Predictive Biomarkers and Personalized Medicine

Excision Repair Cross-Complementing Group 1 May Predict the Efficacy of Chemoradiation Therapy for Muscle-Invasive Bladder Cancer

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Abstract

Purpose: Chemoradiation therapy (CRT) is now widely recognized as bladder-preserving therapy for muscle-invasive bladder cancer (MIBC). However, some patients who fail CRT may miss the chance to be cured by cystectomy. Therefore, it is important to select patients with MIBC who are expected to have a good response to CRT. Several reports indicate that the excision repair cross-complementing group 1 (ERCC1) gene is associated with resistance to cisplatin and radiation therapy. In this study, we examined the correlation between ERCC1 and CRT in vitro and in vivo in bladder cancer.

Experimental Design: Bladder cancer cell lines T24, 5637, Cl8-2 (multidrug-resistant subline of T24), and CDDP10-3 (cisplatin-resistant subline of T24) were used for in vitro assays to measure ERCC1 expression level and growth inhibition with cisplatin or ionizing radiation (IR). We then examined by immunohistochemistry that whether ERCC1 nuclear staining correlates with the efficacy of CRT using cisplatin in 22 patients with MIBC.

Results: Cl8-2 cells expressed ERCC1 mRNA 5.96-fold higher than did T24. Cl8-2 and CDDP10-3 were more resistant to cisplatin or IR than was T24. Resistance to IR, but not to cisplatin, was removed by suppressing ERCC1 using siRNA in both Cl8-2 and CDDP10-3 cells. In immunohistochemistry with ERCC1, 6 of 8 positive cases did not have complete response to CRT, whereas 12 of 14 negative cases had complete response. Sensitivity and specificity were 75% and 85.7%, respectively (P = 0.008).

Conclusion: Although further study is needed, ERCC1 expression level may predict the efficacy of CRT for MIBC. Clin Cancer Res; 17(8); 2561–9. ©2010 AACR.

Introduction

Bladder cancer is the fourth most common cancer in men in the United States (1). It can be divided into 3 major categories: superficial, muscle-invasive, and metastatic. For muscle-invasive bladder cancer (MIBC), radical cystectomy and urinary diversion are the gold standard of therapy in many parts of the world (1–3). Unfortunately, radical cystectomy is not suitable for patients with poor performance status, and, moreover, it reduces patient quality of life. As an alternative, trimodality therapy (conservative surgery and concurrent chemotherapy plus radiation therapy) has been widely recognized as bladder-preserving therapy (4–6). The 5-year survival rate of patients with MIBC is around 50% treated with either trimodality therapy or radical cystectomy (7). However, in various studies, the complete response (CR) rate of chemoradiation therapy (CRT) for MIBC is 60% to 70%. Some patients who fail CRT may miss the chance to be cured by cystectomy (8). Therefore, it is important to select patients with MIBC who are expected to have a good response to CRT by using a simple procedure, such as immunohistochemistry of transurethral resection (TUR) specimens. Levels of Her-2, Ki-67, and the Bax/Bcl-2 ratio were reported to be useful markers to predict the clinical response to CRT for MIBC (9–12). However, none of these markers are presently in clinical use.

The excision repair cross-complementing group 1 (ERC1) gene is located on chromosome 19q13.2-q13.3. The ERCC1 protein, with an apparent molecular weight of about 32.5 kDa, exerts a crucial role in the nucleotide excision repair (NER) pathway (13). Because cisplatin–DNA adducts can be removed by the NER pathway, several reports have shown that high levels of ERCC1 mRNA or protein in different cancer cell lines are associated with resistance to platinum compounds (14). Ahmad and colleagues reported that ERCC1-XPF is required for DNA double-strand break (DSB) repair, and ERCC1-deficient
For muscle-invasive bladder cancer, a method is needed to predict the efficacy of chemoradiation therapy (CRT) as bladder-preserving therapy. In this study, we evaluated the importance of ERCC1 in the response to CRT. We found that knockdown of ERCC1 in bladder cancer cells restored the sensitivity of the cells to ionizing radiation rather than to cisplatin, and ERCC1 immunostaining in human tissue samples showed that it may be a good predictive marker of the efficacy of CRT. Lack of ERCC1 expression may predict well the efficacy of CRT for muscle-invasive bladder cancer. Use of Her-2, Ki-67, or Bax/Bcl-2, which were previously reported as useful markers to predict the clinical response to CRT for muscle-invasive bladder cancer, in combination with ERCC1 may improve the accuracy of predicting the response to CRT.

