

Original Article

Distinct basal and stress-inducible xCT programs define therapeutic vulnerabilities in gastric cancer subtypes

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Abstract: xCT (SLC7A11), a key regulator of cellular redox homeostasis, has emerged as a potential therapeutic target in cancer; however, its regulatory heterogeneity and therapeutic implications in gastric cancer remain poorly understood. In this study, we investigated xCT expression patterns, regulatory states, and their therapeutic relevance across gastric cancer subtypes. Single-cell RNA sequencing analysis revealed higher xCT expression in diffuse-type gastric cancer than in intestinal-type gastric cancer, and elevated xCT expression was associated with poorer clinical outcomes. Consistent with these findings, diffuse-type gastric cancer cell lines exhibited markedly elevated basal xCT expression. In contrast, a subset of intestinal-type gastric cancer cell lines displayed stress-inducible xCT activation following oxidative stress or TGF- β stimulation. This inducible program was associated with increased basal NRF2 activity and coordinated induction of NRF2 target genes. Functionally, NRF2-high intestinal-type cancer cells acquired resistance to cisplatin following stress exposure, whereas NRF2-low intestinal-type cells remained relatively sensitive to cisplatin. In contrast, diffuse-type gastric cancer cells with high basal xCT expression exhibited enhanced sensitivity to combined xCT inhibition and cisplatin treatment. These findings identify distinct xCT regulatory states in gastric cancer, characterized by high basal xCT expression in diffuse-type tumors and NRF2-associated inducible xCT activation in a subset of intestinal-type tumors. Collectively, our results suggest that histologic subtype and NRF2 activity may provide a useful framework for therapeutic stratification and support subtype- and state-specific targeting of the xCT pathway in gastric cancer.

Keywords: Gastric cancer, SLC7A11, xCT, NRF2, histologic subtype, chemotherapy resistance

Introduction

Gastric cancer is a biologically heterogeneous disease composed of histologically and molecularly distinct subtypes with divergent clinical behavior. The Lauren classification broadly divides gastric adenocarcinoma into intestinal and diffuse types, a distinction that remains clinically relevant despite the development of modern genomic classifications. Among these, diffuse-type gastric cancer is characterized by poorly cohesive, infiltrative growth and is frequently associated with stromal-rich or mesenchymal features and poorer prognosis compared with intestinal-type tumors [1]. This growth pattern entails extensive interaction with the surrounding extracellular matrix and stromal components, potentially exposing

tumor cells to increased microenvironmental stresses, including oxidative stress. Accordingly, tumor cells may rely on adaptive programs to withstand such stress conditions, which can in turn influence therapeutic response. Thus, defining subtype-specific adaptive programs that sustain tumor survival and therapeutic resistance remains an important unmet need in gastric cancer.

Among the cellular mechanisms that enable tumor cells to adapt to microenvironmental stress, redox homeostasis has emerged as a critical determinant of cancer cell survival. In particular, ferroptosis, a form of iron-dependent, lipid peroxidation-driven cell death, has gained attention as a therapeutically targetable vulnerability in multiple cancer types [2, 3]. A

central regulator of this process is SLC7A11 (xCT), a cystine/glutamate antiporter that supports intracellular glutathione synthesis and protects cells from oxidative damage. By maintaining intracellular cysteine availability and redox balance, xCT enables tumor cells to withstand oxidative and metabolic stress conditions. Consistently, elevated xCT expression has been associated with increased resistance to oxidative stress and anticancer therapies, whereas its inhibition can sensitize tumor cells to ferroptosis and enhance treatment efficacy [4-7]. Notably, xCT expression is regulated by stress-responsive transcriptional programs, including redox-sensitive signaling pathways, suggesting that its activity may be dynamically modulated in response to environmental and therapeutic pressures [8, 9].

Previous studies have implicated xCT in gastric cancer as a key regulator of ferroptosis suppression, tumor progression, and resistance to anticancer therapies [10-12]. In particular, xCT upregulation has been linked to enhanced redox homeostasis and reduced sensitivity to agents such as cisplatin and 5-fluorouracil, while its inhibition can restore ferroptotic vulnerability and improve therapeutic efficacy [10-12]. However, these studies have largely treated xCT as a static, uniformly acting resistance factor, without considering the dynamic nature of tumor cell adaptation to stress. Tumor cells are continuously exposed to fluctuating microenvironmental and therapeutic pressures, under which xCT expression can be inducibly activated through stress-responsive transcriptional programs [8, 9]. This raises the possibility that tumors may differ not only in their basal xCT activity but also in their capacity to engage stress-inducible xCT programs. In gastric cancer, whether such distinct regulatory states exist across histologic subtypes, and how they shape therapeutic response, remains largely unexplored.

In this study, we sought to define distinct xCT regulatory programs in gastric cancer by integrating basal expression states with stress-inducible responses. Specifically, we aimed to determine whether gastric cancer subtypes differ in their baseline xCT activity as well as in their capacity to activate xCT in response to microenvironmental and therapeutic stress. Furthermore, we investigated how these dis-

tinct regulatory states may influence tumor adaptation and therapeutic response. By establishing a framework that incorporates both basal and stress-inducible xCT programs, this study provides a basis for understanding subtype-specific vulnerabilities in gastric cancer.

Materials and methods

Public dataset analysis

Single-cell RNA-seq data of gastric cancer were obtained from the Gene Expression Omnibus (GSE150290) [13], comprising 52 samples. The data were reanalyzed as previously described [14]. Raw sequencing reads were processed using Cell Ranger v7.0.0 (10x Genomics), and downstream analyses were performed using Seurat v4.1.3 [15]. After quality control and doublet removal, 196,972 cells were retained for analysis. Data were normalized, integrated, clustered, and visualized by UMAP. Cell type annotations and Lauren classification (intestinal or diffuse type) were assigned according to the original study and accompanying metadata [13]. Kaplan-Meier survival analysis was performed using the Kaplan-Meier Plotter database [16]. Patients were stratified into SLC7A11 high- and low-expression groups using the auto-selected best cutoff, and overall survival was analyzed using the default settings.

