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# Small interfering RNA-induced suppression of ERCC1 enhances sensitivity of human cancer cells to cisplatin☆

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**Abstract**

The level of excision repair cross-complementing 1 (ERCC1) gene expression, which is important in the repair of the cisplatin–DNA adducts, is reported to be related to the level of cisplatin resistance in tumor cells. Therefore, ERCC1 is an attractive target to confer increased cellular sensitivity to cisplatin-based chemotherapy. We designed, synthesized, and utilized small interfering RNAs (siRNAs) that were selective for ERCC1 and investigated their effectiveness in altering the repair capacity of the cells to cisplatin–DNA damage as well as the resistance of the cells to cisplatin. Twenty-four and 48 h after transfecting ERCC1 siRNA1 and siRNA2 targeting the two different regions of the ERCC1 transcript, both the ERCC1 mRNA and protein expression were significantly inhibited, whereas the mock or control siRNA had no effect. The suppression of ERCC1 expression in the HeLa S3 cells led to a decrease in the repair activity of cisplatin-induced DNA damage along with a decrease in the cell viability against platinum-based drugs, such as cisplatin, carboplatin, and oxaliplatin. A similar increased sensitivity to cisplatin and decreased repair activity were also observed for siRNA-mediated ERCC1 silencing in the MCF-7 and HCT116 cells. This study is the first to demonstrate the feasibility of utilizing ERCC1 siRNAs to specifically reduce the ERCC1 expression level in human cancer cells and provides direct evidence for the potential use of ERCC1 siRNA as a chemotherapy-sensitizing agent.


Cisplatin is one of the most widely used platinum-containing anticancer drugs and believed to induce tumor cell death as a result of the formation of cisplatin–DNA adducts, which inhibit DNA replication and/or transcription [1–3]. However, the presence of intrinsic or acquired resistance to cisplatin in cancer cells remains a major obstacle to successful cancer chemotherapy. Although cellular resistance to cisplatin is multifactorial, NER appears to be the major mechanism of cisplatin resistance [4–6]. The cytotoxic effect of the anticancer drug cisplatin is principally attributable to the formation of bulky platinum–DNA adducts [2]. The removal of these adducts from the genomic DNA is mediated by the NER pathway [7,8]. The resistance to cisplatin appears to be associated with the increased removal of the cisplatin–DNA adducts and interstrand cross-links [9–11] as well as the enhancement of the host cell reactivation [12,13] and the excision of cisplatin–DNA lesions in cell extracts [14]. This suggests that the enhanced NER contributes to cisplatin resistance. Nucleotide excision repair (NER) is the major cellular

☆ *Abbreviations:* ERCC1, excision repair cross-complementing 1; siRNA, small interfering RNA; HCR, host cell reactivation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NER, nucleotide excision repair.

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repair system that removes the bulky DNA adducts [15,16]. The NER process involves the steps of damage recognition, an opening of the DNA, dual incisions on both sides of the lesion, the removal of oligonucleotides containing the damage, gap-filling DNA synthesis, and ligation [17]. Approximately 30 proteins that participate in this repair process have been identified [18]. Among them, the human excision repair cross-complementing gene 1 (ERCC1), which forms a heterodimer with XPF to make an incision 5′ to the damage site, is the rate-limiting step of the incision process [4,19–21]. High ERCC1 levels are associated with the increased removal of cisplatin-induced DNA adducts, and their expression appears to be related to the level of cisplatin resistance in the tumor cells [22–25].

High ERCC1 levels are associated with the increased removal of the cisplatin-induced DNA adducts as well as the relative cisplatin resistance [5]. In addition, *ERCC1*-knockout mice are highly sensitive to DNA cross-linking agents [22,23], and Chinese hamster ovary cells deficient in the ERCC1 protein (*ERCC1*−/−) transfected with *ERCC1* exhibited an increase in the DNA repair capacity and cisplatin resistance [21]. Moreover, several studies have reported a significant association between *ERCC1* expression and the clinical outcomes for cisplatin-based chemotherapy [24,26–28]. Therefore, controlled targeted inhibition of the ERCC1, combined with cisplatin, could provide attractive anticancer therapeutic benefits for many solid tumors.

Recently, several groups have reported that RNA interference (RNAi) is a form of post-transcriptional control in which the introduction of a double-stranded RNA (dsRNA) into a cell leads to the homology-dependent degradation of its cognate mRNA. Biochemical studies have revealed that cells contain evolutionary conserved RNAi machinery that triggers dsRNA cleavage into the 21- or 22-nucleotide small interfering RNAs (siRNAs). The siRNAs then hybridize into their cognate mRNA, which induces the specific degradation of the target mRNA [29–32]. Although the physiological significance of post-transcriptional gene silencing and RNA interference is currently under investigation, a powerful new technology for the selective inhibition of the specific gene expression employing siRNAs is rapidly evolving [33–36].