Materials and Methods

Antibodies
Monoclonal anti-ERCC1 antibody was purchased from Santa Cruz Biotechnology, Inc. (FL297). The monoclonal anti-phospho H2AX, anti–β-actin, and anti-rabbit IgG, HRP (horseradish peroxidase)-linked antibodies were purchased from Cell Signaling Technology.

Cell culture
Four human high-grade bladder cancer cell lines were studied: 5637, T24, CDDP10-3, and CI8-2. We obtained 5637 cells from the Biomedical Research Institute of Development, Aging and Cancer, Tohoku University (Miyagi, Japan), and the T24 cells from the American Type Culture Collection. CDDP10-3 and CI8-2 are cisplatin-resistant T24 cell lines, which we reported previously (16). All cell lines were grown and maintained as cell monolayers in appropriate medium (RPMI 1640 or Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10%FBS) containing 10 mg/ml gentamicin in a 5% CO2 environment. The medium was changed twice each week.

ERCC1 siRNA transfection
The siRNA for ERCC1 and the nontargeting control siRNA were purchased from QIAGEN. The cells were transfected with the siRNA duplexes using HiPerFect Transfection Reagent (QIAGEN) according to the manufacturer’s protocol.

MTS assay
To confirm the cisplatin resistance, every cell line was seeded at a density of 2 × 10^4 cells/100 µL in 96-well plates and allowed to adhere overnight. Then, the cultures were fed fresh medium containing various concentrations of cisplatin (Nippon Kayaku Co., Ltd.). After a 48-hour incubation, 20 µL of Cell Titer 96 Aqueous One Solution Reagent was added to each well, and the trays were incubated for 2 hours at 37°C, after which the absorbance was measured at a wavelength of 490 nm with a microplate reader. All assays were done at least 3 times.

Quantitative real-time reverse transcriptase PCR
Quantitative real-time PCR for ERCC1 was performed on a Thermal Cycler Dice Real Time System TP800 (TAKARA Bio Inc.) with SYBR premix Ex Taq II (TAKARA Bio). The QuantiTect Primer assay was used to detect ERCC1 (QT00059374, QIAGEN). The expression of β-actin was used as an internal control. The following oligomer was used in the PCR to detect β-actin expression: forward, 5’-CTAAGTCATAGTCCGCCTAGAAGCA-3’ and reverse, 5’-TGCCACCACGACACATTGA-3’. The oligomer was used in the final PCR of ERCC1. A β-actin concentration of 6.25 mol/L was used as an amplification control. The PCR conditions were 50°C for 10 seconds and 95°C for 10 seconds, followed by 32 cycles at 95°C for 15 seconds and 60°C for 1 minute. The ERCC1 level was normalized to the β-actin mRNA level using the comparative Ct method.

Western immunoblotting
Whole-cell protein was purified from each cell line with RIPA Lysis Buffer (sc-24948; Santa Cruz Biotechnology, Inc.). After determining the protein concentration in the supernatant, 15 µg per well of protein was subjected to 10% PAGE and Western blotting of ERCC1 (1:500) for 1 hour at room temperature and β-actin (1:1,000) for 45 minutes at room temperature.

Clonogenic assay
Cells were seeded at 400, 800, and 1,600 cells per well in 6-well plates. After 16 hours, the cells were irradiated at each of the following doses of 2, 4, or 8 Gy with a Gammacell 40 Exactor Research Irradiator (MDS Nordion). Ten to 14 days later, the cultures were fixed and stained with 6% glutaraldehyde and 0.5% crystal violet. Colonies with more than 50 cells were scored as the surviving fraction. The data were plotted as the number ± SEM of colonies that grew on the treated plates relative to untreated plates for at least 3 independent experiments for each cell line.

Fixation and staining of phospho-H2AX foci
Cells were trypsinized and seeded at 2 × 10^4 cells per well on 4-well glass cover slips. Fourteen hours later, the cells were irradiated with 2 Gy of γ-rays. The cells were incubated

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in fresh medium at 37°C for the prescribed amount of time and then fixed with 3.7% paraformaldehyde in PBS for 15 minutes. The cells were permeabilized with 0.1% Triton X-100 in PBS, and the phosphorylated form of H2AX (phospho-H2AX) was detected with monoclonal anti-H2AX (1:100) at 4°C overnight and Alexa Fluor 488–conjugated goat anti-rabbit immunoglobulin (1:500; Molecular Probes) at room temperature for 1 hour in PBS. H2AX foci were counted with a fluorescent microscope with an objective lens (KEYENCE).