Cell lines and culture conditions

Human gastric cancer cell lines MKN1, MKN-45, MKN74, AGS, SNU216, SNU668, and KATO III were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). GCIY and NUGC4 were obtained from the RIKEN BRC Cell Bank (Ibaraki, Japan). Cells were cultured in RPMI-1640 medium (Welgene, Gyeongsanbuk-do, Korea) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO₂.

For the ultra-low attachment culture assay, cells were seeded in 6-well tissue culture-treated plates (SPL, Gyeonggi, Korea) or ultra-low attachment plates (Corning, NY, USA) at densities of 2×10^5 cells per well. Cells were cultured for 48 hrs prior to protein collection.

Oxidative stress and TGF- β treatment

Hydrogen peroxide (H_2O_2 ; Sigma-Aldrich, St. Louis, MO, USA) was used to induce oxidative stress in gastric cancer cells. Two experimental settings were employed depending on the study objective. For oxidative stress pretreatment experiments designed to evaluate persistent adaptive responses, cells were exposed to H_2O_2 at the indicated concentrations (30-200 μM) for 2 hrs. The medium was then replaced with fresh complete medium, and cells were further cultured for the indicated time periods (6-72 hrs). For NRF2 canonical target gene expression analysis, cells were harvested after an additional 4 hrs of incubation following medium replacement. For continuous oxidative stress experiments, cells were maintained in H_2O_2 -containing medium without medium replacement for up to 48 hrs before analysis.

For TGF- β treatment, cells were seeded at a density of 2×10^5 cells per well in 6-well plates. After 24 hrs, culture medium was removed, and cells were washed once with D-PBS (Welgene, Gyeongsan, Korea) before serum starvation in medium containing 1% FBS (Gibco, Grand Island, NY, USA) for 4 hrs. Cells were subsequently treated with 5 ng/mL TGF- β (R&D Systems, Minneapolis, MN, USA) and incubated for 48 hrs or 72 hrs. Protein lysates were then collected for immunoblotting.

Quantitative real-time PCR

Total RNA was isolated from cells using the RNeasy Plus Mini Kit (74136, Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using GoTaq[®] Reverse Transcriptase (A2801, Promega, Madison, WI, USA). Quantitative real-time PCR was performed using GoTaq[®] qPCR Master Mix (A6002, Promega) under standard amplification conditions. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to β -ACTIN and GAPDH as internal reference genes. Primer sequences used in this study are listed in [Supplementary Table 1](#).

Nuclear and cytosolic fractionation

Nuclear and cytosolic protein fractions were prepared using the NE-PER Nuclear and

Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Protease/phosphatase inhibitor cocktail (Cell Signaling Technology, Danvers, MA, USA) was added during the extraction procedure. Fraction purity was verified by immunoblotting using Lamin B1 and GAPDH as nuclear and cytosolic markers, respectively.

Western blot analysis

Cells were lysed in RIPA buffer supplemented with protease/phosphatase inhibitors. Protein concentrations were determined using the Bradford assay. Equal amounts of protein were separated on 4-12% Bis-Tris gels and transferred onto PVDF membranes. After blocking with 5% skim milk, membranes were incubated overnight at 4°C with primary antibodies against xCT, NRF2, GAPDH (Cell Signaling Technology, Danvers, MA, USA), and Lamin B1 and β -actin (GeneTex, Irvine, CA, USA). Protein bands were visualized using an enhanced chemiluminescence detection reagent.

Cell viability assay

Cell viability was assessed using a WST-8 assay. Gastric cancer cells were seeded in 96-well plates at a density of 5×10^3 cells per well and allowed to attach overnight. Cells were then treated with the indicated drugs at the specified concentrations and incubation times. Subsequently, WST-8 reagent (QM1000, Bio-max, Guri, Korea) was added and incubated for 2-4 hrs at 37°C. Absorbance was measured at 450 nm with background correction at 650 nm using a Synergy H1 microplate reader (BioTek Instruments, Winooski, VT, USA). Cell viability was normalized to untreated controls.

For ferroptosis rescue experiments, KATO III and NUGC4 cells were treated with erastin (2 or 10 μM) in the presence or absence of ferrostatin-1 (0.5-2 μM) for 4 hrs, followed by cisplatin treatment (5 or 10 μM) for 48 hrs. Cell viability was then determined using the WST-8 assay.

For analysis of cisplatin sensitivity following transient oxidative stress, cells were pretreated with H_2O_2 for 2 hrs, followed by medium replacement and a 4-hr recovery period. Cells were subsequently treated with the indicated con-

centrations of cisplatin and incubated for the designated time periods prior to cell viability analysis. The effect of H₂O₂ pretreatment on cisplatin sensitivity was calculated as:

$$1 - \frac{\text{Viability}_{\text{H}_2\text{O}_2 + \text{cisplatin}}}{\text{Viability}_{\text{cisplatin}}}$$

where a higher value indicates greater enhancement of cisplatin sensitivity by H₂O₂ pretreatment.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software (GraphPad, Boston, MA, USA). Comparisons between two groups were analyzed using the unpaired Student's t-test or Mann-Whitney U test, whereas comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$ (*) and $P < 0.01$ (**). For single-cell RNA-seq analyses, differences in SLC7A11 expression were assessed using the two-sided Wilcoxon rank-sum test.

