Attenuated Expression of Xeroderma Pigmentosum Group C Is Associated with Critical Events in Human Bladder Cancer Carcinogenesis and Progression

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Abstract

Xeroderma pigmentosum group C (XPC) is an important DNA damage recognition protein that binds to damaged DNA at a very early stage during DNA repair. The XPC protein is also involved in DNA damage–induced cell cycle checkpoint regulation and apoptosis. XPC defects are associated with many types of solid tumors. The mechanism of the XPC protein in cancer progression, however, remains unclear. In this report, we showed the strong correlation between bladder cancer progression and attenuated XPC protein expression using tissues derived from patients with bladder cancer. The results obtained from our immunohistochemical studies further revealed a strong correlation of XPC deficiency, p53 mutation, and the degree of malignancy of bladder tumors. In addition, the results obtained from our studies have also shown that HT1197 bladder cancer cells, which carry a low-level XPC gene cDNA expression vector, exhibited a decreased DNA repair capability and were resistant to cisplatin treatment. When an XPC gene cDNA-expression vector was stably transfected into the HT1197 cells, however, the cisplatin treatment–induced apoptotic cell death was increased. Increased p53 and p73 responses following cisplatin treatment were also observed in HT1197 cells stably transfected with XPC cDNA. Taken together, these results suggest that XPC deficiency is an important contributing factor in bladder tumor progression and bladder cancer cell drug resistance. [Cancer Res 2007;67(10):4578–85]

Introduction

Urinary bladder cancer is the fourth most common neoplasm found in men from western countries, with urothelial cell carcinoma being the most common subtype (1). The established risk factors of bladder cancer include cigarette smoking, exposure to industrially related aromatic amines, and the uptake of chemical drugs such as cyclophosphamide. Because of the nature of bladder as an important void organ, the urothelial cells are continuously exposed to many DNA-damaging reagents contained in the urine. Therefore, DNA repair plays an essential role in preventing deleterious DNA damage–induced effects such as mutation accumulation and tumor occurrence.

Several DNA repair pathways exist in mammalian cells, and each pathway effectively removes particular types of DNA damage. Based on the type of DNA damage, the DNA repair pathways can be categorized into nucleotide excision repair (NER), base excision repair, mismatch repair, and recombinational repair [both homologous recombination (HR) and nonhomologous end joining]. The NER pathway is the major DNA repair pathway for repairing bulky DNA damage generated by most environmental factors, such as UV radiation, chemicals, and therapeutic drugs (e.g., cisplatin and mitomycin). Therefore, the presence of a functional NER pathway is essential for maintaining genetic integrity and for preventing the development of many disease conditions from exposure to these DNA-damaging reagents, such as cancer.

Xeroderma pigmentosum group C (XPC) is a DNA damage recognition protein that plays an important role in the NER process. The XPC protein binds tightly with an HR23B protein to form a stable XPC-HR23B complex (2, 3). Studies indicate that the XPC-HR23B complex is the first protein component that recognizes and binds to the damaged sites (4–9). The XPC protein might also play an important role in other DNA damage–induced cellular responses, including cell cycle checkpoint regulation and apoptosis (10, 11). XPC defects have been found in many types of cancer, including lung and skin cancer (12, 13). XPC knock-out transgenic mice studies reveal the high incidence of a predisposition to many types of cancer (12, 14–17). In addition, genetic studies also reveal a strong association between XPC gene polymorphisms and the occurrence of many types of cancer (18–21). In light of these results, however, no studies have been done to directly investigate the role of XPC defects in bladder cancer progression.

We have studied the relationship of XPC defects with bladder cancer progression and cancer cell drug resistance. The results obtained from our immunohistochemical studies using paraffin wax–embedded tissues from bladder urothelial cell carcinomas revealed a high incidence of reduced XPC protein expression in these tissues. Statistical analysis of these immunohistochemical data showed a strong correlation between XPC deficiency and the degree of malignancy of bladder tumors. The correlation of XPC deficiency/p53 mutation and the degree of malignancy of bladder tumors is also statistically significant. The results obtained from our bladder cancer cell culture studies also suggest a strong correlation between a reduced XPC protein and an increased resistance of bladder cancer cells to cisplatin treatment. These results suggest that the XPC protein plays an important role in preventing the occurrence of bladder cancer, and that the XPC defects lead to a high incidence of bladder cancer occurrence and bladder cancer cell resistance to cisplatin (treatment).

