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Frequent Alteration of p63 Expression in Human Primary Bladder Carcinomas

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Abstract

p63, a recently identified member of the p53 gene family, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. To explore the penetrance of p63 in bladder carcinogenesis, we performed expression and mutation analyses of two major isoforms, TAp63 and ΔNp63, in 63 bladder specimens. In 12 normal tissues, TAp63 was expressed at an easily detectable level whereas ΔNp63 was absent or extremely low. While none of 47 carcinomas showed allelic deletion of the gene, marked reduction of TAp63 and abnormal overexpression of ΔNp63 was found in 25 (53.2%) and 30 (63.8%) carcinomas, respectively. Tumor-specific alteration of TAp63 and ΔNp63 expression was identified in two and three of six matched sets, respectively. In addition, reduced expression of TAp63 showed a correlation with tumor stage and grade. Abnormal expression of TAp63 or ΔNp63 isoform was also observed in three of four cell lines, and treatment with 5-Aza-2-deoxycytidine led to up- or down-regulation of TAp63 and/or ΔNp63 expression, suggesting that the promoters of both isoforms might be affected by DNA methylation, but not in a reciprocal fashion. No sequence alteration of p63 was identified in 47 carcinomas whereas 17 (34.8%) of these showed p53 mutations, and no association between p63 expression and the mutational status of p53 or expression of p21Waf1, MDM2, and 14–3-3σ was recognized. Our data suggest that altered expression of p63 is a frequent event in bladder carcinogenesis and might contribute to the progression of bladder tumors, possibly via the mechanism(s) distinct from the p53 pathway.

Introduction

p53 is the most frequently mutated tumor supressor gene identified in human cancers (1). Tumor suppression functions of p53 stem, in part, from its capabilities to induce cell cycle arrest in late G1 and/or apoptosis in response to genotoxic stress and hypoxia, and mutational inactivation of p53 is associated with an increased risk of tumorigenesis (2). Recently, two members of the p53 family, termed p73 and p63, have been identified at 1p36.3 and 3q27–29, respectively (3–8). p73 and p63 share 80% identity and encode multiple proteins with transactivating, death-inducing, and dominant-negative activities, which are derived from a single gene with two promoters (TAp63 and ΔNp63) and at least three alternative splicing of the transcripts (α, β, and γ; Refs. 4–7). TAp63 isoforms with the acidic NH₂-terminal transactivating domain can activate transcription of p53 target genes such as p21Waf1, whereas ΔNp63 isoforms without the transactivating domain can act as dominant-negative factors toward transactivation by p53 and p63 (4, 6). p63 is highly expressed in proliferating basal cells of epithelial layers, including epidermis, cervix, urothelium, and prostate, and the major p63 isoforms in these basal cells lack the transactivating domain (4, 6). Recent studies revealed that mutational alteration of p63 is uncommon in human cancer cell lines and tumors (15, 16). However, it was demonstrated that expression of p63 is low or absent in a subset of lung cancer and ΔNp63 transcript is dominantly expressed in cell lines with high levels of p63 expression (17). Expression of TAp63γ was also found to associate with tumor growth in cervical carcinogenesis, and numbers of the cells expressing ΔNp63 and their distribution showed a correlation with anaplasia in squamous cell carcinoma (18, 19).

Although the genomic imbalance at 3q27–29 has not been directly implicated in human cancers, several genetic studies using microsatellites and comparative genomic hybridization demonstrated frequent loss of heterozygosity (3) or amplifications at several regions of chromosome 3 in bladder tumors (20, 21). In the present study, we performed expression and mutation analyses of p63 in 63 bladder specimens, including 47 primary carcinomas and four cell lines, to investigate the potential involvement of p63 alteration in the carcinogenesis of bladder cancer. Here, we show that genomic deletion or mutations of p63 is uncommon in primary bladder carcinoma, but its altered expression might contribute to the progression of bladder tumors.

Materials and Methods

Tissue Specimens and Human Cell Lines. Forty-seven carcinoma and 12 noncancerous bladder tissue specimens, including six matched sets, were obtained from 47 bladder cancer patients and 6 noncancer patients by surgical resection in the Kyung Hee University Medical Center (Seoul, Korea). Tissue specimens were snap-frozen in liquid N₂ and stored at −70°C until used. Four human bladder carcinoma cell lines (382, T24, HT1197, and HT1376) were obtained from Korea Cell Line Bank (Seoul National University, Seoul, Korea) or American Type Culture Collection (Manassas, VA). Extraction of total cellular RNA and cDNA synthesis were performed as described previously.