This study presents the data demonstrating that ERCC1 gene expression in human cancer cells is specifically suppressed by ERCC1 siRNA1 and ERCC1 siRNA2, resulting in a significant decrease in the DNA repair capacity as well as an increase in the sensitivity to platinum-based chemotherapeutic agents. These results show the feasibility of using siRNAs to specifically reduce the ERCC1 expression level in human cancer cells and highlight the potential use of ERCC1 siRNA as a new and highly effective strategy to cisplatin-based chemotherapy.

## Materials and methods

*Cell culture*. HeLa S3, MCF-7, and HCT116 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). HeLa S3 cell lines were grown in DMEM, MCF-7 cells in EMEM, and HCT116 cells in McCoy's 5A medium (Life Technologies), supplemented with 10% fetal bovine serum (Life Technologies). All the cultures were grown at 37 °C in a humidified atmosphere of 5% $CO_2$, fed every 5 days with a complete medium, and subcultured when they reached confluence.

*ERCC1-siRNA design, synthesis, labeling, and transfection*. Sequence information regarding the human ERCC1 mRNA was obtained from the NCBI Entrez nucleotide database. Two target sites within the ERCC1 gene were chosen from the human ERCC1 mRNA sequence (GenBank Accession No. NM_0001983). Following selection, each target site was searched using NCBI Blast to confirm the specificity to ERCC1 only. Two different siRNAs designated ERCC1-siRNA1 and ERCC1-siRNA2, which target the nucleotides, 306–326 and 668–688, of the human ERCC1 mRNA sequence, respectively, were prepared using a transcription-based method using a Silencer siRNA construction kit (Ambion, Austin, TX) according to manufacturer's instructions. LacZ siRNA was used as the negative control. The cells were transfected with the siRNA duplexes using Oligofectamine (Invitrogen) according to the manufacturer's protocol.

*Clonogenic cell survival assay*. The cells were seeded at $4 \times 10^5$ cells per 25 cm culture flask and incubated at 37 °C in a 5% $CO_2$ atmosphere. The cells were then treated with cisplatin (Sigma), carboplatin (Sigma), and oxaliplatin (Sanoti Winthrop) for 1 h, washed twice with phosphate-buffered saline (PBS), trypsinized, and then resuspended in fresh medium. They were counted using a Coulter counter, and the number of cells required for plating was obtained by successive dilutions in fresh complete medium. The cells were then allowed to grow at 37 °C in a 5% $CO_2$ atmosphere for 14 days. Fresh medium was added on the fifth day. Fourteen days after the drug treatment, the cultures were fixed using methanol and stained with Giemsa. The number of colonies exceeding 50 cells was counted using a binocular lens. The fraction of surviving cells was estimated using the ratio of the number of colonies observed after treatment to the number of cells seeded and adjusted for the plating efficiency.

*Western blotting*. The cells were centrifuged, washed with PBS, and lysed at 0 °C for 30 min in a lysis buffer (20 mM Hepes, pH 7.4, 2 mM EGTA, 50 mM glycerol phosphate, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM $Na_3VO_4$, and 5 mM NaF). The protein content was determined using a Bio-Rad dye-binding microassay (Bio-Rad, Hercules, CA, USA), and 50 μg of the protein per lane was electrophoresed on 12% SDS–polyacrylamide gels after boiling for 5 min in a Laemmli sample buffer. The proteins were blotted onto the Hybond ECL membranes (Amersham–Pharmacia Biotech). Colored markers (Bio-Rad) were used as the size standards. After electroblotting, the membranes were blocked with Tris-buffered saline with Tween 20 (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, and 0.1% Tween 20) containing 5% milk, and incubated with the anti-human ERCC1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. The membranes were then washed, incubated with the appropriate second antibodies in a blocking buffer for 1 h, and washed again. The blotted proteins were detected using an enhanced chemiluminescence detection system (Amersham–Pharmacia Biotech).

*Host cell reactivation assay*. The host cell reactivation (HCR) of the luciferase activity was determined as described previously [37]. Briefly, the pGL3-Luc plasmid (Promega), where the CMV promoter drives the firefly luciferase gene, was used to estimate the capacity of the cells to reactivate the damaged plasmid. The pRM-CMV plasmid, where CMV promoter drives the *Renilla* luciferase gene, was used as an

internal control for the transfection efficiency. For the host cell reactivation assay, the PGL3-Luc plasmid was damaged in vitro in the cells treated with 200, 400, 600 or 800 nM cisplatin. The mock, control or ERCC1 siRNA transfected cells were then transiently transfected with 1 μg of the treated pGL3-Luc plasmid and 0.1 μg of the pRL-CMV plasmid per well using the LipofectAMINE plus method according to the manufacturer's instructions (Life Technologies). The pRL-CMV plasmid was used to normalize for the total DNA transfected. In all cases, the cells were collected 24 h after transfection, and the cell extracts were used to determine the luciferase activity. The luciferase activity values were quantified using a Luminometer (EG&G, Lumat LB9507).