Patients and samples
Twenty-two patients [18 men, 4 women; median age 75.5 (range = 60–82) years] diagnosed with MIBC and treated by CRT at Osaka University and Sumitomo Hospital between 1998 and 2008 were enrolled in this study (Table 1). Approval for this study was obtained from the local Institutional Review Boards. The stage and histologic grade of the tumors were based on the 5th edition of the tumor node metastasis (TNM) classification. All 22 cases were diagnosed at stages T2 to T4a, and the histologic type was urothelial carcinoma in all cases. Four patients had pelvic lymph node metastasis (TNM) classification. Approval for this study was obtained from the local Institutional Review Boards. The stage and histologic grade of the tumors were based on the 5th edition of the tumor node metastasis (TNM) classification. All 22 cases were diagnosed at stages T2 to T4a, and the histologic type was urothelial carcinoma in all cases. Four patients had pelvic lymph node metastasis (TNM) classification.

Immunohistochemical analysis
ERCC1 protein expression was determined by immunohistochemical staining of paraffin-embedded tissue sections of TUR specimens of the bladder tumor just before CRT. Briefly, 6-μm thick sections were deparaffinized, rehydrated using xylene and alcohol, and incubated with 0.3% H2O2 to block endogenous peroxidase activity. Before immunostaining, antigen was retrieved by immersing the sections in 10 mmol/L citrate buffer (pH 6.0) and boiling in steam for 20 minutes. Immunohistochemistry for ERCC1 was performed with anti-ERCC1 antibody (1:250 dilution) using the EnVision Plus Detection System (DAKO) according to the manufacturer’s instructions. Primary antibody was incubated for 60 minutes at room temperature, and the slides were counterstained with hematoxylin. Bhagwat and colleagues stained ERCC1 both in vitro and in vivo using several commercial antibodies and reported that only the nuclear staining of ERCC1 suggested the manifestation of ERCC1 (18). Therefore, we judged whether nuclear staining for ERCC1 was positive. Two independent investigators well trained in genitourinary pathology independently evaluated the immunostained slides blind to the clinical data. ERCC1 nuclear expression was classified into 4 categories: score 0, no expression in tumor cells; score 1+, faint/barely perceptible nuclear expression in less than 10% of tumor cells; score 2+, weak to moderate expression of the entire nucleus in more than 10% of tumor cells; and score 3+, strong expression of the entire nucleus in more than 10% of tumor cells. Scores 2+ and 3+ were regarded as positive for ERCC1 staining as previously reported (19).

Statistical analysis
Cell viability in vitro in the MTS and clonogenic assays was examined with the t test after obtaining the results of Levene statistics. In the clinical study, associations between the effect just after CRT and the ERCC1 immunoreactivity score were evaluated with Fisher’s exact test. Overall survival was calculated by the Kaplan–Meier method. A value of P < 0.05 was deemed to indicate statistical significance. All statistical analyses were performed with SPSS ver. 16.0 (International Business Machines Corp.).

Results
Cl8-2 was the most resistant to cisplatin and IR exposure
In growth inhibition by cisplatin, the IC50 of the 5637, T24, CDDP10-3, and Cl8-2 cell lines was 14.8, 11.4, 16.5, and 31.1 μg/mL, respectively. We confirmed that Cl8-2 cells established from T24 were more resistant to cisplatin than were T24 cells, as previously reported (Fig. 1A). In the clonogenic assay, the viability of each cell line after IR with 2 Gy was 65.9%, 25.8%, 68.3%, and 82.2%, respectively. Cl8-2 was significantly more resistant to every dose of IR exposure compared with T24 (Fig. 1B).

| Table 1. Clinicopathologic parameters for 22 patients treated by CRT |
|--------------------------|-----------|
| Sex                      | n = 22    |
| Male                     | 18        |
| Female                   | 4         |
| Age (median), y           | 60–82 (75.5) |
| Clinical T stagea         |          |
| 2                        | 6         |
| 3                        | 14        |
| 4a                       | 2         |
| Lymph node metastasis    | 4         |
| Positive                 | 4         |
| Negative                 | 18        |
| Histologic type           | 22        |
| Urothelial carcinoma     | 22        |
| Highest histologic grade | 3         |
| 2                        | 3         |
| 3                        | 19        |
| Total platinum dose, mg   | 100–200   |
| Cisplatin (n = 13)        | 100–200   |
| Nedaplatin (n = 9)        | 100–200   |
| Total radiation dose (median), Gy | 40–66 (50) |

aBased on the 5th edition of the TNM classification.
Cl8-2 had the highest expression levels of ERCC1 mRNA and protein

