

Oncogenic H-Ras Up-Regulates Expression of ERCC1 to Protect Cells from Platinum-Based Anticancer Agents

Cha-Kyung Youn,¹ Mi-Hwa Kim,¹ Hyun-Ju Cho,¹ Hong-Beum Kim,¹ In-Youb Chang,¹ Myung-Hee Chung,³ and Ho Jin You^{1,2}

¹Research Center for Proteineous Materials and ²Department of Pharmacology, School of Medicine, Chosun University, Gwangju, and ³Department of Pharmacology, School of Medicine, Seoul National University, Seoul, Korea

ABSTRACT

Tumors frequently contain mutations in the *ras* genes, resulting in the constitutive activation of the Ras-activated signaling pathway. The activation of Ras is involved not only in tumor progression but also in the development of resistance of the tumor cells to platinum-based chemotherapeutic agents. To investigate the potential mechanisms underlying this resistance, we analyzed the effect of activated H-Ras on the expression of the nucleotide excision repair genes. Here we identified ERCC1, which is one of the key enzymes involved in nucleotide excision repair, as being markedly up-regulated by the activated H-Ras. From promoter analysis of ERCC1, an increase in the Ap1 transcriptional activity as a result of the expression of the oncogenic H-Ras was found to be crucial for this induction. In addition, ERCC1 small interfering RNA expression was shown to reduce the oncogenic H-Ras-mediated increase in the DNA repair activity as well as to suppress the oncogenic H-Ras-mediated resistance of the cells to platinum-containing chemotherapeutic agents. These results suggest that the oncogenic H-Ras-induced ERCC1, which activates the DNA repair capacity, may be involved in the protection of the cells against platinum-based anticancer agents.

INTRODUCTION

Ras is a membrane-bound GTP/GDP-binding (G) protein that serves as a “molecular switch” converting the signals from the cell membrane to the nucleus (1). Under normal conditions the action of Ras and other members of the Ras pathway are strictly regulated during the cell cycle and under different growth conditions (2). In a tumor cell, the oncogenic activation of *ras* is a consequence of point mutations that either impair the GTPase activity or enhance the GTP binding affinity, resulting in a highly active proliferative signal (3). In addition, it is possible that the downstream protein targets of that signal transduction pathway might be expressed abnormally. The *ras* mutations are found in a wide variety of human cancers, with the highest incidences observed in adenocarcinomas of the pancreases (90%), colon (50%), and lung (30%; Ref. 4).

Activated Ras is involved not only in the oncogenic signal process but has also been suggested to be involved in the development of resistance of tumor cells to chemotherapy and ionizing radiation. For example, the transformation of NIH3T3 cells by activated *ras* has been shown in many cases to result in cell lines that are substantially more resistant to cisplatin than the parental cells (5, 6). Several other groups using NIH3T3 cells (7), rat rhabdomyosarcoma (8), human epithelial HBL 100 cells (9), human breast MCF-7 adenocarcinoma (10), and human HT 1080 fibrosarcoma (11) later confirmed this result independently. Although the molecular mechanisms of the oncogenic Ras-mediated resistance to platinum-based chemotherapy need to be

elucidated, evidence suggests that the activated Ras may contribute to cisplatin resistance by stimulating the DNA repair activity (9, 12, 13). Hence, there has been considerable interest in determining which proteins mediate the altered DNA repair capacity in activated Ras-containing cells. However, the downstream target genes of the oncogenic Ras, which are involved in the enhancement of the DNA repair activity, are unclear.

Cisplatin is one of the most effective and widely used anticancer drugs for treating human solid tumors (14). However, its therapeutic efficacy has been limited by the emergence of tumor cell subpopulations with an intrinsic or treatment-induced resistance (15). Therefore, elucidating the mechanisms involved in drug resistance is the key element in the development of new strategies for overcoming this phenomenon and improving the treatment outcome. Whereas several mechanisms of cisplatin resistance have been identified, increased DNA repair is an important contributing mechanism (16). Cisplatin forms a broad spectrum of lesions including monoadducts, intra-, and interstrand cross-links in DNA, which inhibit DNA replication and/or transcription (17, 18). This suggests that DNA is the cytotoxic target of cisplatin and its analogs. Nucleotide excision repair (NER) is the main repair system for eliminating bulky DNA lesions such as platinum/DNA adducts (19). Enhanced DNA repair capacity has been implicated in the cisplatin resistant phenotype (9, 12), whereas DNA repair-defective cells are reportedly hypersensitive to cisplatin (20–22). Therefore, cells that are resistant to these drugs either have the capacity to limit the formation of platinum/DNA adducts or, alternatively, they must be able to repair or tolerate these lesions once they are formed.

Our previous studies have shown that the expression of oncogenic H-Ras protects NIH3T3 cells from cisplatin-induced cytotoxicity and enhances the DNA repair capacity in response to cisplatin (13). Therefore, in this study, expression level analysis of NER genes was performed using ponasterone A regulatable oncogenic H-Ras-expressing NIH3T3 and MCF-7 cells to identify the downstream target genes regulated by activated H-Ras, particularly those that might be also involved in the activation of the DNA repair capacity as well as cisplatin resistance. It was found that ERCC1, which is a mammalian DNA repair gene, of which the gene product has been shown to play a crucial role in the early excision step of damaged DNA by virtue of its intrinsic structure-specific nuclease activity (23, 24), is the ultimate downstream target of oncogenic H-Ras and that the increased Ap1 transcriptional activity as a result of the oncogenic H-Ras expression has a profound positive impact on ERCC1 transcription. In addition, the transient transfection of the activated H-Ras expressing NIH3T3 and MCF-7 cells with ERCC1 small interfering RNA (siRNA) causes the cells to reduce oncogenic H-Ras-mediated increase in the DNA repair activity and to be highly sensitive to platinum-based drugs compared with mock- and control siRNA-transfected cells. These results provide strong evidence that ERCC1 can function directly as a survival effector in the activated H-Ras-containing cells exposed to platinum-based anticancer drugs.

Received 2/3/04; revised 4/27/04; accepted 5/11/04.

Grant support: Ministry of Science and Technology of Korea and the KOSEF through the Research Center for Proteineous Materials, and the National Cancer Control R&D Program 2003, Ministry of Health and Welfare, Republic of Korea.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Ho Jin You, Department of Pharmacology, School of Medicine, Chosun University, 375 Seosuk-dong, Gwangju, 501-759 South Korea. Phone: 82-62-230-6337; Fax: 82-62-233-3720; E-mail: hjyou@mail.chosun.ac.kr.

MATERIALS AND METHODS

Cell Culture. The NIH3T3 and MCF-7 cells (American Type Culture Collection) were maintained in Earle's MEM supplemented with 10% fetal bovine serum, 100 units of penicillin/ml, and 100 μ g of streptomycin/ml (Life Technologies, Inc.). Cells were maintained in 5% CO₂/95% air at 37°C in a humidified incubator.

Preparation of Constructs and Clones. The constructs of the wild-type H-Ras, dominant-positive V12-H-Ras, and dominant-negative V17-H-Ras were described previously (13). The human ERCC1 cDNA were amplified by reverse transcription-PCR using the ERCC1 oligo primer (5'-ATGCCTGCCCGCGCGCTTCTGCC-3', 5'-CTAGCCTTCCGGCCCTTTGGAAC-3') from human fibroblast GM00637 cells. The human ERCC1 promoter (-960 to +30) was amplified by PCR from the genomic DNA prepared from human fibroblast GM00637 cells. PCR was performed with pfu polymerase (Stratagene) using primers (upstream, 5'-gcaggctctaacacaccaa-3'; downstream, 5'-gccaggctcg-cagcactt-3') that introduce *Hind*III at both the 5' and 3' ends of the product. The ERCC1 luciferase reporter was generated by cloning this fragment into the *Hind*III site of the pGL3-Basic vector (Promega). The serial mutagenesis of the region from -960 to +30 was generated using a Erase-a-BaseR system (Promega, Madison, WI). DNA mutagenesis of the region from -537 to -15, as a template with pfu polymerase and mutagenic oligonucleotides, was designed to introduce each specific multibase point mutation. The forward sequences of the oligonucleotides used for mutating different parts of the ERCC1-537 constructs were as follows: proximal Ap1 mut: 5'-CCGGGGCTGAaaCAGGCGCTCCC-3'; Distal Ap1 mut: 5'-TTGGTCA CTGCTGTaaCACCAGCA-3'; Ets1 mut: 5'-GGAA-GAGAGccAGCGCTG-3'. The italicized lowercase letters indicates the nucleotide substitutions the insert mutations. Transfections were carried out using lipofectAMINE (Life Technologies, Inc.). After incubation, cells were lysed; luciferase activity was determined using the dual luciferase reporter assay system (Promega).

Host Cell Reactivation. Host cell reactivation of cisplatin-treated luciferase activity was determined as described previously (13).

Cisplatin Accumulation, DNA Adduct Formation, and Repair. Cells were treated with 10 μ M cisplatin for 0–24 h. 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 μ l 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 μ g/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 mg DNA. To measure the accumulation of intracellular cisplatin, the cell pellets were lysed in 60 μ l 0.1 M NaOH, and the protein content was determined by the modified Bradford method (Bio-Rad, Hercules, CA).

