Hammerhead Ribozyme against γ-Glutamylcysteine Synthetase Attenuates Resistance to Ionizing Radiation and Cisplatin in Human T98G Glioblastoma Cells

Masaharu Tani,1 Shinji Goto,1 Kensaku Kamada,2 Katsuharu Mori,2 Yoshishige Urata,1 Yoshito Ihara,1 Hiroshi Kijima,1 Yoshito Ueyama,1 Shobu Shibata2 and Takahito Kondo1,4

1Department of Biochemistry and Molecular Biology in Disease, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523. 2Department of Neurosurgery, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501 and 3Department of Pathology, Tokai University School of Medicine, Bouseidai, Isehara 259-1193

Glioblastoma cells are highly malignant and show resistance to ionizing radiation, as well as anti-cancer drugs. This resistance to cancer therapy is often associated with a high concentration of glutathione (GSH). In this study, the effect of continuous down-regulation of γ-glutamylcysteine synthetase (γ-GCS) expression, a rate-limiting enzyme for GSH synthesis, on resistance to ionizing radiation and cisplatin (CDDP) was studied in T98G human glioblastoma cells. We constructed a hammerhead ribozyme against a γ-GCS heavy subunit (γ-GCSh) mRNA and transfected it into T98G cells. (1) The transfection of the ribozyme decreased the concentration of GSH and resulted in G1 cell cycle arrest of T98G cells. (2) The transfection of the ribozyme increased the cytotoxicity of ionizing radiation and CDDP in T98G cells. Thus, hammerhead ribozyme against γ-GCS is suggested to have potential as a cancer gene therapy to reduce the resistance of malignant cells to ionizing radiation and anti-cancer drugs.

Key words: γ-Glutamylcysteine synthetase — Hammerhead ribozyme — Glutathione — Cisplatin — Ionizing radiation

Resistance of malignant cells to radiotherapy and chemotherapy is a major problem in the treatment of cancers. Glioblastoma is one of the most malignant forms of neoplasm, and often shows resistance to radio- and chemotherapy.1) This resistance is due to direct factors, such as a low intrinsic radiation sensitivity, a high recovery capacity, an increased number of clonogens, and a high hypoxic fraction.2) Glutathione (γ-glutamylcysteinylglycine, GSH), participates in many biological processes, especially cellular defense against oxidative stress induced by reactive oxygen species (ROS) and anti-cancer drugs.3–5) GSH is synthesized via two ATP-requiring steps that are catalyzed by γ-glutamylcysteine synthetase (γ-GCS), a rate-limiting enzyme for the synthesis of GSH, and GSH synthetase. γ-GCS is composed of a heavy subunit (γ-GCSh) that is catalytic, and a light subunit (γ-GCSI) that is regulatory.6, 7) We previously found that γ-GCS is over-expressed in T98G human glioblastoma cells concomitant with a high concentration of GSH.8) Since ionizing radiation produces ROS inside the cells, over-expression of γ-GCS was thought to be an important factor in the acquisition of T98G glioblastoma cell resistance to ionizing radiation. The over-expression of γ-GCS has also been reported in many human malignant cells resistant to cisplatin [cis-diamminedicloroplatinum (II)] (CDDP) and doxorubicin.9–13) Recently, we reported that the two subunits of γ-GCS are concomitantly expressed in response to CDDP in human cancer cells.9) We also reported that CDDP is transported in the form of a CDDP-GSH adduct14) and that GSH S-transferase π (GSTπ) detoxifies CDDP by forming an adduct with GSH, and is thus important for the efflux of CDDP.15) These findings suggest that a high concentration of GSH is a factor in malignant cell acquisition of resistance to anti-cancer drugs as well as ionizing radiation.

Concerning the expression of γ-GCS, Manna et al. reported that over-expression of γ-GCS heavy subunit suppresses tumor necrosis factor-induced apoptosis and the DNA-binding activity of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1),16) suggesting an important role for γ-GCS in regulating cell proliferation and death.

