Zhang *et al.*
World Journal of Surgical Oncology (2024) 22:345
https://doi.org/10.1186/s12957-024-03626-9

World Journal of Surgical Oncology

RESEARCH

Open Access

Excessive glutathione intake contributes to chemotherapy resistance in breast cancer: a propensity score matching analysis

Zhiyuan Zhang[1,2†], Jiaru Gao[1,2†], Linjiao Jia[1,2†], Shuxin Kong[1,2], Maosen Zhai[1,2], Shuai Wang[1,2], Wenwen Li[1,2], Shoukai Wang[1,2], Yuqing Su[1,2], Wanyue Li[3], Changzheng Zhu[1,2], Wenkang Wang[4], Yuanxiang Lu[1,2*] and Wentao Li[1,2*]

Abstract

Background We aim to explore the impact of excessive glutathione (GSH) intake on chemotherapy sensitivity in breast cancer.

Methods Clinicopathological data were collected from 460 breast cancer patients who underwent adjuvant chemotherapy from January 2016 to December 2019 from Zhengzhou University People's Hospital. The clinicopathological characteristics following GSH treatment were collected and compared with those in Non-GSH group after 1:2 propensity score matching (PSM). Intracellular GSH levels and the expression of antioxidant enzymes (NRF2, GPX4 and SOD1) were evaluated in tumor tissues in 51 patients receiving neoadjuvant chemotherapy.

Results The recurrence rate after adjuvant chemotherapy was significantly higher in the GSH group ($n=28$, 31.8%) than that in the Non-GSH group ($n=39$, 22.2%; $P=0.010$). Additionally, patients in the HGSH group (high GSH intake, ≥ 16 days) exhibited an elevated recurrence rate compared to that in the LGSH group (low GSH intake, < 16 days) ($n=15$ (46.8%) vs. $n=52$ (22.4%); $P=0.003$). Cox regression revealed that High GSH intake, Ki67 ≥ 30%, Triple negative and Lymphovascular invasion were independent risk factors of progression after adjuvant chemotherapy. Among patients receiving neoadjuvant chemotherapy, intracellular GSH levels and the expression levels of antioxidant enzymes (NRF2, GPX4 and SOD1) in the resistant patients were substantially higher ($P<0.001$).

Conclusions Excessive GSH intake may contribute to chemotherapy resistance in breast cancer, and the levels of intracellular GSH and antioxidant enzymes are elevated in resistant patients after neoadjuvant chemotherapy, indicating that the standardization of GSH intake may assist in reducing chemotherapy resistance.

Keywords Glutathione, Breast cancer, Chemotherapy resistance, Prognostic, Propensity score matching

†Zhiyuan Zhang, Jiaru Gao and Linjiao Jia contributed equally to this work.

*Correspondence:
Yuanxiang Lu
luyx220@163.com
Wentao Li
lwt9225@163.com
Full list of author information is available at the end of the article

Introduction

Breast cancer is the most common malignancy among women worldwide. Despite significant advancements in diagnosis and treatment, the prognosis of breast cancer patients remains unsatisfactory [1]. The main treatment options for breast cancer include surgery, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy [2]. Treatment regimen is generally based on the stage of cancer, tumor characteristics, molecular subtype, immunohistochemistry, and the patient's overall health status. Despite 80%–90% of patients are eligible for surgery, 30%–40% will inevitably experience recurrence and metastasis. Additionally, 10%–20% of patients may be deemed unsuitable for surgical intervention at the time of initial treatment [3]. To improve outcomes, around 60%-80% require adjuvant and neoadjuvant chemotherapy to diminish the recurrence risk [4, 5]. However, a large number of patients still exhibit primary or secondary resistance to chemotherapy drugs, leading to poor prognosis. In recent years, the Royal Marsden Hospital (RMH) Score has shown promise as a valuable prognostic tool for assessing patient outcomes, while reliable indicators to predict recurrence and mortality following chemotherapy resistance remain scarce [6].

Chemotherapy-induced apoptosis is widely recognized as a primary mechanism for tumor cell destruction [7]. Chemotherapy promotes the production of Reactive Oxygen Species (ROS), which triggers apoptosis and damages both tumor cells and healthy cells. In response to the oxidative stress-induced damage, tumor cells upregulate their antioxidant systems, upregulating intracellular GSH levels [8]. This adaptation, which mitigates chemotherapy-induced oxidative damage, inadvertently promotes drug resistance and undermines the treatment's efficacy. Besides, chemotherapy may cause various adverse effects, including hepatotoxicity, cardiotoxicity, and neurotoxicity [9]. According to Chinese Society of Clinical Oncology (CSCO) and European Association for the Study of the Liver (EASL) Clinical Practice Guidelines [10, 11], hepatoprotective treatments are recommended when Alanine Aminotransferase (ALT) levels exceed three times the normal value or total bilirubin is more than twice the normal value. The guidelines, however, do not specify whether glutathione can be used or provide recommendations regarding its duration and the use of hepatoprotective drugs in clinical practice is not standardized. Previous studies have documented elevated GSH levels in various tumors, contributing to chemotherapy resistance and counteracting its cytotoxic effects [12]. Nevertheless, there are limited studies investigating the impact of non-standardized GSH intake in chemotherapy for breast cancer.

Utilizing a large cohort of breast cancer patients, we explored the relationship between GSH intake dosage and resistance to adjuvant or neoadjuvant chemotherapy. Furthermore, we assessed the intracellular levels of GSH and antioxidant enzymes in patients undergoing neoadjuvant chemotherapy. In selecting appropriate dosages of GSH as a hepatoprotective agent, we aspire to provide a thoughtful reflection on these considerations.

Materials and methods

Patients

We retrospectively collected clinical data from 460 breast cancer patients who underwent surgery at Zhengzhou University People's Hospital between January 2016 and December 2019. The inclusion criteria were: (I) a pathological diagnosis of breast cancer; (II) female patients; (III) complete and reliable clinical and pathological data. The exclusion criteria were: (I) severe underlying diseases; (II) conditions precluding surgery, such as multiple distant metastases, poor physical condition, or an unfavorable prognosis; (III) loss to follow-up or death from causes irrelevant to the study. Based on these criteria, we ultimately included 366 breast cancer patients. Additionally, 51 patients who received neoadjuvant chemotherapy between January 2016 and December 2019 were analyzed separately. The study was approved by the Ethics Committee of Zhengzhou University People's Hospital. All patients or their families provided written informed consent.

Description of the chemotherapy process and the definition of GSH intake duration

According to the CSCO guidelines, indications for adjuvant chemotherapy involve lymph node metastasis, triple-negative, HER2-positive, and hormone receptor-positive cases with a 21-gene recurrence score (RS) exceeding 26. Most patients in the study received "AC-T" (Adriamycin, Cyclophosphamide followed by Docetaxel or Taxol) for 8 cycles, "TCb" (Docetaxel or Taxol, Carboplatin) for 6 cycles, "TC" (Docetaxel or Taxol, Cyclophosphamide) for 4 cycles, or "AC" (Adriamycin, Cyclophosphamide) for 4 cycles.

According to the CSCO guidelines, indications for neoadjuvant chemotherapy include lymph node metastasis, tumor diameter greater than 5 cm, HER2-positive, triple-negative, and relatively large tumors with a desire to preserve the breast. Most patients receiving neoadjuvant chemotherapy in the study received "TAC" (Docetaxel or Taxol, Adriamycin, Cyclophosphamide) for 6 cycles, or "TCb" (Docetaxel or Taxol, Carboplatin) for 6 cycles.

According to CSCO Guidelines, hepatoprotective treatment with drugs is required when ALT levels exceed three times the normal value. In this study, chemotherapy

patients routinely had their liver function (ALT and AST) assessed post-chemotherapy. If ALT levels exceeded three times the normal value, chemotherapy was paused, and patients initiated oral GSH administration until liver function tests indicated normalized transaminase levels. The duration of GSH administration was defined as the cumulative number of days GSH was taken throughout each chemotherapy cycle. In addition, the dosage of GSH administered to the patient each time was based on the GSH instructions, with 400 mg orally three times a day. The total duration and dosage of glutathione use were recorded by reviewing electronic medical records and through telephone follow-ups.

The evaluation of chemotherapy adverse reactions follows the Common Terminology Criteria for Adverse Events (CTCAE) guidelines.

Follow-up

In this study, patients routinely began adjuvant chemotherapy approximately two weeks after surgery, with monitoring conducted during 4, 6, or 8 cycles of chemotherapy. Follow-up was conducted one month after surgery, with additional visits scheduled every three months during the first two years, and then every six months thereafter through either telephone consultations or outpatient visits. Follow-up assessments involved physical examinations, routine blood tests, serum tumor marker measurements, breast ultrasound, chest and abdominal computed tomography (CT) scans, magnetic resonance imaging (MRI), and ultrasound every three months for the first two years, and every six months afterward. Additionally, a full-body bone scan and positron emission tomography-computed tomography (PET-CT) were performed annually. All follow-up data collection was completed before July 1, 2024. The primary endpoints of this study were Overall Survival (OS) and Disease-Free Survival (DFS). OS was defined as the time from the surgery date to the end date of follow-up or death, while DFS was defined as the time from surgery to either disease recurrence or the end date of follow-up. Postoperative progression was defined as local tumor recurrence or the development of new metastatic lesions. Progression was confirmed through imaging studies, including breast ultrasound, chest and abdominal ultrasound, CT and/or MRI, PET-CT, or biopsy pathology results.

Patients undergoing neoadjuvant chemotherapy started treatment immediately after biopsy confirmation, with chemotherapy typically consisting of 6 or 8 cycles. Follow-up evaluations of the tumor were conducted before each chemotherapy cycle, including breast ultrasound, physical examinations, routine blood tests, and serum tumor marker measurements. Chest and abdominal ultrasound, CT and/or MRI were evaluated every two chemotherapy cycles. After all cycles of neoadjuvant chemotherapy, surgery or biopsy was performed followed by pathological evaluation to assess the efficacy of the treatment. The numbers of patients achieving pathological complete response (pCR), pathological partial response (pPR), and pathological no change (pNC) were recorded.

