Inhibition of Glucosylceramide Synthase Sensitizes Head and Neck Cancer to Cisplatin

Jong-Lyel Roh, Eun Hye Kim, Jin Young Park, and Ji Won Kim

Abstract

Glucosylceramide synthase (GCS) overexpression is associated with multidrug resistance in several human cancers. GCS blockade, which overcomes multidrug resistance by downregulating P-glycoprotein (P-gp), has not been tested in head and neck cancer (HNC). This study investigates whether GCS is targetable in HNC by assessing whether GCS inhibition sensitizes HNC to cisplatin. The effect of genetic or pharmacologic GCS inhibition (using GCS siRNA/shRNA or D,L-threo-PPPMP, respectively) on cisplatin sensitivity was assessed in several human HNC cells and acquired cisplatin-resistant HNC cells by measuring cell viability, cell cycle, death, mRNA and protein expression, ceramide production, and in preclinical tumor xenograft mouse models. GCS and P-gp expression were significantly associated with cisplatin resistance in several HNC cell lines ($P = 0.007$). Both were significantly increased in HN9-cisR cells, which display acquired cisplatin resistance ($P < 0.001$). Genetic or pharmacologic inhibition of GCS induced accumulation of increased ceramide levels. GCS inhibition increased cisplatin-induced cell death in HNC cells via P-gp downregulation and proapoptotic protein activation, which were abrogated by siPUMA transfection. Genetic and pharmacologic GCS inhibition sensitized resistant HNC cells to cisplatin in vitro and in vivo. GCS and P-gp overexpression is associated with acquired cisplatin resistance, suggesting a role for these molecules as therapeutic targets for HNC. Genetic or pharmacologic GCS blockade may have therapeutic benefit in cisplatin-resistant HNC.

Introduction

Cisplatin or cis-diamminedichloroplatinum (II) (CDDP) is a platinum-based compound widely used in various types of human solid neoplasms, including bladder, testicular, ovarian, colorectal, lung, and head and neck cancer (HNC; ref. 1). Cisplatin remains a first-line chemotherapeutic agent and has been continuously used as a primary treatment modality in HNC in combination with radiotherapy in organ preservation strategies (2, 3). It interferes with DNA repair mechanisms by crosslinking with the DNA purine bases, causing DNA damage and inducing apoptosis in cancer cells (4). Unfortunately, the numerous side effects of cisplatin, which affect the kidneys, the gastrointestinal tract, the immune system, peripheral nerves, and the inner ear, have led to the greater use of other platinum-containing anticancer agents such as carboplatin, oxaliplatin, and others (4). In addition, the main limitation to the clinical usefulness of cisplatin is the high incidence of chemoresistance, a major cause of treatment failure in cancer management (1). Combining cisplatin with other drugs may reduce toxicity and circumvent chemoresistance.

Several mechanisms of resistance to chemotherapy have been reported. P-glycoprotein (P-gp), a plasma membrane glycoprotein encoded by the MDRI gene, acts as a drug efflux pump, thereby resulting in the reduced intracellular accumulation and decreased cytotoxicity of anticancer drugs, including cisplatin (5). Furthermore, P-gp regulates cancer proliferation, invasion, and caspase-dependent cell death (6, 7). P-gp overexpression in tumors is associated with poor prognosis in several types of cancers (8–10). The upregulation of rafts and caveolae, including that of the glucosphingolipid-enriched constituents of microdomains, represents another multidrug resistance (MDR) mechanism (11). Ceramide, as a second messenger of apoptosis, participates in cell death signaling, autophagy, and cell-cycle arrest, and defects in ceramide generation and metabolism in cancer cells contribute to resistance to chemotherapy (12, 13).