Clonogenic Cell Survival Assay. Cells were seeded at 4 \times 10⁵ cells/25 cm culture flask and incubated at 37°C in a 5% CO₂ atmosphere. Cells were then treated with various doses of cisplatin (Sigma), carboplatin (Sigma), and oxaloplatin (Sanofi Winthrop) for 1 h, washed twice with PBS, trypsinized, and resuspended in fresh medium. Clonogenic cell survival says were then determined as described previously (13).

Western Blotting. The cells were 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 DTT, 1 mM phenylsulfonyl fluoride, 10 μ g/ml leupeptin, 10 μ /ml aprotinin, 1 mM Na₃VO₄, and 5 mM NaF]. The protein content was determined using the Bio-Rad dye-binding microassay (Bio-Rad); and 20 μ g protein/lane was electrophoresed on the 10% SDS polyacrylamide gels. The proteins were blotted onto the Hybon ECL membranes (Amersham-Pharmacia Biotech), and immunoblotting was carried out with the anti-ERCC1, α -tubulin (Santa Cruz Biotechnology, Santa Cruz, CA), and H-Ras antibodies (BD Biosciences, San Diego, CA). The blotted proteins were detected using an enhanced chemiluminescence detect system (iNtRON Biotech., Seoul, Korea).

Electrophoretic Mobility-Shift Assay. Nuclear extracts were prepared as described previously (25). The electrophoretic mobility shift assay binding reactions were incubated in a 20- μ l final volume for 20 min at room temperature containing 5 μ g of the nuclear extract as well as 20 fmol of the

end-labeled double stranded oligonucleotide and 1 μ g poly[di-dC]-[di-dC]. The binding buffer contained 30 mM HEPES, 0.3 mM EDTA, 1.5 mM MgCl₂, 0.2 mM DTT, 10% glycerol, 1 mM phenylsulfonyl fluoride, 1 mM aprotinin, and 1 mM leupeptin. The complexes were resolved using a 5% nondenaturing polyacrylamide gel in 0.5 \times TBE and electrophoresis at 200 V for 1 h. The gels were dried and exposed to X-ray film overnight at -70°C.

siRNA. The sequences of 21-nucleotide sense and antisense RNA with a 2-nucleotide overhang composed of TT(DNA) were as follows: ERCC1-siRNA1, 5'-UAUGCCAUCU CACAGCCUCdTdT-3' (sense) and 5'-GAG-GCUGUGAGAUGGC AUAdTdT-3' for the ERCC1 gene (nucleotides 306–326); ERCC1-siRNA2, 5'-GGAGCUGGCUAAGAUGUGUdTdT-3' (sense) and 5'-ACACAUCUUAGCCAGCU CcDdTdT-3' (antisense) for the ERCC1 gene (nucleotides 668–688); LacZ siRNA, 5'-CGUACGCGAAUACUUC-GAdTdT-3' (sense), 5'-AAUC GAAGUAUUCGCGUA CgDdTdT-3' (antisense) for the LacZ gene. These siRNAs were prepared by a transcription-based method using the Silencer siRNA construction kit (Ambion, Austin, TX) according to manufacturer's instructions. Cells were transfected with siRNA duplexes by using Oligofectamine (Invitrogen).

Real-Time Quantitative Reverse Transcription-PCR. Real-time quantitative RT-PCR was performed on the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) by monitoring the increase of fluorescence by the binding of SYBR Green to double-stranded DNA. The reaction was run at the default setting program [95°C (15 s), 60°C (1 min), 40 cycles]. ERCC1-specific primers 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'. For quantification of gene expression changes, the $\Delta\Delta$ Ct method was used to calculate relative - fold changes normalized against the glyceraldehyde-3-phosphate dehydrogenase.

Chromatin Immunoprecipitation Assays. NIH3T3 clone-1 cells (1.2 \times 10⁷ cells) were collected by low speed centrifugation and fixed with 1% formaldehyde. After 15 min, cells were washed with ice-cold Tris-buffered saline and lysed with 1 ml of a radioimmune precipitation assay buffer [10 mM Tris-HCl (pH 8.0), 140 mM NaCl, 0.025% Na₃, 1% Triton X-100, 0.1% SDS, and 1% deoxycholic acid]. Chromatin was sheared by sonication to an average size of ~500 bp. The chromatin solution was diluted with 2 volumes of 1% NP40, 350 ml NaCl, and incubated overnight at 4°C with 2 μ g of the anti-c-Jun, anti-c-Fos antibodies or antimouse IgG and then by precipitating the immunocomplexes with protein A-agarose, which was also pretreated with sheared DNA salmon sperm. Input and immunoprecipitated chromatin were incubated overnight at 65°C. After proteinase K digestion, DNA was extracted with phenol-chloroform, precipitated with ethanol-precipitated, allowed to air dry, and then dissolved in 20 μ l of sterile H₂O. Five μ l of the DNA samples were then subjected to amplification by using the primers (5'-GGACTC-CGGGGCTGAGTCA-3', sense; 5'-TCCTGTGCGAGTCCG-3', antisense), which amplified the promoter region of the human ERCC1 (from -535 to -341 respective to the transcription start).

RESULTS

Oncogenic H-Ras Increases DNA Repair Activity. To identify the potential oncogenic H-Ras target genes that might be involved in this DNA repair activity, the effect of oncogenic H-Ras on the DNA repair capacity was initially re-evaluated in the NIH3T3 cells. Therefore, the dominant-active V12-H-Ras was subcloned into the vector pIND and form pIND-Ras to control the generation of oncogenic H-Ras within these cells. Following transfection and double selection using G418 (selection for pIND-Ras) and zeocin (selection for pVg-RXR) for 5 weeks, nine clones were isolated, and the oncogenic H-Ras expression that was able to be turned on or off was analyzed using ponasterone A. Western blot analysis revealed that a treatment of the NIH3T3 clone-1 with ponasterone A for 24 h resulted in the efficient induction of oncogenic H-Ras expression in a dose-dependent manner (Fig. 1A). To examine the effect of oncogenic H-Ras on the DNA repair capacity, host cell reactivation of luciferase activity, which reflects the capacity of the cells to repair plasmids damaged by cisplatin treatment, was used. It was found that the treatment of

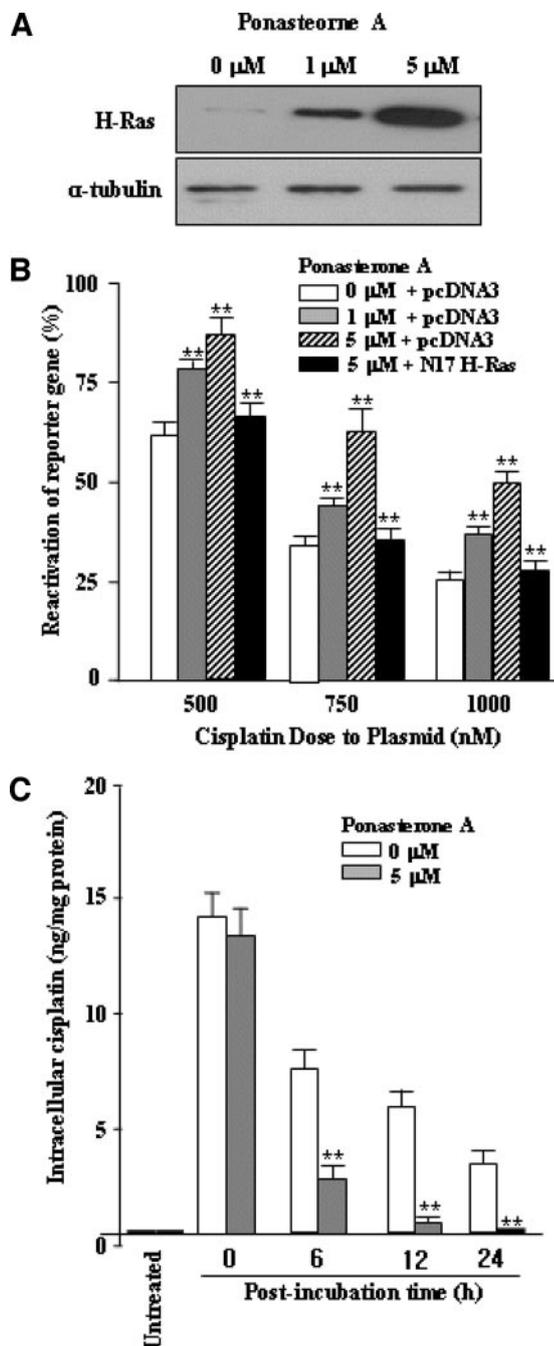


Fig. 1. Oncogenic H-Ras-dependent increase in the DNA repair activity. *A*, the NIH3T3 clone-1 cells were grown in the presence or absence (indicated concentration) of ponasterone A for 24 h. Western blot experiments were performed with the antibody to Ras and an antibody to α -tubulin as a control for an equal loading. *B*, NIH3T3 clone-1 cells were cotransfected with the cisplatin-treated pGL3-Luc reporter plasmid, together with the dominant-negative N17-H-Ras-expressing plasmid (*pN17-Ras*) or the empty expressing plasmid (*pcDNA3*). Each transfection also included the *Renilla* luciferase plasmid (*pRL-CMV*), which was used to normalize the transfection efficiency. The cells were then treated with or without (indicated concentration) ponasterone A for 24 h, and the cell extracts were prepared for the luciferase activity. The values are represented as a mean from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$. *C*, the NIH3T3 clone-1 cells were treated with 5 μ M ponasterone A for 24 h. The cells were then treated with 10 μ M cisplatin for 3 h and postincubated with a drug-free medium for the indicated times. The level of cisplatin adduct removal was measured using an atomic spectrophotometer. The values are represented as a mean from four separate experiments; bars, \pm SD. ** denotes $P < 0.01$.