Clinicopathological indices

Clinicopathological data were collected for all patients, including menopausal status, age, hepatitis infection, preoperative liver function, preoperative blood routine, type of surgery, chemotherapy regimen, histological type and grade, tumor size, number of tumors, lymphovascular invasion, and treatment modalities such as radiotherapy and hormone therapy. Additional factors collected included Estrogen receptor/Progesterone receptor (ER/PR), Human Epidermal growth factor Receptor 2 (HER2), Ki67, molecular subtype, N stage, T stage, tumor-node-metastasis (TNM) stage, adverse reactions during chemotherapy, transaminase levels after chemotherapy, metastasis or recurrence, treatments after recurrence or metastasis, and survival status at the last follow-up.

Quantification of the GSH/GSSG ratio and the relative expression levels of NRF2, GPX4 and SOD1

To investigate the role of GSH in chemotherapy resistance, 51 patients who underwent neoadjuvant chemotherapy between January 2016 and December 2019 were enrolled in this study. None of these patients received neoadjuvant radiotherapy or endocrine therapy. In our study, participants who received neoadjuvant therapy were categorized as the sensitive group (patients with pCR and pPR, $n=30$) or the resistant group (patients with pNC, $n=21$). Moreover, we randomly selected 10 patients from both the sensitive and resistant groups. Tissue samples from both groups were collected post-surgery or biopsy to measure GSH levels, along with the relative expression levels of NRF2, GPX4 and SOD1.

GSH levels and the GSH/GSSG ratio were quantified using the GSH and GSSG Assay Kit (Biovision, K264) in accordance with the manufacturer's instructions. The absorbance of GSH and GSSG at 412 nm was measured using a microplate reader. The results were normalized to protein concentration for accurate comparison. The expression levels of three mRNAs were quantified using qPCR. RNA was isolated with the RNA Isolation Kit V2 (Vazyme) according to the manufacturer's protocol. Reverse transcription was performed using HiScript II Q Select RT SuperMix (+gDNA wiper), following the manufacturer's instructions. Quantitative PCR (qPCR) was subsequently carried out with ChamQ Universal SYBR qPCR Master Mix. Gene expression levels were

normalized to β-actin levels in each sample. Primer sequences are provided in Supplementary Table 1.

Immunohistochemical analysis

The paraffin-embedded slides were first stained with hematoxylin and eosin (HE) prior to immunohistochemistry (IHC) analysis. For IHC staining, the tissue sections were incubated overnight at 4 °C with primary antibodies, including Anti-NRF2, Anti-GPX4 and Anti-SOD1, following the DAB substrate kit protocol (Zsbio Commerce Store). All IHC samples were evaluated by two independent pathologists, who were blinded to the source of the samples and the patient outcomes. Each core was assigned a score from 0 to 3 + based on the expression of NRF2, GPX4 or SOD1 in tumor cells. A score of 0 was assigned when expression was observed in less than 5% of tumor cells, 1 + for expression in 5–50%, 2 + for expression in 50–75%, and 3 + for expression in more than 75% of tumor cells. The final score for each tumor was calculated as the mean score of all tumor cores. Statistical analysis was performed based on the product of staining rate and staining intensity to assess the overall expression levels. The antibodies used in this study are listed in Supplementary Table 2.

Statistical analysis

Statistical analyses were performed using SPSS for Windows version 25.0. Categorical data were expressed as numbers and percentages, continuous variables were presented as the mean ± standard deviation (SD). The normality of continuous variables was assessed using the Shapiro–Wilk test. For normally distributed data, a two-tailed Student's t test was used for comparisons between two groups. For non-normally distributed data, the Mann–Whitney U test was employed. Pearson's chi-square test (χ^2) was used to analyze categorical variables between LGSH (low GSH intake) group and HGSH (high GSH intake) group. Receiver operating characteristic (ROC) curves were applied to determine the cutoff value for the duration of glutathione intake. The Kaplan–Meier (KM) method was utilized to plot survival curves and calculate postoperative survival rates, with the log-rank test used to evaluate differences in survival times between groups. The Cox proportional hazards regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) to identify independent risk factors for patient prognosis. Univariate and multivariate Cox regression analyses were performed to assess predictors of long-term patient survival. In univariate logistic regression analysis, covariates with *P*-values less than 0.05 were considered statistically significant, and these variables were subsequently included in the multivariate analysis. PSM was employed to reduce bias due to differences in observed variables between the two groups in the retrospective observational study, and the analysis was conducted using R version 4.3.0. A *P*-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of breast cancer patients before and after PSM

After three years of follow-up, a total of 366 patients were included in this study, and were separated into GSH group ($n=120$) and Non-GSH group ($n=246$). Among them, 313 patients received mastectomy, while 37 patients underwent breast-conserving surgery. Additionally, 89 patients developed metastasis and recurrence, while 277 patients did not experience either.

PSM analysis was employed to balance the bias between the GSH and Non-GSH groups. Following 1:2 PSM, a total of 264 breast cancer patients were included in the analysis (Table 1 and Supplementary Table 3). The distributions of propensity scores before and after matching were summarized in Supplementary Fig. 1. Among these patients, there were 88 in the GSH group and 176 in the Non-GSH group. The mean age of these patients was 48.5 ± 10.84 years (range: 30—82 years), and the mean tumor size measured post-surgery was 1.819 ± 0.912 cm (range: 0.5—5.8 cm). The study workflow was illustrated in Fig. 1.

Of those receiving GSH treatment, 39 were premenopausal (44.3%) and 49 were postmenopausal (55.7%). Among the 88 patients, 81 underwent mastectomy and 5 underwent breast conserving surgery. All surgical margins were negative. Furthermore, 58 patients received radiation therapy, and 54 received endocrine therapy.

In addition, of the 51 patients who received neoadjuvant chemotherapy, 30 achieved either a pathological partial response ($n=23$, 45% patients) or complete response ($n=7$, 14% patients), while 21 exhibited no pathological response. Moreover, among these patients, 31 were postmenopausal, and 20 were premenopausal (Table 2).

Relationship between glutathione intake and clinicopathological indices in breast cancer patients

Glutathione intake was not significantly associated with preoperative clinical indicators. As shown in Supplementary Table 3, there were no significant differences between the GSH and Non-GSH groups regarding menopausal status, age, preoperative liver function, or other preoperative indicators. Before adjuvant chemotherapy, no significant differences were observed between GSH group and non-GSH group in terms of type of surgery,

Table 1 Clinicopathological characteristics of enrolled breast cancer patients after surgery

Variables	Before PSM			After PSM		
	GSH Group ($n=120$)	Non-GSH Group ($n=246$)	*P* value	GSH Group ($n=88$)	Non-GSH Group ($n=176$)	*P* value
Type of surgery			0.712			0.837
Mastectomy	105(87.6%)	208(84.6%)		81(92.0%)	158(89.8%)	
Breast conserving surgery	11(9.1%)	26(10.6%)		5(5.7%)	13(7.4%)	
Other	4(3.3%)	12(4.8%)		2(2.3%)	5(2.8%)	
Chemotherapy regimen			0.851			0.452
AC-T	60(50.0%)	130(52.8%)		44(50%)	102(57.9%)	
TCb	45(37.5%)	85(34.6%)		28(31.8%)	49(27.9%)	
Other	15(12.5%)	31(12.6%)		16(18.2%)	25(14.2%)	
Histology			0.804			0.976
Infiltrative ductal	90(75.0%)	183(74.4%)		67(76.1%)	132(75.0%)	
Infiltrative lobular	22(18.3%)	50(20.3%)		17(19.4%)	36(20.5%)	
Other	8(6.7%)	13(5.3%)		4(4.5%)	8(4.5%)	
Tumor size			0.527			0.693
≥ 2 cm	18(15.0%)	31(12.6%)		10(11.4%)	23(13.1%)	
< 2 cm	102(85.0%)	215(87.4%)		78(88.6%)	153(86.9%)	
Number of tumors			0.266			0.650
1	110(91.7%)	216(87.8%)		81(92.0%)	159(90.3%)	
> 1	10(8.3%)	30(12.2%)		7(8.0%)	17(9.7%)	
Grade of histology			0.704			0.929
I	6(5.0%)	15(6.1%)		4(4.5%)	9(5.1%)	
II	70(58.3%)	151(61.4%)		49(55.7%)	101(57.4%)	
III	44(36.7%)	80(32.5%)		35(39.8%)	66(37.5%)	
Lymphovascular invasion			0.382			0.702
Yes	80(66.7%)	175(71.1%)		61(69.3%)	126(71.6%)	
No	40(33.3%)	71(28.9%)		27(30.7%)	50(28.4%)	
Radiotherapy			0.137			0.512
Yes	72(60.0%)	167(67.9%)		58(65.9%)	123(69.9%)	
No	48(40.0%)	79(32.1%)		30(34.1%)	53(30.1%)	
Hormone therapy			0.346			0.467
Yes	75(62.5%)	166(67.5%)		54(61.4%)	116(65.9%)	
No	45(37.5%)	80(32.5%)		34(38.6%)	60(34.1%)	
ER/PR			0.346			0.467
Positive	75(62.5%)	166(67.5%)		54(61.4%)	116(65.9%)	
Negative	45(37.5%)	80(32.5%)		34(38.6%)	60(34.1%)	
HER2			0.269			0.492
Positive	35(29.2%)	86(35.0%)		26(29.5%)	45(25.6%)	
Negative	85(70.8%)	160(65.0%)		62(70.5%)	131(74.4%)	
Ki67			0.164			0.330
≥ 30%	63(52.5%)	148(60.2%)		56(63.6%)	101(57.4%)	
< 30%	57(47.5%)	98(39.8%)		32(36.4%)	75(42.6%)	
Molecular subtype			0.640			0.765
Hormone receptor positive	67(55.8%)	150(61.0%)		54(61.4%)	116(65.9%)	
Triple negative	25(20.9%)	46(18.7%)		15(17.0%)	27(15.3%)	
HER2 positive	28(23.3%)	50(20.3%)		19(21.6%)	33(18.8%)	
N stage			0.685			0.994
0	50(41.7%)	109(44.3%)		34(38.6%)	70(39.8%)	
1	31(25.8%)	71(28.9%)		27(30.7%)	55(31.3%)	