Glucosylceramide synthase (GCS), a transmembrane protein encoded by the UGCG gene in humans, is the rate-limiting enzyme that controls the first glycosylation step in the biosynthesis of glucosphingolipids; it catalyzes ceramide glycosylation, converting ceramide to glucosylceramide (14). GCS overexpression confers resistance to apoptosis (15, 16), suggesting a potential role as a biomarker predicting tumor response to chemotherapy (17). Growing evidence shows that a persistent increase in ceramide glycosylation facilitates cancer progression and drug resistance (15, 16). GCS blockade by gene silencing or pharmacologic inhibition leads chemoresistant cancer cells to apoptosis via downregulation of P-gp and restoration of p53-dependent apoptosis (18, 19). GCS blockade overcomes MDR in several cancers but has not been tested in HNC. This study investigates whether GCS is targetable in HNC by assessing whether genetic or pharmacologic GCS inhibition sensitizes chemoresistant HNC cells to cisplatin in vitro and in vivo.
Materials and Methods

Cell culture and establishment of cisplatin-resistant HNC cells

Our experiments used 5 human HNC cells, AMC-HN2, -HN3, -HN4, -HN5, and -HN9 cell lines, previously established from the primary tumors of head and neck in our institution (20). All cancer cell lines were authenticated by DNA (short-tandem repeat, STR) profiling provided by the Korean Cell Line Bank. HNC cells were cultured in Eagle’s minimum essential medium (Life Technologies) supplemented with 10% FBS.

Bank. HNC cells were cultured in Eagle’s minimum essential medium containing 5% CO2. Cisplatin-resistant AMC-HN9 (HN9-cisR) cells were derived from the parental cisplatin-sensitive AMC-HN9 cells by exposure to increasing concentrations of cisplatin [cis-platinum (II) diamine dichloride (CDDP); Sigma-Aldrich; ref. 21]. The cisplatin resistance was evaluated by cell viability assays performed on both the resistant and the parental cells.

Cell viability assay

Cell viability was assessed by trypan blue exclusion and MTT assays. Trypan blue exclusion was performed in HNC cells seeded at 1 × 10^3 in 6-well plates, allowed to reach 60% to 70% confluence, and exposed to d,l-threo-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP; Sigma-Aldrich) for 72 hours. The cells were then trypsinized, stained with 0.4% trypan blue (Life Technologies), and counted using a hemocytometer. MTT assays were performed with HNC cells seeded at 3 × 10^3 to 5 × 10^3 cells per well in 96-well plates, incubated overnight, and exposed to d,l-threo-PPMP and cis-platinum (II) diamine dichloride (cisplatin; Sigma-Aldrich), alone or in combination, for 72 hours. The cells were then exposed to the tetrazolium compound MTT (Sigma-Aldrich) for 4 hours, after which solubilization buffer was added for 2 hours. The absorbance in each well was measured at 570 nm using a SpectraMax M2 microplate reader (Molecular Devices).

Cisplatin cytotoxicity was assessed by MTT assay after 72 hours, and the half maximal inhibitory concentration (IC_{50}) of this drug for each HNC cell line was calculated. The interaction of 2 drugs was considered synergistic when growth suppression was greater than the sum of the suppression induced by either drug alone combination index (CI) was used to evaluate drug interaction: CI = 1, additive interaction; CI < 1, synergistic interaction; CI > 1, antagonistic interaction (22).

Cell-cycle and cell death assays

For cell-cycle assays, the cells were exposed to d,l-threo-PPMP for 72 hours and then trypsinized, fixed overnight in ice-cold ethanol, and stained with 30 minutes with propidium iodide (Sigma-Aldrich) at 37°C. The cellular DNA content was meaured using a FACScalibur flow cytometer (BD Bioscience). For cell death assays, cells were assessed by flow cytometry after staining with Annexin V-FITC and propidium iodide, using an Annexin V-FITC apoptosis detection kit (BD Biosciences). For caspase activity assays, cells were assessed in triplicate wells using the fluorescent Homogeneous Caspase Assay (Roche) after treatment with cisplatin and d,l-threo-PPMP, alone or in combination, for 72 hours. All data were analyzed using the CellQuest software (BD Biosciences). All assays were performed with triplicate samples in 3 separate experiments. For the measurement of mitochondrial membrane potential (ΔΨm), HN9-cisR cells were seeded in a 96-well plate and then exposed to 100-μL medium containing cisplatin and d,l-threo-PPMP, alone or in combination, for 36 hours. The cells were then stained with 200 nmol/L tetramethylrhodamine ethyl ester (TMRE, Life Technologies) for 20 minutes and then analyzed by flow cytometry. The median fluorescent intensity (MFI) of each treatment group was normalized to the control group.