Materials and Methods

Cell lines and plasmid. The HT1197 and T24 bladder carcinoma cells and HeLa cells were purchased from the American Type Culture Collection. The BIU-87 and EJ bladder cancer cells were obtained from the Institute of Urology,
Beijing University. The HT1197 cells were maintained in MEM supplemented with 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 1.0 mmol/L sodium pyruvate, and 10% fetal bovine serum (FBS). The T24 cells were maintained in McCoy's 5A medium supplemented with 10% FBS. The HeLa cells were maintained in DMEM supplemented with 10% FBS. The glu-87 and EB bladder cancer cells were maintained in RPMI 1640 with 10% FBS.

The pXPC-3 plasmid, which carries an XPC gene cDNA, was kindly provided by Dr. R. Legerski (M.D. Anderson Cancer Center, University of Texas, Austin, TX). A 3.4-kb DNA fragment containing the XPC gene cDNA was further removed from the pXPC-3 plasmid DNA by SfiI digestion and inserted into the SfiI site of pcDNA3.1 (+) (Invitrogen) to obtain the pcDNA3-XPC plasmid.

**Tissue sample and immunohistochemistry.** The paraffin-embedded tissues of bladder papillary urothelial carcinoma were collected from patients who underwent transurethral resection of bladder tumor in our department from 2002 to 2004, and a total of 55 tissue specimens were used for this study. Archival materials from the 55 cases were retrieved from the surgical pathology files. These tissues, according to the new WHO classification (2004), were assigned pathologically to papillary urothelial neoplasm of low malignant potential (PUNLMP; 16 cases), low-grade papillary urothelial carcinoma (24 cases), and high-grade papillary carcinoma (15 cases). The research was approved by the ethics board of Southwest Hospital at the Third Medical Military University. Tissue sections of 5 μm were deparaffinized, rehydrated in graded alcohols, and processed using the streptavidin immunoperoxidase method (Zymed). In brief, sections were submitted to antigen retrieval by microwave oven treatment for 10 min in 0.01 mol/L of citrate buffer (pH 6.0). Slides were subsequently incubated in 10% normal serum for 30 min, followed by an overnight incubation at 4°C with the appropriately diluted primary antibody. The goat anti-human XPC (D-18) polyclonal antibody (Santa Cruz Biotechnology) was used at a 1:100 dilution. The mouse monoclonal anti-human p53 (DO-1) antibody (Santa Cruz Biotechnology) recognizing an epitope located between amino acids 11 and 25 was used at a 1:200 dilution. Samples were incubated with biotinylated anti-goat or anti-mouse immunoglobulins for 15 min at 37°C, and high-grade papillary carcinoma (15 cases). The research was approved by the ethics board of Southwest Hospital at the Third Military Medical University. Tissue sections of 5 μm were deparaffinized, rehydrated in graded alcohols, and processed using the streptavidin immunoperoxidase method (Zymed). In brief, sections were submitted to antigen retrieval by microwave oven treatment for 10 min in 0.01 mol/L of citrate buffer (pH 6.0). Slides were subsequently incubated in 10% normal serum for 30 min, followed by an overnight incubation at 4°C with the appropriately diluted primary antibody. The goat anti-human XPC (D-18) polyclonal antibody (Santa Cruz Biotechnology) was used at a 1:100 dilution. The mouse monoclonal anti-human p53 (DO-1) antibody (Santa Cruz Biotechnology) recognizing an epitope located between amino acids 11 and 25 was used at a 1:200 dilution. Samples were incubated with biotinylated anti-goat or anti-mouse immunoglobulins for 15 min at 37°C, followed by streptavidin peroxidase complexes for 15 min at 37°C. The nuclear extracts were prepared as described previously with some modifications (22). Briefly, the cells were harvested and washed twice with PBS. The cells were then resuspended in buffer A [10 mmol/L HEPES (pH 7.9), 1.5 mmol/L MgCl2, 10 mmol/L KCl, and 0.5 mmol/L DTT] five times the packed cell pellet volume and incubated on ice for 10 min. The cell pellets were lysed by 20 strokes of a Kontes glass Dounce homogenizer (B type pestle). The homogenate was centrifuged for 10 min at 25,000 × g (4°C) to pellet nuclei. The nuclei were resuspended in two volumes of buffer B [20 mmol/L HEPES (pH 7.9), 25% glycerol, 0.42 mol/L NaCl, 1.5 mmol/L MgCl2, 0.2 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 0.5 mmol/L DTT] and lysed by 20 strokes of a Kontes glass Dounce homogenizer (B type pestle). The resulting suspension was stirred gently for 30 min at 4°C and then centrifuged for 30 min at 25,000 × g. The clear supernatant was dialyzed against 50 volumes of buffer D [20 mmol/L HEPES (pH 7.9), 20% glycerol, 0.1 mol/L KCl, 0.2 mmol/L EDTA, 0.5 mmol/L PMSF, and 0.5 mmol/L DTT] at 4°C for 6 h. The dialysate was centrifuged at 25,000 × g for 20 min, and the supernatant was frozen as aliquots in liquid nitrogen.

