P53 Expression in Small Cell Carcinoma of the Urinary Bladder: Biological and Prognostic Implications

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Abstract. Small cell carcinoma of the urinary bladder is a rare and highly aggressive tumor. P53 expression has been shown to be associated with poor prognosis in a variety of tumors. This study was undertaken to investigate p53 expression in a large series of small cell carcinomas of the urinary bladder and to correlate the findings with clinicopathologic parameters and clinical outcome. Pathologic findings were reviewed and were correlated with clinical findings and follow-up information. Immunohistochemical staining for p53 was performed on paraffin-embedded tissue sections using the avidin-biotin-peroxidase method. Results were recorded as positive expression (≥10% of cells with nuclear staining) or negative expression (<10% of cells with nuclear staining). The series included 40 males and 10 females. All 50 patients except one had advanced disease (T2 or above) at presentation. Pathologic stages were as follows: T1 in 1, T2 in 25, T3 in 21, and T4 in 3 patients. During a median follow-up of 12 months (range: 1 month to 122 months), 38 patients died of cancer. Two-year and 5-year cancer-specific survival rates were 45% and 16%, respectively. P53 overexpression was present in 27 out of 50 (54%) cases (7 with 10-25% staining, 4 with 25-50% staining, 11 with 50-75% staining and 5 with 75-100% staining); conversely, negative staining for p53 was observed in 23 out of 50 (46%) cases (19 with no staining and 4 with <10% staining). No correlation was demonstrated between the level of p53 expression and survival (p=0.16). The 5-year cancer-specific survival was 16.6% for patients with tumors expressing p53 in greater than or equal to 10% of cells and was 14.7% for patients with tumors expressing p53 in less than 10% of tumor cells. There was no correlation between p53 expression and other clinicopathologic characteristics, including age (p=0.20), gender (p=0.84), history of smoking (p=0.25), pathologic T stage (p=0.38), clinical stage (p=0.60), lymph node metastasis (p=0.17), and distant metastasis (p=0.88). Our data indicate that p53 is frequently expressed in small cell carcinoma of the urinary bladder. However, no correlation is found between p53 expression and clinicopathologic parameters, including long-term survival.

Small cell carcinoma of the urinary bladder is a rare and highly aggressive tumor and is associated with a very poor prognosis, often with a rapidly fatal outcome (1-3). Current knowledge of clinicopathologic parameters, biological behavior and therapy of this disease is derived from a limited number of small series and case reports.

It is well known that activation of proto-oncogenes and inactivation of tumor suppressor genes are involved in bladder cancer tumorigenesis. P53, a well-established tumor suppressor gene on chromosome 17p13, is important in the process of tumorigenesis in a variety of human cancers, including bladder cancer. P53 regulates the cell cycle by causing G1-phase arrest and by inducing expression of DNA repair genes (4, 5). Alteration of the p53 tumor suppressor gene has been demonstrated in nearly half of all human cancers, including those arising in the colon, esophagus, breast, lung, bladder, liver, brain, and other sites (6). Moreover, numerous studies have shown that p53 expression is associated with high grade, high stage, and poor prognosis in malignancies in a variety of sites, including the lung, breast, stomach, prostate and urinary bladder (7-12). However, the prognostic significance of p53 in
small cell carcinoma of the urinary bladder has not been established. The current study was undertaken to investigate the expression of p53 in a large series of small cell carcinomas of the urinary bladder and to correlate p53 expression with clinicopathologic parameters and clinical outcome.

Materials and Methods

This study included 50 cases of small cell carcinoma of the urinary bladder that were obtained from 5 institutions: Indiana University (Indianapolis, IN, USA), Case Western Reserve University (Cleveland, OH, USA), Northwestern University (Chicago, IL, USA), University of Chicago (Chicago, IL, USA) and Cordoba University (Cordoba, Spain). The medical records were reviewed. Formalin-fixed, paraffin-embedded tissue was available for all cases. Hematoxylin and eosin-stained sections were examined. A diagnosis of small cell carcinoma was made only when the morphologic criteria established by the 2004 WHO classification system were met (13). TNM stage was assigned according to the criteria specified in the AJCC Cancer Staging Manual, 6th Edition (2002) (14).

Immunohistochemical staining for p53 was performed on formalin-fixed and paraffin-embedded tissue sections using the peroxidase-labelled streptavidin-biotin method (11, 12). Five-micrometer tissue sections from each patient were chosen for p53 immunohistochemical staining. The sections were deparaffinized in xylene for 5 minutes and then rehydrated through graded ethanol to distilled water. Antigen retrieval was performed by heating sections for 15 minutes (Target Retrieval, DAKO). Endogenous peroxidase was blocked by incubation in 3% H2O2 for 5 minutes. Tissue sections were incubated with primary antibodies against p53 (DAKO, prediluted antibody, 1:100) for 10 minutes, followed by biotinylated secondary antibody (DAKO) and peroxidase-labelled streptavidin (DAKO). 3, 3-Diaminobenzidine was used as the chromogen. In order to evaluate the specificity of the antibody, known positive (breast carcinoma) and negative tissues were used as controls. The extent of staining was evaluated by visual examination microscopically. Each section was scanned at low magnification and nuclear staining of p53 was scored by evaluating the percentage of tumor cells staining positively and assigning each case to one of the following categories: 0%, 0-10%, 10-25%, 25-50%, 50-75% and 75-100% staining, respectively). P53 expression was recorded as positive expression (>10% of tumor cells with intense nuclear staining) or negative expression (<10% of cells with nuclear staining).