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Genomic DNA was extracted from the same cells of the tissues from the DNA phase after RNA was extracted.

**Quantitative PCR Analysis.** For quantitative evaluation by PCR, we initially performed the PCR reaction over a range of cycles (24, 27, 30, 33, 36, 39, and 42 cycles). Diluted cDNA (1:4, 12.5 ng/50 μl, PCR reaction) undergoing 27–36 cycles was observed to be within the logarithmic phase of amplification and yielded reproducible results with the primers used for p63, p40–1 (sense, 5'-CTTCTTCATCACCCTCAGGG-3') and p40–2 (antisense, 5'-TCAGGCTCATTCCACACTTGTT-3'), and an endogenous expression standard gene, GAPDH (14). For isoform-specific quantitation of TAp63 and ΔNp63, primers TAp63–2 (sense, 5'-GACCTAGTCTGGCAACATGTTG-3') and p63–2 (antisense, 5'-TTTCTGTGGGGTACTGGTCTGG-3') were used for amplification and yielded reproducible results with the primers used for p63, ΔNp63–1 (sense, 5'-GGCCCCACAGTCAATTTGACTTG-3') and p63–2 (see above) were used, respectively. PCR was performed for 36 cycles at 95°C (1 min), 60–63°C (0.5 min), and 72°C (1 min) in 1.5 μl MgCl₂ containing reaction buffer (PCR buffer II; Perkin-Elmer Corp.). Ten microliters of PCR products were mixed with 5μl of denaturing loading buffer (95% formamide, 20 mM Tris-HCl, pH 8.0, 0.05% bromphenol blue, and 0.05% xylene cyanol), and 15 μl of ddH₂O. After heating at 95°C for 5 min, samples were loaded in wells and electrophoresed in a 6% polyacrylamide gel containing 7 M urea. The gels were exposed to X-ray film for 1 to 3 days to allow visualization of PCR products. The 5-Aza-2'-deoxycytidine (Sigma Chemical Co., St. Louis, MO) was added to the medium at concentrations of 0.49 μM in duplicate, and cells were harvested after 3 and 5 days.

**Nonisotopic RT-PCR-SSCP Analysis.** Nonisotopic RT-PCR-SSCP analysis was performed as described previously (23). The p63 transcript was amplified with six sets of primers that were designed to cover the entire coding region of the gene. Sequences of the primers used for our PCR–SSCP analysis will be obtained on request. The PCR products of over 300 bp in length were digested with endonuclease(s) to increase the sensitivity of SSCP analysis. Twenty microliters of PCR products were mixed with 5 μl of 0.5% NtIO6, 10 μM EDTA, 10 μl of denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol), and 15 μl of ddH₂O. After heating at 95°C for 5 min, samples were loaded in wells precooled to 4°C. SSCP was performed using 8% nondenaturing acrylamide gels containing 10% glycerol at 4–8°C or 18–22°C.

**5-Aza-2'-Deoxycytidine Treatment.** To assess activation of p63 expression, four bladder carcinoma cell lines were plated in 6-well tissue plates 24 h before treatment. 5-Aza-2'-deoxycytidine (Sigma Chemical Co., St. Louis, MO) was added to the fresh medium at concentrations of 1.0 and 2.0 μM in duplicate, and cells were harvested after 3 and 5 days.

**Results and Discussion**

**Expression of p63 in Noncancerous Bladder Tissues.** To explore the candidacy of p63 as a suppressor in bladder carcinogenesis, we initially evaluated mRNA expression levels of two major isoforms of p63, TAp63, and ΔNp63 in 12 noncancerous bladder tissues by quantitative RT-PCR using isoform-specific primers. As shown in Fig. 1, expression of TAp63 mRNA was easily detectable in all noncancerous tissues we examined, whereas expression of ΔNp63 was extremely low or not detected. No significant variation in expression levels of p63 transcripts was recognized among the specimens (TAp63/GAPDH, 0.83–1.14; ΔNp63/GAPDH, 0.00–0.28). On the basis of this observation, we arbitrarily classified expression levels less than a half of the mean as abnormal expression.

