Cystine/cysteine metabolism regulates the progression and response to treatment of triple-negative breast cancer (Review)

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Abstract. Breast cancer is the most prevalent neoplasm affecting women globally, of which a notable proportion of cases are triple-negative breast cancer (TNBC). However, there are limited curative treatment options for patients with TNBC, despite advancements in the field. Amino acids and amino acid transporters serve vital roles in the regulation of tumor metabolism. Notably, cystine and cysteine can interconvert via a redox reaction, with cysteine exerting control on cell survival and growth and exogenous cystine serving a crucial role in the proliferation of numerous types of cancers. Breast cancer has been reported to disrupt the cystine/cysteine metabolism pathway, as cystine and cysteine transporters affect the development and growth of tumors. The present review aims to provide a comprehensive overview of the metabolic pathways involving cystine and cysteine in normal and TNBC cells. Furthermore, the roles of cystine and cysteine transporters in TNBC progression and metastasis and their potential as therapeutic targets for treatment of TNBC are evaluated.

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1. Introduction

Breast cancer is the most prevalent cancer in the world and the fifth primary cause of cancer-related deaths (1). Triple-negative breast cancer (TNBC) accounts for 15-20% of all cases of breast cancer in women (2). TNBC is highly invasive, prone to metastasis and can easily recur, which leads to a poor clinical prognosis (3,4). Cytotoxic chemotherapy is the primary treatment option for TNBC (5). However, a number of patients with TNBC are resistant to chemotherapy or poly(ADP-ribose) polymerase inhibitor therapy (6). The remaining tumor and metastases following chemotherapy can often lead to tumor recurrence (7,8). Therefore, it is imperative to explore the metabolic characteristics of TNBC to identify novel therapeutic targets.

Unlike healthy cells, tumor cells exhibit notably altered metabolic patterns to obtain increased energy and resources for cell proliferation (9,10). Additionally, the cellular metabolism of tumor cells varies considerably among breast cancer subtypes, due to their highly heterogeneous nature (5). Differences in lipid metabolism among breast cancer subtypes may also be attributed to estrogen receptor status (11). A number of malignancies impact amino acid metabolism and particularly their transport system, which indicates that targeting these pathways could be a potential approach for treating certain types of cancers (12). Normal and cancer cells exhibit distinct amino acid compositions (12). Certain types of cancers rely on specific amino acids for growth. For instance, leucine and cystine are crucial in melanoma (13) and von Hippel-Lindau (VHL)-deficient renal cell carcinoma, respectively (14,15). The association between amino acid metabolism and breast cancer was first evaluated through the investigation of glutamine metabolism. Targeted inhibition of glutamine may represent a possible therapeutic strategy for TNBC, as it demonstrated the potential to increase the antitumor lymphocyte activity specific to TNBC (16). Furthermore, cystine/cysteine serves a crucial role in tumor cells. The involvement of cystine/cysteine metabolism in cancer was first evaluated in chronic lymphocytic leukemia (15,17). Cystine deficiency has been shown to promote the development of ferroptosis in numerous cancer cells, indicating that it may be a treatment target in breast cancer (18). The present study reviews the current research relating to the relationship between TNBC and cystine/cysteine metabolism.

2. Cystine/cysteine and TNBC

Cystine and cysteine. Cysteine and cystine are two interconvertible forms of a sulfur-containing amino acid. Although cysteine is a non-essential amino acid, its significance becomes pronounced during periods of high nutritional demand (18). Typically, cysteine is detected *in vitro* as cystine with disulfide bonds, owing to its susceptibility to redox changes (19). Upon transportation into cells, cystine can be converted into cysteine by the action of reducing agents such as glutathione (GSH) or specific enzymes like thioredoxin. Within cells, cysteine participates in cell metabolism, whereas upon cell exit, cysteine is oxidized into cystine and rejoins the circulatory system. In addition, intracellular cystine can be generated from homocysteine via the transsulfuration pathway, glutathione catabolism or through recycling from protein degradation (Fig. 1) (19-22). Homocysteine is a product of methionine demethylation. When cysteine is in short supply, homocysteine can enter the transsulfuration pathway by combining with serine, thereby providing cysteine (19). For example, the liver produces cysteine via glutathione catabolism or homocysteine through the transsulfuration pathway, following which it can be recycled through protein degradation (18). PIK3-catalytic subunit α mutant breast cancer cell lines also synthesize cysteine via the transsulfuration pathway (23). However, this process of amino acid interchange does not provide adequate cysteine for the rapid growth of malignant cells, thus necessitating the import of exogenous cystine into cells (24).