NIH3T3 clone-1 cells with 1 μ M and 5 μ M ponasterone A resulted in an increase in host cell reactivation in a dose-dependent manner and that this increase in host cell reactivation could be completely inhibited by the transfection of dominant-negative N17-H-Ras (Fig. 1*B*).

To confirm the above observations, we wanted to determine the effect of oncogenic H-Ras on the removal of the cisplatin/DNA adduct in cells. It has been shown that cisplatin induces a broad spectrum of platinum/DNA adducts including inter- and intrastrand cross-links in DNA, which are subjected to repair by the NER pathway (19). Therefore, the removal of the cisplatin/DNA adduct in cells was measured specifically using atomic absorption spectrophotometer assays. NIH3T3 clone-1 cells were treated with 5 μ M ponasterone A for 0–24 h, after which cells were incubated with 10 μ M cisplatin for 3 h and then maintained in culture for various times to allow for repair. As shown in Fig. 1*C*, the repair of the cisplatin/DNA adduct was significantly increased in the oncogenic H-Ras-expressing cells. After 6-h incubation in drug-free medium, the oncogenic H-Ras-expressing NIH3T3 clone-1 cells had removed 2.5 times more adducts than the control cells. By 12 h of incubation in the drug-free medium, the oncogenic H-Ras-expressing cells had removed almost all of the cisplatin/DNA adducts, whereas control cells had removed only \sim 43% of the adducts. This suggests that oncogenic H-Ras contributes to the increase in DNA repair activity in NIH3T3 cells.

Induction of the ERCC1 by Oncogenic H-Ras. In mammalian cells, NER is initiated by six core factors, XPA, RPA, XPC-HR23B, XPG, ERCC1-XPF, and the TFIIH complex, which are essential for damage recognition and dual incision (26). To identify the potential oncogenic H-Ras transcriptional target genes that might be involved in the oncogenic H-Ras-induced DNA repair capacity, we isolated two different mRNA populations, one from the NIH3T3, NIH3T3 clone-1, or the NIH3T3 clone-3 cells, and the other from the same cells generating the oncogenic H-Ras after the treatment with 1 and 5 μ M ponasterone A for 24 h. By real-time quantitative reverse transcription-PCR (reverse transcription-PCR) with the specific primers for XPA, RPA, XPC, XPG, ERCC1, XPF, XPB, and XPD (components of TFIIH complex), we evaluated the mRNA level of six core factors in the presence or absence of ponasterone A and found that the level of ERCC1 mRNA increased as the concentration of ponasterone A was increased (Fig. 2*A*). However, the other NER gene expression levels were unaffected by the treatment with ponasterone A (data not shown). The ERCC1 mRNA level in the parental cell line, NIH3T3, remained unchanged after the treatment with ponasterone A. Experiments using shorter induction times (2 h or 6 h) also failed to produce any significant increase in the ERCC1 mRNA levels (data not shown). Western blot analysis was performed to determine whether the increase in the ERCC1 mRNA level corresponds to an increase in the ERCC1 protein level (Fig. 2*B*). SDS-PAGE was used to separate the whole-cell extracts of the protein from the untreated cells, as well as the protein from two independent cells generating oncogenic H-Ras after the treatment with 1 μ M or 5 μ M ponasterone A. Western blot analysis with the ERCC1 antibody revealed that the ERCC1 protein increased in the two independent NIH3T3 clones in response to ponasterone A. These results demonstrate that ERCC1 is up-regulated via oncogenic H-Ras.

Oncogenic H-Ras Increases ERCC1 Promoter Activity Through Ap1-Binding Sites. The above results suggest that oncogenic H-Ras induces ERCC1 transcription. Therefore, the relationship between oncogenic H-Ras generation and ERCC1 expression was next analyzed using a reporter gene construct containing a 960-bp sequence of the 5'-flanking region of the ERCC1 promoter (ERCC1-960). In transient-transfection assays using the NIH3T3 clone-1 cells, the generation of H-Ras after the treatment with ponasterone A resulted in an increased activity of the ERCC1 promoter (Fig. 3*A*). The greatest induction occurred after the treatment with 5 μ M ponasterone A (7.3 ± 1.1 -fold increase relative to the background expression level; $P < 0.01$). In the control experiment, the parental (untransfected) NIH3T3 cells

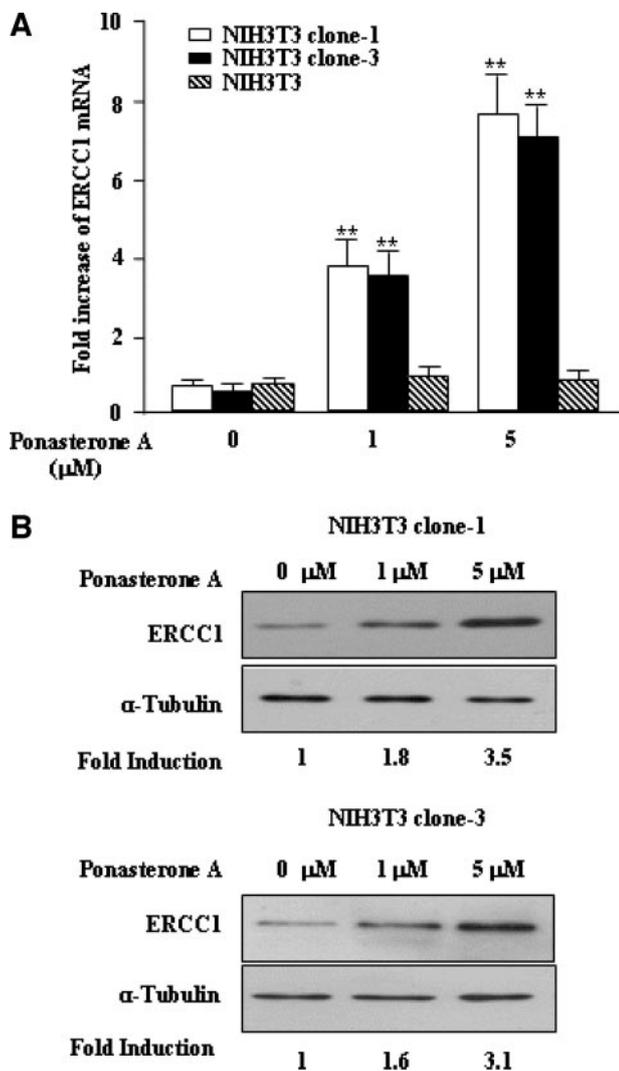


Fig. 2. Oncogenic H-Ras-induced ERCC1 mRNA and protein expression. *A*, the NIH3T3, NIH3T3 clone-1, and clone-3 cells were treated with or without (indicated concentration) ponasterone A for 24 h. Real-time quantitative reverse transcription-PCR was performed using the primers for ERCC1 cDNA. The relative-fold changes were calculated according to a $\Delta\Delta C_t$ method normalized against the glyceraldehydes-3-phosphate dehydrogenase. *B*, the NIH3T3 clone-1 and clone-3 cells were treated with or without (the indicated concentration of) ponasterone A for 24 h. Western blot experiments were performed with the antibody to ERCC1 and an antibody to α -tubulin as a control for an equal loading. The fold induction was calculated from the densitometry measurements of the ERCC1 signals normalized to the α -tubulin loading in the lysates of ponasterone A-stimulated cells compared with the untreated control cells; bars, \pm SD.

treated with ponasterone A showed no induction of ERCC1 promoter activity. To determine the region within the ERCC1 promoter responsible for the transcriptionally active effect of oncogenic H-Ras, a series of 5'-deletion constructs of ERCC1-960 were made with progressively smaller fragments of the 5'-flanking sequence, and these constructs were cloned into the pGL3-Basic vector and tested for promoter activity in the NIH3T3 clone-1 cells in the presence or absence of 5 μ M ponasterone A. A deletion of the 5' end of the ERCC1-960 construct to provide segments -818/30, -724/30, -616/30, and -537/30 were still highly induced by ponasterone A. However, when the deletion was extended to produce segment -413/30, a 42 \pm 6% decrease in the ponasterone A-induced promoter activity was observed. An additional deletion of the 5' end to provide segment -315/30 resulted in a 85 \pm 7% decrease in the ponasterone A-induced promoter activity (Fig. 3B). This indicates that the region between -537 and -315 contains the important controlling elements essential for the

responsiveness of the ERCC1 promoter to oncogenic H-Ras expression.