Table 1 (continued)

Variables	Before PSM			After PSM		
	GSH Group ($n=120$)	Non-GSH Group ($n=246$)	*P* value	GSH Group ($n=88$)	Non-GSH Group ($n=176$)	*P* value
2	22(18.3%)	40(16.3%)		16(18.2%)	30(17.0%)	
3	17(14.2%)	26(10.5%)		11(12.5%)	21(11.9%)	
T stage			0.903			0.937
1	57(47.5%)	112(45.6%)		36(40.9%)	75(42.6%)	
2	55(45.8%)	115(46.7%)		47(53.4%)	90(51.1%)	
3	8(6.7%)	19(7.7%)		5(5.7%)	11(6.3%)	
TNM stage			0.651			0.624
1	20(16.7%)	51(20.7%)		14(15.9%)	35(19.9%)	
2	60(50.0%)	116(47.2%)		46(52.3%)	82(46.6%)	
3	40(33.3%)	79(32.1%)		28(31.8%)	59(33.5%)	
Adverse reaction during chemotherapy [13]			< 0.001			0.002
Low	70(58.3%)	184(74.8%)		53(60.2%)	138(78.4%)	
High	50(41.7%)	62(25.2%)		35(39.8%)	38(21.6%)	
Transaminase after chemotherapy						
ALT	87.830 ± 2.089	30.701 ± 2.262	< 0.001	87.139 ± 2.731	30.246 ± 2.361	< 0.001
AST	71.945 ± 1.233	23.556 ± 1.048	< 0.001	71.270 ± 1.113	23.107 ± 1.611	< 0.001
Nausea and vomiting during chemotherapy			< 0.001			0.023
Yes	79(65.8%)	112(45.5%)		56(63.6%)	86(48.9%)	
No	41(34.2%)	134(54.5%)		32(36.4%)	90(51.1%)	
Metastasis and recurrence			0.022			0.010
Yes	38(31.7%)	51(20.7%)		28(31.8%)	39(22.2%)	
No	82(68.3%)	195(79.3%)		60(68.2%)	137(77.8%)	
Treatment after recurrence and metastasis			0.986			0.364
Conservative treatment	29(76.3%)	39(76.5%)		20(71.4%)	30(76.9%)	
Surgery	9(23.7%)	12(23.5%)		8(28.6%)	9(23.1%)	
Survival status			0.030			< 0.001
Alive	92(76.7%)	211(85.8%)		70(79.5%)	150(85.2%)	
Dead	28(23.3%)	35(14.2%)		18(20.5%)	26(14.8%)	

chemotherapy regimen, tumor size, number of tumors, histological grade, etc. (Table 1). However, GSH group had significantly higher levels of ALT (87.139 ± 2.731; $P<0.001$) and AST (71.270 ± 1.113; $P<0.001$) after chemotherapy and experienced more severe adverse reactions ($P=0.002$), including a notably higher incidence of nausea and vomiting ($P=0.023$). This is consistent with clinical practice, where chemotherapy patients with elevated transaminase levels usually use hepatoprotective drugs. Furthermore, GSH group showed a higher probability of recurrence and progression after adjuvant chemotherapy ($P=0.010$) (Table 1).

Relationship between the duration of glutathione intake and clinical indicators in breast cancer patients

For the 88 participants who took GSH, the mean duration of GSH intake was 18 ± 0.82 days (range: 2—27 days). ROC analysis was carried out to screen the best cutoff value for GSH intake, and the curves with variable cutoff numbers were presented in Fig. 2. The duration of GSH administration was defined as the cumulative number of days GSH was taken throughout each chemotherapy cycle. In addition, the dosage of GSH administered to the patient each time was based on the GSH instructions. Specifically, recurrence after adjuvant chemotherapy was used as the outcome variable to generate the ROC curve. The optimal cutoff point for GSH intake duration was determined to be 16 days, based on the maximum Youden index (Youden index = specificity + sensitivity − 1). The area under the ROC curve was 0.858, with a sensitivity of 84.8% and specificity of 83.3% (Fig. 2).

Patients were divided into HGSH (≥ 16 days, $n=32$) group and LGSH (< 16 days, $n=232$) group based on a cutoff value of 16 days. There were no significant

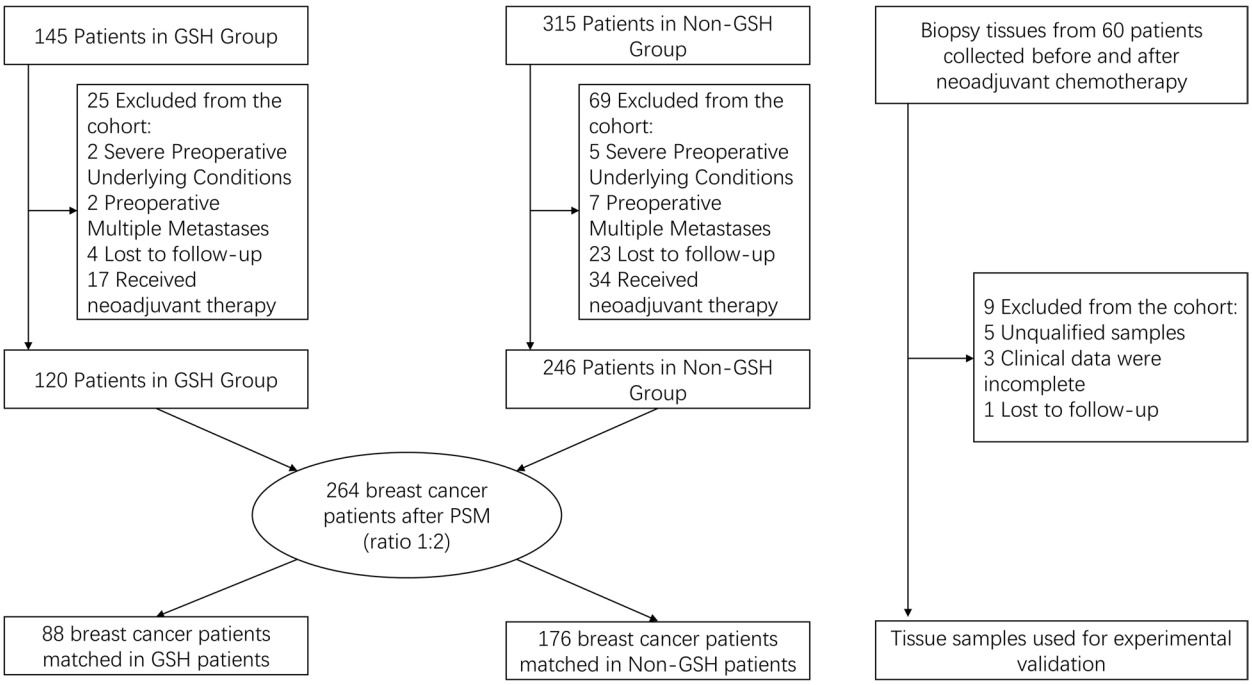

Fig. 1 Flow diagram of the research strategy. Abbreviations: GSH: glutathione; PSM: propensity score matching

associations between the duration of GSH administration and pre-chemotherapy indicators, such as menopausal status and preoperative liver function ($P > 0.05$) (Supplementary Table 4). However, patients in the HGSH group experienced more severe adverse chemotherapy reactions ($P < 0.001$), along with ALT and AST levels ($P < 0.001$). Moreover, patients in the HGSH group showed a higher incidence of postoperative recurrence and metastasis ($P = 0.003$) (Table 3), as well as reduced DFS ($P = 0.001$) and OS ($P = 0.026$) (Fig. 3C and D). These findings suggest that high GSH intake is a significant predictor of recurrence and metastasis in breast cancer patients after chemotherapy.

Survival analysis of patients receiving postoperative chemotherapy for breast cancer

The Kaplan–Meier curves for OS and DFS in the GSH and Non-GSH groups after PSM are presented in Fig. 3A and B. GSH administration was associated with shorter OS and DFS in breast cancer patients, with a median survival of 68 months in the GSH group, compared to 74 months in the Non-GSH group ($P = 0.023$). Additionally, we investigated the effect of the duration of GSH administration on OS and DFS. As illustrated in Fig. 3C and D, patients in the LGSH group exhibited significantly improved OS and DFS compared to those in the HGSH group (median OS: 70 months vs. 64 months, $P = 0.026$).

Risk factors on the prognosis of breast cancer patients receiving postoperative chemotherapy

Univariate logistic regression analysis identified Ki67 ≥ 30%, Lymphovascular invasion, Triple-negative status, TNM stage III, and High GSH intake as independent predictors of recurrence and metastasis in breast cancer patients after chemotherapy ($P < 0.05$). In comparison to Grade I and II histology, Grade III histology was found to be a risk factor for adverse breast cancer outcomes ($P = 0.018$). Multivariate analysis demonstrated that Ki67 ≥ 30% ($P < 0.001$), Lymphovascular invasion ($P = 0.023$), Triple-negative status ($P < 0.001$), and High GSH intake are independent predictors of poor prognosis (Table 4).

Measurement of the levels of intracellular GSH and the relative expression of NRF2, GPX4 and SOD1 in tissue samples following neoadjuvant chemotherapy

To further verify the role of GSH metabolism in chemotherapy resistance, we analyzed breast cancer data from the Gene Expression Omnibus (GEO) dataset (GEO: GSE140494). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Variation Analysis (GSVA) analysis revealed significant activation of the glutathione metabolism pathway in drug-resistant breast cancer tissues compared to sensitive tissues after FEC (5-Fluorouracil, Epirubicin, and Cyclophosphamide) + docetaxel treatment (Fig. 4A and B).