Of four bladder carcinoma cell lines examined, three (J82, T24, and HT1197) and two (HT1197 and HT1197) were identified to express abnormally low TAp63 and high ΔNp63 mRNA, respectively (Table 1).

**Abnormal Reduction of TAp63 Expression in Carcinomas.** We next evaluated expression levels of TAp63 mRNA in 47 primary carcinomas. Quantitative RT-PCR analysis revealed that 25 (53.2%) carcinomas expressed abnormally low level of TAp63, and in 5 of these TAp63 transcripts were nearly undetectable (Fig. 2A and Table 1). In addition, tumor-specific reduction of TAp63 was found in two of six matched sets (Fig. 2B). Interestingly, abnormally low expression (levels 0–2) of TAp63 showed a correlation with tumor stage and grade (Table 1). Whereas abnormal reduction of TAp63 was observed in 28.0% (7 of 25) of superficial tumors (Ta-T1), 81.8% (18 of 22) of invasive tumors (T2-T4) were identified as low or no TAp63 expressions. Low expression of TAp63 was also found in 38.9% (7 of 18) of grade I, 43.8% (7 of 16) of grade II, and 84.6% (11 of 13) of grade III tumors. Thus, these observations demonstrate that altered expression of TAp63 mRNA is a frequent event in bladder carcinogenesis and suggest that inactivation of TAp63 might contribute to the malignant progression of primary bladder tumors.

**Abnormal Overexpression of ΔNp63 in Carcinomas.** Previous studies using ectopic induction of p63 suggested that ΔNp63 could act as a dominant-negative factor toward the G1 cell cycle arrest and apoptosis induction by TAp63 or p53 (4, 6). In this context, we investigated the possible involvement of abnormal ΔNp63 elevation in bladder tumorigenesis. As shown in Fig. 2, abnormally high expression (levels 3–5) of ΔNp63 was detected in 63.8% (30 of 47) of carcinomas and tumor-specific increase of ΔNp63 was observed in three of six matched sets. However, unlike TAp63, abnormal overexpression of ΔNp63 was not associated with tumor stage and grade (Table 1).

**Relationship between TAp63 and ΔNp63 Expression.** If ΔNp63 acts as a dominant-negative factor toward the growth inhibition or apoptosis by TAp63, alteration of TAp63 and ΔNp63 would be expected to be mutually exclusive in cancer cells. Whereas 19 (86.4%) of 22 normal expressors of TAp63 showed abnormally high ΔNp63, 11 (44.0%) of 25 tumors with low TAp63 expression were identified as high ΔNp63 expressors (Table 2). Likewise, although 14 (82.4%) of 17 normal expressors of ΔNp63 showed abnormally low TAp63, only 11 (36.7%) of 30 tumors with high ΔNp63 expression were identified as low TAp63 expressors. Similarly, two (J82 and T24) of the three cell lines with low TAp63 expressed normal levels of ΔNp63, whereas the HT1197 cell line with a normal level of ΔNp63 expressed abnormally high ΔNp63 (Fig. 1). However, simultaneous alteration of TAp63 and ΔNp63 was also found in 11 (23.4%) of the 47 carcinomas and in one (25.0%) of the four cell lines. Thus, a mutually exclusive expression pattern of TAp63 and ΔNp63 was recognized in general.

![Fig. 1. Expressions of p63 isotypes in normal bladder tissues and cell lines. The exons 3–4 region (for TAp63) and exons 3–5 region (for ΔNp63) of p63 transcripts were amplified by quantitative RT-PCR using isoform-specific primers. The GAPDH gene was used as an endogenous control. N1–N8, normal bladder tissues.](image-url)
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Table 1  Expression levels of TAp63 and \( \Delta Np63 \) mRNA in human bladder tissues and cell lines*  