Real-time reverse-transcriptase (RT) PCR showed that Cl8-2 had the highest ERCC1 mRNA level and 5637 had the lowest ERCC1 mRNA level (Cl8-2 5.96-fold and 5637 0.46-fold higher than T24; Fig. 1C). Western immunoblotting revealed that Cl8-2 expressed a greater level of ERCC1 protein than did T24 (Fig. 1D).

Efficacy of ERCC1 siRNA knockdown in Cl8-2

Real-time RT-PCR showed that Cl8-2 transfected with ERCC1 siRNA (Cl8-2<sub>ΔERCC1</sub>) expressed 0.05 times as much ERCC1 mRNA as did Cl8-2, whereas Cl8-2 transfected with control siRNA (Cl8-2<sub>ΔsiRNA</sub>) expressed roughly the same amount of ERCC1 mRNA as did Cl8-2 (Fig. 2A). ERCC1 expression by Cl8-2<sub>ΔERCC1</sub> was almost undetectable by Western immunoblotting (Fig. 2B). CDDP10-3 transfected with ERCC1 siRNA (CDDP10-3<sub>ΔERCC1</sub>) expressed 0.096 times as much ERCC1 mRNA as did CDDP10-3, whereas CDDP 10-3 transfected with control siRNA (CDDP10-3<sub>ΔsiRNA</sub>) expressed roughly the same amount of ERCC1 mRNA as did CDDP10-3 (Fig. 2A). ERCC1 expression by CDDP10-3<sub>ΔERCC1</sub> was almost undetectable by Western immunoblotting (Fig. 2B).

ERCC1 did not affect bladder cancer viability to cisplatin

In the growth inhibition by CDDP, the IC<sub>50</sub> of C18-2<sub>cont</sub> and C18-2<sub>ΔERCC1</sub> was 19.51 and 15.96 μg/mL, respectively. At a cisplatin concentration of 5 μg/mL, the viability of C18-2<sub>cont</sub> (98.8%) was slightly higher than that of C18-2<sub>ΔERCC1</sub> cells (83.1%), but the difference was not statistically significant.
significant ($P = 0.151$; Fig. 2C). As well, there was no statistically significant difference in cell viability between CDDP10-3<sup>Δ<sub>ERCC1</sub></sup> and CDDP10-3<sup>cont</sup> at any cisplatin concentration (Fig. 2D).

Resistance to IR exposure was removed by ERCC1 knockdown

In the clonogenic assay, the respective cell viability of C18-2<sup>cont</sup> and C18-2<sup>Δ<sub>ERCC1</sub></sup> after IR with 2 and 4 Gy was 87.3% and 36.7%, and the difference was statistically significant ($P = 0.022$), and 57.5% and 16.0%, and the difference was also statistically significant ($P = 0.030$; Fig. 2C), respectively. The respective viability of CDDP10-3<sup>cont</sup> and CDDP10-3<sup>Δ<sub>ERCC1</sub></sup> after IR with 2 Gy was 75.3% and 52.4%, also with statistically significant difference ($P = 0.033$). There was no significant difference in cell viabilities after IR with 4 and 8 Gy (Fig. 2D). The effect of ERCC1 knockdown in not only C18-2 cells but
also CDDP10-3 was more prominent against IR than against cisplatin.

**DSB after IR exposure continued longer with ERCC1 knockdown**

To further prove the cause of the radiation sensitivity of ERCC1 knockdown cells, we measured the phosphorylated histone variant H2A.X, a marker of DNA damage, in T24, C18-2, C18-2cont, and C18-2ΔERCC1 at various time points following IR exposure. Phospho-H2A.X foci provide a quantitative measurement of DSB induction and repair at low IR doses. Foci were counted 0, 4, 8, 12, and 24 hours after IR exposure (Fig. 3A). Cells were categorized as having 0 to 1 focus (black), or more than 2 foci (gray) at 0, 4, 8, 12, and 24 hours post-IR. (A, T24; B, C18-2; C, C18-2cont; D, C18-2ΔERCC1). Y-axis, the proportion of the number of phospho-H2A.X (%).