In this study, we developed a hammerhead ribozyme against γ-GCSh to constitutively suppress the γ-GCS gene expression in T98G cells, and examined its effect on γ-GCS and on the resistance to ionizing radiation and CDDP. Hammerhead ribozymes are derived from satellite RNA of tobacco ring spot virus, and are newly developed trans-acting RNA enzymes that modulate specific gene expression.17) The hammerhead ribozymes possess a catalytic core that cleaves the target RNA, and flanking regions that direct the ribozyme core to a specific target site.18) A previous report showed that hammerhead ribo-

4To whom correspondence should be addressed.
E-mail: kondo@net.nagasaki-u.ac.jp
zymes against γ-GCS caused down-regulation of the intracellular GSH concentration in mouse islet cells. Quite recently, we reported that hammerhead ribozymes against γ-GCS not only down-regulate the levels of GSH, but also decrease expression of the ATP-binding cassette superfamily transporters, thereby effectively decreasing the efflux of anti-cancer drugs.

Here we show that a decrease in the levels of GSH induced by anti-γ-GCS ribozyme increased the cytotoxicity of ionizing radiation and CDDP in T98G human cancer cells.

**MATERIALS AND METHODS**

**Materials** Human glioblastoma cells (T98G) were purchased from the Health Sciences Research Resources Bank (Tokyo). The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum at 37°C in 5% CO₂ under 100% humidity.

**Exposure to ionizing radiation** For each experiment the cells were transferred to fresh medium with adjustment to a cell density of 1×10⁶ cells/ml. The cells were preincubated in this fresh medium for 2 h at 37°C in T-75 flasks (Corning International, Corning, NY) prior to exposure to ionizing radiation. Cells were irradiated with 200 kV of X-rays (Toshiba EXS-300, Tokyo) at a fixed dose rate of 1.0 Gy per min at room temperature. Mock-irradiated control cells (0-Gy) were treated identically.

**Estimation of GSH** The concentration of intracellular GSH was estimated enzymatically as described by Beutler.

**Plasmid construction and transfection** The anti-γ-GCS ribozyme was cloned into the pHβ plasmid using two synthetic single-stranded oligodeoxynucleotides, 5′-TCGACTGTATTGCTGATGAGTCCGTGAGGACGAA-3′ and 5′-AGCTTTCCGAGTTTCGTCCTCACGGACTCATCAGCAATACAG-3′ with flanking SalI and HindIII restriction sites. Subconfluent T98G cells were transfected with the pHβ plasmid using Lipofectin Reagent (Gibco BRL, Life Technologies, Gaithersburg, MD). Stably transfected cells were selected for integration of the plasmid in growth media containing 500 µg/ml of G418 sulfate for 4 weeks. Selected G418-resistant colonies were grown and screened for expression of the ribozyme by reverse transcription (RT)-PCR assay. The ribozyme-transfected cells, in which the GSH concentration was decreased by approximately 20%, were further selected and used in these experiments. Disabled hammerhead ribozyme was synthesized by PCR with the following primers: 5′-TCGACTGTATTGCTGATGAGTCCGTGAGGACGAA-3′ and 5′-AGCTTTCCGAGTTTCGTCCTCACGGACTCATCAGCAATACAG-3′. The disabled ribozyme contained a single base exchange (G to A) in the catalytic core compared with the original ribozyme. Fig. 1(A) shows a model of the anti-γ-GCS ribozyme. Fig. 1(B) shows the results of PCR to detect the presence of the ribozyme in the cells. The ribozyme insert sequence was analyzed using the dsDNA Cycle Sequencing System. The sequence primer used was 5′-GTCTGGATCCCTCGAAGC-3′. Subconfluent T98G cells were transfected with the pHβ plasmid using Lipofectin Reagent. Stably transfected cells were selected for integration of the plasmid in growth media containing 500 µg/ml of G418 sulfate for 4 weeks. (A) A model of anti-γ-GCS hammerhead ribozyme. (B) Results of RT-PCR to detect the presence of the ribozyme in the cells. PCR primers to detect ribozyme expression were pHβ-PCR-1,5′-AGCACAGAGCCTCGCCTTT-3′ and pHβ-PCR-2,5′-GTCTGGATCCCTCGAAGC-3′. Lane 1, parent cells; lane 2, vector only; lane 3, ribozyme transfecant, #1; lane 4, ribozyme transfecant, #2.