The data were statistically analyzed with the SAS software. P-values for correlations between patient survival rate and clinical and pathological measures are generated with SAS proc phreg. P-values for correlations between p53 expression and clinical and pathological measures are generated with SAS proc logistic. A p-value less than 0.05 was considered statistically significant.

Results

This series included 40 males and 10 females with small cell carcinoma of the urinary bladder. The patients' ages ranged from 36 to 83 years, with a mean age of 66 years. All 50 patients except one had advanced disease (T2 or above) at presentation. Pathologic stages were as follows: T1 in 1, T2 in 25, T3 in 21, and T4 in 3 patients. During a median follow-up of 12 months (range: 1 month to 122 months), 38 patients died from their disease. Two-year and 5-year cancer-specific survival rates were 45% and 16%, respectively.

P53 expression was present in 27 out of 50 (54%) cases (7 with 10-25% staining, 4 with 25-50% staining, 11 with 50-75% staining, and 5 with 75-100% staining). Conversely, negative staining for p53 was observed in 23 out of 50 (46%) cases (19 with no staining and 4 with <10% staining). No correlation was demonstrated between the level of p53 expression and survival (p=0.16). In patients whose tumors demonstrated p53 expression in more than or equal to 10% of tumor cells, the 5-year cancer-specific survival rate was 16.6%; in patients whose tumors demonstrated p53 expression in less than 10% of tumor cells, the 5-year cancer-specific survival rate was 14.7%. There was no correlation between p53 expression and other clinicopathologic characteristics, including age (p=0.20), gender (p=0.84), history of smoking (p=0.25), pathologic T stage (p=0.38), clinical stage (p=0.60), lymph node metastasis (p=0.17) and distant metastasis (p=0.88).

Discussion

Extra-pulmonary primary small cell carcinoma has been reported in numerous organs, including the breast, larynx, esophagus, stomach, small and large intestine, cervix, prostate, and urinary bladder. Small cell carcinoma of the urinary bladder, like its pulmonary counterpart, has an aggressive clinical course with early and extensive metastases. This tumor is more likely to progress rapidly and the mortality is very high despite radical surgery, radiation and chemotherapy (1-3).

Mutations of the p53 gene have been the most common genetic alterations in human cancers.(5) P53 is a key cell cycle regulator and is a common target of inactivation in human cancers. The p53 gene is located on human chromosome 17p13.1 and is composed of 11 exons encoding a 53 kd nuclear phosphoprotein, which regulates cell growth and proliferation (4, 5). A variety of p53 mutations, including missense mutations, nonsense mutations, deletions and insertions, have been identified in human cancers. Most mutations of p53 are missense mutations and are confined mainly to exons 5 through 8, regions which have been highly conserved throughout evolution (4-6). The abnormal protein coded by mutant p53 is more stable and has a longer half-life than wild-type p53 and, subsequently, accumulates in cells. Therefore, p53 expression can be detected using immunohistochemistry and a variety of other methods including PCR-RFLP, single strand confirmation polymorphism (SSCP) and DNA sequencing. Most studies have shown that there is a good correlation between p53 over-expression and mutation of the p53 gene. Thus, detection of p53 by immunohistochemistry is considered an indication of p53 gene mutation.(15)

Genetic changes of p53 have been studied extensively in pulmonary small cell carcinoma. However, only a few studies
Figure 1. Small cell carcinoma of the urinary bladder (A) showed nuclear accumulation of p53 protein (B).
have focused on extra-pulmonary small cell carcinoma. Parwani et al. reported p53 expression in 5 out of 6 small cell carcinomas of the gall-bladder (83%) (16). Similarly, Maitra et al. studied 12 cases of gall-bladder small cell carcinoma and found 75% had abnormal p53 expression (17).

Very few studies have addressed expression of p53 in small cell carcinoma of the urinary bladder. A flow cytometric study by Atkin and associates showed overexpression of p53 in one case of small cell carcinoma of the urinary bladder (18). Soriano and colleagues demonstrated p53 expression in 8 out of 10 cases (80%) of small cell carcinoma of the urinary bladder (19); whereas Wang et al. found p53 overexpression in 3 out of 8 cases of urinary bladder small cell carcinoma (37%) (20). In this study, we analyzed p53 expression in a much larger series of urinary bladder small cell carcinomas. Our series of 50 cases revealed that p53 was expressed in 27 cases (54%). However, we did not find any correlation between p53 expression and other clinicopathologic characteristics, such as age, gender, history of smoking, pathologic T stage, clinical stage, lymph node metastasis and distant metastasis. To our knowledge, this study is not only the largest series that has addressed the incidence of p53 alteration in small cell carcinoma of the urinary bladder, but also the first study that has demonstrated no correlation in this tumor between p53 overexpression and prognosis.

In summary, p53 is frequently overexpressed in small cell carcinoma of the urinary bladder. Currently, the development of therapeutic modalities aimed at induction of apoptosis in malignant cells is a major goal of cancer therapy. Gene therapy with wild-type p53 might be one of the approaches for treating small cell carcinoma of urinary bladder in the future (4). Further molecular analysis of p53 and other tumor-suppressor genes and the roles of these genes in carcinogenesis may provide potential new targets for management of small cell carcinoma of the urinary bladder.

References


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