Figure 3. A, representative examples of T24 cells treated with 2 Gy of IR and stained for phospho-H2A.X at the indicated time points. (A, 0 h; B, 8 h; C, 12 h; and D, 24 h after IR). B, representative examples of phospho-H2A.X staining in C18-2cont and C18-2ΔERCC1 cells 12 hours after IR. C, quantification of H2A.X foci in T24, C18-2, C18-2cont, and C18-2ΔERCC1 cells exposed to IR. Histograms indicate the fractions of cells with 0 or 1 focus (black), or more than 2 foci (gray) at 0, 4, 8, 12, and 24 hours post-IR. (A, T24; B, C18-2; C, C18-2cont; D, C18-2ΔERCC1). Y-axis, the proportion of the number of phospho-H2A.X (%).
populations of C18-2 and C18-2cont with multiple phospho-H2A.X foci decreased to less than 30%. However, 92% of the T24 and 72% of the C18-2ΔERCC1 populations still had multiple foci, suggesting continued accumulation or persistence of DSBs (Fig. 3B and C). In CDDP10-3 cells, 84.1% of CDDP10-3ΔERCC1 cells had multiple foci, whereas 71.8% of CDDP10-3cont cells had multiple foci by 12 hours after IR exposure (data not shown). Consistency of repair of DSBs correlated with sensitivity to IR in C18-2ΔERCC1.

Finally, we examined the correlation between ERCC1 immunoreactivity and 5-year survival in the MIBC patients undergoing CRT. Overall 5-year survival was 31.2% in the positive and 69.2% in the negative cases ($P = 0.088$; data not shown).

**Discussion**

In the present study, we examined the correlation between bladder cancer outcome and ERCC1 expression in vitro. We postulated that the level of ERCC1 expression predicts the efficacy of CRT for MIBC.

ERCC1 is a crucial gene in the NER pathway. Cisplatin–DNA adducts are removed via the NER pathway, and an association of different cancer cell lines with resistance to platinum compounds has been suggested (14, 20, 21). In a clinical study using immunohistochemistry, Olaussen and colleagues reported that patients with ERCC1-negative non–small cell lung cancer appeared to benefit from adjuvant cisplatin-based chemotherapy, whereas patients with ERCC1-positive tumors did not (22). In a clinical study of bladder cancer, a high ERCC1 mRNA level was reported to be a useful predictor of cisplatin-based chemotherapy against advanced bladder cancer (23). However, the authors did not make a general statement concerning the influence of ERCC1 expression on the outcome of other treatment regimens. In our study, C18-2, the cell line most resistant to cisplatin and that with the highest ERCC1 mRNA levels, expressed ERCC1 protein at a level slightly higher than that of T24, which was the parental cell line of C18-2. CDDP10-3, a cisplatin-resistant subline of T24, had a higher ERCC1 mRNA level, whereas it expressed an ERCC1 protein level less than that of T24. To clarify the association between ERCC1 and cisplatin resistance in bladder cancer cells, we knocked down ERCC1 in C18-2 and CDDP10-3 with siRNA. Our ERCC1 knockdown experiments (C18-2ΔERCC1 and CDDP10-3ΔERCC1) showed that there was no statistical difference in the resistance to cisplatin compared with C18-2cont and CDDP10-3cont. Recently, Usanova and colleagues reported that downregulation of ERCC1-XPF slightly but significantly increased the sensitivity to cisplatin in one bladder cancer cell line (24). We postulate that the

**Table 2. ERCC1 immunohistochemical staining and clinical response**

<table>
<thead>
<tr>
<th>ERCC1 immunoreactive</th>
<th>Clinical response</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>CR</td>
<td>non-CR</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

NOTE: Sensitivity and specificity of clinical response prediction. Prediction of non-CR (ERCC1: positive staining), sensitivity = 75.0%; specificity = 85.7% (Fisher’s exact test: $P = 0.008$). ERCC1 immunoreactivity score: score 0, 1 = negative; score 2, 3 = positive.
resistance to cisplatin is not caused by ERCC1 in CI8-2 but by other mechanisms, and further study is needed to clarify the association between ERCC1 expression and the response to cisplatin in bladder cancer cells.