We used MatInspector software program⁴ to search for the transcription factor binding sites (27) and found that segment -537/-315 contains several putative Ras-responsive transcription factor binding sites, including two Ap1 motifs (proximal Ap1 motif, TGAGTCA: position -523 to -517; distal Ap1 motif, TGTGTCA: -368 and -362) and an Ets1 motif (AGGAA, -330 to -326; Fig. 4A). To investigate that it is indeed these sites that participate in the oncogenic H-Ras-mediated induction of ERCC1, we constructed a variety of mutants of the ERCC1 promoter reporters, wherein the proximal Ap1, distal Ap1, and Ets1 sites were mutated in different combinations by PCR-based mutagenesis. A mutation in the Ets1-binding site (mEts1) led to only 15 \pm 4% reduction in the activation of the ERCC1 promoter after the ponasterone A treatment. However, a mutation in either the proximal Ap1 (mP-Ap1) or distal Ap1 (mD-Ap1) binding site was shown to significantly affect the activation of the ERCC1 promoter caused by H-Ras expression, and the inductions of these reporters were reduced by 39 \pm 7% and 44 \pm 9%, respectively (Fig. 4B). Double mutations in the proximal and distal Ap1 motifs (mP&D-Ap1) resulted in the marked suppression of the ponasterone A-mediated increase in ERCC1 promoter activity (76 \pm 7% decrease by luciferase analysis). Therefore, the presence of these two Ap1 elements was important for the oncogenic H-Ras-mediated activation of the ERCC1 promoter in NIH3T3 cells. As the ERCC1 promoter analysis suggested, the involvement of the proximal and distal Ap1 binding sites in the oncogenic H-Ras-mediated induction of the ERCC1 promoter activity, an electrophoretic mobility-shift assay, using oligonucleotides containing the ERCC1-specific proximal and distal Ap1 binding sites, was used to investigate the possibility that oncogenic H-Ras has a positive regulatory function in the binding of Ap1 to DNA. It was found that the activated H-Ras, which was generated by treating the NIH3T3 clone-1 cells with ponasterone A, significantly increased the proximal and distal Ap1 DNA-binding activity in a dose-dependent manner (Fig. 4C).

The Ap-1 transcription factors are complexes of DNA-binding proteins made up of homodimers of the Jun family members or heterodimers of the Jun and Fos family members (28). To provide evidence of a direct interaction of c-Jun and c-Fos to these Ap1 binding sites of the ERCC1 promoter under the conditions of oncogenic H-Ras expression *in vivo*, the most simple and direct approach was to treat cells with ponasterone A and then perform the chromatin immunoprecipitation assays. Therefore, the NIH3T3 clone-1 cells, which were transfected with the ERCC1-537 promoter fragment, were treated with 5 μ M ponasterone A for 24 h, and the cells were then cross-linked, as described under "Materials and Methods," and protein-DNA complexes were immunoprecipitated with either anti-c-Jun or anti-c-Fos antibodies. The covalent linkage was reversed, and the precipitated double-stranded DNA was amplified by PCR using the binding-specific primers (Fig. 5A). The chromatin immunoprecipitation assay demonstrated that c-Jun and c-Fos protein bind *in vivo* to the ERCC1 promoter. Indeed, the DNA precipitated by anti-c-Jun antibody and anti-c-Fos antibody contains the -536 to -341 sequence of the ERCC1 promoter (Fig. 5B). Importantly, the amount of promoter sequence precipitated by the antibodies increased remarkably after treatment of cells by ponasterone A. The binding was specific, because the negative controls using the samples in which the nonspecific antibody (antimouse IgG) was used did not show a signal. These results of the chromatin immunoprecipitation assays clearly confirmed the *in vitro* studies, demonstrating that the interaction of

⁴ Internet address: <http://www.genomatix.de>.

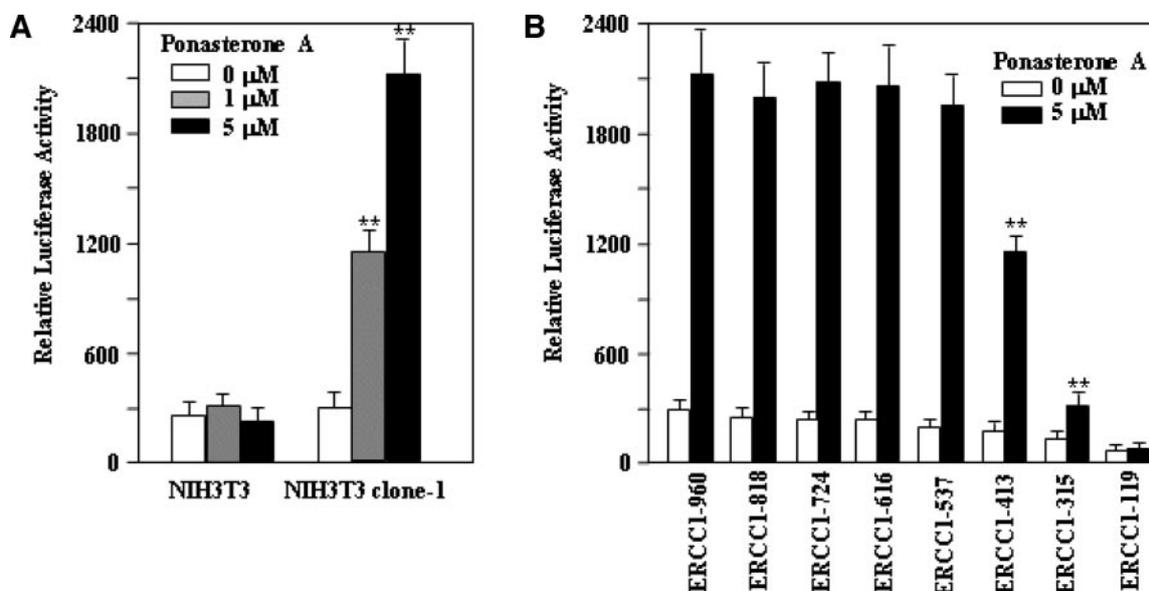


Fig. 3. Oncogenic H-Ras-dependent increases in the ERCC1 promoter activity. *A*, the NIH3T3 and NIH3T3 clone-1 cells were cotransfected with the ERCC1 promoter-reporter plasmid (ERCC1-960) and the internal control plasmid pRL-CMV and treated with or without (the indicated concentration) ponasterone A for 24 h. The luciferase activities were then measured. The graph shows the luciferase activity (relative to that in the cells transfected with pGL3-basic) in the cells treated with or without ponasterone A. The values are represented as a mean from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$. *B*, NIH3T3 clone-1 cells were transfected with the indicated plasmids, i.e., the ERCC1-960, ERCC1-818, ERCC1-724, ERCC1-616, ERCC1-537, ERCC1-413, ERCC1-315, and ERCC1-119 promoter-reporter constructs containing positions -960 to +30, -818 to +30, -724 to +30, -616 to +30, -537 to +30, -413 to +30, -315 to +30, and -119 to +30 of the ERCC1 promoter, respectively. Each transfection also included the *Renilla* luciferase plasmid (pRL-CMV). The graph shows the luciferase activity (relative to that in the cells transfected with pGL3-Basic) in the cells treated with or without 5 μ M ponasterone A. The values represent the means from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$.

c-Jun and c-Fos with the Ap1-binding sites residing in the -537 and -315 of the ERCC1 promoter is important for the oncogenic H-Ras-induced transcription of ERCC1 in NIH3T3 cells.

Down-Regulation of ERCC1 Suppresses the Oncogenic H-Ras-Induced DNA Repair Capacity. To determine whether ERCC1 is involved in the oncogenic H-Ras-mediated increase in the DNA repair capacity, siRNAs in the form of two independent, nonoverlapping, 21-bp RNA duplexes that target ERCC1 were used in an attempt to inhibit its expression. The oncogenic H-Ras expressing NIH3T3 cells

were transfected with the mock, control siRNA oligonucleotide, or the ERCC1-specific siRNA oligonucleotides. The cells were harvested 48 h after transfection, and their protein expression levels were determined (Fig. 6A). Western blot analysis revealed that the ERCC1-specific siRNA oligonucleotide levels decreased by >80% in terms of their overall ERCC1 protein expression level compared with the mock or control siRNA-transfected cells. By 96-h after transfection, the ERCC1 protein levels had increased back to the levels comparable with the mock- and control siRNA-transfected cells (data not shown).

