ERCC1 Expression and Chemosensitivity in Uterine Cervical Adenocarcinoma Cells

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Abstract. *Backgroud/Aim: We previously demonstrated that high protein expression of excision repair cross-complementation group-1 (ERCC1) was associated with poor disease-free survival in patients who received adjuvant cisplatin-based chemotherapy or chemoradiotherapy with cisplatin, and was shown to be an independent prognostic factor. In the present study, we evaluated ERCC1 expression levels in uterine cervical adenocarcinoma cell lines to assess whether they are affected by treatment with cisplatin with and without 5-fluorouracil (5-FU). Materials and Methods: Firstly, half-maximal (50%) inhibitory concentration (IC_{50}) values for cisplatin or 5-FU were calculated in cervical adenocarcinoma, HCA-1, and TCO-2 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazole (MTT) assay. ERCC1 mRNA and protein levels were investigated by reverse transcription-polymerase chain reaction (RT-PCR) and western blotting. Secondly, cisplatin-resistant HCA-1 cells, designated HCA-1R cells were established, and IC_{50} values for cisplatin and 5-FU were calculated by the MTT assay. ERCC1 mRNA expression levels were investigated using quantitative RT-PCR following treatment with cisplatin with and without 5-FU. Results: HCA-1 and TCO-2 cells exhibited similar sensitivity to cisplatin, and 5-FU, and comparable expression of ERCC1 mRNA and protein levels. HCA-1R cells exhibited two-fold higher resistance to cisplatin and a significantly higher level of ERCC1 mRNA expression compared to native HCA-1 cells. ERCC1 expression was significantly elevated by cisplatin treatment, which was reduced by co-administration of 5-FU in HCA-1, TCO-2 and HCA-1R cells. Conclusion: The current study demonstrated an association between ERCC1 expression and sensitivity to cisplatin in cervical adenocarcinoma cells. Co-administration of cisplatin and 5-FU revealed synergistic or additive effects through inhibition of ERCC1 expression in cervical adenocarcinoma cells. Therefore, it is possible that a combination therapy of cisplatin and 5-FU or 5-FU derivatives constitutes an ideal treatment regimen, from the ERCC1 inhibition point of view in cervical adenocarcinoma.*

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Excision repair cross-complementation group-1 (ERCC1) is a protein involved in nucleotide excision repair that forms a heterodimer with xeroderma pigmentosum-F (XPF). ERCC1/XPF complexes are responsible for an incision that cleaves the damaged nucleotide strand at the 5' end of the lesion (1). As such, it has a key role in the response to a range of DNA-damaging chemotherapeutic agents.

ERCC1 has been implicated in resistance to platinum-based chemotherapy or chemoradiotherapy with platinum in various cancer types, including lung (2-4), gastric (5), esophageal (6), bladder (7, 8), head and neck (9), epithelial ovarian (10, 11), and uterine cervical (12, 13) cancer.

We have previously demonstrated an association between ERCC1 expression and clinical outcomes in patients with uterine cervical adenocarcinoma. High ERCC1 protein expression was revealed to be associated with poor disease-free survival in patients who received adjuvant cisplatin-based chemotherapy or chemoradiotherapy with cisplatin, and was shown to be an independent prognostic factor (13).

Moreover, *in vitro* investigations have reported an association between ERCC1 expression and chemosensitivity in various cancer cell lines. Kawashima *et al*. demonstrated

a correlation between ERCC1 expression and resistance to cisplatin or radiotherapy in bladder cancer cell lines using native cell lines and chemoresistant sublines (8). Britten *et al*. also observed a significant correlation between *ERCC1* mRNA levels and cisplatin resistance in uterine cervical cancer cells *in vitro* (14). Takanaka *et al*. reported that combined expression of ERCC1 and RAD51, which is one of the key enzymes in DNA double-strand break repair by homologous recombination, was associated with resistance to cisplatin and carboplatin in a chemosensitivity test using succinate dehydrogenase inhibition (SDI) in non-small cell lung cancer specimens (15). These authors also reported that both ERCC1 and RAD51 expression showed no significant relationship with sensitivity to paclitaxel, etoposide, vinorelbine, gemcitabine, 5-fluorouracil (5-FU), and irinotecan (15).

Furthermore, it has been reported that ERCC1 expression is significantly elevated by cisplatin treatment in human ovarian cancer cell lines, which can be mediated through the modulation of activator protein-1 (AP-1), c-FOS, and c-JUN activities (16). Li *et al*. reported that ERCC1 might be a useful marker to monitor the repair of DNA damage caused by platinum in tumor cells, and further highlighted the potential of pharmacological approaches that specifically inhibit ERCC1 expression to increase cellular sensitivity to cisplatin (17).

