoring LZTS1 expression in SW780 bladder cancer-derived cells, we inhibited cell growth, altered cell cycle progression and suppressed tumorigenicity in immunocompromised mice (10).

In a recent report, Knowles et al. (12) found a new mutation in A1698 bladder cancer cell line and 42% of loss of heterozygosity in the 8p22 region of a large series of bladder cancers. In the same study, a reduction in LZTS1 messenger RNA levels was also observed in the majority of bladder cancer cell lines (12). All these data strongly suggest that LZTS1 is indeed involved in the progression of UC of the urinary bladder.

The exact mechanism whereby LZTS1 contributes to cancer initiation and progression is not known. Recent data suggest that LZTS1 is

Fig. 1. PCR analysis on mouse genomic DNA showing wild-type Lzts1 gene and Lzts1 gene/Neomycin cassette (mutant) products in Lzts1$^{+/+}$, Lzts1$^{+/−}$ and Lzts1$^{−/−}$ mice as indicated.

Fig. 2. Gross anatomy and histopathology of Lzts1 knock-out murine bladder after BBN administration. Macroscopic (A) and hematoxylin and eosin sections (B and C) of an ulcerated tumor protruding inside the peritoneum. Representative examples of a murine normal urothelium in a Lzts1$^{+/+}$ mouse (D) and a moderate dysplasia and an invasive tumor with squamous features in Lzts1$^{+/−}$ mice (E and F). (B, E, F ×100; C and D ×400).

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an important player in the execution of normal M-phase progression (9). Moreover, the absence of Lzts1 leads to impaired chromosome segregation, cell transformation and cancer progression (9). Transgenic mice, where Lzts1 has been knocked out in the germ line, have an increased incidence of both spontaneous and chemically induced cancers (9).

Since the LZTS1 gene is often downregulated in cancer (3–7,10,11), and since BBN-induced bladder cancer is an established model to characterize the tumorigenic process for urinary bladder carcinogenesis (13,14), BBN treatment was used to determine whether Lzts1 modulates carcinogen-induced malignancy development. Treatment of mice with this carcinogen results in the development of transitional cell urinary bladder cancers with a high yield of tumor induction (13,14) and high rate of squamous differentiation (>50% of the induced tumors) (13,14,20). In this study, as in our previous experience (14), we observed an almost complete squamous commitment of the neoplastic lesions. After 13 weeks of BBN administration, the majority of Lzts1-deficient mice (82.3% Lzts1+/− and 93.8% Lzts1−/−) developed invasive bladder cancers (Figure 2). These incidences were significantly higher than those observed in the group of wild-type mice. This confirmed our previous observation that Lzts1-deficient mice develop forestomach tumors more quickly after exposure to N-nitrosomethylbenzylamine (9), further suggesting that Lzts1 absence sensitizes multiple tissues to carcinogenesis. We also confirmed that there is no difference in the incidence of neoplastic lesions between heterozygous+/− versus homozygous−/− mice (82.3 versus 93.8%). Thus, the loss of one single Lzts1 allele can predispose to bladder cancer formation. The difference in the incidence of bladder tumors between these mice is an important indication of the need for the urothelial cell to have both Lzts1 alleles intact to respond efficiently to the damaging action of a chemical carcinogen. Therefore, as observed for other tumor suppressor gene (21–23), we hypothesize that Lzts1 gene could be haploinsufficient in tumor suppression.

In conclusion, we have shown for the first time that Lzts1 null mouse is a useful and valid animal model for investigating the biology of bladder cancer. We further discovered that both Lzts1 alleles are necessary for a proper host response to chemical carcinogens such as BBN. Thus, further studies on this interesting gene are warranted to understand how Lzts1 participates in bladder carcinogenesis. Elucidation of the molecular mechanisms of LZTS1 activity might help not only the diagnosis but also the treatment of patients with this devastating disease.

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**References**