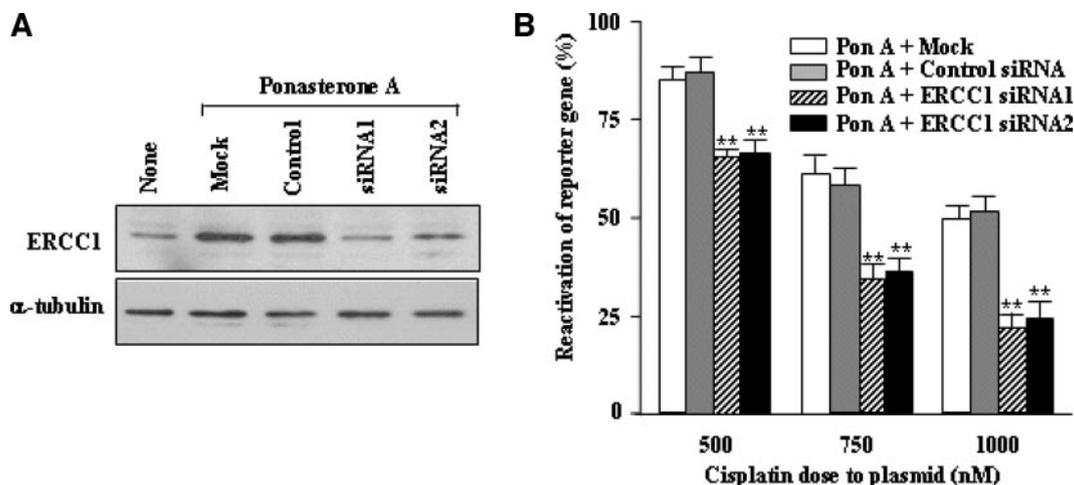


Fig. 4. Oncogenic H-Ras-triggered increase in the DNA binding activity of Ap1 to the ERCC1 promoter. *A*, ERCC1-537 promoter has two putative Ap1 binding sites and one putative Ets1 binding site. The *underlined* GT in Ap1 and GA in Ets1 were mutated to AA (*mP-Ap1* and *mD-Ap1*) and CC (*m-Ets*), respectively. *B*, the ERCC1-537 reporter constructs containing the indicated mutations, in the proximal Ap1 site (*mP-Ap1*), in the distal Ap1 site (*mD-Ap1*), in the proximal and distal Ap1 sites (*mP&D-Ap1*), or in the Ets1 site (*mEts1*) were transfected together with pRL-CMV into the NIH3T3 clone-1 cells. The cells were then treated with or without 5 μ M ponasterone A for 24 h, and the luciferase activities were measured. The graph shows the luciferase activity (relative to that in the cells transfected with pGL3-Basic) in the cells treated with or without 5 μ M ponasterone A. The values represent the means from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$. *C*, electrophoretic mobility-shift assays were performed using a radio-labeled probe from the ERCC1 promoter region containing the proximal or distal Ap1 sites and the nuclear extracts from the ponasterone A-treated or untreated (-) NIH3T3 clone-1 cells. Lane 1, NIH3T3 clone-1 nuclear extract treated with a 50-fold excess of the unlabeled consensus Ap1 oligonucleotide; lane 2, untreated NIH3T3 clone-1 cells; lane 3, NIH3T3 clone-1 cells treated with 1 μ M ponasterone A for 24 h; lane 4, NIH3T3 clone-1 cells treated with 5 μ M ponasterone A for 24 h; lane 5, NIH3T3 clone-1 nuclear extract treated with a 50-fold excess of the unlabeled consensus Ap1 oligonucleotide; lane 6, untreated NIH3T3 clone-1 cells; lane 7, NIH3T3 clone-1 cells treated with 1 μ M ponasterone A for 24 h; lane 8, NIH3T3 clone-1 cells treated with 5 μ M ponasterone A for 24 h.

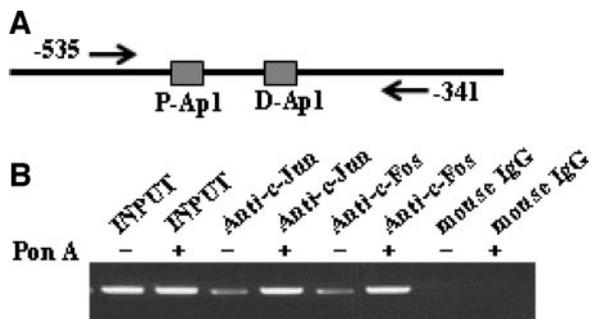


Fig. 5. Chromatin immunoprecipitation analysis of Ap1 binding to the human ERCC1 promoter in the NIH3T3 cells cultured in the presence or absence of ponasterone A. *A*, schematic of the annealing position of the PCR primers used to specifically amplify the ERCC1 promoter containing the proximal (*P-Ap1*) and distal Ap1 (*D-Ap1*); product size 194 bp. *B*, the NIH3T3 clone-1 cells transfected with ERCC1-537 were treated with (+) or without (–) 5 μ M ponasterone A (*Pon A*) for 24 h, then cross-linked with formaldehyde and sonicated. The fragmented chromatin was immunoprecipitated by the addition of anti-c-Jun or anti-c-Fos antibodies or antimouse IgG (nonspecific). The samples were then amplified by PCR using the primers flanking the Ap1-binding sites, and the PCR products were resolved on a 1.5% agarose gel. The input chromatin from the untreated and treated cells was also amplified by PCR (INPUT) to compare the relative amounts of chromatin used in immunoprecipitation. The data are representative of several similar experiments.

The DNA repair capacity after ERCC1-siRNA transfection was next examined, and it was found that the oncogenic H-Ras-expressing cells with the reduced levels of ERCC1 had significantly lower levels of host cell reactivation when compared with the mock- or control siRNA-transfected cells (Fig. 6*B*). To additionally verify the effect of ERCC1 on the DNA repair activity, the removal of the cisplatin/DNA

adducts in the mock-, control siRNA-, and ERCC1 siRNA-transfected NIH3T3 clone-1 cells in the presence of ponasterone A was measured. As shown in Fig. 6*C*, the oncogenic H-Ras-expressing cells with reduced levels of ERCC1 exhibited a significant decrease in the repair of the cisplatin/DNA adduct. By 6 h of incubation in a drug-free medium, the mock and control siRNA-treated cells had removed >80% cisplatin/DNA adducts, whereas the ERCC1 siRNA-transfected cells exhibited a defect in the repair of the cisplatin/DNA adduct, with only 31–34% of the lesions repaired by 6 h after the cisplatin treatment and 44–49% of the lesions repaired by 12 h after the cisplatin treatment (Fig. 6*C*). These findings demonstrate that ERCC1 expression is essential for the oncogenic H-Ras-mediated increase in the DNA repair activity.

ERCC1 Plays an Essential Role in Oncogenic H-Ras-Mediated Cell Survival against Platinum-Based Agents. To determine whether this increase in ERCC1 expression contributes to the cisplatin resistance in oncogenic H-Ras-transfected cells, the oncogenic H-Ras-expressing cells were transfected with the mock, control siRNA oligonucleotides, or ERCC1-specific siRNA oligonucleotides. At 24 h after transfection, the cells were treated with various doses of cisplatin for 1 h, and the cellular sensitivity was determined by a clonogenic survival assay. Those cells treated with the ERCC1 siRNA oligonucleotides exhibited hypersensitivity to the lethal effects of cisplatin when compared with the mock or control siRNA-transfected cells (Fig. 7*A*). In the control experiments, NIH3T3 clone-1 cells in the absence of ponasterone A showed similar poor levels of protection. In another series of experiments, the response of ERCC1-inhibited cells