Sato *et al*. reported that combination of oxaliplatin and 5-FU had additive or synergistic effects in ovarian mucinous adenocarcinoma cell lines, and significantly prolonged survival in a xenograft model in nude mice (18). They also demonstrated that ERCC1 expression was down-regulated by exposure to 5-FU, and concluded that combination chemotherapy of 5-FU and oxaliplatin was an effective treatment for ovarian mucinous adenocarcinoma (18). Moreover, it was also reported that 5-FU treatment in combination with radiation gradually inhibited *ERCC1* mRNA expression in colon cancer (19).

To our knowledge, the association between ERCC1 expression and sensitivity to cisplatin in uterine cervical adenocarcinoma has not yet been investigated *in vitro*. Induction of *ERCC1* expression by cisplatin treatment has not been previously reported for uterine cervical adenocarcinoma. In the present investigation, we evaluated ERCC1 expression levels in uterine cervical adenocarcinoma cell lines, and assessed whether expression levels are affected by treatment with cisplatin-alone, 5-FU-alone, and in combination treatments of cisplatin with 5-FU.

Materials and Methods

Cell lines and cell culture. Two uterine cervical adenocarcinoma cell lines, HCA-1 (20, 21) and TCO-2 (22), were used in the current study. The HCA-1 cell line was purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan) and the TCO-2 cell line was obtained from Dr. T. Hidaka, Toyama University School of Medicine (Toyama, Japan), who established the line. These two cell lines were grown in Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere with 5% CO_2.

To establish a cisplatin-resistant cell line, we used the HCA-1 cell line as a source. A clone of cisplatin-resistant HCA-1 cells was selected by incubating parental HCA-1 cells in the presence of 0 to 5 μg/ml cisplatin, and gradually increasing the dose of cisplatin over a period of two months. This cell line was designated HCA-1R.

Analysis of chemosensitivity and ERCC1 expression in cervical adenocarcinoma cell lines. Half-maximal (50%) inhibitory concentration (IC_{50}) values for cisplatin and 5-FU in HCA-1 and TCO-2 cells were calculated using 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, yellow tetrazole (MTT) assay. *ERCC1* mRNA and protein levels were investigated using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and western blotting in HCA-1 and TCO-2 cells, according to the protocols described below. The same protocol was performed in both parental and resistant HCA-1 cells.

Western blotting. Cultured cells were collected using a cell scraper followed by two washings with ice-cold phosphate buffered saline (PBS). The cell pellets were prepared in 200 μl of lysis buffer and homogenized with a sonicator for 10 min at 4˚C. Homogenates were centrifuged at 10,000 rpm for 30 min at 4˚C, and the supernatant was collected. Protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA). Protein aliquots (25 μg) were separated on 16% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to Immunobilon-P membranes (EMD Millipore Corporation, Billerica, MA, USA). The blots were blocked in 5% (w/v) non-fat dry milk in 1% Tween-PBS for 3 h and incubated with appropriate antibodies: ERCC1, mouse monoclonal antibody (clone 8F1; Thermo Scientific, San Jose, CA, USA) (1:100 dilution) for 1 h, and β-actin, mouse monoclonal antibody (clone AC-15; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:500 dilution) for 1 h. Primary antibody binding was detected by blotting with goat anti-mouse secondary antibody linked to horseradish peroxidase, and visualized with diaminobenzidine (DAB). Quantification of protein was performed with a Lumivision IMAGER (Aishin, Japan). Each value represents the mean of triple experiments, and is presented as the relative density of protein bands normalized to that of β-actin.

Semi-quantitative RT-PCR and quantitative RT-PCR. Semi-quantitative RT-PCR: HCA-1 and TCO-2 cells were seeded in 5-cm dishes at 1×10^5 cells/well and grown in culture medium without chemotherapeutic drugs. Total RNA was extracted from cells in each well using an RNeasy kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Semi-quantitative RT-PCR for *ERCC1* was performed using the Ready-To-Go RT-PCR Beads kit (Amersham Bioscience, Piscataway, NJ, USA) following the manufacturer's instructions. The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control. The following primers were used: *ERCC1*: forward, GGGAATTTGGCGACGTAATTC, reverse, GCGGAGGCTGAG GAACAG; GAPDH: forward, GGGAGTCAA CGGATTTGGTCG TAT, reverse, AGCCTTCTCCATGGTGGT GAAGAC. Cycling conditions for *ERCC1* were 40 cycles of