Results

SLC7A11 is preferentially expressed in diffuse-type gastric cancer

To begin to define xCT regulatory states in gastric cancer, we first examined the cellular and subtype-specific expression landscape of SLC7A11 using publicly available single-cell and clinical transcriptomic datasets. Analysis of single-cell RNA sequencing data (GSE150-290) identified diverse epithelial, stromal, and immune cell populations within the gastric tumor microenvironment (**Figure 1A**). SLC7A11 expression was detected across multiple cellular compartments, with particularly prominent expression observed in monocyte/macrophage populations as well as tumor epithelial cells (**Figure 1B**). Importantly, despite expression in several non-neoplastic cell types, tumor epithelial cells displayed significantly higher SLC7A11 expression compared with normal gastric epithelium (**Figure 1C**), suggesting activation of xCT-associated programs during malignant transformation.

We next asked whether basal SLC7A11 activity differs according to gastric cancer histolo-

gy. Comparative analysis of tumor cells demonstrated significantly elevated SLC7A11 expression in diffuse-type gastric cancer relative to intestinal-type tumors (**Figure 1D**). To validate this observation experimentally, we examined a panel of gastric cancer cell lines representing intestinal and diffuse histologic subtypes. Consistent with the transcriptomic findings, diffuse-type cell lines, including KATO III, SNU668, and NUGC4, exhibited markedly higher SLC7A11 protein and mRNA expression, whereas most intestinal-type cell lines showed relatively low or nearly undetectable basal expression (**Figure 1E, 1F**).

Given these subtype-associated differences, we further evaluated the clinical relevance of SLC7A11 expression in gastric cancer. In the overall cohort, elevated SLC7A11 expression was significantly associated with inferior overall survival (HR = 1.32, $P = 0.0098$; **Figure 1G**). Given that diffuse-type gastric cancer is generally associated with poorer survival [17] and exhibits substantially higher basal SLC7A11 expression, this association may partly reflect differences in subtype composition within the overall cohort.

Diffuse-type gastric cancer exhibit xCT-dependent therapeutic vulnerability

Following the discovery of elevated basal SLC7A11 expression in diffuse-type gastric cancer, we next investigated whether diffuse type-associated growth conditions could influence xCT regulation and therapeutic response. Because diffuse-type gastric cancer is characterized by poorly cohesive growth and reduced cell-matrix attachment, we first modeled this phenotype using ultra-low attachment (ULA) culture conditions in gastric cancer cells (**Figure 2A**). Under ULA conditions, SLC7A11 expression was markedly reduced in intestinal-type MKN45 and MKN74 cells (**Figure 2B**). Among diffuse-type cells, KATO III maintained robust SLC7A11 expression, whereas NUGC4 showed reduced expression under the same condition (**Figure 2C**). These findings suggest that loss of cell attachment does not uniformly induce xCT expression and therefore cannot fully explain the elevated basal SLC7A11 expression observed in diffuse-type gastric cancer. Rather, xCT maintenance under anchorage-independent conditions appears to be cell-line dependent, indicating that additional

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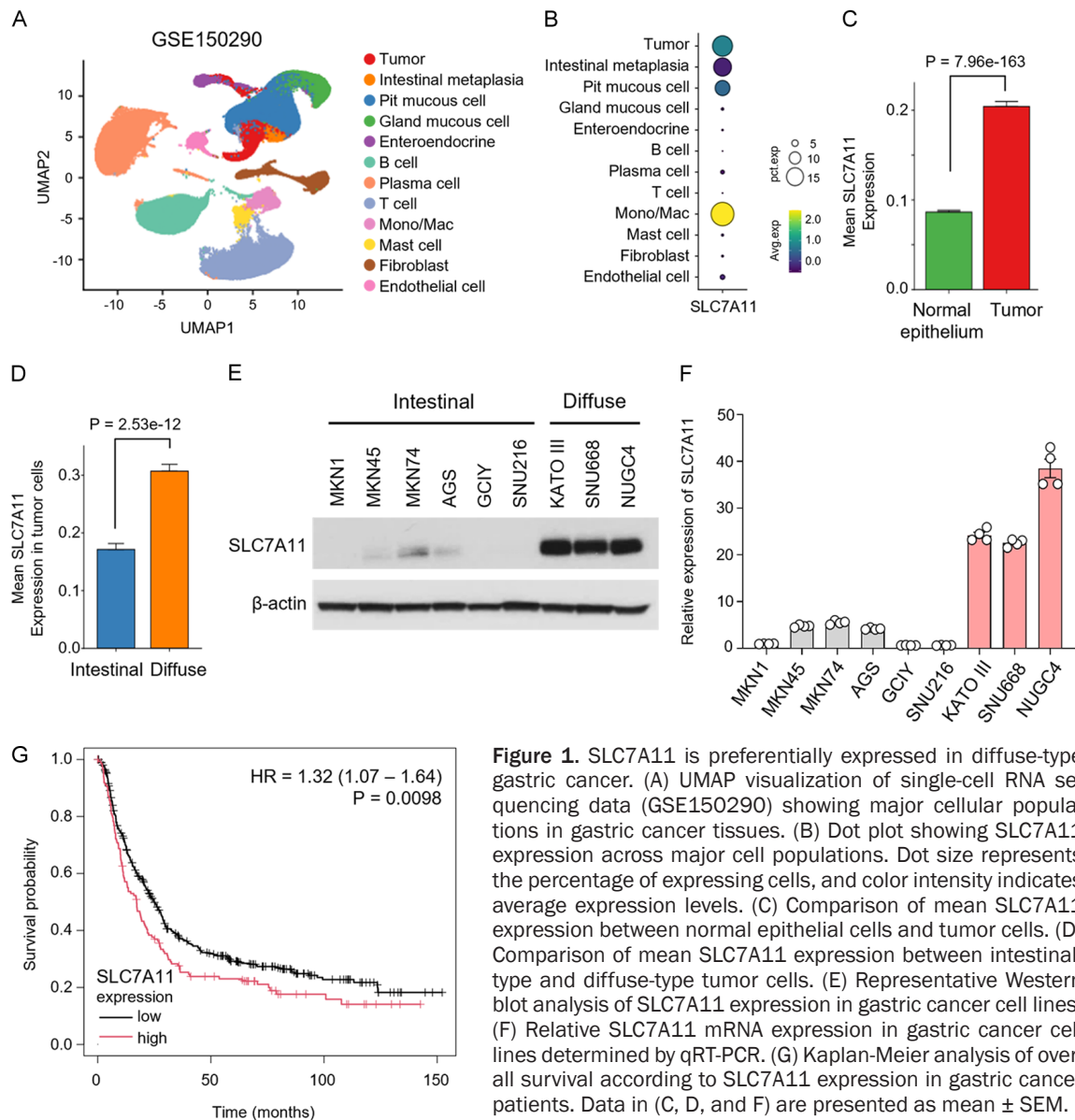