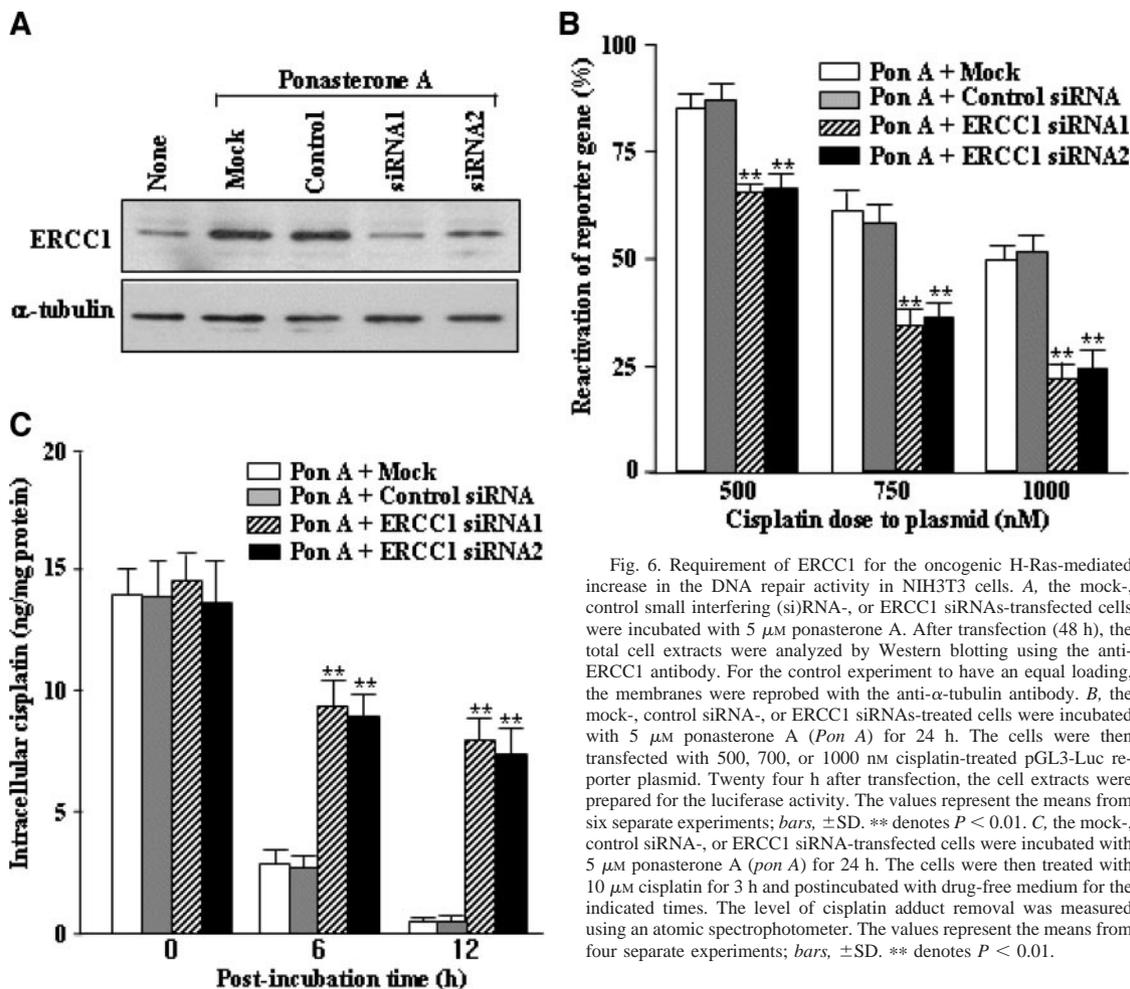


Fig. 6. Requirement of ERCC1 for the oncogenic H-Ras-mediated increase in the DNA repair activity in NIH3T3 cells. *A*, the mock-, control small interfering (si)RNA-, or ERCC1 siRNAs-transfected cells were incubated with 5 μ M ponasterone A. After transfection (48 h), the total cell extracts were analyzed by Western blotting using the anti-ERCC1 antibody. For the control experiment to have an equal loading, the membranes were reprobbed with the anti- α -tubulin antibody. *B*, the mock-, control siRNA-, or ERCC1 siRNAs-treated cells were incubated with 5 μ M ponasterone A (*Pon A*) for 24 h. The cells were then transfected with 500, 700, or 1000 nM cisplatin-treated pGL3-Luc reporter plasmid. Twenty four h after transfection, the cell extracts were prepared for the luciferase activity. The values represent the means from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$. *C*, the mock-, control siRNA-, or ERCC1 siRNA-transfected cells were incubated with 5 μ M ponasterone A (*pon A*) for 24 h. The cells were then treated with 10 μ M cisplatin for 3 h and postincubated with drug-free medium for the indicated times. The level of cisplatin adduct removal was measured using an atomic spectrophotometer. The values represent the means from four separate experiments; bars, \pm SD. ** denotes $P < 0.01$.

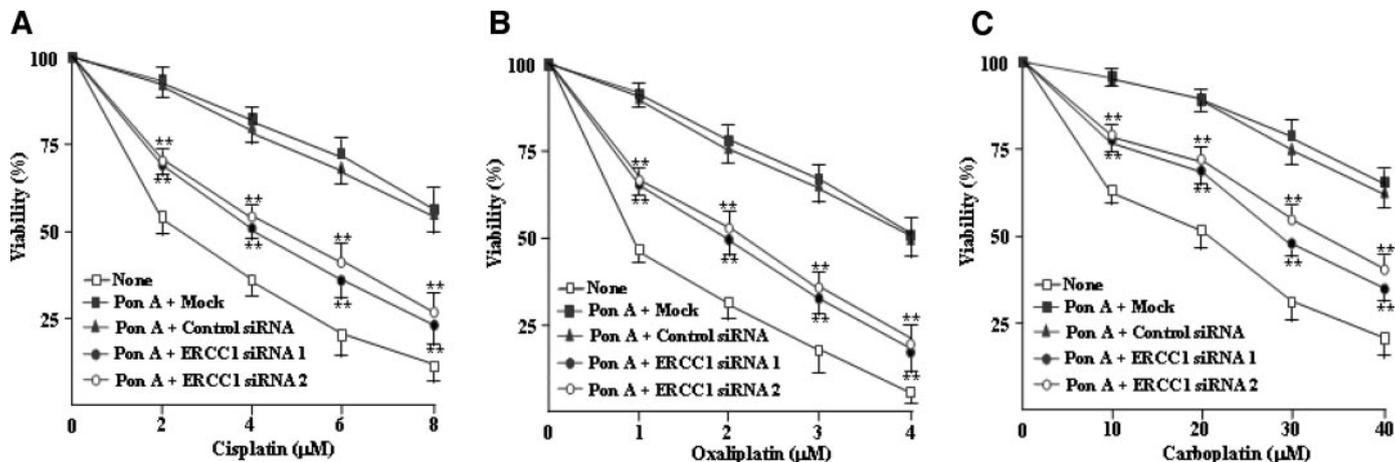


Fig. 7. Reduced ERCC1 expression results in cisplatin sensitivity in oncogenic H-Ras-expressing NIH3T3 cells. The mock-, control small interfering (si)RNA-, or ERCC1 siRNA-transfected cells were incubated with or without (*None*) 5 μ M ponasterone A (*Pon A*) for 24 h. Subsequently, the cells were then treated with the indicated doses of cisplatin (A), oxaliplatin (B), or carboplatin (C) for 1 h, and the cell viability was determined by a clonogenic survival assay. The values represent the means from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$.

to other platinum-based anticancer agents, such as oxaliplatin and carboplatin, was examined and it was found that the transfection of the oncogenic H-Ras-expressing cells with ERCC1-specific siRNA also reduced the viability response to these anticancer agents compared with the mock- and control siRNA-transfected cells (Fig. 7, B and C).

To additionally investigate if the oncogenic H-Ras-mediated protection against platinum-based chemotherapeutic agents, resulting from ERCC1 expression, can occur in cancer cells, human breast carcinoma MCF-7 cells were used to determine whether ERCC1 expression is a determining factor in the ability of oncogenic H-Ras to regulate the resistance of cancer cells to platinum-based drugs. Therefore, a pIND-Ras construct was transfected into the MCF-7 cells. After double selection using G418 and zeocin for 5 weeks, five clones were isolated, and the oncogenic H-Ras expression, which could be turned on or off using ponasterone A, was analyzed. As shown in Fig. 8A, treatment of ponasterone A enhanced H-Ras expression. In addition, oncogenic H-Ras-expressing MCF-7 clone-2 cells exhibited high

ERCC1 expression levels, as well as resistance to cisplatin and oxaliplatin, and these protective effects were almost abolished after transfecting the cells with the ERCC1-specific siRNA oligonucleotides (Fig. 8B).

DISCUSSION

The accumulated evidence suggests that activated Ras plays an important role in the development of resistance of tumor cells to cisplatin-based chemotherapy. However, the molecular mechanisms underlying this effect are unclear. This study found that activated H-Ras increases the DNA repair capacity in response to cisplatin. In addition, it was demonstrated that oncogenic H-Ras could induce ERCC1 and that the enhanced interaction of c-Jun and c-Fos with the Ap1 motifs caused by oncogenic H-Ras was important for the ERCC1 induction. Furthermore, ERCC1 induction was essential for the on-

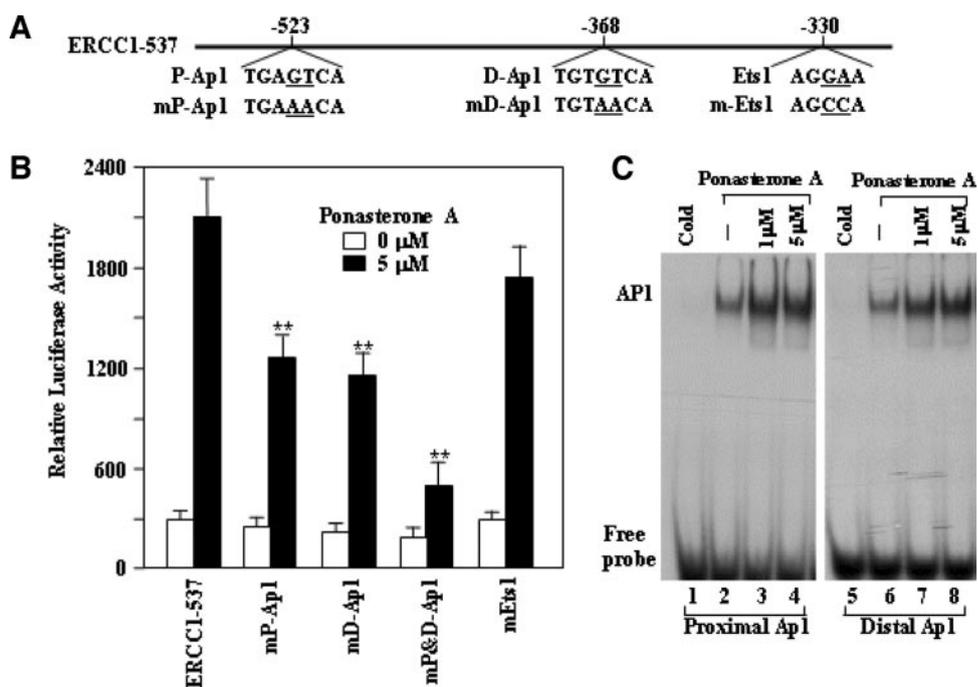


Fig. 8. Effect of small interfering (si)RNA-mediated ERCC1 depletion on the resistance to the platinum-based agents of MCF-7 cells conferred by the expression of oncogenic H-Ras. A, the mock-, control small interfering (si)RNA-, or ERCC1 siRNA-transfected MCF-7 clone-2 cells were treated with or without (*None*) 5 μ M ponasterone A and harvested 24 h later. Immunoblots were performed with the antibodies to Ras, ERCC1, and α -tubulin, and α -tubulin expression level was used as the control experiment with an equal protein loading. B, the mock-, control siRNA-, or ERCC1 siRNA-transfected MCF-7 clone-2 cells were incubated with or without (*None*) 5 μ M ponasterone A (*Pon A*) for 24 h. Subsequently, the cells were treated with either 40 μ M cisplatin or 10 μ M oxaliplatin for 1 h, and the cell viability was then determined by a clonogenic survival assay. The values represent the means from six separate experiments; bars, \pm SD. ** denotes $P < 0.01$.

cogenic H-Ras-mediated resistance cells to platinum-based chemotherapeutic agents.