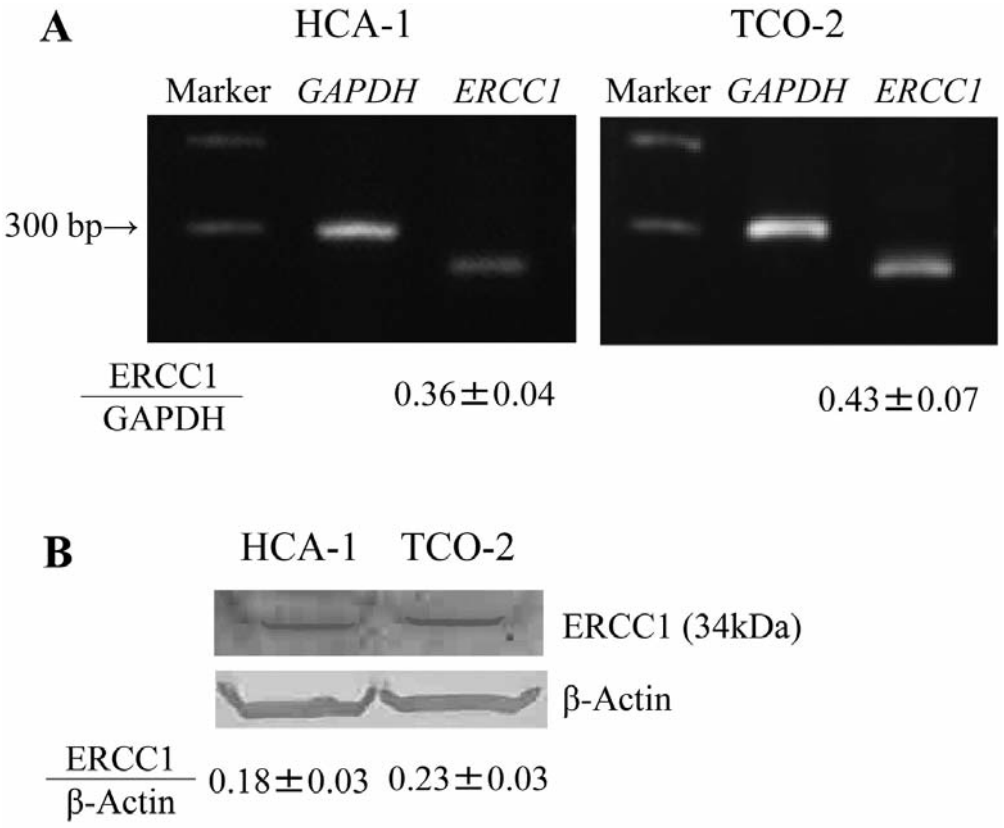

Figure 1. *Excision repair cross-complementation group 1 (ERCC1) mRNA and protein expression in HCA-1 and TCO-2 cells. A: Reverse transcription-polymerase chain reaction (RT-PCR) and B: western blotting of ERCC1/ glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA and ERCC1/β-actin protein ratios in HCA-1 and TCO-2 cells.*

denaturation, annealing, and extension (94˚C for 1 min, 55˚C for 2 min, and 72˚C for 3 min); conditions for *GAPDH* were 27 cycles of denaturation, annealing, and extension (94˚C for 1 min, 55˚C for 1 min, and 72˚C for 1 min), followed by an additional extension step (72˚C for 10 min). Amplified PCR products were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining and ultraviolet transillumination.

Quantitative RT-PCR: HCA-1 and TCO-2 cells were seeded in 5-cm dishes at 1×10^5 cells/well and grown in culture medium containing cisplatin alone (0, 5, and 10 μg/ml), 5-FU alone (0, 5, and 10 μg/ml), or a combination of cisplatin and 5-FU for 24 h. In addition, HCA-1 and HCA-1R cells were cultured in culture medium containing cisplatin alone (0 and 3.75 μg/ml), 5-FU alone (0, 5, and 10 μg/ml), or a combination of cisplatin and 5-FU for 24 h. Total RNA was extracted from cells in each well using an RNeasy kit. Total RNA was then subjected to complementary DNA (cDNA) synthesis using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). cDNA was subsequently used for fluorescence-based real-time quantitative RT-PCR (TaqMan PCR) with an ABI Prism 7900 Sequence Detector System (Applied Biosystems) according to methods described elsewhere (23, 24). The housekeeping gene, *GAPDH*, served as an internal control due to its stable expression in different cell lines. *ERCC1* (assay ID: Hs01012158_m1) and *GAPDH* (assay ID: Hs99999905_m1) primers and TaqMan probes were purchased from Applied Biosystems. TaqMan probes were labeled with the reporter dye 6-carboxyfluorescein (FAM) at the 5'-end of the oligonucleotide and with the quencher dye 6-carboxytetramethylrhodamine (TAMRA) at the 3'-end. The PCR conditions were as follows: 50˚C for 2 min and 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 s and 60˚C for 1 min. All assays were run in triplicate. Data were analyzed by the delta-delta Ct method for comparing relative expression (ratio: $2^{-[Ctsample-CtGAPDH]}$), where Ct is the threshold cycle. The relative quantity of mRNA was represented as the mean±standard deviation (SD). The *ERCC1/GAPDH* mRNA ratio in each sample without treatment was defined as 1.0 (control).