**Fig. 1.** Construction and transfection of anti-γ-GCS ribozyme. Anti-γ-GCS hammerhead ribozyme was constructed. The anti-γ-GCS ribozyme was cloned into the pHβ plasmid using two synthetic single-stranded oligodeoxynucleotides with flanking SalI and HindIII restriction sites. These primers were used for screening cell lines to detect the presence of pHβ/anti-γ-GCS ribozyme. The ribozyme insert sequence was analyzed using a dsDNA Cycle Sequencing System. The sequence primer used was 5′-GTCTGGATCCCTCGAAGC-3′. Subconfluent T98G cells were transfected with the pHβ plasmid using Lipofectin Reagent. Stably transfected cells were selected for integration of the plasmid in growth media containing 500 µg/ml of G418 sulfate for 4 weeks. (A) A model of anti-γ-GCS hammerhead ribozyme. (B) Results of RT-PCR to detect the presence of the ribozyme in the cells. PCR primers to detect ribozyme expression were pHβ-PCR-1,5′-AGCACAGAGCCTCGCCTTT-3′ and pHβ-PCR-2,5′-GTCTGGATCCCTCGAAGC-3′. Lane 1, parent cells; lane 2, vector only; lane 3, ribozyme transfecant, #1; lane 4, ribozyme transfecant, #2.
Sequencing System (Gibco BRL). The sequence primer used was 5′-GTCTGGATCCCTCGAAGC-3′. PCR primers to detect ribozyme expression were pH β-PCR-1, 5′-AGCACAGAGCCTGCCTTT-3′ and pH β-PCR-2, 5′-GTCTGGATCCCTCGAAGC-3′. The following experiments were performed using the ribozyme transfectant #1.

**RT-PCR assay** RT-PCR was performed according to a commercial protocol (Perkin-Elmer Cetus, Norwalk, CT), and was used to detect the ribozyme and γ-GCSH gene expression using Superscript II (Gibco BRL) and Taq DNA polymerase (Perkin-Elmer Cetus) as described previously. Briefly, the specific PCR products were size-fractionated by horizontal agarose gel electrophoresis, and stained with ethidium bromide. The authentic sense primer for γ-GCSH was 5′-CCAGTTCCTGCACATCTACCAG-3′, and the antisense primer was 5′-GTCTGGATCCCTCGAAGC-3′.

**Cytophagic assay** The sensitivity of the cells to ionizing radiation and CDDP was assayed by tetrazolium salt assay (Cytotoxicity assay) and was used to detect the ribozyme and commercial protocol (Perkin-Elmer Cetus, Norwalk, CT), RT-PCR assay experiments were performed using the ribozyme transfectant #1.

**RESULTS**

**Suppression of GSH levels by the ribozyme** Fig. 2 shows the characteristics of T98G cells transfected with hammerhead ribozyme against γ-GCSH. The anti-γ-GCSH ribozyme down-regulated the expression of γ-GCSH mRNA in T98G-transfected cells (Fig. 2A). The transfection resulted in a significant decrease in the level of GSH of approximately 26% in cloned cells (P<0.05 vs. the cells transfected with disabled ribozyme). The concentration of GSH was 81.2±5.1 nmol/10⁶ cells in T98G cells, 39.9±1.5 nmol/10⁶ cells in the cells transfected with disabled ribozyme, and 21.5±3.1 nmol/10⁶ cells in the cells transfected with anti-γ-GCSH ribozyme (Fig. 2B).

**Proliferation rate and sensitivity to ionizing radiation** The effect of transfection with anti-γ-GCSH ribozyme on the proliferation rate of T98G cells and on the sensitivity to ionizing radiation was studied. The cells were treated with ionizing radiation and the cytotoxicity was estimated by MTT assay. Fig. 3 shows the findings of MTT assay. The proliferation of T98G cells was expressed in terms of absorbance at 570 nm. (A) During culture, cells proliferated approximately 20-fold by the 7th day in the cases of control cells and the cells transfected with disabled ribozyme. The cells transfected with anti-γ-GCSH ribozyme showed suppressed proliferation (only 10-fold on the 7th day). Two grays of ionizing radiation had no apparent
Anti-γ-GCS Ribozyme for Cancer Therapy

effect on cell survival. On the 7th day, 10-Gy radiation decreased the survival of these cells. (B) The data in (A) were analyzed using a two-factor factorial ANOVA test. On the 7th day, the survival rate of anti-γ-GCS ribozyme-transfected cells exposed to 10-Gy radiation was significantly less than that of disabled ribozyme-transfected cells \( (P<0.01) \).