**In vitro DNA repair assay.** The in vitro DNA repair synthesis assay was done as described previously with some modification (23). Briefly, the pcDNA3.1 plasmid was treated with 20 μmol/L cisplatin (Sigma) for 4 h to introduce DNA damage. The DNA repair assay was done in a 25-μL reaction mixture containing 20 mmol/L HEPES (pH 7.9), 0.1 mol/L KCl, 0.2 mmol/L EDTA, 1 μg plasmid DNA, 250 μmol/L each of dATP, dGTP, and dTTP, 10 μCi [α-32P]dCTP (ICN Pharmaceuticals), and 50 μg nuclear extract. The reactions were incubated at 30°C for 2 h and then digested with proteinase K (100 μg/mL) at 50°C for 30 min. The reaction products were extracted with phenol/chloroform once, and the plasmid DNA was purified using a Centricon 30 apparatus (Millipore). The plasmid DNA was then digested with XhoI and analyzed by agarose gel electrophoresis using a 1% gel. Visualization of the plasmid DNA and the incorporated [α-32P]dCTP was achieved by ethidium bromide (EB) staining and autoradiography. Quantification of the incorporated [α-32P]dCTP was determined using Scion image densitometry software.

**Colonogonic survival assay.** Cytotoxicity was evaluated using colonogonic survival. Exponentially growing cells were plated at appropriate densities in 100-mm dishes and exposed to varying concentration of cisplatin (0, 5, 10, 20, and 40 μmol/L) for 2 h after overnight attachment. The cell were washed and cultured for 14 days in normal growth medium. The cells were fixed and stained with 0.1% crystal violet. Colonies of >50 cells were counted, and the survival adjusted for plating efficiency of cell types was calculated and plotted.

**DNA fragmentation analysis.** The adhered cells were gently trypsinized, mixed with any unattached cells removed with the medium, and pelleted by centrifugation. DNA was isolated from the pelleted cells using a unique differential cellular lysis procedure that separates fragmented DNA released only by apoptotic cells from the native intact DNA of unaffected cells by centrifugation. DNA was isolated from the pelleted cells using a unique differential cellular lysis procedure that separates fragmented DNA released only by apoptotic cells from the native intact DNA of unaffected cells by centrifugation. The 13.000 × g supernatant was treated with RNase A for 30 min at 37°C followed by proteinase K (100 μg/mL) for 1 h at 45°C. Aliquots of DNA corresponding to 1 × 106 cells were resolved by gel (1.2% agarose) electrophoresis and visualized by EB staining. The apoptotic index was calculated from the amounts determined for the fraction of released DNA containing DNA damage.