Figure 1. SLC7A11 is preferentially expressed in diffuse-type gastric cancer. (A) UMAP visualization of single-cell RNA sequencing data (GSE150290) showing major cellular populations in gastric cancer tissues. (B) Dot plot showing SLC7A11 expression across major cell populations. Dot size represents the percentage of expressing cells, and color intensity indicates average expression levels. (C) Comparison of mean SLC7A11 expression between normal epithelial cells and tumor cells. (D) Comparison of mean SLC7A11 expression between intestinal-type and diffuse-type tumor cells. (E) Representative Western blot analysis of SLC7A11 expression in gastric cancer cell lines. (F) Relative SLC7A11 mRNA expression in gastric cancer cell lines determined by qRT-PCR. (G) Kaplan-Meier analysis of overall survival according to SLC7A11 expression in gastric cancer patients. Data in (C, D, and F) are presented as mean $\pm$ SEM.

intrinsic regulatory programs may contribute to the xCT-high phenotype in diffuse-type gastric cancer.

Given the elevated basal SLC7A11 expression observed in diffuse-type gastric cancer, we next investigated whether this feature could be therapeutically exploited. Previous studies have suggested that high SLC7A11 expression may confer increased susceptibility to erastin, a pharmacologic inhibitor of xCT. However, erastin monotherapy produced heterogeneous responses across gastric cancer cell lines and did not show a simple correlation with histolog-

ic subtype or basal SLC7A11 expression (**Figure 2D**). These findings suggest that basal xCT expression alone may be insufficient to predict response to xCT inhibition as a single-agent strategy. We therefore asked whether the functional importance of xCT might become more apparent under chemotherapy-induced stress conditions. Given the established role of xCT in maintaining redox homeostasis and limiting oxidative damage, we examined whether pharmacologic xCT inhibition could enhance the therapeutic efficacy of cisplatin, a standard chemotherapeutic agent widely used in gastric cancer. To this end, diffuse-type gastric cancer

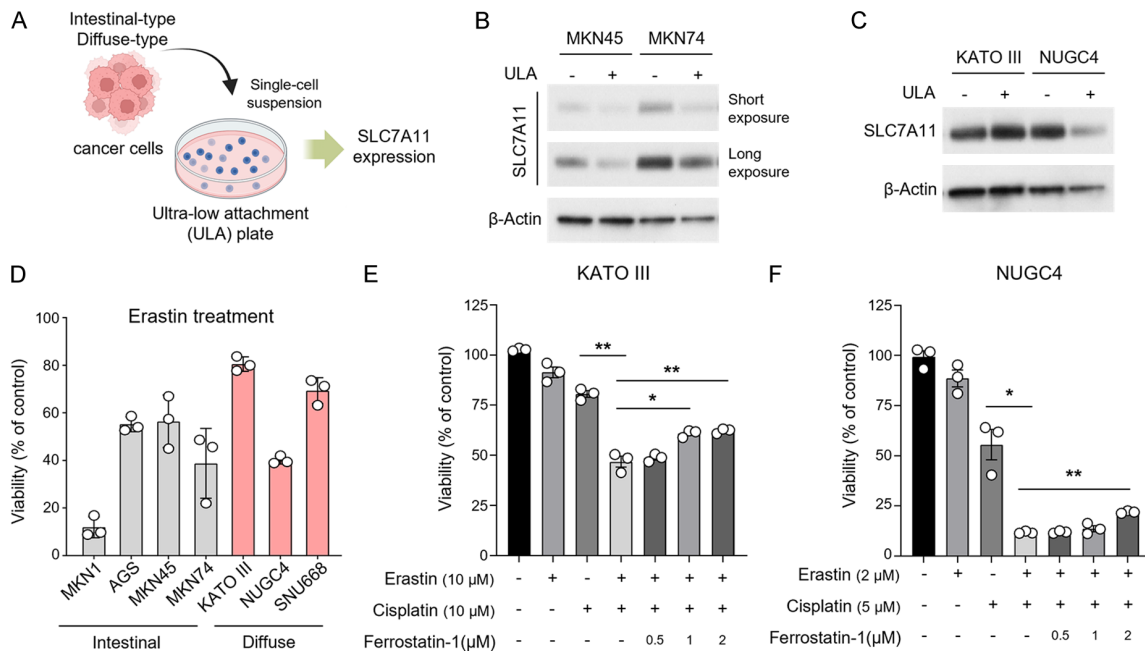


Figure 2. Diffuse-type gastric cancer exhibit xCT-dependent therapeutic vulnerability. (A) Schematic illustration of ultra-low attachment (ULA) culture conditions used to model anchorage-independent growth in gastric cancer cells. (B) Representative Western blot analysis of SLC7A11 expression in intestinal-type gastric cancer cells (MKN45 and MKN74) cultured under adherent or ULA conditions. Short- and long-exposure images are shown to visualize low-abundance signals. (C) Representative Western blot analysis of SLC7A11 expression in diffuse-type gastric cancer cells (KATO III and NUGC4) cultured under adherent or ULA conditions. (D) Cell viability of gastric cancer cell lines following treatment with erastin (2 μ M). Cell viability was assessed 48 hrs after treatment using the WST-8 assay. (E, F) Cell viability of KATO III and NUGC4 cells following treatment with erastin, cisplatin, the erastin-cisplatin combination, or ferrostatin-1 rescue. Erastin and cisplatin were used at 10 μ M and 10 μ M in KATO III cells and 2 μ M and 5 μ M in NUGC4 cells, respectively. Cell viability was assessed 48 hrs after treatment using the WST-8 assay. Data are presented as mean $\pm$ SEM. * P < 0.05, ** P < 0.01. (A) was created with BioRender.com.