Several lines of evidence have suggested recently that activated Ras may be associated with the regulation of the DNA repair activity. For example, transformations by an activated Ras of the human epithelial HBL 100 cells resulted in less formation of cisplatin-induced interstrand cross-links as well as an increase in the DNA repair synthesis (9). Similarly, oncogenic Ras-transfected Syrian hamster Osaka-Kanazawa cells exhibited an increased resistance to cisplatin as well as a decrease in the intracellular platinum binding to DNA (29). In addition, the expression of the *erbB-2* proto-oncogene, which encodes a 185 kDa transmembrane glycoprotein (p185) with a tyrosine kinase activity homologous to the epidermal growth factor receptor, led to the direct regulation of the DNA repair mechanism, and the Ras-coupled pathway is important for modulating the DNA repair induced by *erbB-2* (30). Using the host cell reactivation of the reporter gene expression from the cisplatin-damaged plasmid and the unscheduled DNA synthesis after the cisplatin treatment of the cells, we demonstrated previously that activated H-Ras expression in NIH3T3 cells increased the DNA repair activity (13).

To address the question as to what kind of DNA repair protein might be involved in the oncogenic H-Ras-mediated increase in the DNA repair activity, we analyzed the effect of activated H-Ras overexpression on the expression of NER genes. NER is a versatile DNA repair mechanism that removes a wide variety of lesions, such as UV-induced lesions and platinum/DNA adducts (19). The principal steps in the NER reaction mechanism are: (a) recognition and demarcation; (b) chromatin-remodeling; (c) endonuclease-mediated incision at both sides of the lesion and the removal of the damaged oligonucleotides; and (d) repair DNA synthesis and ligation. In human cells, the minimum set of components involved in performing this reaction comprises the replication protein A, XPA, XPC-HR23B, XPG, ERCC1-XPF, TFIIH, the replication factor C, proliferating cell nuclear antigen, DNA polymerase δ or ϵ , and DNA ligase I (26). The results showed that treating the NIH3T3 clone-1 and clone-3 cells with ponasterone A leads to a dose-dependent increase in the ERCC1 mRNA and ERCC1 protein expression levels (Fig. 2), and two Ap-1 motifs appear to be critically important in the oncogenic H-Ras-mediated increase in ERCC1 transcription (Fig. 4). Ap1 is the class of transcription factors that are believed to act as a nuclear target for Ras activation (28). A recent report suggested that Ap1 transactivation is important for inducing ERCC1 in response to cisplatin and phorbol ester (31–33). Moreover, we showed previously that an oncogenic H-Ras enhanced DNA repair capacity was required, at least in part, for an increase in ROS production (13). Because Ap-1 acts as a redox-sensitive transcription factor in several cell types (34) and is also activated by either the Ras/extracellular signal-regulated kinase or Ras/c-Jun NH₂-terminal kinase signal transduction process (35, 36), these studies are consistent with our data in that Ap1 is a key positive regulator of ERCC1 transcription in activated H-Ras-expressing cells.

Previous studies have shown that the expression of c-Jun and c-Fos has been implicated in the enhancement of DNA repair activity. Treatment of human ovarian cancer cells with cisplatin induces expression of the c-Fos, as well as a number of genes involved in DNA repair (37), and a dominant-negative c-Jun reduces the repair of the cisplatin adducts (38). In addition, ERCC1 mRNA expression has been shown recently to be reduced by the down-regulation of c-Jun expression after the treatment with herbimycin A or by expression of TAM-67, which is a known inhibitor of the c-Jun function (39). Similarly, cisplatin-resistant cells exhibited higher levels of c-Jun and c-Fos, and the down-regulation of c-Jun and c-Fos expression sensitizes the cells to cisplatin (40, 41). Moreover, the cells derived from

c-Jun knockout mice are more resistant to cisplatin toxicity than normal cells (42). The present finding, that the induced binding of c-Jun and c-Fos to the Ap1 motifs is important for the oncogenic H-Ras-induced transcription of the *ERCC1* gene (Fig. 5), is consistent with previous studies that indicated that ERCC1 is a downstream target gene of oncogenic H-Ras, and this increased ERCC1 may be involved in the oncogenic H-Ras-mediated increase in the DNA repair capacity.

The cytotoxic effect of the platinum-based anticancer drugs has been attributed to the formation of bulky platinum-DNA adducts (17, 18). The removal of these adducts from the genomic DNA are repaired primarily by the NER (19, 43). Cisplatin resistance appears to be associated with the increased removal of the cisplatin-DNA adducts and the interstrand crosslinks (9, 12) in addition to the enhancement of the host cell reactivation (44). This suggests that NER is a major mechanism of cisplatin resistance (16). ERCC1 is known to play a key role in NER, because ERCC1 defect cells exhibit the most severe DNA repair-deficient phenotype (20). ERCC1 forms a heterodimer with XPF, and the ERCC1/XPF complex is responsible for the incision to cleave the damaged strand at the phosphodiester bonds between the 22 and 24 nucleotides 5' to the lesion (45). According to the literature, the increase in ERCC1 expression is likely to cause the observed cisplatin resistance phenotype. Indeed, accumulating evidence from a series of biochemical and clinical experiments demonstrated that the level of ERCC1 is important for the repair of the platinum/DNA adducts and for the response to cisplatin-based chemotherapy. Mice with a deletion of the gene for ERCC1 are hypersensitive to cisplatin due to their decreased ability to repair the cisplatin/DNA adducts (46), and Chinese hamster ovary deficient in the ERCC1 protein (*ERCC1*^{-/-}) transfected with human *ERCC1* exhibited a 5-fold higher resistance than *ERCC1*^{-/-} cells (20). In addition, high tumor tissue levels of ERCC1 mRNA in human ovarian and gastric cancer patients have been associated with the clinical resistance to platinum-based agents (47, 48), whereas low mRNA levels are associated with the clinical sensitivity. Similarly, expressing antisense ERCC1 showed a decreased DNA repair capacity of cisplatin resistance ovarian cancer cells and increased their sensitivity to cisplatin (49). Moreover, several studies reported recently a significant association between *ERCC1* expression and the clinical outcomes for cisplatin-based chemotherapy (50, 51). This suggests that ERCC1 expression might contribute to the oncogenic H-Ras-mediated increase in DNA repair activity and cell viability in response to cisplatin-based chemotherapeutic agents. In the present study, we found that the ERCC1-targeted siRNA oligonucleotides caused an inhibition of the activated H-Ras-induced DNA repair activity and a reduction of the activated H-Ras-mediated increase in the viability response to platinum-based anticancer agents (Figs. 6 and 8). Therefore, it is concluded that oncogenic H-Ras-mediated up-regulation of ERCC1 is involved in the resistance of the platinum-based anticancer agents when oncogenic H-Ras is expressed.

In summary, ERCC1 expression is essential for the oncogenic H-Ras-mediated increase in the capacity of the cancer cells to initiate DNA repair and to afford protection against cisplatin, oxaliplatin, and carboplatin, which are platinum-based chemotherapeutic agents that are widely used for treating human solid tumors. Therefore, the up-regulation of ERCC1 induced by Ras activation may offer a selective advantage to tumor cells when they are exposed to platinum-based drugs. Although platinum-based chemotherapeutic agents are highly effective at treating many types of cancer, an acquired or intrinsic resistance of the cells to the drug limits their therapeutic efficacy (15). Because an oncogenic Ras mutation occurs in 30% of all human cancers (4), the inhibition of ERCC1 may provide a novel

approach for improving platinum-based chemotherapeutic treatments of such cancers.