By cell cycle analysis, as shown in Fig. 4, it was found that transfection with anti-γ-GCS ribozyme increased the percentage of G0/G1 phase (75.7%) and decreased the percentage of cells in S phase (15.1%) compared with disabled ribozyme (59.5% of G0/G1 phase, and 29.2% of S phase), suggesting that transfection with anti-γ-GCS ribozyme causes G1 cell cycle arrest.

Sensitivity to CDDP The effect of different concentrations of CDDP on cell survival was studied. The cells were treated with various concentrations of CDDP for 72 h and the cytotoxicity was estimated by MTT assay (Fig.

Fig. 3. The sensitivity of T98G cells to ionizing radiation. The sensitivity of T98G cells to ionizing radiation was examined by MTT assay. Cells (1500) were placed in 100 µl medium/well in 96-well plates. Up to 7 days after treatment with 2 or 10 Gy of radiation, the cells were incubated for 4 h at 37°C with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (652 µg/ml) and lysed as described in “Materials and Methods.” (A) The absorbance at 570 nm was measured for control (upper panel), + disabled ribozyme (middle panel) and + anti-γ-GCS ribozyme (lower panel). Open circle, control/0 Gy; open triangle, control/2 Gy; open square, control/10 Gy; closed circle, disabled ribozyme/0 Gy; closed triangle, disabled ribozyme/2 Gy; closed square, disabled ribozyme/10 Gy; slashed circle, ribozyme/0 Gy; slashed triangle, ribozyme/2 Gy; slashed square, ribozyme/10 Gy. (B) Cell survival (%) was shown using the same data, and comparing the cell numbers on day 0 and on the 7th day. T, T98G cells; D, disabled ribozyme; R, ribozyme. The data are ±SD of three independent analyses. \( * P<0.01 \) vs. each cell at 0 Gy; \( ** P<0.01 \) vs. + disabled ribozyme.
The number of survivors (%) among the control cells as well as the cells transfected with disabled ribozyme decreased gradually, depending on the concentration of CDDP. As in the case of the data for ionizing radiation, the cell number increased by 7-fold at 3 days of pre-incubation, but this decreased to only 5-fold after treatment with 25 µM CDDP for 3 days. In contrast, the cell number of T98G cells transfected with anti-γ-GCS ribozyme showed an increase of only 2.5-fold at 3 days of pre-incubation, and this was decreased to 25% by the treatment with 25 µM CDDP for 3 days.

**DISCUSSION**

In this study, we clarified the effects of continuous suppression of GSH synthesis on the resistance of T98G human glioblastoma cells to ionizing radiation and CDDP. We transfected anti-γ-GCS ribozyme into T98G cells to effectively and consistently decrease the levels of GSH (Fig. 2). The decrease in GSH levels was associated with a decrease in the cell survival after exposure to ionizing radiation (Fig. 3) and CDDP (Fig. 5) in T98G cells. CDDP metabolism consists of various processes, such as binding of CDDP to cellular DNA to cause damage, detoxification of CDDP by coupling with GSH to form adducts and ATP-dependent efflux of CDDP-GSH adducts through the multidrug resistance related protein II.15, 24) According to reports on the over-expression of γ-GCS in CDDP-resis-

---

**Fig. 4.** Flow cytometric analysis of cell cycle. The effect of anti-γ-GCS ribozyme on the cell cycle was analyzed using a FACScan flow cytometer. Cells in the steady state were harvested and employed for the cell cycle analysis. The proportion of cells in each stage was calculated using Modifit-LT software. Upper plot, T98G; middle plot, T98G cells transfected with disabled ribozyme; lower plot, T98G cells transfected with anti-γ-GCS ribozyme.