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>CL (^b)</th>
<th>N</th>
<th>Ca</th>
<th>Stage</th>
<th>T1-T1</th>
<th>T2-T4</th>
<th>Grade</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
</table>
| Expression /GAPDH/  
\( \Delta Np63 \) | 4-5 (>0.99) | 0 | 7 (58.3) | 11 (23.4) | 8 (36.0) | 3 (13.6) | 6 (33.3) | 4 (25.0) | 1 (7.9) |
| 3 (0.49–0.98) | 1 | 5 (41.7) | 11 (23.4) | 10 (40.0) | 1 (4.5) | 5 (27.8) | 5 (31.3) | 1 (7.9) |
| 2 (0.26–0.48) | 3 | 0 (0.0) | 17 (36.2) | 6 (24.0) | 11 (50.0) | 6 (33.3) | 5 (31.3) | 6 (46.2) |
| 0-1 (<0.25) | 0 | 0 (0.0) | 8 (17.0) | 1 (4.0) | 7 (31.8) | 1 (5.6) | 2 (12.5) | 5 (38.5) |
| \( \Delta Np63 \) | 4-5 (>0.79) | 2 | 0 (0.0) | 14 (29.8) | 7 (28.0) | 7 (31.8) | 5 (27.8) | 5 (31.3) | 4 (30.8) |
| 3 (0.39–0.78) | 0 | 0 (0.0) | 16 (34.0) | 9 (36.0) | 7 (31.8) | 6 (33.3) | 6 (37.5) | 4 (30.8) |
| 2 (0.19–0.38) | 0 | 7 (58.3) | 8 (17.0) | 4 (16.0) | 4 (18.2) | 3 (16.7) | 3 (18.8) | 2 (15.4) |
| 0-1 (<0.19) | 2 | 5 (41.7) | 9 (19.1) | 5 (20.0) | 4 (18.2) | 4 (22.2) | 2 (12.5) | 3 (23.1) |

* Numbers in parentheses are percentage.
\(^b\) CL, cell lines; N, normal; Ca, carcinoma.
\(^c\) Expression levels (0–5) were classified based on the ratio of \( TAp63 \)/GAPDH.

Expression of \( p63 \) and \( \Delta Np63 \) in Bladder Tissues. To evaluate expression of alternatively spliced variants \( p63\alpha \), \( p63\beta \), and \( p63\gamma \), we performed PCR amplification of the COOH-terminal portion of \( p63 \) transcripts using variant-specific primer sets. \( p63\alpha \) and \( p63\gamma \) transcripts were easily detectable in all \( p63\alpha \)-positive tissues, and no significant difference in expression levels of these two variants was recognized. In contrast, expression of \( p63\beta \) transcripts was not detected under our experiment conditions (data not shown).

Absence of \( p63 \) Mutations and No Correlation with \( p53 \) Status. To investigate the allelic deletion or mutational alteration of the \( p63 \) gene, we performed quantitative DNA/PCR and RT-PCR-SSCP analyses of \( p63 \) for 47 primary carcinomas, four cell lines, and 10 noncancerous tissues. Compared with normal tissues, no significant difference was detected in \( p63 \) gene levels in tumors, indicating that abnormal expression of \( p63 \) mRNA is not associated with allelic alteration of the gene (Figs. 1 and 2). For SSCP analysis, the entire coding region of the transcripts was amplified using six different sets of primers, digested with several different restriction endonucleases, and subjected to electrophoresis under two different running conditions. However, we failed to detect any types of mutation leading to amino acid substitutions or frameshifts, whereas 36.2% (17 of 47) of the same set of primary carcinomas was identified to carry \( p53 \) mutations. Thus, this result indicates that, unlike \( p53 \), mutational alteration of \( p63 \) is not a main genetic event in the bladder carcinogenesis. In addition, no correlation was identified between altered expression of \( p63 \) isoforms and \( p53 \) status in tumors we analyzed (Table 2). To further define the possible association of \( p63 \) with the \( p53 \) pathway, we examined expression of \( p53 \) target genes such as \( p21^{\text{Waf1}} \), \( MDM2 \), and \( 14–3−3\text{z} \). Whereas low expression of \( p21^{\text{Waf1}} \) mRNA was more frequently observed in tumors with \( p53 \) mutation (11 of 17, 64.7%) than tumors with wild-type \( p53 \) (4 of 30, 13.3%), alteration of \( TAp63 \) or \( \Delta Np63 \) showed no association with mRNA expression of \( p53 \) target genes (Table 2). Taken together, these data demonstrate that alteration of \( p63 \) expression does not correlate with the mutational status of \( p53 \) and its target gene expression in bladder carcinomas.