**Flow cytometry assay.** Cells were plated in 60-mm dishes and incubated at 37°C overnight. The cells were treated with either 20 or 40 μmol/L cisplatin for 2 h. After washing completely the cells were cultured in normal growth medium. The cells were harvested at various time points (16, 24, and 48 h) and fixed with 70% ethanol. The cells were treated with propidium iodide (Sigma) and RNase A (400 units) for 30 min at 4°C. Cellular DNA content was analyzed using a flow cytometer (FACSCalibur, Becton Dickinson), and cell cycle analysis was carried out using ModFit 2.0 software (Becton Dickinson).
Results

Attenuated XPC expression coupled with an increased p53 mutation was correlated to the malignancy of bladder tumors. To study the correlation of XPC defects to bladder cancer progression, we first determined the levels of XPC protein in paraffin-embedded bladder tumor tissues collected by our hospital in the past 2 years. Because of the prevalence of p53 mutation in bladder cancer occurrence, we also determined the p53 mutation in these paraffin-embedded bladder tumor tissues to define the relationship of XPC deficiency and p53 mutation in bladder cancer progression. We have taken advantage of immunohistochemistry in this study because of the availability of paraffin-fixed clinical samples and the reliability of the immunohistochemistry procedures. In addition, the immunohistochemistry analysis can also help determine the p53 mutation because a nuclear localization of p53 represents p53 mutations, which is a widely accepted consensus in most p53 studies (24, 25).

The results obtained from our immunohistochemical studies revealed that a majority of the XPC protein was localized nuclei with both the nucleus and nuclear membrane stained with the XPC antibody (Fig. 1). Only a small amount of XPC protein was present in the cytoplasm as evidenced by the very faint stain of the cytoplasm with the XPC antibody (Fig. 1). We further analyzed the correlations of XPC deficiency and p53 mutation in bladder cancer occurrence. The results obtained from the immunohistochemical studies indicated that the XPC-deficient phenotype [XPC(−)] was present in 12.5% of PUNLMP cases (2/16), 33.3% of low-grade cases (8/24), and 80% of high-grade cases (12/15), and the p53 mutation phenotype [p53(+)] was observed in 6.2% of PUNLMP cases (1/16), 33.3% of low-grade cases (8/24), and 66.7% of high-grade cases (10/15; Table 1). In the case of co-occurrence of both XPC deficiency and p53 mutation, the results obtained from the immunohistochemistry stain revealed that the XPC(−)/p53(+) phenotype was present in 8.3% of low-grade cases (2/24) and 53.3% of high-grade cases (8/15) for the tested bladder tumor tissues. Interestingly, the XPC(−)/p53(+) phenotype was not detected in any of the PUNLMP cases (0/16). The statistical analysis of the immunohistochemistry data indicated that the occurrence rate of attenuated XPC expression was the highest in the high-grade tumors (compared with both the PUNLMP and the low-grade tumors; \( P < 0.01 \)). No statistical difference was observed between the PUNLMP and low-grade tumors \(( P > 0.05)\). The incidence of p53 mutation was also higher in the high-grade tumors than that of the PUNLMP tumors \(( P < 0.01)\), but no statistical difference between the high- and low-grade tumors was seen \(( P > 0.05)\); Table 1). Interestingly, the coincidence of both XPC deficiency and p53 mutation was also higher in the high-grade tumors than in those of the PUNLMP and low-grade tumors \(( P < 0.01)\). These results suggest that an attenuated expression of the XPC protein is indeed correlated with the degree of malignancy of bladder tumors. These results also suggest a possible synergistic effect of attenuated XPC expression and p53 mutation in the progression of bladder cancer.

The expression level of XPC protein was varied among individual bladder cancer cell lines. To further investigate a molecular mechanism of XPC protein in the carcinogenesis process, we extended our studies to bladder cancer cell lines.

Table 1. The associations of attenuated XPC expression/p53 mutation with tumor grade and malignancy