Table 2 Clinicopathological characteristics of patients receiving neoadjuvant chemotherapy

Variables	Neoadjuvant chemotherapy Patients ($n=51$)
Menopausal status	
Premenopausal	20(39.2%)
Postmenopausal	31(60.8%)
Age	
≥ 50 years	34(66.7%)
< 50 years	17(33.3%)
Hepatitis Infection	
No	45(88.2%)
Yes	6(11.8%)
ALT (U/L)	19.611 ± 2.056
AST (U/L)	24.127 ± 2.088
Chemotherapy regimen	
TCb	21(41.2%)
TAC	21(41.2%)
AC-T	5(9.8%)
TP	2(3.9%)
Other	2(3.9%)
Histology	
Infiltrative ductal	29(56.9%)
Infiltrative lobular	20(39.2%)
Other	2(3.9%)
Tumor size	
≥ 2 cm	45(88.2%)
< 2 cm	6(11.8%)
Number of tumors	
1	36(70.6%)
> 1	15(29.4%)
Grade of histology	
I	15(29.4%)
II	23(45.1%)
III	13(25.5%)
Lymphovascular invasion	
Yes	25(49%)
No	26(51%)
ER/PR	
Positive	35(68.6%)
Negative	16(31.4%)
HER2	
Positive	17(33.3%)
Negative	34(66.7%)
Ki67	
≥ 30%	26(51%)
< 30%	25(49%)
Molecular subtype	
Hormone receptor positive	35(68.6%)
Triple negative	9(17.6%)
HER2 positive	7(13.7%)
N stage	
0	19(37.3%)
1	12(23.5%)
2	15(29.4%)
3	5(9.8%)
T stage	
1	6(11.8%)
2	31(60.8%)
3	14(27.5%)
TNM stage	
1	2(3.9%)
2	33(64.7%)
3	16(31.4%)
Pathological response after neoadjuvant chemotherapy	
pPR	23(45.1%)
pCR	7(13.7%)
pNC	21(41.2%)

Abbreviations: *pPR* pathological partial response, *pCR* pathological complete response, *pNC* pathological no change

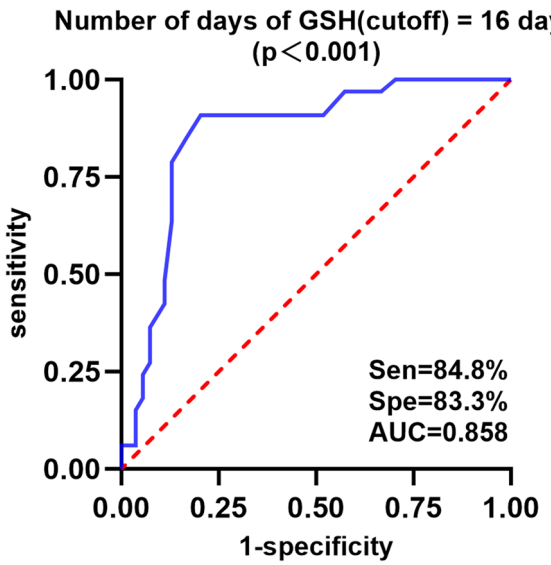

Fig. 2 ROC curve indicating that the highest sensitivity and specificity can be achieved using the average of 16 days, $P < 0.001$

Therefore, we assessed the levels of GSH and GSH metabolism-related antioxidant enzymes in the tissues of patients undergoing neoadjuvant chemotherapy. In our study, 51 participants who received neoadjuvant therapy were categorized as the sensitive group (patients with

Table 3 Postoperative clinicopathological characteristics in the LGSH (Low GSH intake) group and the HGSH (High GSH intake) group

Variables	LGSH Group ($n=232$)	HGSH Group ($n=32$)	t/χ^2	*P* value
Type of surgery			0.801	0.670
Mastectomy	209(90.1%)	30(93.8%)		
Breast conserving surgery	17(7.3%)	1(3.1%)		
Other	6(2.6%)	1(3.1%)		
Chemotherapy regimen			0.482	0.786
AC-T	130(56.0%)	16(50.0%)		
TCb	67(28.9%)	10(31.3%)		
Other	35(15.1%)	6(18.7%)		
Histology			0.661	0.719
Infiltrative ductal	176(75.9%)	23(71.9%)		
Infiltrative lobular	45(19.4%)	8(25.0%)		
Other	11(4.7%)	1(3.1%)		
Tumor size			1.300	0.254
≥ 2 cm	27(11.6%)	6(18.8%)		
< 2 cm	205(88.4%)	26(81.2%)		
Number of tumors			3.250	0.071
1	212(91.4%)	28(87.5%)		
> 1	20(8.6%)	4(12.5%)		
Grade of histology			0.804	0.669
I	11(4.7%)	2(6.3%)		
II	130(56.1%)	20(62.5%)		
III	91(39.2%)	10(31.2%)		
Lymphovascular invasion			0.306	0.580
Yes	163(70.3%)	24(75.0%)		
No	69(29.7%)	8(25.0%)		
Radiotherapy			0.011	0.980
Yes	159(68.5%)	22(68.8%)		
No	73(31.5%)	10(31.2%)		
Hormone therapy			0.889	0.346
Yes	147(63.4%)	23(71.9%)		
No	85(36.6%)	9(28.1%)		
ER/PR			0.889	0.346
Positive	147(63.4%)	23(71.9%)		
Negative	85(36.6%)	9(28.1%)		
HER2			0.351	0.553
Positive	61(26.3%)	10(31.2%)		
Negative	171(73.7%)	22(68.8%)		
Ki67			0.010	0.991
≥ 30%	138(59.5%)	19(59.4%)		
< 30%	94(40.5%)	13(40.6%)		
Molecular subtype			1.276	0.528
Hormone receptor positive	147(63.4%)	23(71.9%)		
Triple negative	37(15.9%)	5(15.6%)		
HER2 positive	48(20.7%)	4(12.5%)		
N stage			0.639	0.887
0	93(40.1%)	11(34.3%)		
1	72(31.1%)	10(31.3%)		
2	40(17.2%)	6(18.8%)		
3	27(11.6%)	5(15.6%)		

Table 3 (continued)

Variables	LGSH Group ($n=232$)	HGSH Group ($n=32$)	t/χ^2	*P* value
T stage			0.420	0.811
1	100(43.1%)	11(34.3%)		
2	119(51.3%)	18(56.3%)		
3	13(5.6%)	3(9.4%)		
TNM stage			0.478	0.788
1	43(18.6%)	6(18.8%)		
2	111(47.8%)	17(53.1%)		
3	78(33.6%)	9(28.1%)		
Adverse reaction during chemotherapy			30.745	< 0.001
Low	181(78.0%)	10(31.3%)		
High	51(22.0%)	22(68.7%)		
Transaminase after chemotherapy				
ALT	55.089 ± 2.237	88.106 ± 2.502	-2.401	< 0.001
AST	50.970 ± 1.096	73.312 ± 1.915	-3.028	< 0.001
Nausea and vomiting during chemotherapy			8.677	0.022
Yes	117(50.4%)	25(78.1%)		
No	115(49.6%)	7(21.9%)		
Metastasis and recurrence			8.885	0.003
Yes	52(22.4%)	15(46.8%)		
No	180(77.6%)	17(53.2%)		
Treatment after recurrence and metastasis			0.057	0.811
No surgery	39(75.0%)	11(73.3%)		
Surgery	13(25.0%)	4(26.7%)		
Survival status			8.222	0.014
Alive	199(85.8%)	21(65.6%)		
Dead	33(14.2%)	11(34.4%)		

pCR and pPR, $n=30$) or the resistant group (patients with pNC, $n=21$) (Table 2). Subsequently, we randomly selected 10 patients from both the sensitive and resistant groups to measure the expression levels of intracellular antioxidant enzymes and antioxidant substrate — GSH. The results indicated that, compared to the sensitive group, intracellular GSH levels in the resistant group were significantly higher, (Fig. 4C) and the relative expression levels of NRF2, GPX4 and SOD1 were substantially elevated (Fig. 4D). Applying the modified Response Evaluation Criteria in Solid Tumors (mRECIST), we observed that patients with higher intracellular GSH levels responded worse to neoadjuvant chemotherapy, as indicated by MRI scans, than those with lower intracellular GSH levels (Fig. 4E). Immunohistochemistry analysis indicated that chemotherapy-resistant patients had significantly higher expression levels of NRF2, GPX4 and SOD1 in tumor tissues compared to the sensitive ones. These results collectively confirmed that enhanced expression of GSH metabolism and antioxidant enzymes contributes to chemotherapy resistance in breast cancer patients (Fig. 4F).

Additionally, the ROC curve demonstrated strong predictive performance in distinguishing between drug-resistant and sensitive cells in the context of neoadjuvant chemotherapy (Fig. 4G). A cutoff value of 10.2 nmol/mg for GSH was identified, and the area under the ROC curve was 0.913, with a sensitivity of 90.2% and a specificity of 81.8%, demonstrating significant predictive capability in differentiating between the resistant group and sensitive group. Based on the ROC curve, we defined the IGH group (intracellular GSH high, ≥ 10.2 nmol/mg) and the IGL group (intracellular GSH low, < 10.2 nmol/mg). Detailed clinicopathological characteristics were presented in Table 5. Specifically, 28 of the 32 patients (87.5%) in the IGL group achieved pPR/pCR, which was significantly greater than the 4/19 (21.1%) in the IGH group ($P<0.001$) (Fig. 4H). Notably, only four patients of pNC were observed in the IGL group, whereas the majority were in the IGH group (Fig. 4I). Moreover, Kaplan–Meier survival curve revealed that the IGH group had poorer DFS ($P=0.038$) and OS ($P=0.037$) compared with the IGL group (Fig. 4J).