*Cisplatin accumulation, DNA adduct formation, and repair.* Cells were transfected with control siRNA, ERCC1 siRNA1 or ERCC1 siRNA2 for 24 h and then treated with 0, 25, and 75 μM cisplatin for 3 h. The cells were washed three times with ice-cold PBS and collected immediately to determine the total intracellular accumulation of cisplatin, using total cell extract or genomic DNA extract to measure the initial amount of DNA adduct formed. Cells from duplicate flasks were maintained in culture in drug-free complete medium to allow DNA repair. At the indicated times, genomic DNA of the cells was isolated by the phenol/chloroform method and then dissolved in Tris/EDTA. The DNA content was measured by absorbance at 260 nm, and the cisplatin content binding to DNA was determined by injecting a volume of 20 μl of sample into a pyrocoated graphite cuvette using a Hitachi polarized Zeeman Model z-8100 flameless atomic absorption spectrophotometer. Standards were prepared from a commercial atomic absorption platinum standard (1000 μg/ml in 5% HCl; Sigma) and a calibration curve was established using standard platinum solutions. The amount of cisplatin binding to DNA was assayed in triplicate and was expressed per milligram DNA. To measure the accumulation of intracellular cisplatin, the cell pellets were lysed in 60 μl of 0.1 M NaOH, and the protein content was determined by the modified Bradford method (Bio-Rad, Hercules, CA, USA). The cisplatin content of the mixture was measured using a Hitachi polarized Zeeman Model z-8100 flameless atomic absorption spectrophotometer. The cisplatin accumulation was determined in triplicate and was expressed in per milligram protein. Comparison between of DNA adducts in control siRNA- and ERCC1 siRNA-transfected cells was done by Student's *t* test.

*RNA isolation and analysis by real-time reverse transcriptase-PCR.* The cells were lysed in 1 ml of the Tri-reagent with 5 μl Glyco-Blue (Ambion) added as a co-precipitant and stored at room temperature for 5 min. After adding 0.1 ml of 1-bromo-3-chloro-propane, the samples were vigorously shaken, incubated for 15 min at room temperature, and centrifuged at 12,000*g* for 15 min at 4 °C. The aqueous phase was transferred to a new tube, and 0.5 ml of isopropyl alcohol was then added. The samples were mixed, stored for 10 min, and centrifuged at 12,000*g* for 15 min at 4 °C. After removing the supernatant, the RNA pellet was washed with 75% EtOH and repelleted by centrifugation at 12,000*g* for 15 min at 4 °C. The RNA was resuspended in a RNA Secure solution (Ambion), and the concentration and purity were determined spectroscopically. Real-time quantitative RT-PCR was performed using an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) by monitoring the increase in fluorescence by the binding of SYBR Green to the double-stranded DNA. The PCR primers were designed using Primer Express software (Applied Systems) at the default setting for a small amplicon of the PCR product. The first-strand cDNA template was synthesized from 5 μg of the total RNA using oligo(dT) and diluted into 500 μl water. For a 50 μl PCR, a 5 μl cDNA template was mixed with the forward and reverse primers (250 nM of each primer at final concentration), the probe primer (300 nM), and 2× SYBR Green PCR Master Mix (Applied Biosystem). The reaction was run at the default setting program (95 °C (15 s), 60 °C (1 min), 40 cycles). Gene-specific PCR was performed in the triplicate accession numbers in parentheses that were as follows: forward primer 5′-GGC GAC GTA ATT CCC GAC TA-3′; reverse primer 5′-AGT TCT TCC CCA GGC TCT GC-3′; and probe 5′-ACC ACA ACC TGC ACC CAG ACT ACA TCC A-3′. In order to quantify the gene expression changes, the $\Delta\Delta C_t$ method was used to calculate the relative fold-changes normalized against the glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