**Fig. 5.** Cytotoxicity of CDDP. The effect of anti-γ-GCS ribozyme on the cytotoxicity of CDDP was examined by MTT assay. Seventy-two hours after treatment with various concentrations of CDDP, the cells were incubated for 4 h at 37°C with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (652 µg/ml). The cells were lysed with 100 µl of 20% SDS/50% N,N-dimethylformamide (pH 4.7) in each well. After overnight incubation at 37°C, the absorbance at 570 nm was measured. The data were expressed as the number of survivors (%), with those at day 0 as 100%. Wells without cells served as blanks. Data are the mean±SD of three independent analyses.
tant cancer cells, an increase in GSH synthesis is closely related to resistance of cancer cells to CDDP. Our findings show that GSH plays an important role in CDDP resistance and ribosome-mediated specific modulation of γ-GCS expression causes an increase in the cytotoxicity of CDDP to cancer cells.

In the present study, transfection of cells with anti-γ-GCS ribozyme resulted in G1 cell cycle arrest and suppression of cell growth (Fig. 4). Depletion of GSH using buthionine sulfoximine (BSO), a specific inhibitor of GSH synthesis, is known to inhibit cell growth by arresting the cell cycle at G1. The data in this study are in good agreement with these reports.

Previously, we reported that depletion of GSH by exposure to BSO enhanced the ionizing radiation-dependent induction of γ-GCS mediated by NF-κB. It was thought that the treatment with BSO caused an increase in the NF-κB-DNA binding activity suppressed by GSH. A role for GSH in the redox regulation of transcriptional factors and intracellular signal transduction pathways has been suggested. In such cases, cysteine residues may be modified by GSH at the phosphorylation site of transcriptional factors or protein kinases. We reported the importance of GSH in regulating the phosphorylation of IκB and the NF-κB-mediated expression of NF-κB subunits by tumor necrosis factor-α and in Fas-mediated apoptosis. Similarly, it has been reported that an increase in the intracellular GSH concentration induced by the transfection of γ-GCS caused down-regulation of cytokine-induced activation of NF-κB and AP-1. Klatt et al. reported that the DNA binding activity of c-Jun is down-regulated by GSH through its reversible S-glutathionylation and protein disulfide formation. Cell growth arrest by the transfection of anti-γ-GCS ribozyme observed in the present study suggests that a decrease in GSH level negatively attenuates intracellular factors for cell growth. However, the mechanism of regulation of cell growth by GSH remains to be clarified.

In the present study, sensitivity of ionizing radiation was attenuated by the transfection of T98G cells with anti-γ-GCS ribozyme (Fig. 3). This suggests a role of GSH in the protection of cells against damage by ionizing radiation. According to other reports, radiosensitivity is determined by GSH and other anti-oxidants such as superoxide dismutase and GSH S-transferase. The contributions of other anti-oxidants to the sensitivity to ionizing radiation should be further studied.

The findings suggest that the acquisition of resistance to ionizing radiation and CDDP by cancer cells, which leads to difficulty in anti-cancer chemotherapy, can be attenuated to some extent using the anti-γ-GCS hammerhead ribozyme.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

(Received September 21, 2001/Revised March 15, 2002/ Accepted March 22, 2002)