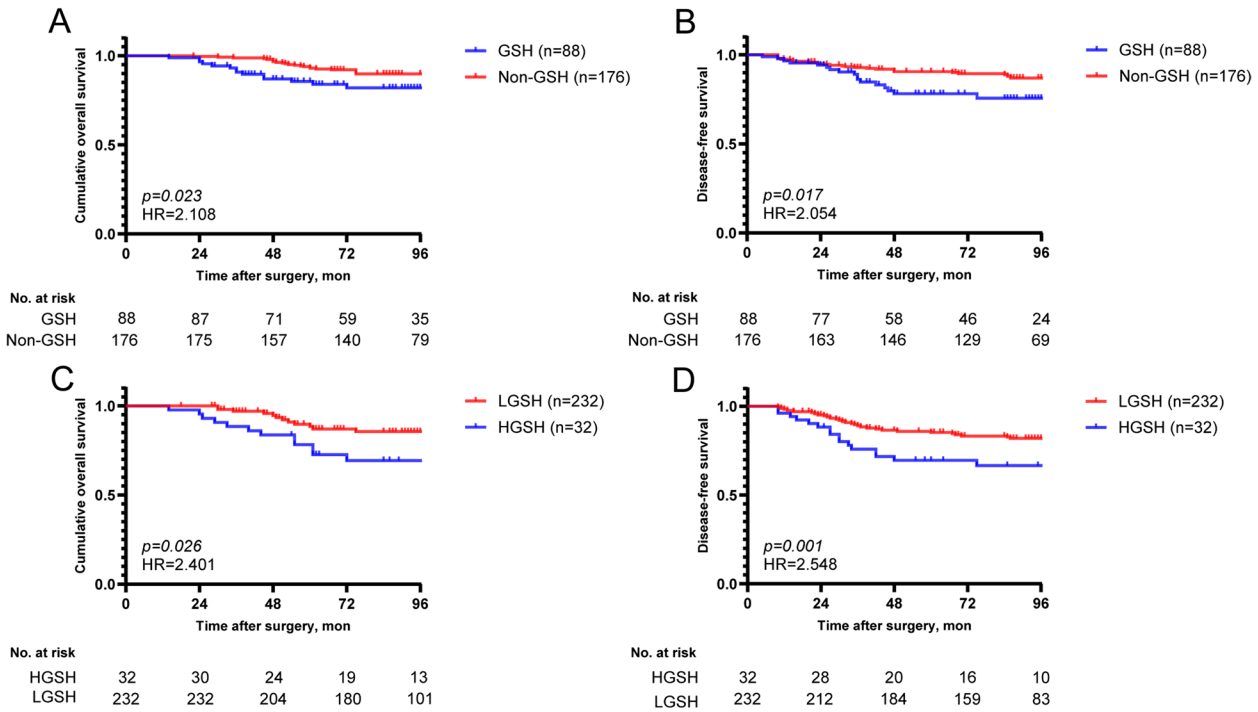

Fig. 3 Kaplan–Meier analysis for patients after adjuvant chemotherapy for breast cancer. **A** Survival curves of OS for the GSH group and the Non-GSH group. **B** Survival curves of DFS for the GSH group and the Non-GSH group. **C** Survival curves of OS for the LGSH (Low GSH intake) group and the HGSH (High GSH intake) group. **D** Survival curves of DFS for the LGSH group and HGSH group

Table 4 Univariate and multivariate Cox regression analyses of risk factors in breast cancer patients undergoing chemotherapy

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Menopausal status at diagnosis (Premenopausal vs. Postmenopausal)	1.328 (0.836–2.109)	0.229		
Age (< 50 years vs. ≥ 50 years)	1.051 (0.107–2.335)	0.159		
Hepatitis Infection (yes vs. no)	1.366 (0.921–1.810)	0.135		
Grade of histology (III vs. I-II)	1.344 (1.017–1.603)	0.018		
Lymphovascular invasion (yes vs. no)	3.474 (1.869–6.458)	< 0.001	2.484 (1.472–3.570)	0.023
Radiotherapy (yes vs. no)	2.136 (0.253–4.042)	0.405		
Ki67 (≥ 30% vs. < 30%)	2.578 (1.496–3.441)	< 0.001	1.485 (1.234–1.749)	< 0.001
Triple negative	3.065 (1.748–4.374)	< 0.001	2.542 (1.586–4.107)	< 0.001
TNM stage (3 vs. 1–2)	3.137 (1.965–5.008)	< 0.001		
Treatment after recurrence and metastasis (surgery vs. no surgery)	2.110 (0.112–4.307)	0.072		
High GSH intake	3.487 (1.176–5.790)	< 0.001	2.927 (1.344–4.589)	< 0.001

Discussion

Research on the influence of GSH on breast cancer chemotherapy sensitivity remains limited. In this study, by collecting clinicopathological data from patients receiving adjuvant chemotherapy, we found that high GSH intake increases the risk of recurrence after adjuvant chemotherapy. Additionally, our molecular experiments confirmed that chemotherapy drugs increase the expression levels of intracellular GSH and antioxidant enzymes, leading to chemotherapy resistance during neoadjuvant chemotherapy. We propose that the dosage and duration of GSH administration in clinical treatment be carefully evaluated and standardized to prevent overuse and mitigate the risk of chemotherapy resistance.

Increasing evidence indicates that elevated GSH level may contribute to chemotherapy resistance. Nevertheless, GSH is commonly used as a liver-protective drug after chemotherapy for breast cancer patients to mitigate chemotherapy-induced hepatotoxicity. Although many types of hepatoprotective drugs are available, GSH is often overused by clinicians, with its effects on tumors being overlooked. Previous studies have shown that the mechanism by which chemotherapy drugs induce tumor cell death primarily involves the promotion of ROS production [14]. An increase in ROS accelerates tumor cell death, while GSH, as a key component in redox homeostasis, plays a crucial role in scavenging ROS and neutralizing exogenous metabolites in tumor cells [15]. Thus, GSH is essential for tumor cell survival. Previous literature has demonstrated that GSH levels are elevated in various tumors, including breast, colon, laryngeal, gastric, and lung cancers [16]. Moreover, elevated GSH levels can protect tumor cells by reducing the cytotoxic effects of several chemotherapy drugs, including cisplatin, doxorubicin, melphalan, and paclitaxel, ultimately leading to drug resistance [17]. In clinical practice, during chemotherapy for patients with impaired liver function, the liver-protective effect of GSH is often prioritized, while the potential risk of chemotherapy resistance due to its non-standardized intake is frequently overlooked. This issue warrants deeper consideration. However, no systematic studies have been published to investigate the relationship between GSH intake and post-chemotherapy recurrence in breast cancer patients. Our study provides compelling speculation that GSH intake may adversely affect the prognosis of breast cancer patients undergoing chemotherapy.

After PSM, our study identified significant differences in transaminase levels, recurrence rates, and mortality after adjuvant chemotherapy between breast cancer patients between GSH group and Non-GSH group, indicating that GSH intake may have adverse effects on chemotherapy outcomes. To enhance risk stratification and optimize treatment selection, we divided the patients into two groups based on GSH intake duration: HGSH (≥ 16 days, $n=32$) group and LGSH (<16 days, $n=232$) group. Patients in the HGSH group exhibited significantly higher recurrence rates, and mortality compared to the LGSH group. Kaplan–Meier survival analysis also revealed a significantly decreased survival rate in the HGSH group. These findings suggest that high GSH intake correlates with poorer prognosis in breast cancer patients who underwent adjuvant chemotherapy. Specifically, the use of chemotherapy drugs resulted in elevated levels of ALT and AST, and an increase in the severity of side effects such as nausea and vomiting, contributing to high GSH intake. This, in turn, increased the likelihood of chemotherapy resistance, recurrence, and worsened prognosis. Previous in vitro studies demonstrated that reduced GSH levels in tumor cells promoted cell death, while elevated levels inhibited cell death [18]. Our findings align with these previous studies, leading us to hypothesize that GSH levels are higher in the tumor tissues of chemotherapy-resistant breast cancer patients compared to chemotherapy-sensitive patients.

In these retrospective studies, Triple-negative status, Lymphovascular invasion, Ki67 $\geq$ 30%, and High GSH intake were identified as independent risk factors for poor postoperative prognosis in breast cancer chemotherapy patients. Ki67 is commonly used as a marker for breast cancer proliferation [19]. In early-stage breast cancer, high Ki67 expression is associated with poor prognosis [20]. Additionally, elevated Ki67 levels are considered high-risk factors when selecting chemotherapy regimens [21]. It is well established that triple-negative breast cancer (TNBC) is highly aggressive [22], with significantly higher rates of metastasis and recurrence compared to other breast cancer subtypes [23], especially in isceral and

(See figure on next page.)

Fig. 4 High levels of intracellular GSH contribute to chemotherapy resistance in breast cancer. **A** KEGG analysis highlighted the glutathione metabolism pathway as the most significantly upregulated in the resistant patients. **B** GSVA revealed notable upregulation of the glutathione metabolism pathway in the resistant patients. **C** The levels of intracellular GSH in chemotherapy-resistant and chemotherapy-sensitive patients. **D** Relative expression levels of NRF2, GPX4 and SOD1 in chemotherapy-resistant and chemotherapy-sensitive tumor tissues. All assays were performed independently in triplicate. **E** Representative case illustrates the comparison between chemotherapy-sensitive (pCR + pPR, $n=32$) and chemotherapy-resistant (pNC, $n=19$) breast cancer patients of representative before and after neoadjuvant chemotherapy assessed by MRI images. Scale bar: 2 cm. **F** Representative case illustrates the comparison between high and low expression levels of NRF2, GPX4 and SOD1 in chemotherapy-sensitive (pCR + pPR, $n=32$) and chemotherapy-resistant (pNC, $n=19$) breast cancer tissues assessed by immunohistochemistry(IHC) staining. Scale bar: 200 μm. **G** ROC curves were used to determine the optimal cutoff value for intracellular GSH levels distinguishing between drug-resistant and sensitive cells in the context of neoadjuvant chemotherapy. **H** Evaluation of the pathological response to neoadjuvant chemotherapy in 51 patients based on modified RECIST criteria. **I** Waterfall plots displaying pathological responses in subgroups with IGH ($n=19$) and IGL ($n=32$) groups after neoadjuvant chemotherapy. **J** Kaplan–Meier survival curves depicting OS and DFS for the IGH group and the IGL group were generated using SPSS 25.0. Statistical significance was assessed via two-tailed unpaired Student's t test or one-way analysis of variance (ANOVA). Each bar represents the mean ± SD. Statistical notation: ns (not significant), $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$