Figure 1. GCS and P-gp expression is associated with cisplatin resistance. A, cell viability was assessed by trypan blue exclusion after exposure to cisplatin for 72 hours. B, GCS mRNA was measured by quantitative real-time PCR, and cisplatin cytotoxicity was assessed by MTT at 72 hours. The correlation between GCS mRNA copy numbers and IC_{50} was examined by Pearson correlation coefficient (r²). P = 0.017. C, Western blot analysis showing different levels of GCS, P-gp, and p53 proteins in untreated AMC-HNC cells and cisplatin-resistant HN9 (HN9-cisR) cells. β-Actin was assessed as a loading control. D, change in gene expression of GCS and MDR1 between HN9 and HN9-cisR. The error bars represent SD from 3 independent experiments, each performed with triplicate samples. * P < 0.001 relative to HN9 cells.
Ceramide production measurements

Total ceramide levels were measured in HNC cells and tumors of the transplanted mice. After treatment, cells were homogenized, and total protein concentrations were measured, and lipids were then extracted from samples. Sphingolipids were separated on an Agilent 1200 high-performance liquid chromatography system (Agilent Technologies) and analyzed by electrospray ionization-tandem mass spectrometry on a 400 QTRAP (AB Sciex). The peak areas for different sphingolipid subspecies were quantified by internal standards and then normalized to protein concentrations (23). Total ceramide levels in the cells exposed for 24 hours to 10 μmol/L PPMP, 10 μmol/L cisplatin, or their combination were calculated as the percentage of those in untreated HNC controls and compared between groups. Total ceramide levels were also measured in the tumors of each group in mice after implantation of tumor cells.

Transfection and infection

For knockdown experiments of GCS and PUMA, AMC-HN2 with mutant p53 and HN9-cisR were seeded onto 60-mm plates in medium without antibiotics and 18 hours later were transfected with 100 nmol/L siRNA targeting human UGCS or PUMA or a scrambled control siRNA (Life Technologies). After 72 hours, the cells were exposed to D,L-threo-PPMP for an additional period of 72 hours and then analyzed for protein expression. For knockdown of GCS gene, AMC-HN9-cisR cells were stably transfected with shRNA directed against UGCS or control shRNA in a lentiviral vector (Life Technologies). At 72 hours after transfection, cells at 60% to 70% confluence were infected with virus-containing media supplemented with 4 μg/mL polybrene (EMD Millipore) and incubated overnight. Selection was performed using 2 μg/mL puromycin (Sigma-Aldrich). Protein expression and knockdown was confirmed by Western blotting using anti-GCS and anti-PUMA antibodies.

Quantitative real-time PCR

Total cellular RNA was extracted using the QIAzol lysis reagent and the RNAeasy Mini Kit (Qiagen). cDNA was generated from the purified RNA using the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer’s instructions. The cDNAs corresponding to GCS, MDR1, and ACTB were amplified by PCR using the primers (Supplementary Data). Quantitative real-time PCR was performed using SYBR Green Mix (Qiagen) on a 7900HT Fast Real-time PCR System (Applied Bioscience). Relative target mRNA levels were normalized to β-actin transcript.
levels. Quantification was performed using GCS DNA standard curves generated by serial dilution of pcDNA 3.1-GCS plasmid (GenScript; ref. 24).

**Immunoblotting**

Cells were lysed and immunoblotting was then performed according to standard procedures. Briefly, 50 μg protein was resolved by SDS-PAGE on 10% to 12% gels, transferred to nitrocellulose or polyvinylidene difluoride membranes, and probed with primary and secondary antibodies. The following primary antibodies were used: GCS (H-300) and p53 (DO1; Santa Cruz Biotechnology), P-gp (EMD Millipore) and p21WAF1/CIP1, primary antibodies were used: GCS (H-300) and p53 (DO1; Santa Cruz Biotechnology), PUMA, cleaved PARP, phospho-p53-Ser15, cleaved caspase-3, BAX, and Bcl-2 (Cell Signaling Technology). β-Actin (Sigma-Aldrich) was used as the loading control. All antibodies were diluted at between 1:250 and 1:5,000.