## Results and discussion

### *siRNA-mediated down-regulation of ERCC1*

To investigate the effects of ERCC1 siRNA on the ERCC1 expression level, the DNA repair activity, and the cell viability, three different 21-basepair siRNA constructs, ERCC1-siRNA1, ERCC1-siRNA-2, and control siRNA, were transfected into the HeLa S3 cells to test their ability to suppress ERCC1 expression. The ERCC1 mRNA and protein levels in the HeLa S3 cells transfected with 200 nM each for the two different ERCC1 siRNAs and the control siRNA are shown in Fig. 1. In order to obtain the quantitative ERCC1 mRNA expression values, quantitative real-time RT-PCR experiments were performed 24 h after treating the cells with either the ERCC1 siRNAs or control siRNA. Treatment with the ERCC1 siRNA1 resulted in a decrease in the ERCC1 mRNA level to $34 \pm 4\%$, and treatment with ERCC1 siRNA2 decreased the ERCC1 mRNA level to $38 \pm 5\%$, compared to either the mock or control siRNA-transfected cells (Fig. 1A). The protein extracts were obtained from the 24- to 96-h post-transfection, and Western blot analyses were performed for ERCC1 protein and then normalized for α-tubulin expression. We found that both siRNAs to the different sequences within the ERCC1 gene effectively inhibited ERCC1 protein expression 48 h after transfection (Fig. 1B). Transfection of either the ERCC1-siRNA1 or siRNA2 reduces the expression level of the targeted ERCC1 by 66–73% and 60–64%, respectively, compared to either the mock- or control siRNA-transfected cells. By 96-h post-transfection, the ERCC1 protein levels had risen back to the levels comparable to the control siRNA-transfected and untransfected cells (data not shown). These results demonstrate that both ERCC1 siRNA1 and ERCC1 siRNA2 were specific to ERCC1. Because there is a strong correlation between ERCC1 mRNA and protein suppression by siRNA, these results suggest that ERCC1 silencing in the HeLa S3 cells results from a reduction in the amount of ERCC1 mRNA available for translation. This suggests that the ERCC1-siRNAs were highly specific and efficient in ERCC1 gene silencing in the HeLa S3 cells.

### *Silencing of ERCC1 expression via siRNA leads to a decrease in the DNA repair activity*

Because ERCC1 is involved in the NER pathway and is known to play a crucial role in the repair of the

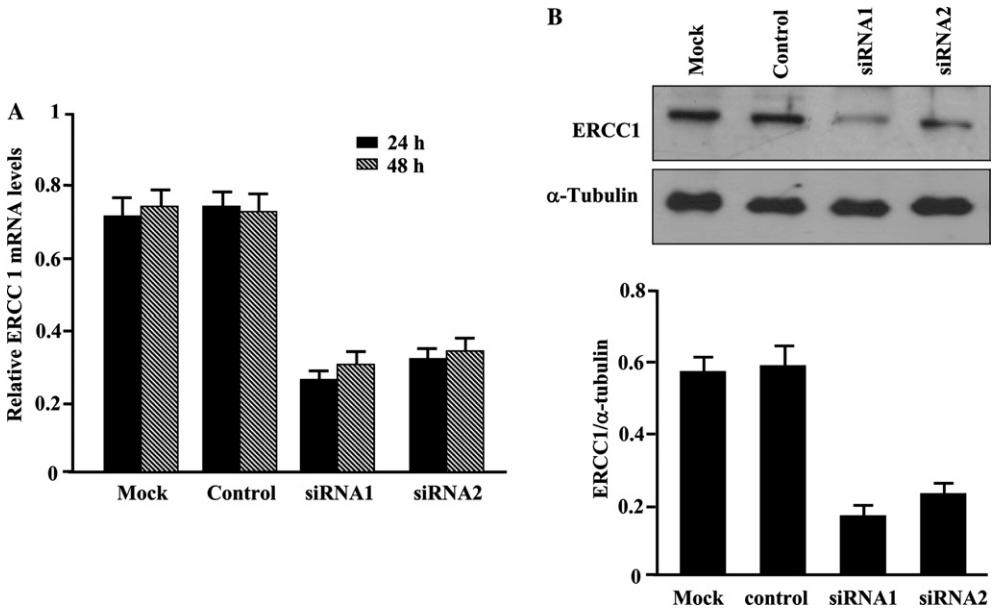


Fig. 1. Effect of siRNA on ERCC1 mRNA and protein expression in the HeLa S3 cells. (A) The cells were transfected with the mock, control siRNA or ERCC1 siRNAs. Twenty-four hours (black bar) and 48 h (hatched bar) after transfection, the total RNA was extracted from the cells and analyzed using real-time quantitative RT-PCR, as described under Materials and methods. Each PCR was run in triplicate. The relative fold-changes were calculated according to the $\Delta\Delta C_t$ method normalized against the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. The values are represented as means ± SD from triplicate RT-PCRs. (B) The cells were treated with the mock, control siRNA or ERCC1 siRNAs. Forty-eight hours later, the cell lysates were prepared from the mock-, control siRNA- or ERCC1 siRNA-treated cells. Equal amounts (50 μg proteins) of the cell lysates were separated by 12% SDS–PAGE and then transferred onto a nitrocellulose membrane. The membrane was immunoblotted with either anti-ERCC1 or anti-α-tubulin antibodies. The ERCC1 and α-tubulin were detected using the enzyme-linked chemiluminescence. The protein expression levels were quantified using a Bio-Rad Versa-Doc imager and Quantity One analysis software. The results are representative of three similar experiments.