<table>
<thead>
<tr>
<th>Grade</th>
<th>XPC(−)</th>
<th>p53(+)</th>
<th>XPC(−)/p53(+)</th>
<th>Total tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary neoplasm of low malignant potential</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Low-grade papillary carcinoma</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>High-grade papillary carcinoma</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>( P ) value</td>
<td>( P &lt; 0.05, P^\Delta &lt; 0.01, P^\gamma &lt; 0.01 )</td>
<td>( P &lt; 0.05, P^\Delta &lt; 0.05, P^\gamma &gt; 0.05 )</td>
<td>( P &lt; 0.05, P^\Delta &lt; 0.01, P^\gamma &lt; 0.01 )</td>
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NOTE: \( P^\Delta \) value is the comparison between the group of low-grade papillary carcinoma and the group of papillary neoplasm of low malignant potential; \( P^\gamma \) value is the comparison between the group of high-grade papillary carcinoma and the group of papillary neoplasm of low malignant potential; \( P^\gamma \) value is the comparison between the group of high-grade papillary carcinoma and the group of low-grade papillary carcinoma.
repaired in the HeLa nuclear extracts as confirmed by the incorporation of \([\alpha-^32P]dCTP\) into the plasmid DNA (an 8.3-fold increase over the undamaged plasmid; (Fig. 2B, lane 2 versus lane 1). In contrast, a lower DNA repair activity was detected when the cisplatin-damaged plasmid DNA was incubated with HT1197 nuclear extracts (a 4-fold increase over the undamaged plasmid DNA; Fig. 2B, lane 4 versus lane 3). This result suggests that the presence of low-level XPC protein in the HT1197 cells significantly reduced its DNA repair capacity for cisplatin-caused DNA damage.

Although the XPC gene polymorphism has been associated with a risk of urinary bladder cancer, no studies have been conducted to determine the expression level of the XPC protein in various bladder cancer cells. Therefore, we determined the XPC protein level in four bladder cancer cell lines, HT1197, T24, BIU-87, and EJ (Fig. 2A). HeLa cells were used as a positive control for this study because the HeLa cells are proficient in DNA repair, especially in NER. Three of the tested bladder cancer cells, T24, BIU-87, and EJ, displayed similar levels of XPC protein when compared with the HeLa cells (Fig. 2A). In contrast, the level of XPC protein was much lower in HT1197 bladder cancer cells than that of the HeLa cells (Fig. 2A). Being equivalent to high-grade tumor cells, the HT1197 cells are an advanced and aggressive form of bladder cancer. These results suggest that different bladder cancer cells carry different levels of XPC protein. Because the heterogenicity is the hallmark for bladder cancer cells, this result suggests that the expression level of the XPC protein in bladder cancer cells is related not only to cell types but also to the heterogenicity or pathology grade of the bladder tumors.

The decreased DNA repair abilities of HT1197 cells. Although XPC gene mutations and polymorphisms have been linked to defective DNA repair and the predisposition of many types of cancer (12, 18–21), it is unknown if an attenuated XPC expression level would affect the DNA repair capacity of the cells. To determine if the low-level XPC protein in HT1197 is sufficient for DNA repair of the cells, the DNA repair abilities of HT1197 cells were measured using an in vitro DNA repair assay (22), and the HeLa nuclear extracts were used as a positive control for this study (Fig. 2). The cisplatin-damaged plasmid DNA was effectively

Figure 2. Detection of the cisplatin DNA damage–induced DNA repair synthesis in HeLa and HT1197 nuclear extracts. A, Western blot analysis of XPC expression in various bladder cancer cell lines. Top, probed with XPC antibody. Bottom, probed with \(\alpha\)-tubulin antibody. The \(\alpha\)-tubulin was used to confirm the equal loading of cell lysates on the gel. \(B\), the cisplatin treatment–induced DNA repair synthesis of pcDNA3 plasmid DNA in nuclear extracts prepared from HT1197 and HeLa cells. The pcDNA3 plasmid DNA was treated with 20 \(\mu\)mol/L cisplatin to generate cisplatin DNA damage into the plasmid template. Top, visualization of plasmid DNA by EB staining. Bottom, autoradiogram of the same gel showing labeled nucleotide incorporation indicative of DNA repair synthesis. The amount of incorporated \([\alpha-^32P]dCTP\) in pcDNA3 in the nuclear extracts was taken as the background. Incorporated \([\alpha-^32P]dCTP\) in the other reactions was calculated as a percentage to the background. Lane 1, pcDNA3 plasmid DNA + HeLa nuclear extract; lane 2, cisplatin-damaged pcDNA3 plasmid DNA + HeLa nuclear extract; lane 3, pcDNA3 plasmid DNA + HT1197 nuclear extract; lane 4, cisplatin-damaged pcDNA3 plasmid DNA + HT1197 nuclear extract. Densitometric values are indicated below each lane.
Overexpression of XPC protein resulted in an increased sensitivity of HT1197 bladder cells with cisplatin treatment. To further investigate the role of XPC protein in bladder cancer cell resistance to cisplatin treatment, we transfected the HT1197 cells with an XPC gene cDNA-expressing vector, and the cells that stably overexpressed the XPC protein were selected (Fig. 3A). The results obtained from our Western blotting indicated that clones 2 and 3 expressed much higher levels of XPC protein than the untransfected HT1197 or the empty vector–transfected HT1197 cells (Fig. 3A). Therefore, clone 2 was used to further study the role of XPC overexpression in cisplatin treatment–induced cell killing.