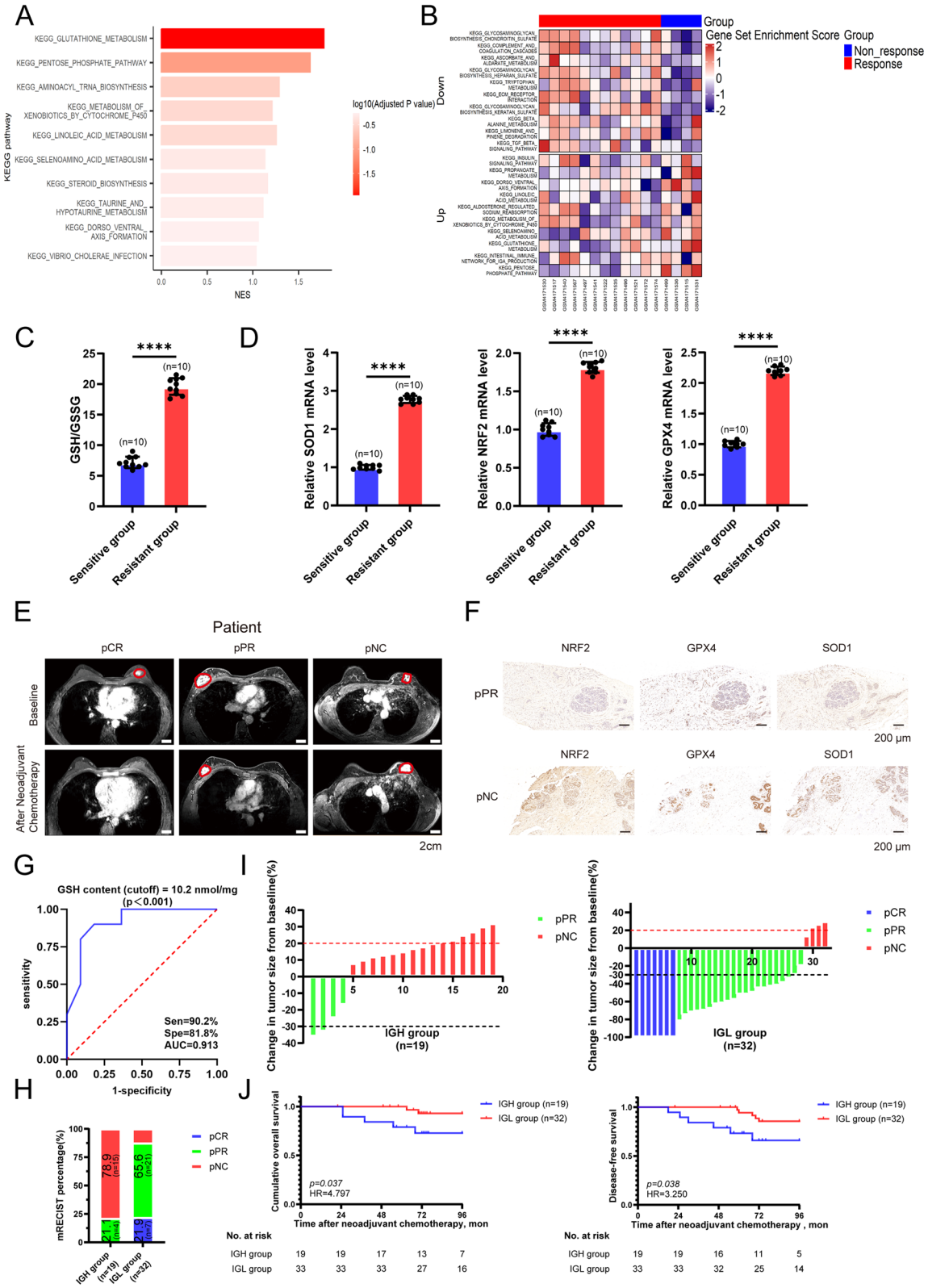

Fig. 4 (See legend on previous page.)

Table 5 Clinicopathological characteristics in the IGL (intracellular GSH low) group and the IGH (intracellular GSH high) group

Variables	IGL group ($n=32$)	IGH group ($n=19$)	t/χ	*P* value
Menopausal status			0.106	0.745
Premenopausal	12(37.5%)	8(42.1%)		
Postmenopausal	20(62.5%)	11(57.9%)		
Age			0.042	0.838
≥ 50 years	21(65.6%)	13(68.4%)		
< 50 years	11(34.4%)	6(31.6%)		
Hepatitis Infection			0.045	0.832
No	28(87.5%)	17(89.5%)		
Yes	4(12.5%)	2(10.5%)		
ALT (U/L)	19.503 ± 2.028	19.667 ± 2.419		
AST (U/L)	24.093 ± 2.512	24.146 ± 2.117		
Chemotherapy regimen			1.763	0.779
TCb	12(37.5%)	9(47.4%)		
TAC	14(43.7%)	7(36.8%)		
AC-T	3(9.4%)	2(10.5%)		
TP	1(3.1%)	1(5.3%)		
Other	2(6.3%)	0(0)		
Histology			0.800	0.670
Infiltrative ductal	17(53.1%)	12(63.1%)		
Infiltrative lobular	14(43.8%)	6(31.6%)		
Other	1(3.1%)	1(5.3%)		
Tumor size			0.473	0.492
≥ 2 cm	29(90.6%)	16(84.2%)		
< 2 cm	3(9.4%)	3(15.8%)		
Number of tumors			1.019	0.313
1	21(65.6%)	15(78.9%)		
> 1	11(34.4%)	4(21.1%)		
Grade of histology			0.141	0.932
I	10(31.3%)	5(26.3%)		
II	14(43.7%)	9(47.4%)		
III	8(25.0%)	5(26.3%)		
Lymphovascular invasion			0.954	0.329
Yes	14(43.8%)	11(57.9%)		
No	18(56.3%)	8(42.1%)		
ER/PR			0.001	0.980
Positive	22(68.8%)	13(68.4%)		
Negative	10(31.3%)	6(31.6%)		
HER2			0.671	0.413
Positive	12(37.5%)	5(26.3%)		
Negative	20(62.5%)	14(73.7%)		
Ki67			0.033	0.856
≥ 30%	16(50.0%)	10(52.6%)		
< 30%	16(50.0%)	9(47.4%)		
Molecular subtype			0.398	0.820
Hormone receptor positive	21(65.6%)	14(73.7%)		
Triple negative	6(18.8%)	3(15.8%)		
HER2 positive	5(15.6%)	2(10.5%)		
N stage			0.925	0.819
0	13(40.6%)	6(31.6%)		

Table 5 (continued)

Variables	IGL group ($n=32$)	IGH group ($n=19$)	t/χ	*P* value
1	8(25.0%)	4(21.1%)		
2	8(25.0%)	7(36.8%)		
3	3(9.4%)	2(10.5%)		
T stage			0.269	0.874
1	4(12.5%)	2(10.5%)		
2	20(62.5%)	11(57.9%)		
3	8(25.0%)	6(31.6%)		
TNM stage			0.645	0.724
1	1(3.1%)	1(5.3%)		
2	22(68.8%)	11(57.9%)		
3	9(28.1%)	7(36.8%)		
Pathological response after neoadjuvant chemotherapy			23.117	< 0.001
pPR	21(65.6%)	4(21.1%)		
pCR	7(21.9%)	0(0)		
pNC	4(12.5%)	15(78.9%)		
Survival status			4.044	0.044
Alive	29(90.6%)	13(68.4%)		
Dead	3(9.4%)	6(31.6%)		

brain metastasis [24]. To improve the prognosis of metastatic TNBC patients, antibody–drug conjugates (ADCs), such as sacituzumab govitecan, have been utilized to specifically deliver potent chemotherapy agents directly to cancer cells expressing targeted antigens, demonstrating promising efficacy [25]. Furthermore, lymphovascular invasion is a critical step in tumor dissemination and a strong adverse prognostic factor for breast cancer survival [26]. These pathways enable tumor cells to metastasize to distant organs [27]. Our study is consistent with previous research, revealing that Triple-negative status, Lymphovascular invasion, and Ki67 ≥ 30% are risk factors for recurrence after chemotherapy. Additionally, our study confirmed that High GSH intake is an independent risk factor for poor prognosis, offering critical cautionary note for clinical interventions in postoperative breast cancer treatment strategies.

To demonstrate the role of GSH in chemotherapy resistance, we analyzed transcriptomic data from chemotherapy patient samples in the GEO database and collected post-chemotherapy samples from patients who underwent neoadjuvant chemotherapy, assessing the expression levels of GSH and GSH synthesis-related antioxidant enzymes. Our study revealed a significant upregulation of the GSH metabolic pathway in chemotherapy-resistant patients. Moreover, in fresh samples from patients who underwent neoadjuvant chemotherapy, the GSH/GSSG levels in the resistant group were significantly higher than those in the sensitive group, which is consistent with previous research [28]. Additionally, studies have shown that antioxidant enzymes GPX4 and SOD1, along with the transcription factor NRF2, are involved in intracellular GSH redox reactions [29, 30]. The upregulation of NRF2 and SOD1, along with the activation of ROS scavenging pathways, can lead to resistance to chemotherapy or targeted therapy in various tumors. For instance, activation of NRF2 promotes ROS clearance in drug-resistant liver cancer cell lines [31]. Specifically, NRF2 is a transcription factor that governs the antioxidant pathway by targeting GSH metabolism-related genes. In chemotherapy-sensitive patients, Keap1 (Kelch-like ECH-associated protein 1) binds to NRF2 and mediates NRF2 ubiquitination via the Cul3-E3 ligase complex, promoting its degradation and maintaining NRF2 at low levels [32]. However, in resistant patients, oxidative stress causes a conformational change in Keap1, leading to the release of NRF2, which translocates to the nucleus and binds to antioxidant response element (ARE), activating the expression of downstream antioxidant genes, including GPX4, NQO1, HO-1 and SOD1 [33], and promoting the synthesis of GSH, as illustrated in Fig. 5.