REFERENCES

1) Nagane, M., Shibui, S., Oyama, H., Asai, A., Kuchino, Y. and Nomura, K. Investigation of chemoresistance-related genes mRNA expression for selecting anticancer agents in successful adjuvant chemotherapy for a case of recurrent glioblastoma. Surg. Neurol., 44, 462–470 (1995).
2) Taghian, A., Gioioso, D., Budach, W. and Shuit, H. In vitro split-dose recovery of glioblastoma multiforme. Radiat. Res., 134, 16–21 (1993).
3) Meister, A. Methods for the selective modification of glutathione metabolism and study of glutathione transport. Methods Enzymol., 113, 571–585 (1985).
4) Kondo, T., Yoshida, K., Urata, Y., Goto, S., Gasu, S. and Taniguchi, N. Gamma-Glutamylcysteine synthetase and active transport of glutathione S-conjugate are responsive to heat shock in K562 erythroid cells. J. Biol. Chem., 268, 20366–20372 (1993).
5) Urata, Y., Yamamoto, H., Goto, S., Teushima, H., Akazawa, S., Yamashita, S., Nagataki, S. and Kondo, T. Long exposure to high glucose concentration impairs the responsive expression of gamma-glutamylcysteine synthetase by interleukin-1beta and tumor necrosis factor-alpha in mouse endothelial cells. J. Biol. Chem., 271, 15146–15152 (1996).
6) Beutler, E., Gelbart, T., Kondo, T. and Matsunaga, A. T. The molecular basis of a case of gamma-glutamylcysteine synthetase deficiency. Blood, 94, 2890–2894 (1999).
7) Yan, N. and Meister, A. Amino acid sequence of rat kidney γ-glutamylcysteine synthetase. J. Biol. Chem., 265, 1588–1593 (1990).
8) Iwanaga, M., Mori, K., Iida, T., Urata, Y., Matsuo, T., Yasunaga, A., Shibata, S. and Kondo, T. Nuclear factor kappa B dependent induction of gamma glutamylcysteine synthetase by ionizing radiation in T98G human glioblastoma cells. Free Radic. Biol. Med., 24, 1256–1268 (1998).
9) Iida, T., Mori, E., Mori, K., Goto, S., Urata, Y., Oka, M., Kohno, S. and Kondo, T. Co-expression of gamma-glutamylcysteine synthetase sub-units in response to cisplatin and doxorubicin in human cancer cells. Int. J. Cancer., 82, 405–411 (1999).
10) Godwin, A. K., Meister, A., Odwyer, P. J., Huang, C. S., Hamilton, T. C. and Anderson, M. E. High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc. Natl. Acad. Sci. USA, 89, 3070–3074 (1992).
11) Yao, K. S., Godwin, A. K., Johnson, S. W., Ozols, R. F., O’Dwyer, P. J., Hamilton, T. C. and Kao, M. T. Evidence for altered regulation of gamma-glutamylcysteine synthetase gene expression among cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines. *Cancer Res.*, 55, 4367–4374 (1995).

12) Kao, M. T., Yao, K., Kijima, H., Tsuchida, T., Kondo, H., Iida, T., Oshika, Y., Savaraj, N., Masuzawa, T. and Ishikawa, T. Frequent coexpression of MRP/GS-X pump and gamma-glutamylcysteine synthetase mRNA in drug-resistant cells, untreated tumor cells, and normal mouse tissues. *Biochem. Pharmacol.*, 55, 605–615 (1998).

13) Ogretmen, B., Bachoudi, H. R., McCauley, M. D., Boylan, A., Green, M. R. and Saffa, A. R. Co-ordinated overexpression of the MRP and gamma-glutamylcysteine synthetase genes, but not MDR1, correlates with doxorubicin resistance in human malignant mesothelioma cell lines. *Int. J. Cancer*, 75, 757–761 (1998).

14) Goto, S., Yoshida, K., Morikawa, T., Urata, Y., Suzuki, K. and Kondo, T. Augmentation of transport for cisplatin-glutathione adduct in cisplatin-resistant cancer cells. *Cancer Res.*, 55, 4297–4301 (1995).

15) Goto, S., Iida, T., Cho, S., Oka, M., Kohno, S. and Kondo, T. Over-expression of glutathione S-transferase π enhances the adduct formation of cisplatin with glutathione in human cancer cells. *Free Radic. Res.*, 31, 549–558 (1999).

16) Manna, S. K., Kuo, M. T. and Aggarwal, B. B. Overexpression of gamma-glutamylcysteine synthetase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappa B and activator protein-1. *Oncogene*, 18, 4371–4382 (1999).

17) Scanlon, K. J., Ishida, H. and Kashani-Sabet, M. Ribozyme-mediated reversal of the multidrug-resistant phenotype. *Proc. Natl. Acad. Sci. USA*, 91, 11123–11127 (1994).

18) Haseloff, J. and Gerlach, W. L. Simple RNA enzymes with new and highly specific endoribonuclease activities. *Nature*, 334, 585–591 (1988).