cells were treated with erastin, either alone or in combination with cisplatin. Combined treatment with erastin and cisplatin produced substantially greater growth inhibition than either monotherapy in both KATO III and NUGC4 cells (Figure 2E, 2F). In contrast, in intestinal-type cells such as MKN1 and GCIY, erastin-cisplatin co-treatment did not result in a meaningful additional reduction in cell viability compared with either monotherapy (Supplementary Figure 1). To determine whether ferroptosis contributes to this therapeutic effect, we assessed the impact of ferrostatin-1. In both KATO III and NUGC4 cells, ferrostatin-1 partially rescued cell viability following erastin-cisplatin co-treatment (Figure 2E, 2F), supporting the contribution of ferroptosis to the combinatorial response. Collectively, these findings indicate that diffuse-type gastric cancer cells exhibiting high basal xCT expression may be therapeutically vulnerable to combined xCT inhibition and cisplatin treatment.

Distinct stress-inducible xCT programs exist across gastric cancer cell subtypes

Having established subtype-associated differences in basal xCT activity, we next investigated whether gastric cancer cells differ in their stress-responsive regulation of xCT. Interestingly, intestinal-type gastric cancer cells exhibited heterogeneous responses to oxidative stress, with some cell lines displaying marked sensitivity whereas others showed relative tolerance following H_2O_2 exposure (Figure 3A). In contrast, diffuse-type cell lines generally maintained greater resistance to oxidative stress, consistent with their elevated basal xCT activity.

We next examined whether oxidative stress induces SLC7A11 activation across gastric cancer cells. H_2O_2 exposure revealed marked heterogeneity in stress-responsive SLC7A11 regulation, with strong induction observed in