We determined the role of XPC overexpression in cisplatin treatment–induced cell killing using a colonogenic survival assay. Both HT1197 and XPC gene cDNA-stably transfected HT1197 cells were treated with cisplatin and plated onto tissue culture dishes to allow for surviving cells to form colonies. As a control, the empty vector–stably transfected HT1197 cells were also treated with cisplatin and plated onto tissue culture dishes to allow for surviving cells to form colonies (Fig. 3B). The cell survival rate was determined with the number of colonies formed after the cisplatin treatment (Fig. 3B). All three groups of HT1197 cells displayed a dose-dependent decrease in the number of surviving colonies following cisplatin treatment. However, the XPC stable–expressing HT1197 cells (HT1197/XPC) were relatively more sensitive to cisplatin treatment than the untransfected or the empty vector–transfected HT1197 cells. No apparent difference was found between the untransfected and the empty vector–transfected HT1197 cells with regard to their sensitivity to the cisplatin treatment. This result suggests that the overexpression of the XPC protein resulted in an increased sensitivity of HT1197 cells to cisplatin treatment.

Overexpression of XPC protein caused an increased apoptotic cell death of HT1197 cells after cisplatin treatment. One of the important mechanisms for cisplatin's killing effect is the induction of apoptosis (26). Because the HT1197 cells with overexpressed XPC protein displayed an increased sensitivity to cisplatin, we further determined whether this increased sensitivity was caused by apoptotic cell death. Both HT1197 and HT1197/XPC cells were treated with cisplatin (0, 10, and 20 μmol/L) for 2 h and then cultured for 48 h. The cells were harvested, and the cell lysates were evaluated for DNA fragmentation analysis (Fig. 3C). A negligible amount of DNA fragmentation was seen in HT1197 cells treated with 10 μmol/L cisplatin (Fig. 3C, lane 6). In comparison, a greatly increased amount of DNA fragmentation was detected in the HT1197/XPC cells treated with cisplatin at the same concentration (Fig. 3C, lane 3 versus lane 6). This result suggests that the increased sensitivity of the HT1197/XPC cells was caused by the cisplatin treatment–induced apoptosis.

The XPC protein was required for cisplatin treatment–induced cell cycle regulation. The DNA damage checkpoint is a multistep cellular response, including cell cycle arrest, DNA repair,
and/or apoptosis. Because XPC is the first protein component that binds to damage DNA sites, we postulated that the XPC protein might serve as an important sensor protein in the DNA damage checkpoint process. Therefore, we determined the role of the XPC protein in cisplatin treatment–mediated cell cycle regulation using a flow cytometry assay (Fig. 4). Consistent with many reports, no obvious G1-S arrest response was observed in either HT1197 or HT1197/XPC cells; instead, a predominant S-phase arrest was seen in both HT1197 and HT1197/XPC cells following the cisplatin treatment (Fig. 4–C). The S-phase arrested cell population reached the highest point (80%) at 16 h after 40 μmol/L cisplatin exposure. Comparison of the flow cytometry data indicated no significant difference between HT1197 and HT1197/XPC cells in the cisplatin treatment–induced S-phase arrest, suggesting that the XPC protein does not play an important role in the cisplatin treatment–induced S-phase checkpoint (P > 0.05).