Statistical analysis. All experiments were carried out in triplicate. All results are presented as the mean±standard deviation (SD). Statistical analysis was performed using the Chi-square test or Student's *t*-test. Differences with a *p*-value of less than 0.05 were considered statistically significant.

Results

Characterization of cervical adenocarcinoma cell lines. The doubling times of HCA-1 and TCO-2 cells, calculated from growth curves, were 21 h and 69 h, respectively (growth curve not shown).

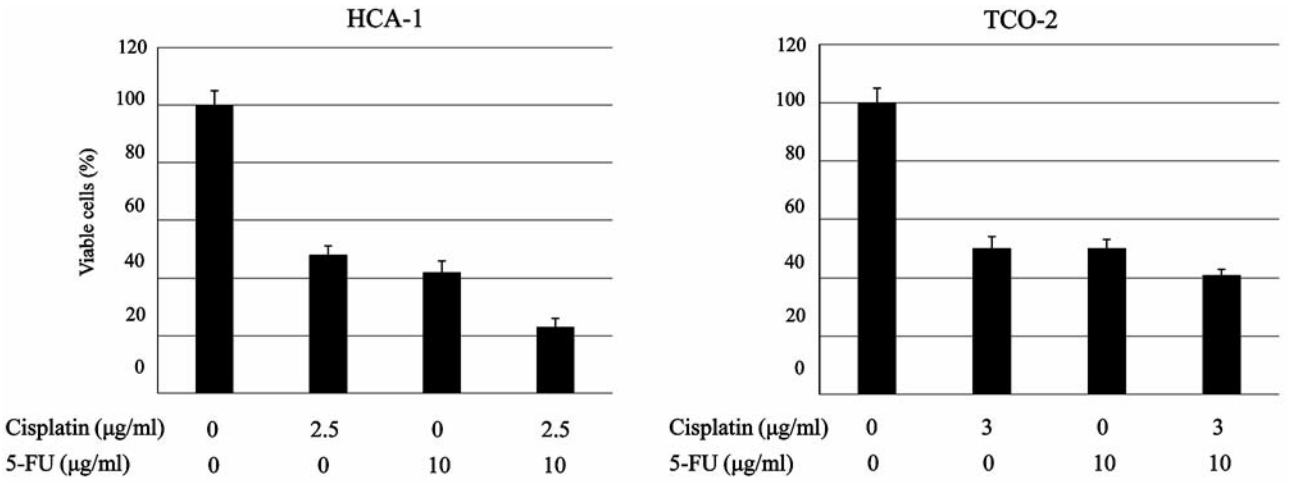

Figure 2. *Sensitivity to cisplatin and 5-fluorouracil (5-FU) in HCA-1 and TCO-2 cells (MTT assay). The chemosensitivity to cisplatin and 5-FU in HCA-1 cells was somewhat higher than that in TCO-2 cells, but there are no remarkable differences in chemosensitivity in the cell lines.*