cisplatin–DNA adducts [15,16], this study examined the DNA repair capacity in response to cisplatin in the ERCC1 depleted HeLa S3 cells, using the host cell reactivation of the luciferase activity, which reflects the capacity of the cells to repair the plasmids damaged by cisplatin. The mock, control or ERCC1 siRNA-treated HeLa S3 cells were transfected with cisplatin-treated reporter plasmid. Twenty-four hours after transfection, the cell extracts were then prepared for the luciferase activity. As shown in Fig. 2, the ERCC1 siRNA-treated cells significantly decreased the host cell reactivation of the cisplatin-treated luciferase activity, compared to the mock or control siRNA-transfected cells. To confirm that the ERCC1 is essential for the repair of cisplatin–DNA adducts, experiments were performed to measure specifically the removal of the cisplatin–DNA adducts in cells by atomic absorption spectrophotometry. The mock, control siRNA or ERCC1 siRNA-transfected HeLa S3 cells were incubated with cisplatin for 3 h and then maintained in culture for various periods of time to allow for repair. As shown in Fig. 3A, following incubation with cisplatin, intracellular cisplatin levels were similar in all four cell lines and increased with increasing levels of the drug. At 75 μM cisplatin, intracellular cisplatin levels were approximately: mock 912 ± 116, control siRNA 887 ± 102, ERCC1 siRNA1

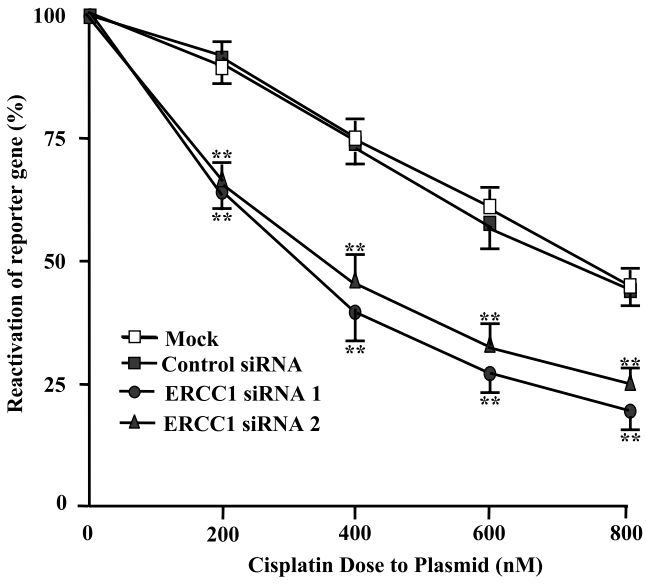


Fig. 2. Inhibition of ERCC1 expression associated with the reduced host cell reactivation in HeLa S3 cells. The mock, control siRNA- or ERCC1 siRNA-treated cells were transfected with the cisplatin-treated (0–800 nM) pGL3-Luc reporter plasmid. Twenty-four hours after transfection, the cell extracts were prepared for the luciferase activity. Cotransfection with the *Renilla* luciferase plasmid (pRL-CMV) was used to normalize for the transfection efficiency. Values are means ± SD from six separate experiments. $^{**}p < 0.01$.

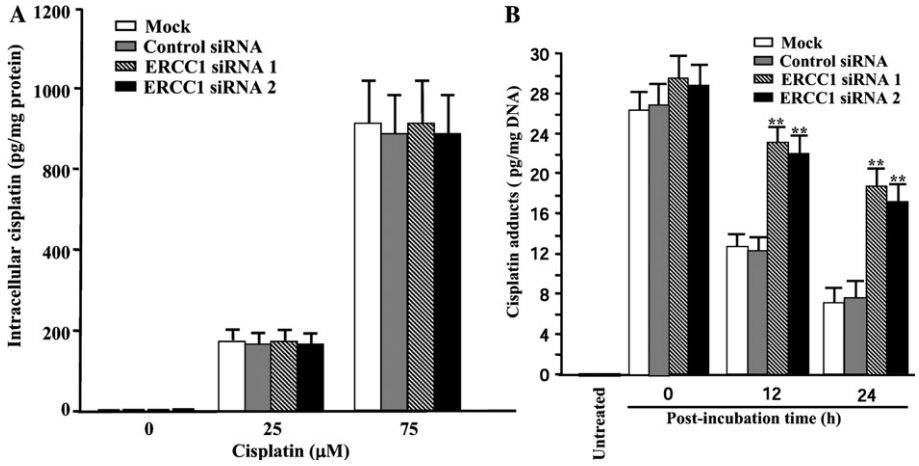


Fig. 3. Reduced ERCC1 expression leads to decrease in platinum adduct removal in HeLa S3 cells. (A) Cisplatin accumulation in the mock-, control siRNA- or ERCC1 siRNA-treated cells. Cells were treated with 0–75 μM cisplatin for 3 h, harvested, and processed for atomic absorption spectrometry as described under Material and methods. (B) Removal of cisplatin/DNA adducts in the mock-, control siRNA- or ERCC1 siRNA-treated cells. Cells were treated with 25 μM cisplatin for 3 h and post-incubated with drug-free medium for the indicated times. Platinum adduct removal was measured by atomic spectrophotometry. Values are means ± SD from four separate experiments. $^{**}p < 0.01$.