**Preclinical studies**

All mouse study procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of our institution. Six-week-old athymic male BALB/c nude mice (nu/nu) were purchased from Central Lab Animal Inc. AMC-HN9-cisR cells (5 × 10⁶) were injected subcutaneously into the flank of nude mice. Mice were randomized into each treatment group. In the other in vivo experiments, AMC-HN9-cisR cells were injected subcutaneously into the flank of nude mice. Mice were randomized into four treatment groups: vehicle, cisplatin, D,L-threo-PPMP, and cisplatin plus D,L-threo-PPMP. Mice were treated by intraperitoneal (i.p.) injection of 5 mg/kg cisplatin once per week, by i.p. injection of 5 mg/kg D,L-threo-PPMP once per day, or with a combination of cisplatin and D,L-threo-PPMP according to the same schedules. Tumor volume and body weights were measured every 3 days. Tumors were measured using a caliper, and volume was calculated as (length × width²)/2. The mice were sacrificed, and tumors were harvested and analyzed by immunoblotting, ceramide production measurements, and in situ terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay (R&D Systems). The number of apoptotic bodies was counted blindly in 10 randomly selected high-power fields. The statistical significance of differences observed between treatment groups was assessed by using the 2-tailed Mann–Whitney U test or the Student t test.

**Results**

GCS and P-gp expression is associated with cisplatin resistance in HNC cells

The cytotoxic effect of cisplatin was tested in cultured human HNC cells (Fig. 1A). The half maximal inhibitory concentration (IC₅₀) of cisplatin in different HNC cell lines was positively correlated with the GCS mRNA copy number [correlation coefficient (r²) = 0.796, P = 0.017; Fig. 1B]. Cisplatin-resistant HN9 (HN9-cisR) cells showed an 18-fold increase in IC₅₀ compared with parental HN9 cells and a 4-fold increase in GCS mRNA copy number compared with HN9 cells. The expression of GCS and P-gp proteins varied between HNC cell lines, but the 2 values remained inversely correlated (Fig. 1C). In addition, GCS and MDR1 gene expression significantly increased in HN9-cisR cells compared with parental HN9 cells (P < 0.001; Fig. 1D).
Genetic or pharmacologic inhibition of GCS increases cisplatin-induced cell death in HNC cells

When AMC-HN2 cells, which express mutant p53, were transfected with scrambled or GCS siRNA, the protein and mRNA expression of both GCS and P-gp decreased significantly (Fig. 2A and B). PPMP also inhibited GCS mRNA and protein expression and subsequently increased the expression of the proapoptotic protein BAX. GCS suppression appeared to be associated with decreased expression of P-gp. Furthermore, GCS inhibition upon shRNA transfection in HN9-cisR cells was confirmed by Western blotting (Fig. 2C). Both the genetic and pharmacological (L-threo-PPMP) blockade of GCS induced P-gp inhibition and increased cisplatin cytotoxicity in HN9-cisR cells. GCS inhibition increased ceramide production in both HN9 and HN9-cisR cells. Ceramide production also increased by both GCS inhibition and cisplatin treatment (Fig. 2D).

GCS inhibition sensitizes cisplatin-resistant HNC cells to cisplatin in vitro and in vivo

Cisplatin (10 μmol/L) did not induce significant cytotoxicity or apoptotic protein expression in cisplatin-resistant HNC (HN9-cisR) cells compared with parental cisplatin-sensitive HN9 cells (Fig. 4A). D,L-threo-PPMP induced the expression of apoptotic proteins and increased cisplatin-induced cytotoxicity and apoptotic protein expression in HN9-cisR cells. Furthermore, genetic blockade of GCS also increased cisplatin cytotoxicity and the

GCS Inhibition in HNC
induction of apoptotic proteins; that effect was weakened by PUMA gene inhibition (Fig. 4B). In combination, cisplatin and GCS inhibition (genetic or pharmacological) increased the cytotoxicity of cisplatin in HN9-cisR cells by increasing caspase activity to an extent greater than the sum of the effects of either agent alone (Fig. 4C). The mitochondrial membrane potential (Δφm) was higher in the cisplatin-resistant HN9-cisR cells than in the cisplatin-sensitive parental HN9 cells [(MFI of Δφm: 1 ± 0 vs. 0.42 ± 0.07, P < 0.001) and reduced by PPMp or by the combination of cisplatin and PPMP; the effect was abrogated by PUMA gene inhibition (Fig. 4D).