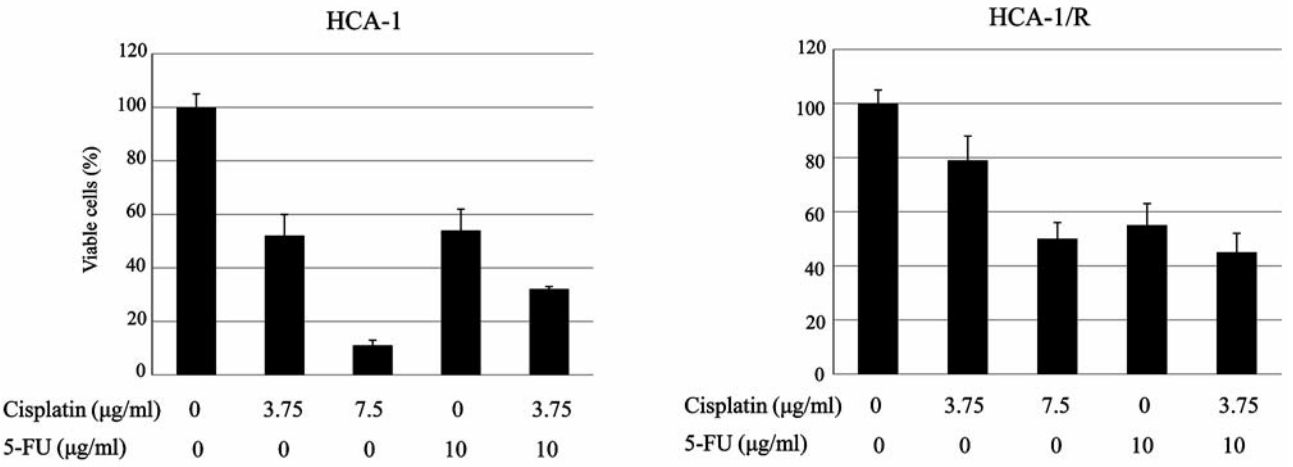

Figure 3. *Sensitivity to cisplatin and 5-FU in HCA-1 and HCA-1R cells (MTT assay). Half-maximal (50%) inhibitory concentration (IC_{50}) values for cisplatin in HCA-1 and HCA-1R cells were 3.7±0.2 and 7.5±0.2 μg/ml, respectively, demonstrating that HCA-1R cells are two-fold more resistant to cisplatin than parental cells.*

IC_{50} values for cisplatin and 5-FU in HCA-1 and TCO-2 cells cultured for 72 h were calculated by the MTT assay. IC_{50} values for cisplatin in HCA-1 and TCO-2 cells were 2.5±0.2 and 3.0±0.2 μg/ml, respectively. IC_{50} values for 5-FU in HCA-1 and TCO-2 cells were 10.0±0.3 and 10.0±0.5 μg/ml, respectively. *ERCC1*/*GAPDH* mRNA ratios determined by RT-PCR in HCA-1 and TCO-2 cells were 0.36±0.04 and 0.43±0.07, respectively (Figure 1). Moreover, ERCC1/β-actin protein ratios determined by western blotting in HCA-1 and TCO-2 cells were 0.18±0.03 and 0.23±0.05, respectively (Figure 1). Although the chemosensitivity to cisplatin with and without 5-FU of HCA-1 cells was somewhat higher than that of TCO-2 cells, there were no remarkable differences in chemosensitivity of the cell lines (Figure 2). Furthermore, *ERCC1* mRNA and protein expression in both cell lines were also very similar.

Subsequently, we established a cisplatin-resistant HCA-1 cell line, designated HCA-1R. The doubling time of HCA-1R cells calculated from growth curves was 20 h, similar to that of parental cells (21 h; growth curve not shown).

IC_{50} values for cisplatin and 5-FU in HCA-1 and HCA-1R cells when cultured for 72 h were calculated by MTT assay and 3.7±0.2 and 7.5±0.2 μg/ml, respectively, for cisplatin demonstrating that HCA-1R cells are two-fold more