891 ± 98, and ERCC1 siRNA2 875 ± 94. However, the repair of cisplatin–DNA adduct was significantly decreased in ERCC1 siRNA-transfected cells. Mock and control siRNA-transfected cells demonstrated efficient removal of cisplatin–DNA adduct, with 54 ± 8% and 56 ± 7% of the lesions repaired by 12 h, respectively, and 73 ± 5% and 71 ± 6% of the lesions repaired by 24 h after cisplatin treatment, respectively. In contrast, ERCC1 siRNA-transfected cells exhibited a defect in repair of cisplatin–DNA adduct, with only 33–37% of the lesions being repaired even after 24 h (Fig. 3B). These results suggested that the transfection and subsequent expression of the ERCC1-targeted siRNAs resulted in the suppression of the DNA repair capacity in response to cisplatin.

### *siRNA silencing of the ERCC1 protein renders the human cancer cells sensitive to platinum containing agents*

Previous studies have demonstrated that the down-regulation of ERCC1 expression resulted in increased cellular sensitivity to DNA-damaging agents such as cisplatin [22–25]. In order to determine if siRNA-mediated attenuation of ERCC1 expression results in a subsequent sensitizing effect to cisplatin, HeLa S3 cells were transfected with the ERCC1-targeted, siRNA1 and 2, which were shown to have the greatest inhibition of ERCC1 protein expression. At 48 h post-transfection where the protein levels were shown to be the lowest, the resulting transfected cultures were treated with cisplatin, and the cellular sensitivity was measured by

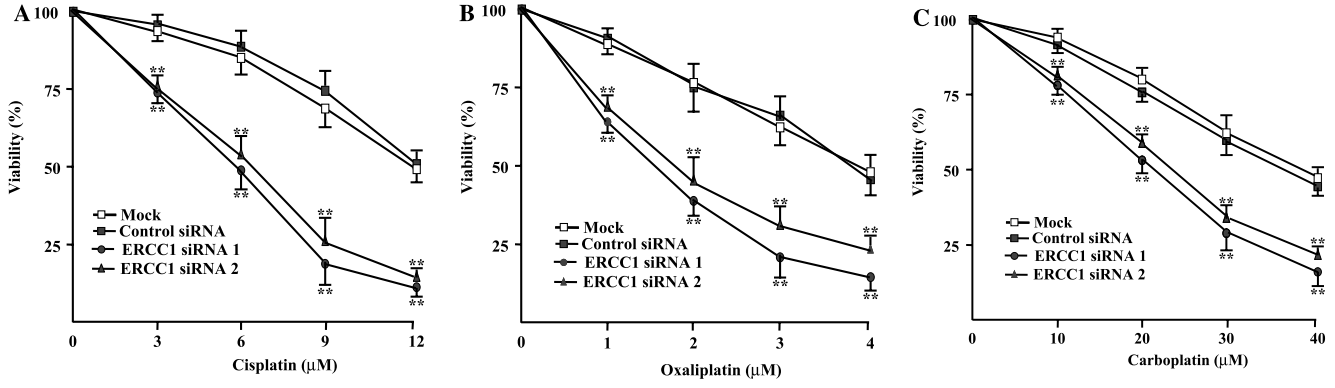


Fig. 4. Reduced ERCC1 expression results in sensitivity to platinum-based drugs in the HeLa S3 cells. The mock-, control siRNA- or ERCC1 siRNA-transfected cells were treated with various doses of cisplatin (A), oxaliplatin (B) or carboplatin (C) for 1 h, and the cell viability was determined by a clonogenic survival assay. Values are means ± SD from six separate experiments. $^{**}p < 0.01$.

clonogenic survival assays. The siRNA-mediated inhibition of the ERCC1 protein conferred an increased sensitivity to ERCC1 in the siRNA-transfected cell populations compared to the mock or control siRNA-transfected cells (Fig. 4A). In contrast to cisplatin, the mechanisms of resistance of the other platinum-based drugs, such as oxaliplatin and JM216, have not yet been reported. However, as the active metabolite of cisplatin is identical to those of the other platinum complexes or it reacts in an identical manner, all the mechanisms are probably common to all the platinum derivatives [38]. Moreover, the ability of NER to remove the cisplatin and oxaliplatin adducts showed no significant differences [8]. Therefore, the cellular capacity to repair the platinum adducts of DNA may also be important to the ability of the tumor cells to resist the platinum-based