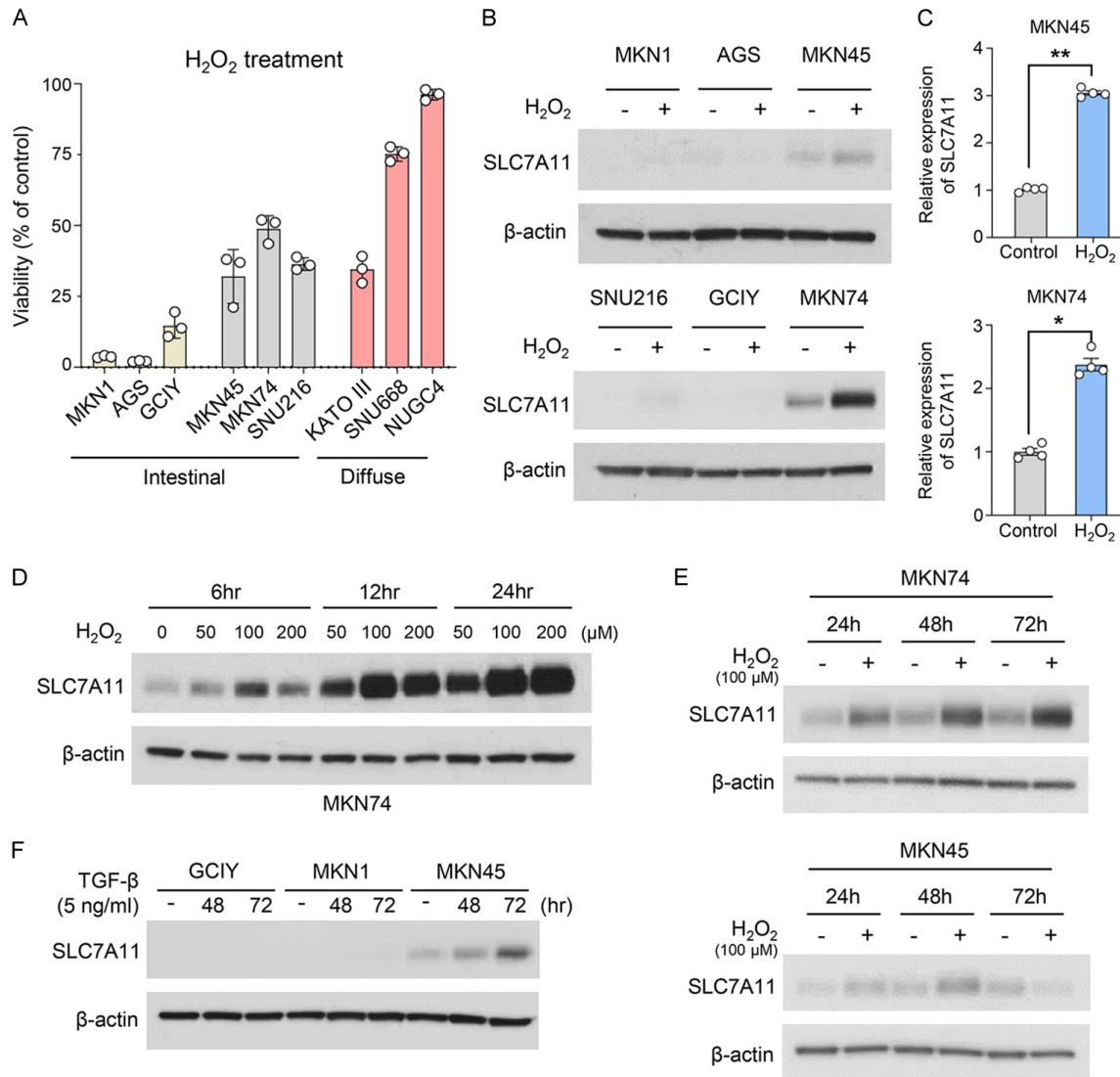


Figure 3. Distinct stress-inducible SLC7A11 programs in gastric cancer cells. (A) Cell viability of gastric cancer cell lines following H_2O_2 (100 μM) treatment. Cell viability was assessed 48 hrs after treatment using the WST-8 assay. (B) Representative Western blot analysis of SLC7A11 expression in gastric cancer cell lines following H_2O_2 (100 μM) treatment. (C) Relative SLC7A11 mRNA expression in MKN45 and MKN74 cells following H_2O_2 (100 μM) treatment, determined by qRT-PCR. (D) Representative Western blot analysis of SLC7A11 expression in MKN74 cells treated with the indicated concentrations of H_2O_2 for 6, 12, or 24 hrs. (E) Representative Western blot analysis of SLC7A11 expression in MKN74 and MKN45 cells at the indicated time points following a 2-hr H_2O_2 (100 μM) exposure. (F) Representative Western blot analysis of SLC7A11 expression in GCIY, MKN1, and MKN45 cells following TGF- β (5 ng/mL) treatment for 48 or 72 hrs. Data in (A) and (C) are presented as mean $\pm$ SEM. * P < 0.05, ** P < 0.01.

MKN74 cells and moderate responses in MKN45, whereas MKN1, AGS, SNU216, and GCIY showed limited or negligible induction (Figure 3B, 3C). In MKN74 cells, SLC7A11 expression increased progressively with escalating H_2O_2 concentrations (Figure 3D), supporting the presence of a dose-responsive stress adaptation program. Notably, following a brief 2-hour H_2O_2 exposure, SLC7A11 induc-

tion remained detectable for up to 72 hours in both MKN74 and MKN45 cells, with greater induction observed in MKN74 (Figure 3E), indicating sustained activation of stress-responsive xCT programs. Beyond oxidative stress, TGF- β stimulation also induced SLC7A11 expression in selected gastric cancer cells (Figure 3F), suggesting that inducible xCT activation may be engaged through multiple stress-

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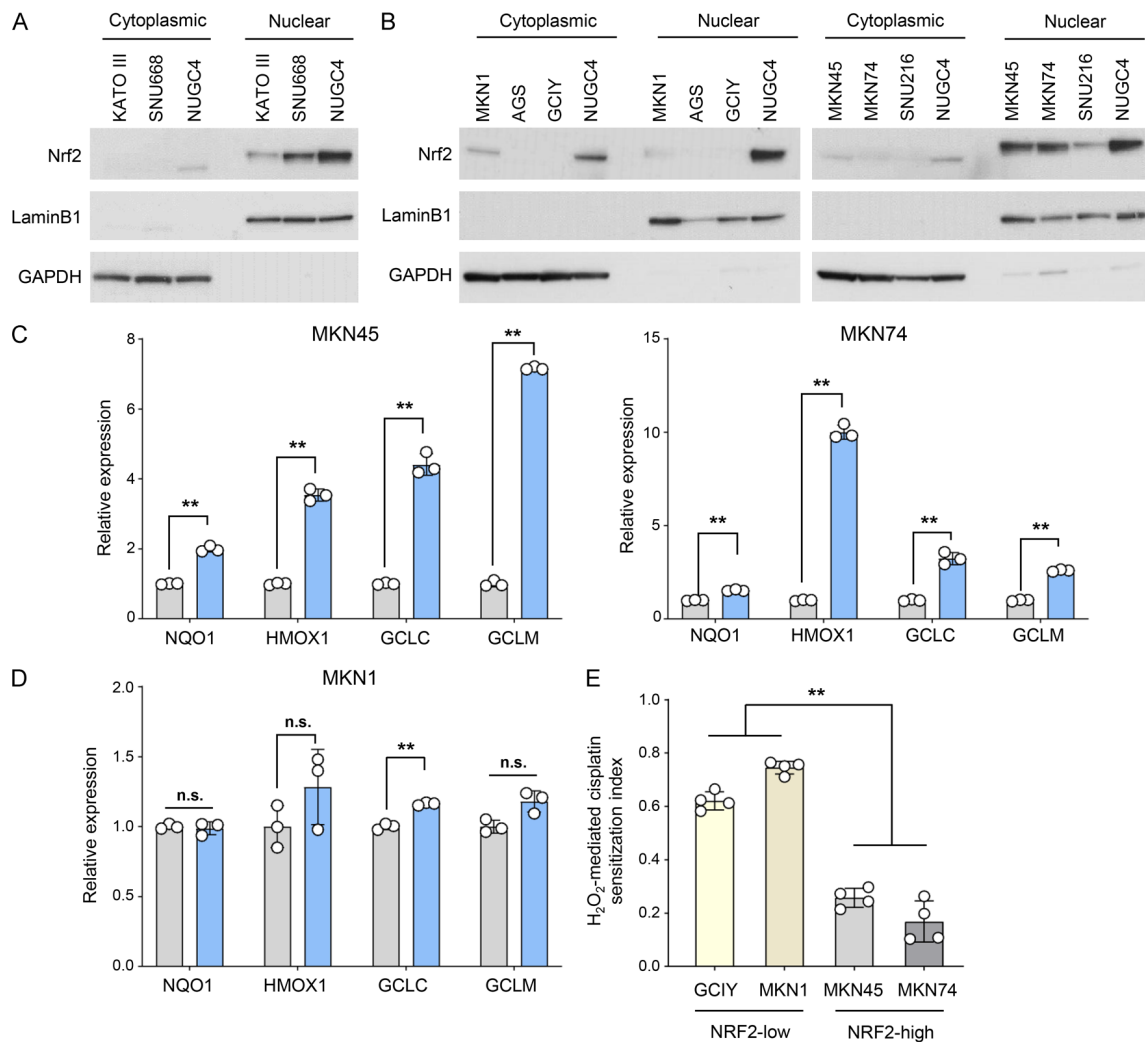