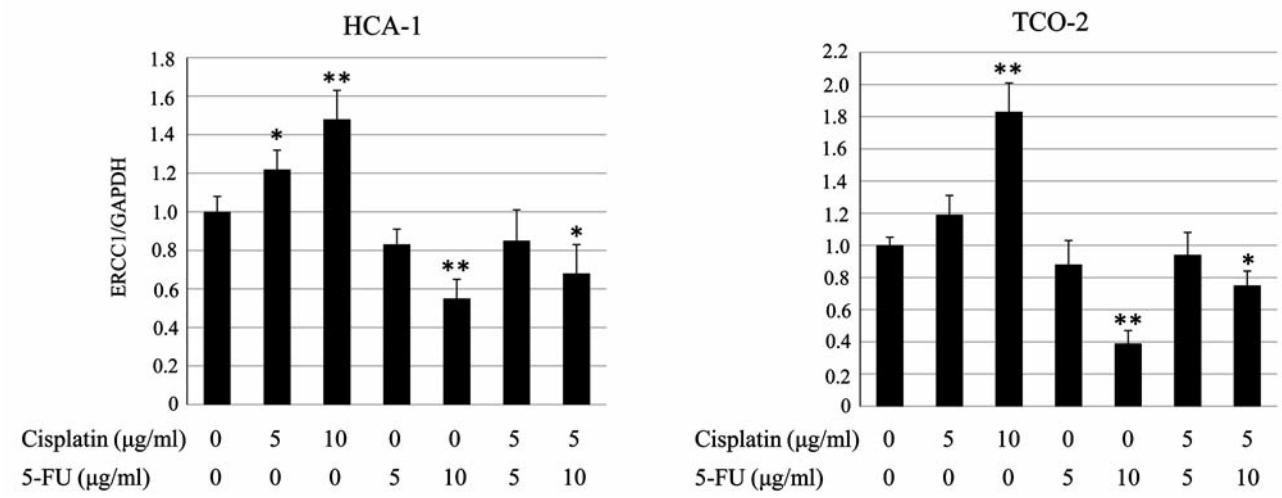

Figure 4. *Excision repair cross-complementation group 1 (ERCC1) mRNA expression in HCA-1 and TCO-2 cells following treatment with cisplatin and 5-fluorouracil (5-FU). In HCA-1 cells, the ratio of ERCC1/ glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was significantly elevated after cisplatin treatment according to the concentration (5 μg/ml cisplatin, p=0.04; 10 μg/ml cisplatin, p=0.008). The ratio was significantly reduced by 5-FU treatment (10 μg/ml 5-FU, p=0.003). ERCC1 elevation by cisplatin treatment was reduced by co-administration with 5-FU (5 μg/ml cisplatin plus 10 μg/ml 5-FU, p=0.03). A similar tendency was observed in TCO-2 cells, which showed comparable chemosensitivity to cisplatin and 5-FU. Statistical analysis was performed by comparison to the controls. *p<0.05, **p<0.01.*

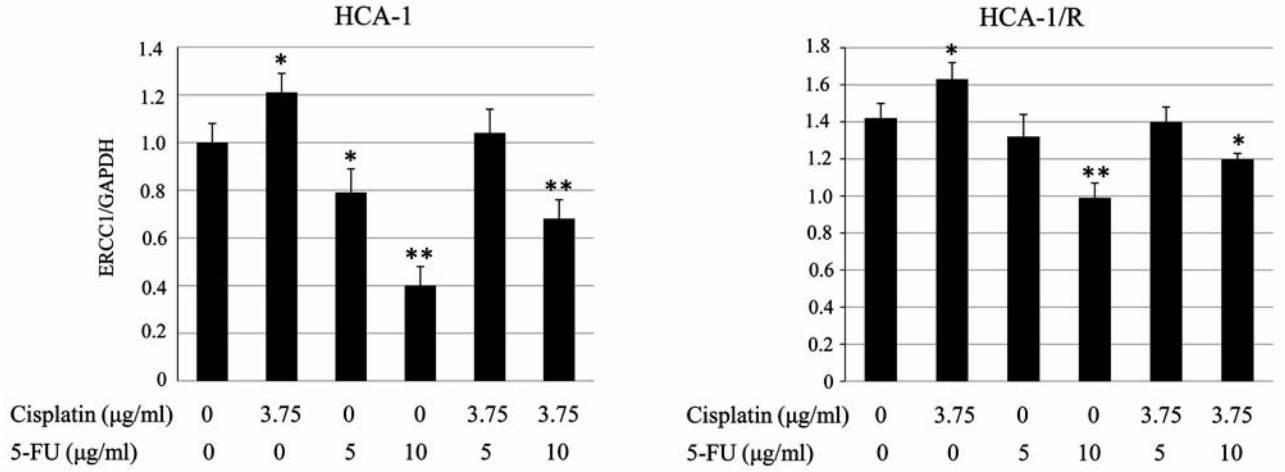

Figure 5. *Expression excision repair cross-complementation group 1 (ERCC1) mRNA in HCA-1 and HCA-1R following treatment with cisplatin and 5-fluorouracil (5-FU). Control ERCC1/ glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA ratios in HCA-1 and HCA-1R cells without treatment were 1.0±0.03 and 1.42±0.11, respectively, when the mean ERCC1 expression in HCA-1 cells was defined as 1.0. ERCC1 mRNA expression in HCA-1R cells was significantly elevated (p=0.003). The ratio after cisplatin treatment (3.75 μg/ml) was significantly elevated compared with the control (p=0.03), whereas this elevation was lost by co-administration with 5-FU (10 μg/ml) (p=0.008). In HCA-1R, the ratio after cisplatin treatment (3.75 μg/ml) was also significantly elevated compared with the control (p=0.04), whereas this elevation was also lost by co-administration with 5-FU (10 μg/ml) (p=0.01). Statistical analysis was performed comparing to controls. *p<0.05, **p<0.01.*

resistant to cisplatin than parental cells (Figure 3); for 5-FU, IC_{50} was 10.0±0.3 and 12.5±0.4 μg/ml, respectively.

