**P53 Overexpression in Bladder Urothelial Neoplasms**  
**New Aspect of World Health Organization/International Society of Urological Pathology Classification**  
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**Introduction:** The aim of this study was to investigate the probable differences in P53 expression between papillary urothelial neoplasm of low malignant potential (PUNLMP) and varying grades of transitional cell carcinoma (TCC) of the bladder.

**Materials and Methods:** Ten biopsy specimens of the patients with PUNLMP, 20 of the patients with papillary low-grade TCC, 20 of those with invasive high-grade TCC, and 10 of healthy individuals were stained for P53 protein by immunohistochemical methods. Histological grading was performed according to the World Health Organization/International Society of Urological Pathology consensus classification of urothelial neoplasms of the urinary bladder.

**Results:** Nuclear P53 protein in invasive high-grade TCC was slightly more frequent than that in noninvasive low-grade papillary TCC ($P = .35$). Ten percent of specimens with PUNLMP had nuclear P53 accumulation, while in low-grade and high-grade TCCs, 75% and 85% of the specimens were positive for P53 protein accumulation ($P < .001$). Expression of P53 was nil in all normal transitional epithelium specimens.

**Conclusion:** Overexpression of P53 in papillary low-grade TCC and invasive high-grade TCC, while lacking of expression in PUNLMP indicates that mutations of P53 gene are not usually associated with the development of urothelial neoplasms and they may play a crucial role only in progression of PUNLMP to low-grade TCC.

Keywords: tumor suppressor protein P53, bladder, transitional cell carcinoma, immunohistochemistry

**INTRODUCTION**

Mutations in P53 gene are the most common genetic abnormality in human cancers. P53 acts as a tumor suppressor gene and the major functional activities of the P53 protein are cell-cycle regulation and initiation of apoptosis in response to DNA damage. Wild-type P53 protein has a short half-life; however, the protein encoded by mutated P53 remains active for a long period. Therefore, mutation of P53 gene results in P53 protein accumulation in cells’ nuclei. This accumulation is detectable with immunohistochemical methods and correlates with P53 gene mutation. Mutated P53 gene is a common genetic abnormality in transitional cell carcinoma (TCC) of the bladder. Previous studies have depicted that overexpression of P53 occurs in higher stages and grades of TCC. In this study, we investigated whether there are immunohistochemical differences in the P53 expression between papillary urothelial neoplasm...
of low malignant potential (PUNLMP), varying grades of papillary noninvasive TCC, and invasive TCC.

**MATERIALS AND METHODS**

Biopsy specimens of 10 patients with PUNLMP, 20 with papillary low-grade TCC, 20 with invasive high-grade TCC, and 10 with normal transitional mucosa and no cystoscopic and microscopic pathologic findings were selected. Histological grading was performed according to the World Health Organization/International Society of Urological Pathology (WHO/ISUP) consensus classification of urothelial neoplasms of the urinary bladder. Immunohistochemical staining for $P_53$ was performed on formalin-fixed, paraffin-embedded sections using avidin-biotin technique (Dako, Carpinteria, California, USA). Samples of the bladder carcinoma with known $P_53$ mutations and documented accumulations of $P_53$ protein by immunohistochemical analysis were used as positive controls. Nonepithelial cells (lymphocytes, stromal cells, and endothelial cells), used as internal negative controls, demonstrated no immunoreactivity. Only nuclear localization of immunoreactivity was evaluated. Samples demonstrating at least 10% nuclear reactivity were considered to be positive for $P_53$ (have a mutation in $P53$ gene; Figure). The immunohistochemical analysis was performed blindly to the tumor grade and stage.

The chi-square test was used to evaluate the association of $P53$ protein accumulation in the nuclei of the urothelial cells with pathologic stage and histological grade of TCC. A $P$ value less than .05 was considered significant.

**RESULTS**

Analysis of 50 tumoral and 10 normal transitional epithelium specimens revealed that nuclear $P53$ protein was identified more frequently in invasive high-grade TCC in comparison with noninvasive low-grade papillary TCC, but this association was not statistically significant ($P = .35$). In contrast, the difference of nuclear $P53$ accumulation between PUNLMP and low and high grade TCC (invasive or noninvasive) was statistically significant ($P < .001$; Table). Actually, about 90% of PUNLMP specimens were $P53$-negative. Expression of $P53$ was nil in all normal transitional epithelium specimens.