Figure 4. NRF2 activity is associated with stress-inducible xCT programs in gastric cancer. (A, B) Representative Western blot analysis of NRF2 expression in cytoplasmic and nuclear fractions of gastric cancer cell lines. Lamin B1 and GAPDH were used as nuclear and cytoplasmic markers, respectively. (C, D) Relative mRNA expression of NRF2 target genes following H₂O₂ pretreatment (100 μ M, 2 hrs) and subsequent recovery for 4 hrs, determined by qRT-PCR. (E) H₂O₂-mediated cisplatin sensitization index following transient H₂O₂ pretreatment (50 μ M, 2 hrs), recovery for 4 hrs, and subsequent cisplatin (10 μ M) treatment. Data in (C-E) are presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01. n.s., not significant.

associated pathways. Together, these findings indicate that gastric cancer cells differ not only in basal xCT activity but also in their capacity and magnitude of stress-inducible xCT activation.

NRF2-associated programs underlie heterogeneous stress-inducible xCT activation in gastric cancer

Given the distinct basal and stress-inducible xCT programs observed across gastric cancer subtypes, we next investigated whether differ-

ential NRF2 activity underlies these regulatory states. Consistent with the elevated basal xCT expression observed in diffuse-type gastric cancer, diffuse-type cell lines exhibited prominent NRF2 activation, as evidenced by increased nuclear NRF2 accumulation (**Figure 4A**). Notably, among intestinal-type gastric cancer cells, NRF2 activation was also observed in MKN45 and MKN74 cells, both of which displayed inducible SLC7A11 responses following oxidative stress, whereas MKN1 and GCIY exhibited relatively weak NRF2 activation (**Figure 4B**). These findings suggest that differ-

ential basal NRF2 activation is associated with distinct xCT regulatory states in gastric cancer. As expected, brief H_2O_2 treatment induced coordinated upregulation of representative NRF2 target genes in MKN45 and MKN74 cells (**Figure 4C**), whereas MKN1 showed minimal transcriptional changes under the same conditions (**Figure 4D**). Together, these findings suggest that differential basal NRF2 activation is associated with distinct xCT regulatory states in gastric cancer.

We next examined whether distinct xCT regulatory programs influence chemotherapy responses within intestinal-type gastric cancer cells. Brief H_2O_2 exposure enhanced cisplatin-induced growth suppression in MKN1 and GCIY cells, whereas this sensitizing effect was attenuated in MKN45 and MKN74 cells (**Figure 4E**). These findings suggest that differential basal NRF2 activity is associated with distinct stress adaptation capacities and differential susceptibility to oxidative stress-mediated cisplatin sensitization, even among intestinal-type gastric cancer cells. Collectively, these results indicate that heterogeneous basal NRF2 activity shapes stress-inducible xCT programs and influences subsequent responses to chemotherapeutic stress across gastric cancer cells.

Discussion

In the present study, we identified distinct xCT regulatory states across gastric cancer subtypes and found that diffuse-type gastric cancer is characterized by elevated basal SLC7A11 expression and poor clinical outcome. Although the mechanisms underlying this subtype-associated xCT activation remain incompletely understood, several biological features of diffuse-type gastric cancer may provide a plausible framework. Diffuse-type gastric cancer is characterized by poorly cohesive growth, stromal enrichment, and mesenchymal-associated features, all of which are linked to altered redox homeostasis and stress adaptation [1, 18]. Previous studies have shown that mesenchymal transition and ferroptosis-related pathways are closely interconnected, with stress-adaptive programs frequently engaged in tumors exhibiting mesenchymal or invasive phenotypes [19, 20]. Furthermore, diffuse or stromal-mesenchymal gastric cancers are often associated with unfavorable prognosis

and enhanced stromal signaling, suggesting a biological context in which antioxidant defense mechanisms such as xCT may be preferentially maintained or activated. Our findings therefore support the notion that elevated basal xCT expression may represent an adaptive redox state associated with diffuse-type gastric cancer biology rather than merely a passive subtype marker.

While diffuse-type gastric cancer exhibited elevated basal SLC7A11 expression, our findings indicate that xCT regulation is not uniform but instead comprises heterogeneous regulatory states within intestinal-type gastric cancer, as a subset of cells exhibited inducible SLC7A11 expression following oxidative or stress-associated stimulation. This observation suggests that xCT activity should not be viewed as a static feature, but rather as a dynamic stress-adaptive program. Furthermore, we found that NRF2 is associated with this heterogeneous inducible xCT program. NRF2 is a central regulator of cellular antioxidant defense and directly controls transcription of SLC7A11 and other redox-associated genes involved in glutathione metabolism and oxidative stress adaptation [5, 21]. Consistent with this established role, cells exhibiting inducible xCT responses showed increased basal NRF2 activation and coordinated induction of NRF2 target genes following stress exposure. Notably, TGF- β and oxidative stress did not universally activate xCT across gastric cancer cells, suggesting that stress-associated signaling alone is insufficient to induce xCT and instead requires a permissive cellular state characterized by basal NRF2 activity. These findings support a model in which NRF2-associated programs contribute to heterogeneous, stress-inducible xCT regulation across gastric cancer cells.