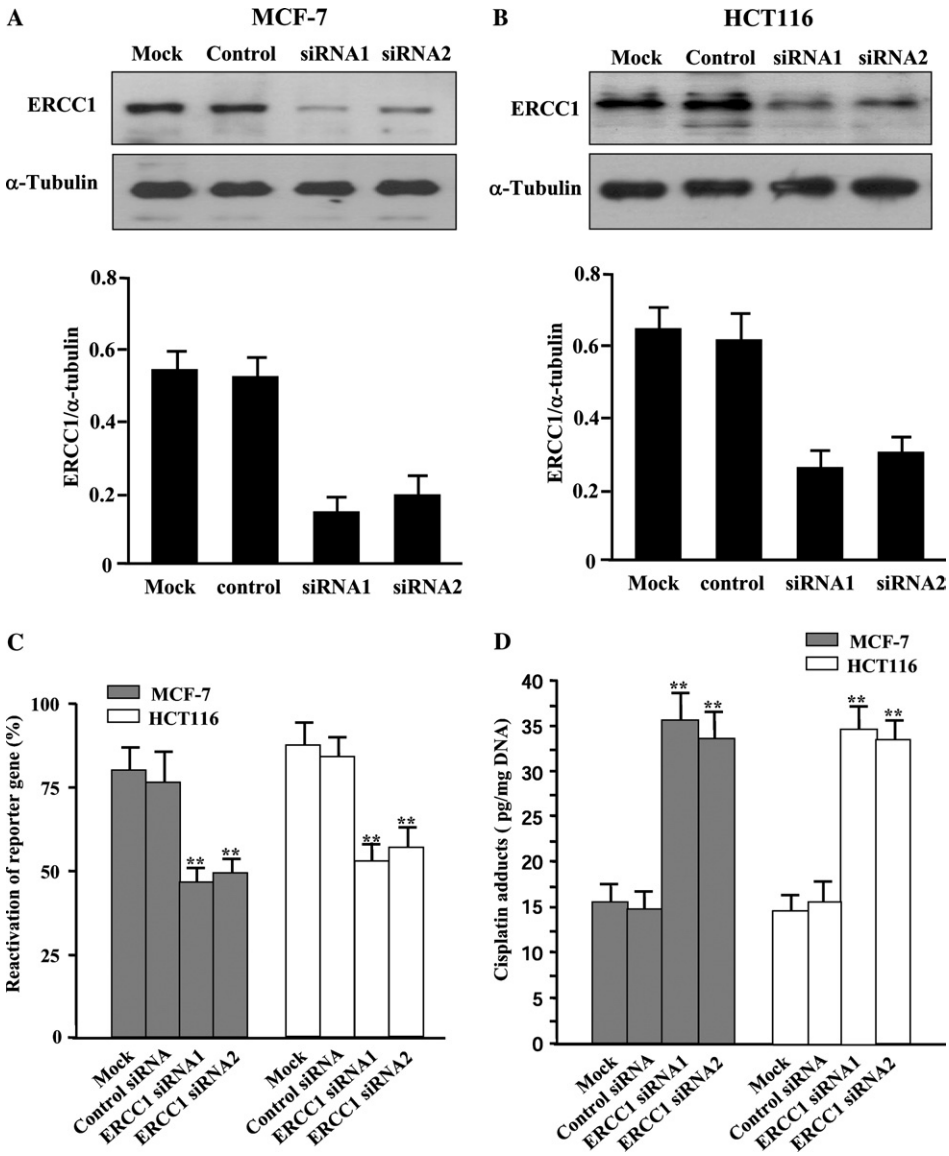


Fig. 5. siRNA-mediated down-regulation of the ERCC1 protein results in cisplatin sensitivity in MCF-7 and HCT116 cells. (A) The cells were transfected with the mock, control siRNA or ERCC1 siRNAs and harvested 48 h later. The α-tubulin expression level was used as the control of equal protein loading. (B) The cells were transfected with the mock, control siRNA or ERCC1 siRNAs and subsequently treated with 10 μM cisplatin for 1 h. The cell viability was determined by a clonogenic survival assay. Values are means ± SD from six separate experiments. (C) Decreased host cell reactivation in ERCC1 si-RNA-transfected MCF-7 and HCT116 cells. The mock, control siRNA- or ERCC1 siRNA-treated cells were transfected with the cisplatin-treated pGL3-Luc reporter plasmid. Twenty-four hours after transfection, the cell extracts were prepared for the luciferase activity. Each transfection also included the *Renilla* luciferase plasmid (pRL-CMV), which was used to normalize the transfection efficiency. Values are means ± SD from six separate experiments. $^{**}p < 0.01$. (D) Reduced ERCC1 expression leads to decrease in platinum adduct removal in MCF-7 and HCT116 cells. The mock-, control siRNA- or ERCC1 siRNA-treated cells were treated with 25 μM cisplatin for 3 h and incubated in fresh medium for 24 h. Cisplatin–DNA adduct formation and removal was measured by atomic spectrophotometry. Values are means ± SD from four separate experiments. $^{**}p < 0.01$.

chemotherapeutic agents. In order to determine if ERCC1 siRNA could also increase the cellular sensitivity to other platinum-based drugs, including carboplatin and oxaliplatin, HeLa S3 cells were transfected with the mock, control or ERCC1 siRNA and then treated with various doses of carboplatin or oxaliplatin. A clonogenic survival assay showed that the siRNA-mediated inhibition of ERCC1 expression results in an increased sensitivity to oxaliplatin and carboplatin (Figs. 4B and C), whereas the transfection of the mock or control siRNA in these cells failed to cause any obvious oxaliplatin and carboplatin sensitization. These results suggest that the ERCC1 expression level is important for the cell viability against platinum-based drugs and the expression of ERCC1 siRNA effectively increased the sensitivity to these drugs.