**Nuclear P53 Immunoreactivity in Normal and Neoplastic Urothelial Specimens**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of Specimens</th>
<th>$P53$ positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal urothelium</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>PUNLMP</td>
<td>10</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Papillary low-grade TCC</td>
<td>20</td>
<td>15 (75)</td>
</tr>
<tr>
<td>Invasive high-grade TCC</td>
<td>20</td>
<td>17 (85)</td>
</tr>
</tbody>
</table>

*Values in parentheses are percents. PUNLMP indicates papillary urothelial neoplasm of low malignant potential and TCC, transitional cell carcinoma.*

Immunochemistry staging for $P_53$ protein reactivity. **Left,** There is no nuclear reactivity in a specimen diagnosed with papillary urothelial neoplasm of low malignant potential ($\times 100$). **Right,** Moderate to severe nuclear reactivity in about 80% of tumoral cells in a specimen with high-grade invasive transitional cell carcinoma ($\times 100$).
DISCUSSION

In spite of short half-life of wild-type P53 protein, the half-life of a mutated P53 product is long.(6) This characteristic results in accumulation of the mutated P53 product, and thus, detection of P53 protein in the nuclei of cells by immunohistochemical methods. However, in 15% to 20% of tumors, despite of P53 gene mutation, its product does not accumulate in the nucleus.(6) On the other hand, in a proportion of tumors, despite the nuclear accumulation of P53 protein, there is no mutation in P53 gene.(3) In the first condition, some P53 gene mutations (such as point mutations) may result in lack of or severe decrease in P53 protein synthesis, and in the second condition, it has been shown that some cellular oncogenic products, such as mouse double minute 2 (MDM2), which bind to and inactivate wild-type P53 protein, result in a long half-life of P53 protein. In fact, recent studies have revealed that overexpression of MDM2 leads to overexpression of P53, without any detectable P53 mutation.(7,8)

In early stages of bladder cancer, deletion of chromosome 9 may be the only genetic abnormality, suggesting an initial role in development of the urothelial cancer.(9-12) Deletion in chromosome 9 is thought to be associated with loss of genes that have a tumor suppression role.(13-15) Carcinomas with only chromosome 9 aberration do not show progression. However, addition of other genetic abnormalities such as P53 defects may indicate potential for progression.

Comparative studies on the molecular genetics of Ta urothelial carcinomas and nonpapillary flat urothelial carcinoma in situ, which is a full-thickness proliferation of malignant urothelial cells confined to the epithelium, have revealed that these tumors are probably derived from a distinctly different genetic pathway.(16-18) Whereas, the earliest genetic aberration in papillary TCC may involve chromosome 9 deletion, nonpapillary flat urothelial carcinoma in situ is characterized by abnormalities of the P53 genes. Simon and colleagues, using comparative genomic hybridization, showed that low-grade noninvasive papillary neoplasms (Ta) are not associated with major genomic aberrations, except for chromosome 9 losses.(19) These authors also showed that there is clearly a higher number of genetic alterations in T1 than in Ta tumors. Most of all, a much higher degree of genetic instability is suggested in T1 than in Ta tumors.(19)

Papillary low-grade TCC and PUNLMP present the first step of tumor development. Although the only difference between these tumors is the presence or absence of mild anaplasia and dysplasia, there may be other differences which are not apparent on histological evaluation alone. In our study, a significant genetic difference (P53 overexpression) was found between PUNLMP and papillary low-grade TCC; Only 10% of PUNLMPs were P53 positive, suggesting that P53 mutation does not play a role in development of transitional tumors. Conversely, 75% of the papillary low-grade TCC tumors revealed P53 overexpression that shows a crucial role for P53 mutation in further tumor progression from PUNLMP to low-grade TCC. Moreover, multiple genomic alterations may be needed for transformation of papillary TCC (Ta) to invasive forms, but P53 mutation is most probably not such an alteration, since there was no significant statistical difference between low-grade papillary TCC and high-grade invasive TCC in nuclear P53 protein accumulation. However, our data were on a very small sample size. The significant differences we observed between the stages of tumor progression encourage us to perform future research to confirm these findings.

CONCLUSION

Our findings of P53 overexpression in papillary low-grade TCC and invasive high-grade TCC together with lack of its expression in PUNLMP support the notion that mutation of P53 gene might be unrelated to the development of urothelial neoplasm. Whereas, we can speculate that mutation of this gene may play a crucial role in further progression of PUNLMP to low-grade TCC. In our opinion, recent changes in urothelial neoplasm classification from triple-staging systems to WHO/ISUP are in agreement with our findings. Thus, this study shows the importance of WHO/ISUP classification in renaming of grade 1 TCC to PUNLMP.

CONFLICT OF INTEREST

None declared.
REFERENCES