Our findings suggest that ferroptosis-related therapeutic strategies should be stratified according to distinct xCT regulatory states in gastric cancer. Diffuse-type gastric cancers, which are characterized by elevated basal xCT expression, may be particularly suitable for treatment strategies incorporating xCT inhibition in combination with conventional chemotherapy. In contrast, a subset of intestinal-type gastric cancers exhibited NRF2-associated inducible xCT activation and acquired resistance to cisplatin following stress exposure. In

these tumors, inhibition of the NRF2-xCT axis may represent a strategy to prevent or overcome adaptive therapeutic resistance. In contrast, intestinal-type gastric cancers lacking this inducible NRF2-xCT program remained relatively sensitive to cisplatin, suggesting that conventional chemotherapy may be sufficient without the need for additional targeting of xCT-mediated stress adaptation.

Several limitations of this study should be acknowledged. First, although we identified distinct basal and inducible xCT regulatory states in gastric cancer, the molecular mechanisms responsible for elevated basal xCT expression in diffuse-type gastric cancer remain incompletely understood. Second, our findings were primarily derived from in vitro models, and additional validation using patient-derived models or in vivo systems will be required to confirm the therapeutic relevance of these observations. Finally, although our data suggest that histologic subtype and NRF2 activity may serve as useful frameworks for therapeutic stratification, further clinical studies are needed to determine whether these xCT regulatory states can predict treatment response in patients with gastric cancer.

Conclusion

In conclusion, our study demonstrates that xCT regulation is heterogeneous across gastric cancer and comprises distinct constitutive and stress-inducible regulatory states. Diffuse-type gastric cancers are characterized by elevated basal xCT expression, whereas a subset of intestinal-type gastric cancers exhibits NRF2-associated inducible xCT activation in response to stress. These distinct xCT regulatory states are associated with differential therapeutic vulnerabilities and may provide a framework for subtype- and NRF2-guided therapeutic stratification. Collectively, our findings highlight the NRF2-xCT axis as a potential therapeutic target and support the development of personalized ferroptosis-related treatment strategies in gastric cancer.

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Disclosure of conflict of interest

None.

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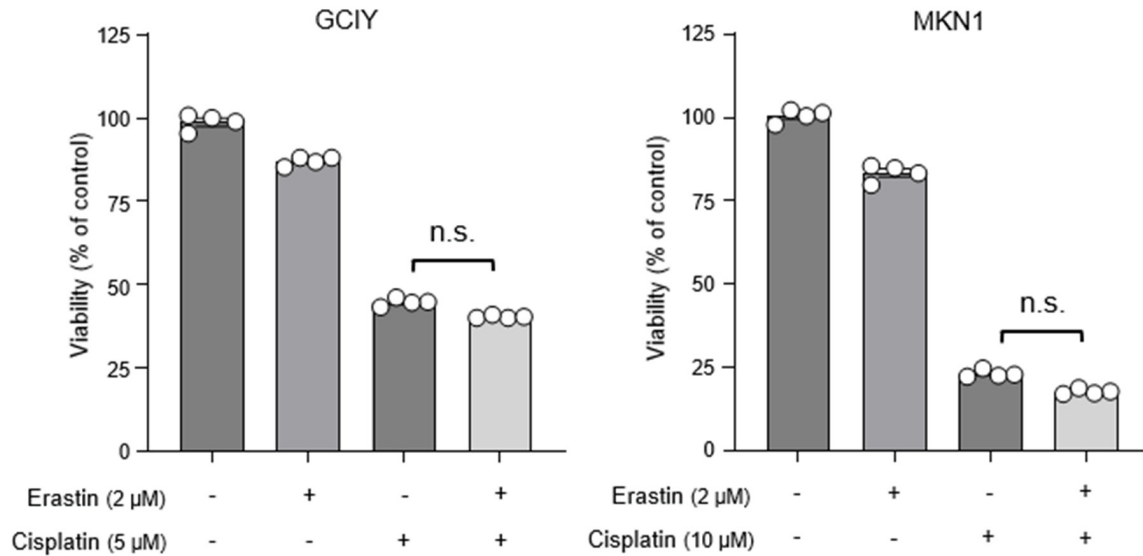
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Distinct xCT regulatory states in gastric cancer

Supplementary Table 1. Target genes and primer sequences

Gene	Primer	Sequence (5' to 3')	NCBI sequence
<i>SLC7A11</i>	Forward	CATTATCATTGGCACCATCATTGGA	NM_014331.4
	Reverse	GTTGTTCCCAATTCAGCATAAGACA	
<i>NQO1</i>	Forward	TAGGAGAGTTTGCTTACACTTACGC	NM_000903.3
	Reverse	CTCTGAATTGGCCAGAGAATGACA	
<i>HMOX1</i>	Forward	GCAGAGAATGCTGAGTTCATGAGG	NM_002133.3
	Reverse	AGCTCTTCTGGGAAGTAGACAGG	
<i>GCLC</i>	Forward	ATGGAGGTGCAATTAACAGACTTTG	NM_001498.4
	Reverse	TCTCTTTTCTGTGCTACCTTCATGT	
<i>GCLM</i>	Forward	AGACGGGGAACCTGCTGAAC	NM_002061.4
	Reverse	GGTTGATTGGGAACTCCATTCATT	
β -ACTIN	Forward	AACTGGGACGACATGGAGAAAA	NM_001101.5
	Reverse	ATAGCACAGCCTGGATAGCAA	
<i>GAPDH</i>	Forward	ATGTTCCAATATGATTCCACCCATG	NM_002046.7
	Reverse	TTGCATACTTCTCATGGTTCACAC	



Supplementary Figure 1. Cell viability of GCIY and MKN1 cells following treatment with erastin, cisplatin, the erastin-cisplatin combination. Cell viability was assessed 48 hrs after treatment using the WST-8 assay. Data are presented as mean $\pm$ SEM. *P < 0.05, **P < 0.01, n.s., not significant.