REFERENCES

- Katz ME, McCormick F. Signal transduction from multiple Ras effectors. *Curr Opin Genet Dev* 1997;7:75–9.
- Macaluso M, Russo G, Cinti C, Bazan V, Gebbia N, Russo A. Ras family genes: an interesting link between cell cycle and cancer. *J Cell Physiol* 2002;192:125–30.
- White MA, Nicolette C, Minden A, et al. Multiple Ras functions can contribute to mammalian cell transformation. *Cell* 1995;80:533–41.
- Bos JL. Ras oncogenes in human cancer: a review. *Cancer Res* 1989;49:4682–9.
- Sklar MD. Increased resistance to cis-diamminedichloroplatinum(II) in NIH3T3 cells transformed by ras oncogenes. *Cancer Res* 1988;48:793–7.
- Sklar MD. The ras oncogenes increase the intrinsic resistance of NIH3T3 cells to ionizing radiation. *Science* 1988;239:645–7.
- Isonishi S, Hom DK, Thiebaut FB, et al. Expression of the c-Ha-ras oncogene in mouse NIH3T3 cells induces resistance to cisplatin. *Cancer Res* 1991;51:5903–9.
- Hermens AF, Bentvelzen PA. Influence of the H-ras oncogene on radiation responses of a rat rhabdomyosarcoma cell line. *Cancer Res* 1992;52:3073–82.
- Levy E, Baroche C, Barret JM, et al. Activated ras oncogene and specifically acquired resistance to cisplatin in human mammary epithelial cells: induction of DNA cross-links and their repair. *Carcinogenesis* 1994;15:845–50.
- Fan J, Banerjee D, Stambrook PJ, Bertino JR. Modulation of cytotoxicity of chemotherapeutic drugs by activated H-ras. *Biochem Pharmacol* 1997;53:1203–9.
- Bernhard EJ, Stanbridge EJ, Gupta S, et al. Direct evidence for the contribution of activated N-ras and K-ras oncogenes to increased intrinsic radiation resistance in human tumor cell lines. *Cancer Res* 2000;60:6597–600.
- Dempke W, Voigt W, Grothey A, Hill BT, Schmoll HJ. Cisplatin resistance and oncogenes. *Anticancer Drugs* 2000;11:225–36.
- Cho HJ, Jeong HG, Lee JS, et al. Oncogenic H-Ras enhances DNA repair through the Ras/phosphatidylinositol 3-kinase/Rac1 pathway in NIH3T3 cells. Evidence for association with reactive oxygen species. *J Biol Chem* 2002;277:19358–66.
- O'Dwyer PJ, Hamilton TC, Johnson SW. In: V. J. DeVita, Principles and practice of oncology, Cisplatin and its analogue. Philadelphia: J. B. Lippincott, 1997. p. 418–32.
- Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. *Mutat Res* 2001;478:23–43.
- Crul M, Schellens JH, Beijnen JH, Maliepaard M. Cisplatin resistance and DNA repair. *Cancer Treat Rev* 1997;23:341–66.
- Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. *Cancer Treat Rev* 1998;24:331–44.
- Suo Z, Lippard SJ, Johnson KA. Single d(GpG)/cis-diammineplatinum(II) adduct-induced inhibition of DNA polymerization. *Biochemistry* 1999;38:715–26.
- Batty DP, Wood RD. Damage recognition in nucleotide excision repair of DNA. *Gene* 2000;241:193–204.
- Lee KB, Parker RJ, Bohr V, Cornelison T, Reed E. Cisplatin sensitivity/resistance in UV repair-deficient Chinese hamster ovary cells of complementation groups I and III. *Carcinogenesis* 1993;14:2177–80.
- Damia G, Imperatori L, Stefanini M, D'Incalci M. Sensitivity of CHO mutant cell lines with specific defects in nucleotide excision repair to different anti-cancer agents. *Int J Cancer* 1996;66:779–83.
- Dunkern TR, Fritz G, Kaina B. Cisplatin-induced apoptosis in 43–3B and 27–1 cells defective in nucleotide excision repair. *Mutat Res* 2001;486:249–58.
- Mu D, Park CH, Matsunaga T, Hsu DS, Reardon JT, Sancar A. Reconstitution of human DNA repair excision nuclease in a highly defined system. *J Biol Chem* 1995;270:2415–8.
- Aboussekhra A, Biggerstaff M, Shivji MK, et al. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* 1995;80:859–68.
- Lee MR, Kim SH, Cho HJ, et al. Transcription factors NF-YA regulate the induction of human OGG1 following DNA-alkylating agent methylmethane sulfonate (MMS) treatment. *J Biol Chem* 2004;279:9857–66.
- Sancar A. DNA excision repair. *Annu Rev Biochem* 1996;65:43–81.
- Quandt K, Frech K, Karas H, Wingender E, Werner T. MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res* 1995;23:4878–84.
- Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1991;1072:129–57.
- Kinashi Y, Akaboshi M, Masunaga S, Ono K, Watanabe M. Resistance to 195mPt-radiolabeled cis-diamminedichloroplatinum (II) of SHOK cells transfected with various oncogenes. *Radiat Med* 1998;16:233–7.
- Yen L, Nie ZR, You XL, Richard S, Langton-Webster BC, Alaoui-Jamali MA. Regulation of cellular response to cisplatin-induced DNA damage and DNA repair in cells overexpressing p185(erbB-2) is dependent on the ras signaling pathway. *Oncogene* 1997;14:1827–35.
- Li Q, Gardner K, Zhang L, Tsang B, Bostick-Bruton F, Reed E. Cisplatin induction of ERCC-1 mRNA expression in A2780/CP70 human ovarian cancer cells. *J Biol Chem* 1998;273:3419–25.
- Li Q, Tsang B, Bostick-Bruton F, Reed E. Modulation of excision repair cross complementation group 1 (ERCC-1) mRNA expression by pharmacological agents in human ovarian carcinoma cells. *Biochem Pharmacol* 1999;57:347–53.
- Li Q, Zhang L, Tsang B, Gardner K, Bostick-Bruton F, Reed E. Phorbol ester exposure activates an AP-1-mediated increase in ERCC-1 messenger RNA expression in human ovarian tumor cells. *Cell Mol Life Sci* 1999;55:456–66.
- Abate C, Patel L, Rauscher FJ, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* 1990;249:1157–61.
- Campbell SL, Khosravi-Far R, Rossman KL, Clark GJ, Der CJ. Increasing complexity of Ras signaling. *Oncogene* 1998;17:1395–413.
- Xiao L, Lang W. A dominant role for the c-Jun NH2-terminal kinase in oncogenic ras-induced morphologic transformation of human lung carcinoma cells. *Cancer Res* 2000;60:400–8.
- Kashani-Sabet M, Wang W, Scanlon KJ. Cyclosporin A suppresses cisplatin-induced c-fos gene expression in ovarian carcinoma cells. *J Biol Chem* 1990;265:11285–8.
- Gjerset RA, Lebedeva S, Haghghi A, Turla ST, Mercola D. Inhibition of the Jun kinase pathway blocks DNA repair, enhances p53-mediated apoptosis and promotes gene amplification. *Cell Growth Differ* 1999;10:545–54.
- Potapova O, Haghghi A, Bost F, et al. The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. *J Biol Chem* 1997;272:14041–4.
- Zhao R, Rabo YB, Eghyazi S, et al. Apoptosis and c-jun induction by cisplatin in a human melanoma cell line and a drug-resistant daughter cell line. *Anticancer Drugs* 1995;6:657–68.
- Moorehead RA, Singh G. Influence of the proto-oncogene c-fos on cisplatin sensitivity. *Biochem Pharmacol* 2000;59:337–45.
- Potapova O, Basu S, Mercola D, Holbrook NJ. Protective role for c-Jun in the cellular response to DNA damage. *J Biol Chem* 2001;276:28546–53.
- Johnson SW, Laub PB, Beesley JS, Ozols RF, Hamilton TC. Increased platinum-DNA damage tolerance is associated with cisplatin resistance and cross-resistance to various chemotherapeutic agents in unrelated human ovarian cancer cell lines. *Cancer Res* 1997;57:850–6.
- Sheibani N, Jennerwein MM, Eastman A. DNA repair in cells sensitive and resistant to cis-diamminedichloroplatinum(II): host cell reactivation of damaged plasmid DNA. *Biochemistry* 1989;28:3120–4.
- Houtsmuller AB, Rademakers S, Nigg AL, Hoogstraten D, Hoeijmakers JH, Vermeulen W. Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* 1999;284:958–61.
- Melton DW, Ketchen AM, Nunez F, et al. Cells from ERCC1-deficient mice show increased genome instability and a reduced frequency of S-phase-dependent illegitimate chromosome exchange but a normal frequency of homologous recombination. *J Cell Sci* 1998;111:395–404.
- Dabholkar M, Vionnet J, Bostick-Bruton F, Yu JJ, Reed E. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest* 1994;94:703–8.
- Metzger R, Metzger R, Leichman CG, et al. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998;16:309–16.
- Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC. Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. *Cancer Res* 2003;63:1311–6.
- Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin Oncol* 2001;19:4298–304.
- Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res* 2002;8:2286–91.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Oncogenic H-Ras Up-Regulates Expression of ERCC1 to Protect Cells from Platinum-Based Anticancer Agents

Cha-Kyung Youn, Mi-Hwa Kim, Hyun-Ju Cho, et al.

Cancer Res 2004;64:4849-4857.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/64/14/4849>

Cited articles This article cites 49 articles, 22 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/64/14/4849.full#ref-list-1>

Citing articles This article has been cited by 12 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/64/14/4849.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/64/14/4849>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.