Expression and mutational status of \( p63 \) and \( p55 \) in human bladder tumors*  

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>( TAp63 ) expression</th>
<th>( \Delta Np63 ) expression</th>
<th>( p53 ) status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Ab. (^a) Low</td>
<td>Normal</td>
<td>Ab. High</td>
</tr>
<tr>
<td>22</td>
<td>17</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Ab. Low</td>
<td>3 (13.6)</td>
<td>14 (56.0)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>( \Delta Np63 ) expression Normal</td>
<td>3 (13.6)</td>
<td>14 (56.0)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>Ab. Low</td>
<td>7 (31.8)</td>
<td>8 (32.0)</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td>Low ( p21^{\text{Waf1}} )</td>
<td>0 (0.0)</td>
<td>1 (4.0)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>High ( MDM2 )</td>
<td>4 (18.2)</td>
<td>5 (20.0)</td>
<td>4 (23.5)</td>
</tr>
<tr>
<td>Low 14–3−3\text{z} )</td>
<td>4 (18.2)</td>
<td>5 (20.0)</td>
<td>4 (23.5)</td>
</tr>
</tbody>
</table>

* Expression level of \( p63 \): less than half (<0.49; levels 0–2 for \( TAp63 \)) and more than 2-fold (>0.38; levels 3–5 for \( \Delta Np63 \)) of means of normal tissues were classified as abnormal expression.
\(^a\) Ab, abnormal.

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that altered expression of \( TAp63 \) is correlated with tumor progression and patient survival but no further increase of \( \Delta Np63 \) alteration is observed in advanced tumors, suggesting its possible contribution to an initial step of bladder tumorigenesis. Further study will be required to gain understanding for the biological significance of isoform-specific roles of \( p63 \) in human tumorigenesis.

It has been hypothesized that disruption of normal \( p53 \) function results in compensatory or deleterious up-regulation of other members of the \( p53 \) gene family or that overexpressed \( \Delta Np63 \) may bind \( p53 \) DNA target sites in a competitive manner or mimic mutant \( p53 \), thus acting as a dominant-negative factor in wild-type \( p53 \)-carrying tumor cells (3–6). However, recent studies demonstrated that \( p63 \) transactivates the \( p53 \) target genes but the degree of the transactivation by \( p63 \) differed from that by \( p53 \), and the tumor-derived \( p63 \) missense mutations were found to retain their ability to transactivate the \( MDM2 \) and/or the \( Bax \) promoter but not the \( p21^{Waf1} \) promoter, indicating that the cellular signal on \( p63 \) cross-talks partially, but not completely, with that of the \( p53 \) pathway (24, 25). It has been also observed that \( p53 \) in cancer cells was not able to interact with endogenous or exogenous \( p63 \) or \( p73 \) via their respective oligomerization domains while the multiple isoforms of \( p63 \), as well as those of \( p73 \), are capable of interacting via their common oligomerization domain (26). Moreover, recent studies showed that \( p53 \)-inactivating viral oncopro-

**Biphasic Effect of 5-Aza-2’-Deoxycytidine on \( p63 \) Expression.** To explore whether abnormal methylation is associated with the altered expression of \( p63 \), we treated the four cell lines with a demethylating agent, 5-Aza-2’-deoxycytidine, and analyzed expression levels of \( TAp63 \) and \( \Delta Np63 \). Whereas expression of \( TAp63 \) mRNA was induced in T24 by 5-Aza-2’-deoxycytidine treatment, up- and down-regulation of both \( TAp63 \) and \( \Delta Np63 \) transcriptions were observed in HT1376 and HT1197, respectively, and no change in \( p63 \) levels was detected in J82 (Fig. 3). These results suggest that abnormal hypermethylation would be one of the causes for the altered expression of \( p63 \), but other factors might be implicated in the reciprocal regulation of \( TAp63 \) and \( \Delta Np63 \) in bladder carcinoma.

**Expression of \( p63 \) and Patient Survival.** To further characterize the possible relationship of \( p63 \) alteration with disease progression, we performed Kaplan-Meier survival analysis for 36 patients whose follow-up history was available. As shown in Fig. 4, expression of \( TAp63 \) (\( P = 0.06 \)) but not \( \Delta Np63 \) (\( P = 0.88 \)) was recognized to correlate with cumulative survival of the patients after operation.