We next investigated if the ERCC1 expression level contributed to the cisplatin resistance in the other cancer cells, such as MCF-7 and HCT116. MCF-7 and HCT116 cells were transfected with the mock, control siRNA oligonucleotide or ERCC1-specific siRNA oligonucleotides, harvested 48 h after transfection, and their protein expression levels were determined. Western blot analysis revealed that ERCC1-specific siRNA oligonucleotides decreased by more than 80% in terms of their overall ERCC1 protein expression level in MCF-7 and HCT116 cells, compared with the mock or control siRNA-transfected cells (Figs. 5A and B). To examine whether ERCC1 contributed to DNA repair capacity, we used the host cell reactivation of luciferase activity, and found that reduced levels of ERCC1 exhibited significantly decreased levels of host cell reactivation when compared with the mock- or control siRNA-transfected cells (Fig. 5C). To further verify the effect of ERCC1 on DNA repair capacity, we measured the removal of cisplatin–DNA adducts in control and ERCC1 siRNA-transfected MCF-7 and HCT116 cells. Fig. 5D demonstrates that there are no significant differences in cisplatin–DNA adduct formation between mock and control siRNA-transfected cells. However, the rates of repair of cisplatin–DNA adducts were significantly impeded in ERCC1 siRNA-transfected cells. After 4 h incubation in drug-free medium, ERCC1 siRNA-transfected cells had removed approximately 2.3-fold less adducts than their control siRNA-transfected cells. To test the functional significance of the ERCC1 expression, the ERCC1 siRNA-transfected MCF-7, and HCT116 cells were treated with various doses of cisplatin for 12 h, and the cellular sensitivity was determined by a clonogenic survival assay. Those cells transfected with ERCC1 were more resistant to cisplatin when compared to the mock and control siRNA-transfected cells (Fig. 6), which suggests that suppression of ERCC1 leads to an increase in the sensitivity to cisplatin in human carcinoma cells.

In summary, high tumor tissue levels of ERCC1 in cancer patients have been associated with the clinical

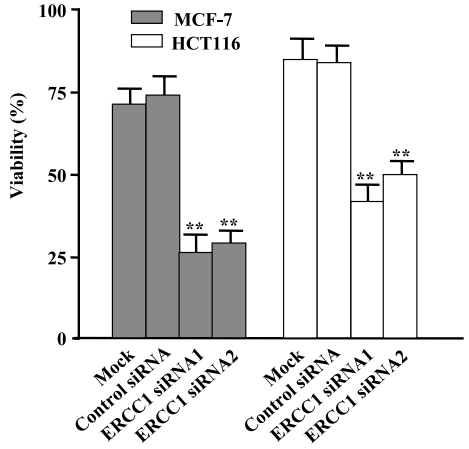


Fig. 6. siRNA-mediated down-regulation of the ERCC1 protein results in cisplatin sensitivity in MCF-7 and HCT116 cells. The MCF-7 and HCT116 cells were transfected with the mock, control siRNA or ERCC1 siRNAs and subsequently treated with 10 μM cisplatin for 1 h. The cell viability was determined by a clonogenic survival assay. Values are means ± SD from six separate experiments. $^{**}p < 0.01$.

resistance to platinum-based agents, whereas low ERCC1 levels are associated with the clinical sensitivity [35–38], suggesting that the ERCC1 protein inside the cells is quite unstable and that the level of ERCC1 is important for the response to cisplatin-based chemotherapy. This study designed and utilized siRNAs to ERCC1 that markedly diminished the expression level of this protein in human tumor cells, including HeLa S3, MCF-7, and HCT116 cells. As a result, the DNA repair activity to cisplatin and the cell viability in response to platinum-based chemotherapeutic agents, such as cisplatin, oxaliplatin, and carboplatin, were significantly reduced, suggesting that the ERCC1 expression level contributed to the sensitivity of the platinum-containing anticancer drugs in human tumor cells. The work presented here demonstrates the first reported use of ERCC1 siRNA as a novel tool for modulating the killing of human cancer cells by platinum-based drugs. Although cisplatin is highly effective in many types of cancer, an acquired or intrinsic resistance of cells to the drug limits its therapeutic efficacy. Therefore, the down-regulation of ERCC1 by ERCC1 siRNA may be relevant to the development of new strategies for improving the anticancer therapeutic benefits, involving the administration of platinum-based anticancer drugs along with ERCC1 siRNA.

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