Analysis of ERCC1 expression and chemosensitivity in cervical adenocarcinoma cell lines. ERCC1/GAPDH mRNA ratios in HCA-1 and TCO-2 cells treated with cisplatin with and without 5-FU are shown in Figure 4. In HCA-1 cells, the ratios were significantly elevated by cisplatin treatment according to its concentration (1.22±0.15 by 5 μg/ml cisplatin treatment, *p*=0.04; 1.48±0.15 by 10 μg/ml cisplatin

treatment, p=0.008). The ratios were significantly reduced by 5-FU treatment (0.55±0.1 by 10 μg/ml 5-FU treatment, p=0.003). Interestingly, the increase in *ERCC1* expression levels in cisplatin-treated cells was reduced by the co-administration of 5-FU (0.68±0.15 by 5 μg/ml cisplatin plus 10 μg/ml 5-FU treatment, p=0.03). A very similar tendency in ERCC1 expression was observed in TCO-2 cells, which showed comparable chemosensitivity to cisplatin, and 5-FU (Figure 4).

After consideration of IC_{50} values for cisplatin and 5-FU in HCA-1 and HCA-1R cells, *ERCC1*/*GAPDH* mRNA ratios in HCA-1 and HCA-1R cells treated with cisplatin with and without 5-FU were calculated (Figure 5). Control *ERCC1*/*GAPDH* mRNA ratios in HCA-1 and HCA-1R cells without treatment were 1.0±0.03 and 1.42±0.11, respectively, when the mean ERCC1 expression in HCA-1 cells was defined as 1.0. *ERCC1* mRNA expression in cisplatin-resistant HCA-1R cells was significantly elevated (p=0.003). In HCA-1R cells, the ratio after cisplatin treatment (3.75 μg/ml) was significantly elevated compared with the control (p=0.03), whereas this elevation was lost following co-administration of 5-FU (10 μg/ml) (p=0.008) (Figure 5). In HCA-1R cells, the ratio after cisplatin treatment (3.75 μg/ml) was also significantly elevated compared to that of the control (p=0.04), whereas this elevation was lost by co-administration with 5-FU (10 μg/ml) (p=0.01). In conclusion, we observed very similar *ERCC1* expression levels in HCA-1 and HCA-1R cells (Figure 5).

Discussion

ERCC1 protein overexpression and increased mRNA expression have been implicated in resistance to platinum, and may act as prognostic markers for poor survival in patients with various types of malignant neoplasms treated with platinum-based chemotherapy or chemoradiotherapy with platinum (2-14).

Park *et al*. reported that determination of ERCC1 protein expression levels in pre-treatment specimens might facilitate the prediction of response to cisplatin-based neoadjuvant chemotherapy in International Federation of Gynaecology and Obstetrics stage IIB uterine cervical cancer, and that low ERCC1 expression is a significant favorable prognostic factor for disease-free survival (12). Our retrospective study indicated that immunostaining for ERCC1 expression may be useful for predicting survival in patients with uterine cervical adenocarcinoma receiving platinum agent-based chemotherapy or chemoradiotherapy with cisplatin, and can provide additional information for planning individualized chemotherapy for uterine cervical adenocarcinoma.

In *in vitro* investigations, the association between *ERCC1* expression in various cancer cell lines and chemosensitivity has been reported (8, 14, 15). Moreover, it has been reported that ERCC1 expression in an ovarian cancer cell line was significantly elevated by cisplatin treatment (16). Thus, these observational studies and *in vitro* investigations suggest that there is a possibility that inhibition of ERCC1 could be used to overcome chemoresistance in many kinds of cancers.

Recently it was reported that the mitogen-activated protein kinase (MAPK) pathway also has an important role in the regulation of ERCC1 expression (25-27). Cisplatin treatment increased extracellular signal-regulated kinase (ERK) activation in melanoma cell lines and enhanced drug resistance (25). Andrieux *et al*. reported that *ERCC1* induction by epidermal growth factor was dependent on regulation of MAPK signaling by the GATA transcription factor (26). Li and Melton also reported MAPK pathway-dependent increased levels of both ERCC1 and XPF proteins after cisplatin treatment in melanoma cells (27). Thus, many findings of molecular interactions associated with ERCC1/XPF expression have accumulated, and we should consider which of these interactions might be targeted in order to overcome chemoresistance of cancer cells.

Recently, there have been reports that ERCC1 expression was down-regulated by 5-FU treatment *in vitro* (18, 19). These reports concluded that combination chemotherapy with oxaliplatin and 5-FU or 5-FU derivatives was an effective treatment for ovarian mucinous adenocarcinoma (18), and 5-FU treatment with radiation might be effective in colon cancer through ERCC1 inhibition (19).