19) Kijima, H., Tsuchida, T., Kondo, H., Iida, T., Oshika, Y., Nakamura, M., Scanlon, K. J., Kondo, T. and Tamaoki, N. Hammerhead ribozymes against gamma-glutamylcysteine synthetase mRNA downregulate intracellular glutathione concentration of mouse islet cells. *Biochem. Biophys. Res. Commun.*, 247, 697–703 (1998).

20) Iida, T., Kijima, H., Urata, Y., Goto, S., Ihara, Y., Oka, M., Kohno, S., Scanlon, K. J. and Kondo, T. Hammerhead ribozyme against γ-glutamylcysteine synthetase sensitizes human colon cancer cells to cisplatin by down-regulating both the glutathione synthesis and the expression of multidrug resistance proteins. *Cancer Gene Ther.*, 8, 803–814 (2001).

21) Beutler, E. A manual of biochemical methods. In “Red Cell Metabolism,” 3rd Ed., ed. E. Beutler, pp. 77–78 (1984). Grune and Stratton, Orlando, FL.

22) Nagata, J., Kijima, H., Hatanaka, H., Asai, S., Miyachi, H., Takagi, A., Miwa, T., Mine, T., Yamazaki, H., Nakamura, M., Kondo, T., Scanlon, K. J. and Ueyama, Y. Reversal of cisplatin and multidrug resistance by ribozyme-mediated glutathione suppression. *Biochem. Biophys. Res. Commun.*, 286, 406–413 (2001).

23) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65, 55–63 (1983).

24) Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kogotani, K., Okumura, K., Akiyama, S. and Kuwano, M. A human canalicul multi-specific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. *Cancer Res.*, 56, 4124–4129 (1996).

25) Tipnis, S. R., Blake, D. G., Shepherd, A. G. and MucLellan, L. I. Over-expression of the regulatory subunit of γ-glutamylcysteine synthetase in HeLa cells increases γ-glutamylcysteine synthetase activity and confers drug resistance. *Biochem. J.*, 337, 559–566 (1999).

26) Sawyer, T. E. and Bonner, J. A. The interaction of buthionine sulfoximide (BSO) and the topoisomerase I inhibitor CPT-11. *Br. J. Cancer Suppl.*, 27, S109–S113 (1996).

27) Dethlefsen, L. A., Biaglow, J. E., Peck, V. M. and Ridinger, D. N. Toxic effects of extended glutathione depletion by buthionine sulfoximine on murine mammary carcinoma cells. *Int. J. Radiat. Oncol. Biol. Phys.*, 12, 1157–1160 (1986).

28) Aruoma, O. I. and Halliwell, B., eds. “Molecular Biology of Free Radicals in Human Diseases” (1998). Oica International, London.

29) Cho, S., Urata, Y., Iida, T., Goto, S., Yamaguchi, M., Sumikawa, K. and Kondo, T. Glutathione downregulates the phosphorylation of I kappa B: autoolop regulation of the NF-kappa B-mediated expression of NF kappa B subunits by TNF-alpha in mouse vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, 253, 104–108 (1998).

30) Kohno, T., Yamada, Y., Hata, T., Morii, H., Yamamura, N., Tomonaga, M., Urata, Y., Goto, S. and Kondo, T. Relation of oxidative stress and glutathione synthesis to CD95 (Fas/APO-1)-mediated apoptosis of adult T cell leukemia cells. *J. Immunol.*, 156, 4722–4728 (1996).

31) Klatt, P., Molina, E. P., De Lacoba, M. G., Padilla, C. A., Martinez-Galesteo, E., Barcena, J. A. and Lamas, S. Redox regulation of c-Jun DNA binding by reversible S-glutathionylation. *FASEB J.*, 13, 1481–1490 (1999).

32) Poppenborg, H., Munstermann, G., Knupfer, M. M., Hotfilder, M., Hacker-Klom, U. and Wolff, J. E. Cisplatin induces radioprotection in human T98G glioma cells. *Anti-cancer Res.*, 17, 1131–1134 (1997).

33) Yoshii, Y., Saito, A., Hyodo, A., Tsurushima, H. and Sun, L. Expression of enzymes and oncogene induced after radiotherapy and/or chemotherapy in patients with brain tumors. *Hum. Cell*, 14, 95–103 (2001).