Accompanied with the initial S-phase arrest, the G2-M arrest appeared 24 h after the cisplatin treatment, and it reached the peak point at 48 h after the treatment. For the controls, only a small proportion of the untreated HT1197 and HT1197/XPC cells were at the G2 phase (10% and 14%, respectively; Fig. 4D and E). However, the cisplatin treatment led to an increase in G2 cell population. Importantly, more G2-arrested cell population was observed in the HT1197/XPC cells than the HT1197 cells following the cisplatin treatment (Fig. 4B and C): the cisplatin treatment caused an increase of the G2-arrested cells from 29% to 36% in HT1197 cells, whereas the same treatment resulted in an increase of the G2-arrested cells from 40% to 55% in the HT1197/XPC cells (Fig. 4C; P < 0.05). The increased G2-arrested cell population in the HT1197/XPC cells suggests that the XPC protein plays an important role in the G2-M checkpoint process in response to cisplatin treatment, and a decrease in XPC protein results in reduced G2-M arrest under cisplatin treatment.

The XPC deficiency was correlated with attenuated p53 and p73 induction following cisplatin treatment. The results described above suggest an important role of the XPC protein in the cisplatin treatment–induced cellular response. However, the signal transduction pathway initiated from the XPC protein DNA damage recognition signal is not well understood. Although the tumor suppressor protein p53 is known to play an important role in DNA damage–induced cell cycle checkpoint regulation and apoptosis, at least two different signaling pathways, one dependent on p53 and the other independent of p53 but dependent on p73, exist in mammalian cells (27). To elucidate the mechanism of XPC protein in cisplatin treatment–induced checkpoint and apoptotic response, the cisplatin treatment–mediated p53 and p73 responses were determined in both HT1197 and HT1197/XPC cells (Fig. 5A and B). Both p53 and p73 displayed a dose-dependent response following cisplatin treatment (Fig. 5). However, the cisplatin treatment–induced p53 and p73 responses were much weaker in HT1197 cells than in HT1197/XPC cells. As an internal control, the level of α-tubulin remained unchanged in both HT1197 cells and HT1197/XPC cells with or without the cisplatin treatment. These data are consistent with the results obtained from our previous studies, which revealed that the XPC protein is involved in the cisplatin treatment–induced p53 responses (10). The results obtained from this study provide the initial clue that the XPC protein DNA damage recognition signal causes cell cycle arrest and apoptosis through the activation of both p53- and p73-dependent apoptotic pathways. However, further work needs to be done for elucidating the mechanism by which XPC regulates p53 and p73 responses to cisplatin treatment.

Discussion
In this report, we have studied the role of XPC deficiency in bladder tumor progression. Our results showed a strong correlation between attenuated XPC expressions and the degree of malignancy of bladder tumor cells. In addition, we have also studied the role of the XPC protein in DNA damage–induced cellular responses. The results obtained from our study suggest that the XPC protein plays an essential role in cisplatin treatment–induced cellular responses, including DNA repair, cell cycle arrest, and apoptosis.

It is known that the genomes of living organisms are insulted continuously by DNA-damaging agents both endogenously and exogenously. In response to this DNA damage, two important mechanisms, DNA repair and cell apoptosis, are used to overcome the DNA damage–induced deleterious effects such as mutation accumulation and uncontrolled cell proliferation. The initial response following DNA damage detection is cell cycle arrest, which allows DNA repair to take place and DNA damage to be removed. However, if DNA damage exceeds the capacity of DNA repair, an appropriate switching mechanism takes place that allows the damaged cells to undergo an apoptotic cell death process. DNA repair is important for restoring normal function in the damaged cells, and apoptosis is essential in eliminating the severely damaged cells. Given the fact that reduced apoptosis is observed in most types of cancer, it is important to understand the mechanism by which DNA damage leads to apoptosis. In recent years, a subset of DNA repair proteins has been identified that serve as triggering proteins for cell cycle arrest, apoptosis, and DNA repair. Some apoptotic proteins have also been linked with the DNA repair process. These proteins include ATM, ATR, BRCA1, DNA-PK, XBP, XPD, p53, ING1, ING2, PARP, MLH1, PMS2, MSH2, and MSH6, which are termed as DNA repair/proapoptotic dual-role proteins (28). Regarding the XPC protein, the results obtained from our recent study suggest an important role in cisplatin treatment–induced cell cycle regulation and apoptosis (10). This result,
together with the results obtained from our mutagenesis study (23) and other reports using XPC gene knock-out mice studies (12, 15, 16, 29), strongly suggests that the XPC protein plays a critical role in preventing cancer progression and malignancy through its important functions in the DNA repair, cell cycle checkpoint regulation, and apoptosis pathways.