Despite its high degree of structural similarity to \( p53 \) and a growth inhibitory role by epigenetic control of expression, there are currently few evidences that inactivation of \( p63 \) is required for transformation or malignant progression of human tumors. In this study, we first demonstrate that altered expression of \( TAp63 \) and \( \Delta Np63 \) is a frequent event and might contribute to the progression of bladder tumors. Our study also suggests that alteration of \( p63 \) expression might be caused by more complicated mechanisms, including epigenetic factors rather than allelic or mutational alteration of the gene. It has been previously reported that expression of \( p63 \), predominantly \( \Delta Np63 \), is highly expressed in basal cells with high proliferative potential and is absent from the cells that are undergoing terminal differentiation (4, 6). A recent study also showed that \( p63 \) expression is absent or low in a considerable proportion of lung cancers and \( \Delta Np63 \) transcript is dominantly expressed in cell lines with high levels of \( p63 \) expression, whereas only 1 of 44 cell lines but none of 45 primary tumors has \( p63 \) mutation (15). In squamous cell carcinoma of the skin, the number and distribution of cells expressing \( \Delta Np63 \) was found to correlate with anaplasia (19). In this context, it is noteworthy that a mutually exclusive alteration of \( TAp63 \) and \( \Delta Np63 \) was recognized in a substantial fraction of bladder tumors we analyzed, and low \( TAp63 \) rather than high \( \Delta Np63 \) expression showed a correlation with tumor stage and grade. However, our finding of simultaneous alteration of \( TAp63 \) and \( \Delta Np63 \) expression in a subset of tumors also raises the possibility that two isoforms of \( p63 \) might carry their specific roles in bladder carcinogenesis. This hypothesis is partly supported by our observation

![Fig. 3. Effect of 5-Aza-2'-deoxycytidine treatment on p63 expression in bladder cell lines. Four carcinoma cell lines were treated with the demethylating agent 5-Aza-2'-deoxycytidine (2 μM) for 72 h, and expressions of TAp63 and ΔNp63 isotypes were evaluated by quantitative RT-PCR. C, untreated control; T, treated.](image-url)

![Fig. 4. Expression of p63 in bladder carcinomas and cumulative survival of patients after operation. Thirty-six bladder cancer patients were subjected to Kaplan-Meier survival analysis to evaluate the possible association with the mRNA expression status of TAp63 and ΔNp63. Expression levels 0–2 and 3–5 were classified as low and high expression, respectively (see text).](image-url)
teins such as SV40 T antigen, human papilloma virus E6, and adenovirus E1B do not directly interact with p63 and do not inhibit p63-mediated transcription, suggesting that unlike p53, p63 does not seem to be a necessary target in virus-induced cell transformation (27, 28). Consistent with these observations, we identified no association of TAp63 or ΔNp63 expression with the mutational status of p53 and the expressions of p53 target genes such as p21Waf1, MDM2, and 14–3–3σ in primary bladder tumors and cell lines. Our preliminary work also showed that transient overexpression of wild-type p53 or p73, treatment with a DNA-damaging agent (etoposide), or cell growth under growth factor-deprived culture condition did not significantly affect the expression levels of both p53 and ΔNp63 mRNA in bladder carcinoma cell lines (data not shown). Taken together, these results suggest that p63 may not exert a role comparable with p53 and be involved in an unknown tumor suppressor pathway distinct from that of p53.

Recent studies demonstrated that mutations in the p63 gene are rare in human cell line and tumors. Hagiwara et al. (15) reported that only 2 of 54 human cell lines have either heterozygous mutations or polymorphisms in the putative DNA binding domain of p63. Other investigators identified only four distinct missense mutations of p63 after screening >200 tumors and cell lines (5, 16). In the present study, we also failed to detect allelic deletion or any type of mutation leading to amino acid change of p63 in bladder cancers whereas 36.2% of the same tumors were identified to carry p53 mutations. This result indicates that mutation alteration of p63 may not be a main genetic event in the bladder carcinogenesis and suggests that p63 is unlikely to be a tumor suppressor gene that conforms to a two-hit model of tumorigenesis.

In conclusion, the evidences we obtained here clearly demonstrate that p63 is not a target of sequence alterations in bladder carcinogenesis, but abnormal reduction of TAp63 and/or overexpression of ΔNp63 are frequent and might contribute to the progression of bladder cancer, although the functional significance of p63 alteration was not defined in the present work. Additional studies will be required to elucidate the roles of altered p63 expression in growth and apoptosis of bladder epithelial cells.

References

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