These findings were further examined in vivo. BALB/c athymic nude mice received AMC-HN9-cisR xenograft cells transfected with GCS shRNA or control shRNA. The tumors were treated with i.p. injections of vehicle or cisplatin. GCS genetic blockade increased the cisplatin sensitivity of HN9-cisR cells (Fig. 5A). GCS inhibition increased ceramide production in HN9-cisR cells in vivo, and ceramide production much increased by both GCS inhibition and cisplatin treatment (Fig. 5B). This was further examined by pharmacologic inhibition of GCS using d,l-threo-PPMP in HN9-cisR tumors. d,l-threo-PPMP alone decreased the growth rate of HN9-cisR tumors (Fig. 6A). Notably, the combination of cisplatin and d,l-threo-PPMP synergistically suppressed in vivo tumor growth. In situ apoptosis assays showed that TUNEL-positive apoptotic bodies were more frequently seen in tumors treated with d,l-threo-PPMP and cisplatin plus d,l-threo-PPMP than in those treated with vehicle (Fig. 6B). Ceramide production much increased by both PPMP and cisplatin treatment (Fig. 6C). Western blot analyses of tumor tissues showed that apoptotic protein levels were increased to a greater extent in HN9-cisR cells treated with the combination of cisplatin and d,l-threo-PPMP than in cells treated with single agents (Fig. 6D).

Discussion

The present study shows that GCS and P-gp overexpression is associated with an aggressive HNC phenotype showing cisplatin resistance. Increased expression of GCS is correlated with the progression of glioblastoma, melanoma, leukemia, and ovarian, lung, colon, urinary, and breast cancers (13). The levels of GCS mRNA and protein are elevated 4-fold in approximately 80% of metastatic breast cancers, and GCS overexpression is associated with lymph node metastasis in bladder cancers (25, 26). In addition, P-gp overexpression is associated with unfavorable prognosis in leukemia, lymphoma, and breast, colon, pancreatic, and gastric cancers (8–10). The clinical significance of P-gp overexpression has also been studied in HNC. P-gp accumulation is associated with chemotherapy resistance and progression of HNC (27, 28). Unlike that of P-gp, the prognostic value of GCS overexpression in patients with HNC has been rarely studied. Our study demonstrates that GCS overexpression and its association with chemoresistance in vitro and in vivo. GCS and P-gp are closely linked with each other and predictive of response to chemotherapy in HNC cells, which has been noted in several types of human cancers (7, 12, 13). These findings suggest that GCS and P-gp may be suitable therapeutic targets in HNC.

In our study, blockade of GCS by gene silencing or PPMP increased the cisplatin-induced cell death of HNC cells. GCS overexpression is frequently correlated with P-gp (MDR1) levels in chemotherapy-resistant cells and tumors. Glycosphingolipids, produced by ceramide glycosylation, upregulate MDR1 expression...
via cSrc signaling and TCF4/β-catenin recruitment (29). Increased GCS and P-gp expression in response to anticancer drugs confers cancer cell resistance by augmenting ceramide glycosylation and MDR1-mediated drug efflux (24, 30). Furthermore, GCS silencing, achieved by transfection of GCS siRNA or mixed-backbone oligonucleotides, delays the clearance of ceramide generated in response to chemotherapeutic drugs and reverses cell resistance by suppression of MDR1 (18, 31). Close correlation between GCS and P-gp expression was also observed in our study. In addition, limiting GCS activity by either GCS siRNA/shRNA transfection or PPMP treatment downregulates P-gp expression and restores the ceramide-mediated death cascade, thereby heightening chemosensitivity in cisplatin-resistant HNC cells. Although cisplatin is a substance that is untransportable by P-gp, the sensitivity of cisplatin is affected by P-gp expression via inhibition cisplatin-induced apoptosis without any contribution the drug efflux activity of P-gp (32). GCS blockade downregulates P-gp and restores p53-dependent apoptosis in chemoresistant cancer cells (18, 19). In addition to targeting GCS, PPMP appeared to downregulate P-gp by acting as an antagonist (18, 33), contributing to resensitize cisplatin-resistant HNC cells to cisplatin.