In the current study, we demonstrated that two cervical adenocarcinoma cell lines, HCA-1 and TCO-2, showed similar sensitivity to cisplatin and comparable expression of ERCC1 mRNA and protein. In addition, a cisplatin-resistant cell line HCA-1R was established from HCA-1 cells in order to compare chemosensitivity and ERCC1 expression in both cell lines. HCA-1R had a significantly higher level of *ERCC1* mRNA expression than native cells (p=0.003). Cisplatin treatment induced a significant elevation of ERCC1 expression, and co-administration of 5-FU reduced *ERCC1* expression levels in both HCA-1 and HCA-1R cells. Co-administration of cisplatin and 5-FU revealed synergistic or additive effects, which was not specified at this study in cervical adenocarcinoma cells. Therefore, there is a possibility that this combination therapy may be an ideal treatment regimen for cervical adenocarcinoma, from the point of view of ERCC1 inhibition. However, the precise mechanism for down-regulation of *ERCC1* expression by 5-FU treatment in cervical adenocarcinoma, as well as in other cancer cells, has not been identified. Further investigations are needed to clarify this mechanism through the elucidation of interactions between molecules involved in ERCC1 expression or modulation, especially the MAPK pathway.

In uterine cervical cancer treatment guidelines, concurrent chemoradiotherapy with cisplatin is indicated for locally advanced cancer, and is also used as an adjuvant therapy for high-risk early cervical cancer, particularly for those with positive lymph node metastases. For patients with distant metastases or recurrence, no optimal combination chemotherapy regimen has yet been defined, although cisplatin is frequently used, not only for squamous cell carcinoma, but also for adenocarcinoma (28). From the results of a recent randomized control study for advanced or recurrent cervical cancer, combination cisplatin and paclitaxel chemotherapy is currently considered to be one of the most optimal regimens (29). Moreover, it was reported that the overall survival rate was significantly worse in patients with adenocarcinoma than in those with squamous cell carcinoma (30). The poor prognosis of adenocarcinoma may be due to a higher incidence of lymph node metastasis, and relatively low sensitivity to radiotherapy compared to squamous cell carcinoma. Therefore, the role of chemotherapy in the treatment of adenocarcinoma is thought to be important for improving prognosis. Furthermore, defining the various factors that predict and influence chemosensitivity to cisplatin may enable for individualized treatment and have important clinical implications.

In uterine cervical cancer, chemoradiotherapy combined with cisplatin and 5-FU, as well as chemoradiotherapy with cisplatin alone, has been used for locally advanced cancer (31, 32). In patients with distant metastases or recurrence, combination chemotherapy with cisplatin and 5-FU has also been reported (33, 34). However, the clinical significance of the addition of 5-FU to cisplatin is controversial. Some reports have shown that the combination therapy with cisplatin and 5-FU was more toxic than cisplatin monotherapy, but combination therapy did not appear to significantly impact the outcomes of patients with locally advanced cancer (35, 36). In contrast, a few reports showed a significant clinical benefit of the combination therapy compared to cisplatin monotherapy (37). However, the clinical benefit of the addition of 5-FU to cisplatin has not been sufficiently demonstrated in cervical adenocarcinoma.

In a recent study by our group, high ERCC1 protein expression was revealed to be associated with poor disease-free survival in patients who received adjuvant cisplatin-based chemotherapy or chemoradiotherapy with cisplatin (13). This suggests that the suppression or modulation of ERCC1 expression may be a potential therapy for cervical adenocarcinoma, and it is possible that ERCC1 inhibition therapy can become an alternative therapy for patients with tumors highly expressing ERCC1. The current study suggests a clinical advantage of combining cisplatin with 5-FU or 5-FU derivatives for suppression of ERCC1 in cervical adenocarcinoma cells. However, there have been no reports on the clinical efficacy of the combination chemotherapy of cisplatin with 5-FU or 5-FU derivatives, compared to that of other cisplatin-containing regimens in cervical adenocarcinoma. Therefore, further investigations using a larger number of patients are required to confirm the efficacy of combination chemotherapy of cisplatin with 5-FU or 5-FU derivatives. In particular, this combination chemotherapy should be evaluated along with evaluation of ERCC1 expression level in tumors.

Moreover, the relationships of ERCC1 expression level and chemosensitivity to other chemotherapeutic drugs, such as paclitaxel, etoposide, vinorelbine, gemcitabine, and irinotecan, have not been fully-studied, and so this relationship should be investigated *in vitro*. Furthermore, other molecules involved in ERCC1/XPF expression or modulation may be particularly attractive as molecular targets to overcome resistance to cisplatin in cervical adenocarcinoma, as well as in other kinds of cancer. To facilitate the development of ERCC1/XPF inhibitors, many *in vitro* and *in vivo* investigations are warranted for future study.

Conflicts of Interest

No conflicts of interest to declare.

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