In regard to the association between ERCC1 and IR sensitivity, Ahmad and colleagues reported that ERCC1-XPF is required for DNA DSB repair, and ERCC1-deficient cells are sensitive to IR exposure (15). Liu and colleagues reported that methylation of the ERCC1 promoter correlates with radiosensitivity in glioma cell lines (25). There are no reports on ERCC1 expression and IR in bladder cancer. In the present study, CI8-2 expressed the highest level of ERCC1 and was also the most resistant to IR exposure of the 4 bladder cancer cell lines. When we knocked down ERCC1 in CI8-2 and CDDP10-3, the sensitivity to IR exposure recovered significantly in CI8-2<sup>ΔERCC1</sup> and CDDP10-3<sup>ΔERCC1</sup>. Although CI8-2<sup>ΔERCC1</sup> could eliminate the resistance to every dose of IR exposure, the resistance only to 2 Gy of IR exposure was eliminated in CDDP10-3<sup>ΔERCC1</sup>. DSBs are the most lethal form of IR-induced DNA damage. Failure to repair such breaks results in tumor cell death. Recent studies have observed a close correlation between the number of phospho-H2A.X foci and the number of expected DSBs after IR. Phospho-H2A.X prompts DSB repair and amplifies DSB signaling by ATM (ataxia-telangiectasia mutated; ref. 26). CI8-2<sup>ΔERCC1</sup> and CDDP10-3<sup>ΔERCC1</sup> recovered more slowly in terms of the number of phospho-H2A.X foci than did CI8-2<sup>cont</sup> and CDDP10-3<sup>cont</sup>, suggesting continued accumulation or persistence of DSBs. Based on our <i>in vitro</i> data, ERCC1 may play greater roles in IR resistance in some bladder cancers.

Our <i>in vitro</i> data suggest that ERCC1 expression level might be a better predictor of the efficacy of CRT against bladder cancer than cisplatin-based chemotherapy, as in other malignancies (27, 28). Here, we examined the correlation between ERCC1 staining and the response to CRT for MIBC in a clinicopathologic study to confirm our <i>in vitro</i> data, especially in regard to whether low ERCC1 expression resulted in a better response to CRT.

For MIBC, the gold standard treatment is radical cystectomy and urinary diversion. Concurrent CRT using cisplatin or other agents is an alternative therapy that preserves bladder function (4–6, 17, 29–34). Because the CR rate of CRT for MIBC is 60% to 70%, a simple procedure is needed to select patients with MIBC who are expected to have a good response to CRT so that they will not miss the chance to be cured by immediate cystectomy (8). Although the sample size of our study was small, lack of ERCC1 expression may predict the efficacy of CRT for MIBC, as was shown in our <i>in vitro</i> data.

In terms of a predictor of the clinical response of CRT for MIBC, Chakravarti and colleagues reported that by multivariate analysis, only Her-2 expression was significantly associated with a reduced rate of CR after CRT (10). Rödel and colleagues reported that the apoptotic index and Ki-67 expression, but not p53 or bcl-2 expression, were significantly related to an initial CR after CRT (11). Matsumoto and colleagues suggested that only the Bax/Bcl-2 ratio was associated with the CR rate, although Bax and Bcl-2 individually were not significantly associated with the CR rate (12). The specificity of all of these markers was almost 50%; therefore, a molecular marker is needed that is more specific. In our study, ERCC1 immunohistochemistry had a sensitivity of 75% and specificity of 85.7%. Moreover, ERCC1 expression tended to correlate with the survival rate after CRT for MIBC, although not significantly so. ERCC1 may be a better predictor than the markers previously reported. Use of Her-2, Ki-67, or the Bax/Bcl-2 ratio in combination with ERCC1 may further improve the accuracy of predicting the response to CRT, although we have not performed experiments to verify this hypothesis.

One limitation of the present study is that we have not shown a direct correlation between ERCC1 and IR resistance in bladder cancer cells. However, our ERCC1 staining results with clinical samples suggest that the lack of ERCC1 is associated with the response to CRT. Another limitation of this study is that sample size for ERCC1 immunohistochemical analysis was very small. More detailed studies are needed to address these limitations. In conclusion, our results suggest that in some bladder cancer cells, ERCC1 expression correlates with IR resistance but not with cisplatin resistance. Moreover, the lack of ERCC1 expression correlated well with the efficacy of CRT, and especially with that of IR, in our clinical study. Although the sample size of our study was quite small, the results indicate that ERCC1 might become a new molecular marker for predicting both the clinical response to, and the survival rate after, CRT for MIBC. Further careful study is needed to confirm our preliminary results.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Excision Repair Cross-Complementing Group 1 May Predict the Efficacy of Chemoradiation Therapy for Muscle-Invasive Bladder Cancer

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