The present study shows that downregulation of P-gp and ceramide generation by GCS inhibition drives the ceramide-induced apoptosis pathway and consequently caspase-dependent mitochondrial apoptosis. Cisplatin-resistant HNC cells present elevated expression of the antiapoptotic protein Bcl-2, commonly linked to cancer cell survival and resistance to chemotherapy (34), but decreased Bcl-2 expression upon blockade of ceramide glycosylation via inhibition of GCS (35). The blockade of GCS elevates the proapoptotic protein BAX and reverses the Bcl-2/BAX ratio (35). Furthermore, silencing of GCS by siRNA or PPMP treatment substantially increases the levels of phosphorylated p53 in both cisplatin-sensitive and -resistant HNC cells. This recovered the transactivation of p53-responsive genes, such as p21WAF1/CIP1, PUMA, and BAX. This is supported by the recent finding that suppression of GCS can restore p53-dependent apoptosis in drug-resistant ovarian cancer cells with p53 mutants (19). Ceramide accumulation promotes p53 activation and inhibits Bcl-2

Figure 6.
GCS pharmacologic inhibition sensitizes cisplatin-resistant HNC cells to cisplatin in vivo. A, increased cisplatin sensitivity by GCS pharmacologic inhibition in vivo. Nude mice were injected with 5 x 10^6 AMC-HN9-cisR cells in the flank. Treatments with vehicle (control), cisplatin, PPMP, or the combination of both drugs. Each group included 8 mice. B, quantification from in situ TUNEL assays in tumor sections from each group. TUNEL-positive apoptotic bodies were counted blindly in 10 randomly selected high-powered fields. The error bars represent SE. Two-tailed Student t test: *P < 0.01. C, change in ceramide production to cisplatin and/or PPMP in vivo. Total ceramide levels were measured by mass spectrometry in tumors of each group. *P < 0.001. D, Western blot analysis of cleaved PARP, PUMA, and GCS proteins obtained from tumors treated with vehicle control, cisplatin, PPMP, or the combination of both drugs. β-Actin served as internal loading control.

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phosphorylation through the activation of protein phosphatase 2A (PP2A), leading to increased p53/Bcl2 binding and apoptotic cell death (36). Our study suggests that the accumulation of p53 triggered by GCS inhibition is followed by a decrease in Bcl-2 levels and an increase in BAX levels, leading to apoptotic cell death in cancer cells. This is further supported by the fact that the expression of p53-responsive proteins induced by GCS silencing is abrogated by siPUMA transfection. Therefore, suppression of GCS is associated with restoration of p53 tumor suppression in mutant p53-bearing cancer cells (19, 31). Mutant p53 proteins not only lose the tumor-suppressive function of wild-type p53 but also gain new functions promoting tumorogenesis (37). Mutation of TP53 is associated with aggressive cancer, poor prognosis, and drug resistance in a growing array of malignancies (37, 38). Our and prior studies indicate that GCS inhibition may restore p53 function at the level of posttranscriptional processing (19), supporting a new approach to targeting mutant p53 for cancer therapy.

Our study revealed that the pharmacologic GCS inhibitor PPMP or GCS siRNA/shRNA transfection synergized with cisplatin and thereby circumvented resistance to cisplatin in HNC cells. As cisplatin is a first-line chemotherapeutic agent used in HNC, the combination of cisplatin and PPMP may be effective in the clinical setting at reducing toxicity and overcoming drug resistance. The present study is the first to show that PPMP or GCS genetic silencing recovers the cytotoxic effect of cisplatin in drug-resistant HNC cells in vitro and in vivo. PPMP induced a robust increase in cisplatin-mediated apoptosis via BAX, PLUMA, and PARP activation in cisplatin-resistant HNC cells. Genetic or pharmacologic inhibition of GCS sensitizes drug-resistant HNC cells to cisplatin, leading to increased cytotoxicity and more effective therapy for aggressive HNC. Taken together, these findings may be of paramount clinical significance: by inducing the death of resistant cells with GCS inhibition, PPMP treatment, or siRNA/shRNA transfection could reduce the dose of cisplatin required in the clinical setting and thereby minimize the potential adverse effects of cisplatin chemotherapy.

In conclusion, our data suggest that GCS and P-gp overexpression is significantly associated with cisplatin resistance in several HNC cell lines, suggesting that GCS and P-gp may be suitable therapeutic targets in HNC. Genetic or pharmacologic inhibition of GCS increases cisplatin-induced cell death in cisplatin-resistant HNC cells via downregulation of P-gp and activation of proapoptotic proteins in vitro and in vivo. This study supports the need for further investigation of GCS inhibition as a potential cancer therapy, particularly in HNC with aggressive phenotypes.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: J.-L. Roh, E.H. Kim
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