We have studied the mechanisms by which XPC defects lead to cancer progression. The results obtained from our previous studies have revealed the requirement of the p53 signaling pathway in the XPC protein DNA damage recognition–mediated signaling process (10). The results obtained from this study further show a reduced p53 response of HT1197 bladder cancer cells following cisplatin treatment, and this p53 response was partially restored when an XPC gene cDNA expression vector was stably transfected into the HT1197 cells. Therefore, these data are consistent with the results obtained from our previous studies. In addition, the results obtained from this study also reveal that the XPC protein is required for regulating p53 in bladder cancer cells following cisplatin treatment, which suggests that the XPC protein may act as one of the upstream activators of the p53 protein for the cisplatin treatment–induced cellular responses. Many studies have shown the important role of the p53 protein in DNA damage–induced cell cycle checkpoint regulation. Studies also revealed that defects of the p53 protein attributed to the increased resistance attenuated cell cycle arrest, reduced DNA repair activity, and decreased apoptosis following cisplatin treatment. The results obtained from this study provide further evidence to suggest the important role of the XPC protein in cisplatin treatment–induced apoptosis of bladder cancer cells (Figs. 3–5).

In addition, the results obtained from this study also suggest the involvement of the p73 signaling pathway, a p53-independent signaling pathway, in the cisplatin treatment–induced cellular responses. Because frequent p53 mutations were found in the majority of cancer patients, these results suggest an important alternative mechanism for cisplatin treatment–induced cell killing in these p53-mutant cancer cells. Therefore, the knowledge obtained from this study has important clinical importance in the treatment of p53-mutant cancer cells.

Although our studies focused on determining the roles of the XPC protein in bladder cancer progression and bladder cancer cell resistance to cisplatin treatment, it is worthy to mention that the p53 protein can also regulate the XPC protein following DNA damaging treatment (30–32). It is possible that both the XPC and p53 proteins can regulate each other in response to DNA damaging treatment. Because the XPC-HR23B complex quickly recognizes and binds to the damaged DNA template, it is possible that the XPC protein may serve as a DNA damage sensor to activate the p53 protein. As a consequence, the active p53 protein may further regulate the transcription of important DNA damage–responsive genes, including XPC, resulting in cell cycle arrest to allow for DNA repair and apoptosis to take place.

The hallmarks of cancer cells are high recurrence, high genetic instability, and frequent drug resistance. Cancer progression is associated with multiple gene defects or mutations. One of the characteristics of bladder cancer is the heterogenesis of the recurrent tumors, which is usually accompanied with a gradual progressed malignancy and genetic alterations when compared with the original tumors (33–36). The cause of this disease, however, remains unclear. The results obtained from this study have shown the strong correlation between XPC deficiency/p53 mutation and the degree of malignancy of bladder cancer. It is possible that either attenuated XPC expression or p53 mutation will reduce the proper cellular responses of bladder epithelial cells to the continuous DNA damaging exposure, leading to an accumulation of mutations in other genes and resulting in an acceleration in bladder tumor progression. The results obtained from our recent studies using breast cancer cells also support this hypothesis: the breast cancer cells that overexpress an error-prone DNA polymerase iota result in elevated mutation accumulation following DNA damaging treatment (37).

Although we determined the role of XPC deficiency in bladder cancer progression, the evoking factors that lead to XPC deficiency are still unknown. Besides the observed XPC gene polymorphism, several potential mechanisms may affect the level of XPC expression: the XPC gene mutation, the transcriptional regulation, and the protein degradation. The recent discovery of small ubiquitin-related modifier–related post-translational modification of XPC may provide some new insight into the stabilization of the XPC protein (38). Regarding the XPC gene defects, a loss of heterozygosity of chromosomes 3p, 17p, and 9p are frequently observed in human bladder cancer patients. Some tumor suppressor genes have already been identified within these sites, such as p53, which is located on chromosome 17p, and p16, which is located on chromosome 9p. However, no candidate genes associated with bladder cancer are identified at the chromosome 3p region. It is possible that the XPC gene, which is located on the 3p25 region, can be one of the candidate genes for the 3p deletion that leads to bladder tumor progression (39, 40).

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