GSH is synthesized in two steps: First, gamma-glutamylcysteine (γ-GC) is formed from glutamate and cysteine, which is the rate-limiting step in GSH production. In the second step, glutathione synthetase (GS) adds glycine to γ-GC to produce GSH. NRF2 plays a critical role in this process by upregulating the expression of

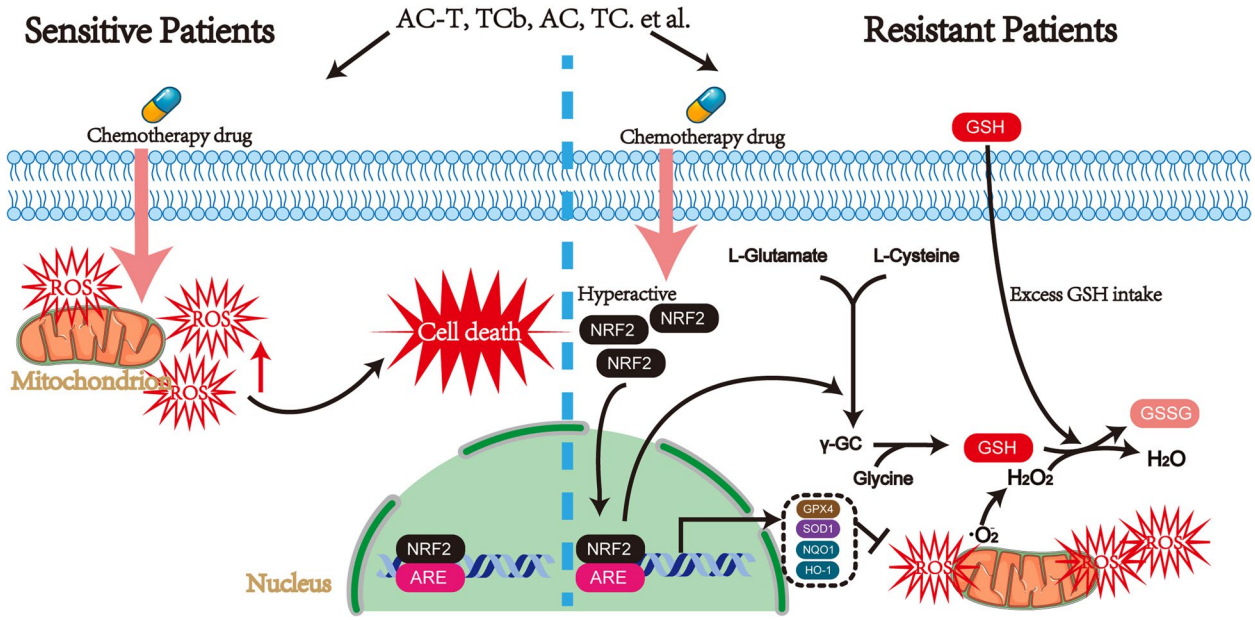

Fig. 5 Diagram of antioxidant mechanisms in chemotherapy-resistant and chemotherapy-sensitive patients

genes involved in the synthesis of γ-GC, thereby increasing the overall production of GSH [34]. Elevated GSH levels help neutralize ROS by reacting with hydrogen peroxide (H_2O_2), which is produced from superoxide radicals ($\cdot O_2^-$). This reaction converts H_2O_2 and GSH into water and GSSG, thereby reducing ROS-induced cellular damage [35]. Through NRF2-driven GSH synthesis, tumor cells are protected from oxidative stress, which contributes to chemotherapy resistance by reducing the cytotoxic effects of ROS generated during treatment.

In addition, the high expression of NRF2 enhances the expression of downstream SOD1, an important antioxidant enzyme that plays a crucial role in protecting cells from oxidative damage [36]. Specifically, SOD1 catalyzes the conversion of $\cdot O_2^-$ into H_2O_2, thereby reducing the levels of ROS and mitigating oxidative stress [37]. By activating the expression of SOD1, NRF2 helps maintain cellular redox balance and protects cells from oxidative injury [38].

GPX4 is an antioxidant enzyme that primarily reduces lipid peroxides, especially lipid hydroperoxides, into their corresponding alcohols, thus protecting cell membranes from oxidative damage [39]. This reaction is essential in lipid-rich environments, such as cellular membranes, where lipid peroxidation can lead to cell death. GPX4 works in conjunction with GSH, as it utilizes GSH as a cofactor to catalyze the reduction of lipid peroxides. By promoting the expression of GPX4 and GSH, the cellular defense mechanisms of the cell membrane are enhanced, reducing damage from lipid peroxides and maintaining cellular integrity [40].

Immune regulation within the tumor microenvironment plays a critical role in tumor progression and chemotherapy resistance. Zhang's study demonstrated that the synthesis of GSH is intricately linked to serine metabolism, with its metabolic byproducts lactate and 2-hydroxyglutarate (2-HG) suppressing the cytotoxic function of T cells. The metabolic pathways promote the development of an immunosuppressive vascular microenvironment, thereby facilitating resistance to chemotherapy [41]. In addition, Yang et al. found that active GSH metabolism within tumors further suppresses immune responses, such as IFN-γ signaling and PD-1/PD-L1 interactions, while reducing the number of M1 macrophages and increasing the proportion of M2 macrophages. Moreover, GPX4 promotes immune evasion by inhibiting ferroptosis. The combination of GPX4 inhibitors with immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis increases the proportion of CD8 + T cells, enhances their cytotoxic activity and induces ferroptosis, thereby promoting chemotherapy resistance [42]. In addition, the incorporation of ICIs into neoadjuvant chemotherapy regimens has been shown to increase the pCR rate [43]. However, despite their significant therapeutic potential in cancer treatment, it is essential to also focus on managing immune-related adverse events (irAEs). These adverse effects, including peripheral neuropathy and hearing loss, can have a profound impact on patient quality of life and require careful monitoring throughout treatment [44, 45].

Our study also found that NRF2, GPX4 and SOD1 expression levels were higher in the neoadjuvant chemotherapy-resistant group compared to the sensitive group,

further supporting our hypothesis and demonstrating the adverse effects of GSH on chemotherapy in breast cancer patients. Additionally, we discovered that a GSH threshold of 10.2 nmol/mg effectively differentiates resistant cells from sensitive cells, with an AUC of 0.913, providing a robust distinction between chemotherapy-resistant and sensitive patients. Patients were classified into IGH and IGL groups according to the cutoff value of intracellular GSH levels. Kaplan–Meier survival analysis revealed that the IGH group exhibited significantly poorer survival outcomes compared to the IGL group, suggesting that GSH may contribute to neoadjuvant chemotherapy resistance and lead to a worse prognosis in breast cancer patients. These findings offer a theoretical basis for precision treatment of breast cancer chemotherapy involving GSH.

Further investigation into the role and mechanisms of GSH and its associated antioxidant enzymes is essential for reversing chemotherapy resistance [46]. Several research teams have targeted GSH synthesis inhibitors as potential therapeutic agents. For instance, Li et al. examined the impact of GSH synthesis inhibitors on the cytotoxicity of cisplatin and gemcitabine in bile duct cancer cells [47]. Their findings revealed that low concentrations of GSH synthesis inhibitors significantly enhanced cancer cell death. This effect was similarly observed in a separate study on ovarian cancer [48]. Additionally, researchers have developed a range of glutathione-depleting nanodrugs for efficient cancer treatment [49], particularly in addressing chemotherapy resistance in TNBC [50]. Collectively, these studies underscore the significance of investigating the role of GSH in breast cancer patient prognosis, stressing the necessity of regulating GSH usage to prevent non-standardized intake and decrease post-chemotherapy recurrence.

However, our study has several limitations. As a single-center retrospective analysis, it may be subject to selection bias, and conclusions should be validated in large-scale multicenter prospective studies. Furthermore, the precise mechanisms of glutathione's impact remain unclear, and future research should investigate its specific roles in chemotherapy resistance and cell survival. Additionally, we primarily focus on the duration of GSH intake, without examining the effect of specific dosage on the prognosis of breast cancer patients undergoing chemotherapy. Future studies should employ stratified analyses or dose–response curves to explore the influence of different GSH doses on prognosis, offering guidance for more precise clinical application.

Conclusion

In conclusion, this study reveals that excessive GSH intake may contribute to chemotherapy resistance in breast cancer patients, identifying high GSH intake as an independent risk factor for tumor recurrence. Chemotherapy may induce tumor cells to produce GSH to counteract oxidative damage. These findings urge researchers and clinicians to assess the implications of GSH in promoting chemotherapy resistance and to evaluate the duration of GSH intake during treatment to optimize outcomes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-024-03626-9.

Supplementary Material 1.

Synopsis

This study investigates the impact of excessive glutathione intake on breast cancer chemotherapy resistance, showing that higher intake is linked to increased recurrence rates and poor prognosis, emphasizing the need for standardized glutathione usage during treatment.

Authors' contributions

Wentao Li, Yuanxiang Lu conceived and designed the experiments. Zhiyuan Zhang, Jiaru Gao, Wenwen Li, Shoukai Wang, Yuqing Su, Changzheng Zhu performed the experiments and analyzed the data. Zhiyuan Zhang, Jiaru Gao wrote the manuscript. Yuanxiang Lu, Linjiao Jia, Shuxin Kong contributed to project administration and revised the paper. Maosen Zhai, Wanyue Li, Wenkang Wang contributed to data collection and data analysis.

Funding

This work was supported by the Henan Provincial Breast Cancer Precision Prevention and Control Engineering Research Center (ZC20220050), the Henan Provincial People's Hospital—23456 Talent Engineering Project (ZC23456026) and the study on the Effect and Mechanism of Macrophage-Derived Exosomes Loaded with Doxorubicin for Targeted Therapy of Triple-Negative Breast Cancer (ZC20220114).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Zhengzhou University People's Hospital. All patients or their families provided written informed consent.

Consent for publication

We confirm that the manuscript has been read and approved by all named authors, and that there are no other persons who satisfied the criteria for authorship but are not listed. We also confirm that the order of the authors listed in the manuscript has been approved by all of us.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Breast Surgery, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, No.7 Weiwu Road, Zhengzhou, Henan 450003, China. [2]Henan Provincial Engineering Research Center of Breast Cancer Precise Prevention and Treatment, Zhengzhou, Henan 450003, China. [3]Department of Radiology, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, Zhengzhou, Henan 450003, China. [4]Department of Breast Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China.

Received: 2 November 2024 Accepted: 15 December 2024
Published online: 21 December 2024

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. Lancet. 2021;397(10286):1750–69.
3. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, et al. Breast cancer statistics, 2019. CA Cancer J Clin. 2019;69(6):438–51.
4. Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379(9814):432–44.
5. Li J, Jiang Z. Chinese Society of Clinical Oncology Breast Cancer (CSCO BC) guidelines in 2022: stratification and classification. Cancer Biol Med. 2022;19(6):769–73.
6. Sahin TK, Rizzo A, Aksoy S, Guven DC. Prognostic Significance of the Royal Marsden Hospital (RMH) score in patients with cancer: a systematic review and meta-analysis. Cancers (Basel). 2024;16(10):1835.
7. Lake RA, van der Most RG. A better way for a cancer cell to die. N Engl J Med. 2006;354(23):2503–4.
8. Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Reactive oxygen species and cancer paradox: to promote or to suppress? Free Radic Biol Med. 2017;104:144–64.
9. Xu Z, Qian J, Meng C, Liu Y, Ding Q, Wu H, et al. TME-targeting theranostic agent uses NIR tracking for tumor diagnosis and surgical resection and acts as chemotherapeutic showing enhanced efficiency and minimal toxicity. Theranostics. 2022;12(6):2535–48.
10. Mao Y, Ma S, Liu C, Liu X, Su M, Li D, et al. Chinese guideline for the diagnosis and treatment of drug-induced liver injury: an update. Hepatol Int. 2024;18(2):384–419.
11. European Association for the Study of the Liver. EASL clinical practice guideline: occupational liver diseases. J Hepatol. 2019;71(5):1022–37.
12. Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci. 2006;43(2):143–81.
13. Freites-Martinez A, Santana N, Arias-Santiago S, Viera A. Using the common terminology criteria for adverse events (CTCAE - Version 5.0) to evaluate the severity of adverse events of anticancer therapies. Actas Dermosifiliogr (Engl Ed). 2021;112(1):90–2.
14. Cui Q, Wang JQ, Assaraf YG, Ren L, Gupta P, Wei L, et al. Modulating ROS to overcome multidrug resistance in cancer. Drug Resist Updat. 2018;41:1–25.
15. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, et al. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev. 2013;2013:972913.
16. Yeh CC, Hou MF, Wu SH, Tsai SM, Lin SK, Hou LA, et al. A study of glutathione status in the blood and tissues of patients with breast cancer. Cell Biochem Funct. 2006;24(6):555–9.
17. Zhang W, Tung CH. Redox-responsive cisplatin nanogels for anticancer drug delivery. Chem Commun (Camb). 2018;54(60):8367–70.
18. Zhang C, Liu Z, Wang F, Zhang B, Zhang X, Guo P, et al. Nanomicelles for GLUT1-targeting hepatocellular carcinoma therapy based on NADPH depletion. Drug Deliv. 2023;30(1):2162160.
19. Matsubara N, Mukai H, Itoh K, Nagai S. Prognostic impact of Ki-67 overexpression in subgroups categorized according to St. Gallen with early stage breast cancer. Oncology. 2011;81(5–6):345–52.
20. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11(2):174–83.
21. Polley MY, Leung SC, McShane LM, Gao D, Hugh JC, Mastropasqua MG, et al. An international Ki67 reproducibility study. J Natl Cancer Inst. 2013;105(24):1897–906.
22. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007;13(15 Pt 1):4429–34.
23. Sharma P. Biology and management of patients with triple-negative breast cancer. Oncologist. 2016;21(9):1050–62.
24. de Ruijter TC, Veeck J, de Hoon JP, van Engeland M, Tjan-Heijnen VC. Characteristics of triple-negative breast cancer. J Cancer Res Clin Oncol. 2011;137(2):183–92.
25. Caputo R, Buono G, Piezzo M, Martinelli C, Cianniello D, Rizzo A, et al. Sacituzumab Govitecan for the treatment of advanced triple negative breast cancer patients: a multi-center real-world analysis. Front Oncol. 2024;14:1362641.
26. Gujam FJ, Going JJ, Edwards J, Mohammed ZM, McMillan DC. The role of lymphatic and blood vessel invasion in predicting survival and methods of detection in patients with primary operable breast cancer. Crit Rev Oncol Hematol. 2014;89(2):231–41.
27. Kikuchi E, Margulis V, Karakiewicz PI, Roscigno M, Mikami S, Lotan Y, et al. Lymphovascular invasion predicts clinical outcomes in patients with node-negative upper tract urothelial carcinoma. J Clin Oncol. 2009;27(4):612–8.
28. Xu X, Wang C, Zhang P, Gao X, Guan W, Wang F, et al. Enhanced intracellular reactive oxygen species by photodynamic therapy effectively promotes chemoresistant cell death. Int J Biol Sci. 2022;18(1):374–85.
29. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the hallmarks of cancer. Cancer Cell. 2018;34(1):21–43.
30. Osman AA, Arslan E, Bartels M, Michikawa C, Lindemann A, Tomczak K, et al. Dysregulation and epigenetic reprogramming of NRF2 signaling axis promote acquisition of cisplatin resistance and metastasis in head and neck squamous cell carcinoma. Clin Cancer Res. 2023;29(7):1344–59.
31. Zhang Y, Zhang Y, Tao H, Zhu J, Lu Y, Cheng F, et al. Targeting LINC01607 sensitizes hepatocellular carcinoma to Lenvatinib via suppressing mitophagy. Cancer Lett. 2023;576:216405.
32. Done AJ, Traustadóttir T. Nrf2 mediates redox adaptations to exercise. Redox Biol. 2016;10:191–9.
33. Liu P, Tian W, Tao S, Tillotson J, Wijeratne EMK, Gunatilaka AAL, et al. Non-covalent NRF2 activation confers greater cellular protection than covalent activation. Cell Chem Biol. 2019;26(10):1427–35.e5.
34. Jiang Y, Wang X, Hu D. Furanodienone induces G0/G1 arrest and causes apoptosis via the ROS/MAPKs-mediated caspase-dependent pathway in human colorectal cancer cells: a study in vitro and in vivo. Cell Death Dis. 2017;8(5):e2815.
35. Liu J, Tang H, Chen F, Li C, Xie Y, Kang R, et al. NFE2L2 and SLC25A39 drive cuproptosis resistance through GSH metabolism. Sci Rep. 2024;14(1):29579.
36. Boo SJ, Piao MJ, Kang KA, Zhen AX, Fernando P, Herath H, et al. Comparative study of autophagy in oxaliplatin-sensitive and resistant SNU-C5 colon cancer cells. Biomol Ther (Seoul). 2022;30(5):447–54.
37. Eggler AL, Small E, Hannink M, Mesecar AD. Cul3-mediated Nrf2 ubiquitination and antioxidant response element (ARE) activation are dependent on the partial molar volume at position 151 of Keap1. Biochem J. 2009;422(1):171–80.
38. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284(20):13291–5.
39. Huang YB, Jiang L, Liu XQ, Wang X, Gao L, Zeng HX, et al. Melatonin alleviates acute kidney injury by inhibiting NRF2/Slc7a11 axis-mediated ferroptosis. Oxid Med Cell Longev. 2022;2022:4776243.
40. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, et al. Regulation of ferroptotic cancer cell death by GPX4. Cell. 2014;156(1–2):317–31.
41. Zhang D, Li AM, Hu G, Huang M, Yang F, Zhang L, et al. PHGDH-mediated endothelial metabolism drives glioblastoma resistance to chimeric antigen receptor T cell immunotherapy. Cell Metab. 2023;35(3):517–34.e8.
42. Yang F, Xiao Y, Ding JH, Jin X, Ma D, Li DQ, et al. Ferroptosis heterogeneity in triple-negative breast cancer reveals an innovative immunotherapy combination strategy. Cell Metab. 2023;35(1):84–100.e8.
43. Rizzo A, Cusmai A, Acquafredda S, Giovannelli F, Rinaldi L, Misino A, et al. KEYNOTE-522, IMpassion031 and GeparNUEVO: changing the paradigm of neoadjuvant immune checkpoint inhibitors in early triple-negative breast cancer. Future Oncol. 2022;18(18):2301–9.
44. Rizzo A, Santoni M, Mollica V, Logullo F, Rosellini M, Marchetti A, et al. Peripheral neuropathy and headache in cancer patients treated with immunotherapy and immuno-oncology combinations: the MOUSEION-02 study. Expert Opin Drug Metab Toxicol. 2021;17(12):1455–66.
45. Guven DC, Erul E, Kaygusuz Y, Akagunduz B, Kilickap S, De Luca R, et al. Immune checkpoint inhibitor-related hearing loss: a systematic review and analysis of individual patient data. Support Care Cancer. 2023;31(12):624.

46. Liu J, Zhang Y, Yang B, Jia Y, Liu RT, Ding L, et al. Synergistic glutathione depletion and STING activation to potentiate dendritic cell maturation and cancer vaccine efficacy. Angew Chem Int Ed Engl. 2024;63(10):e202318530.
47. Li Q, Yin X, Wang W, Zhan M, Zhao B, Hou Z, et al. The effects of buthionine sulfoximine on the proliferation and apoptosis of biliary tract cancer cells induced by cisplatin and gemcitabine. Oncol Lett. 2016;11(1):474–80.
48. Sarkhosh-Inanlou R, Molaparast M, Mohammadzadeh A, Shafiei-Irannejad V. Sanguinarine enhances cisplatin sensitivity via glutathione depletion in cisplatin-resistant ovarian cancer (A2780) cells. Chem Biol Drug Des. 2020;95(2):215–23.
49. Cheng X, Xu HD, Ran HH, Liang G, Wu FG. Glutathione-depleting nanomedicines for synergistic cancer therapy. ACS Nano. 2021;15(5):8039–68.
50. Chen W, Hu F, Gao Q, Zheng C, Bai Q, Liu J, et al. Tumor acidification and GSH depletion by bimetallic composite nanoparticles for enhanced chemodynamic therapy of TNBC. J Nanobiotechnology. 2024;22(1):98.

Publisher's Note