Cystine and TNBC. Exogenous cystine facilitates the survival and proliferation of numerous types of cancers. Basal-like breast cancer (BLBC) accounts for 70-80% of TNBC cases (25,26). A previous study demonstrated that cystine deprivation induces rapid necrosis in BLBC cells (27). It was demonstrated that cystine deprivation can alter the phenotype of TNBC cells, as cystine deprivation instigated the development of necroptosis and ferroptosis in TNBC cells, and triggered mitochondrial fragmentation and reactive oxygen species (ROS) production (28). By contrast, luminal-type breast cancer cells, which are commonly found in patients with breast cancer with hormone receptor-positive status, are cystine-independent and therefore less affected by cystine deprivation (27,28). The role of cystine in TNBC may be associated with the epithelial-mesenchymal transition (EMT) of cancer cells (7). EMT endows TNBC cells with characteristics of cancer stem cells (CSC), such as heightened metastatic potential and increased chemotherapy resistance (29). Furthermore, the mechanisms and behavioral traits of cell death mediated by cystine deletion have been shown to be similar to those in renal cell carcinoma cells with VHL deletion (13). Further research is warranted to elucidate the molecular mechanisms by which cystine deprivation affects TNBC cells.

Cysteine and TNBC. Cysteine serves a significant role in tumor progression, growth and the development of resistance to treatment (21). Both clinical and animal studies have indicated that cysteine might impede cancer development by enhancing cellular detoxification from carcinogens (23,30). Clinical investigations have established associations between cysteine levels and certain types of cancers, such as esophageal and gastric cancers (30). In a prospective case-control study, increased plasma cysteine levels were positively correlated with an increased risk of breast cancer. The TNBC patient subgroup did not exhibit a significant elevation in cysteine levels compared with the estrogen and progesterone-receptor positive breast cancer cases (30). Furthermore, decreased folate levels, which potentially resulted in the accumulation of homocysteine and its subsequent conversion to cysteine, were reported to increase the positive correlation between plasma cysteine levels and the risk of breast cancer. Consequently, the buildup of homocysteine due to folate deficiency may intensify its detrimental effects on breast cancer development (30).

In addition, *in vitro* studies have found positive associations between homocysteine levels and proliferation, as well as oxidative damage in certain types of cancers, including breast cancer, which suggests its potential as a tumor marker for monitoring tumor activity (31,32). Increased homocysteine expression levels may be related to the cysteine metabolism pathways, as homocysteine is required for cysteine synthesis via the transsulfuration process (30). Furthermore, increased homocysteine and cysteine expression levels have been associated with several metabolic conditions, such as obesity, high triglyceride levels and hypertension (30), which may promote the development of numerous cancers, including breast cancer (33,34). Therefore, further in-depth research should be performed to determine whether cysteine contributes to metabolic dysfunction related to breast cancer.

3. Cystine/cysteine transporters and breast cancer

Cystine transporters and breast cancer. System x_c^- is a cystine and glutamate antiporter responsible for the transportation of intracellular glutamate out of the cell and extracellular cystine into the cell at a 1:1 ratio (35). System x_c^- is expressed in most mammalian cultured cells. Cystine influx serves as a precursor for glutathione biosynthesis and is found to be upregulated in certain types of cancers (36). The influxing cystine consists of two chains: The light chain solute carrier family 7 member 11 (SLC7A11) and the heavy chain solute carrier family 3 member 2 (SLC3A2, also termed CD98), which are bound together by disulfide bonds (19,35,36). Cystine-glutamate antiporter activity is mediated by the catalytic component SLC7A11. xCT regulates the import of cystine into the cells and thus eliminates ROS, mitigates oxidative stress and promotes the survival of malignant cells (37). SLC3A2 maintains the stability of the SLC7A11 protein and helps locate SLC7A11 on the plasma membrane (36). SLC3A2 is a chaperone protein for numerous other subunits of heterotrimeric amino acid transporter systems, such as SLC7A5 and SLC7A8 (38).

Although xCT expression is confined to the plasma membrane in normal tissues (36,39), it is upregulated in certain types of cancer, such as colorectal cancer, malignant glioma and non-small cell lung cancer, which increases cystine uptake (40-42). These cancer cells can also modulate xCT expression either individually or in combination through cystine starvation-induced activation of the nuclear factors erythroid 2-related factor 2 and activation of transcription factor 4. Specifically, xCT expression levels are low in normal breast tissue samples but increased in hyperplastic breast

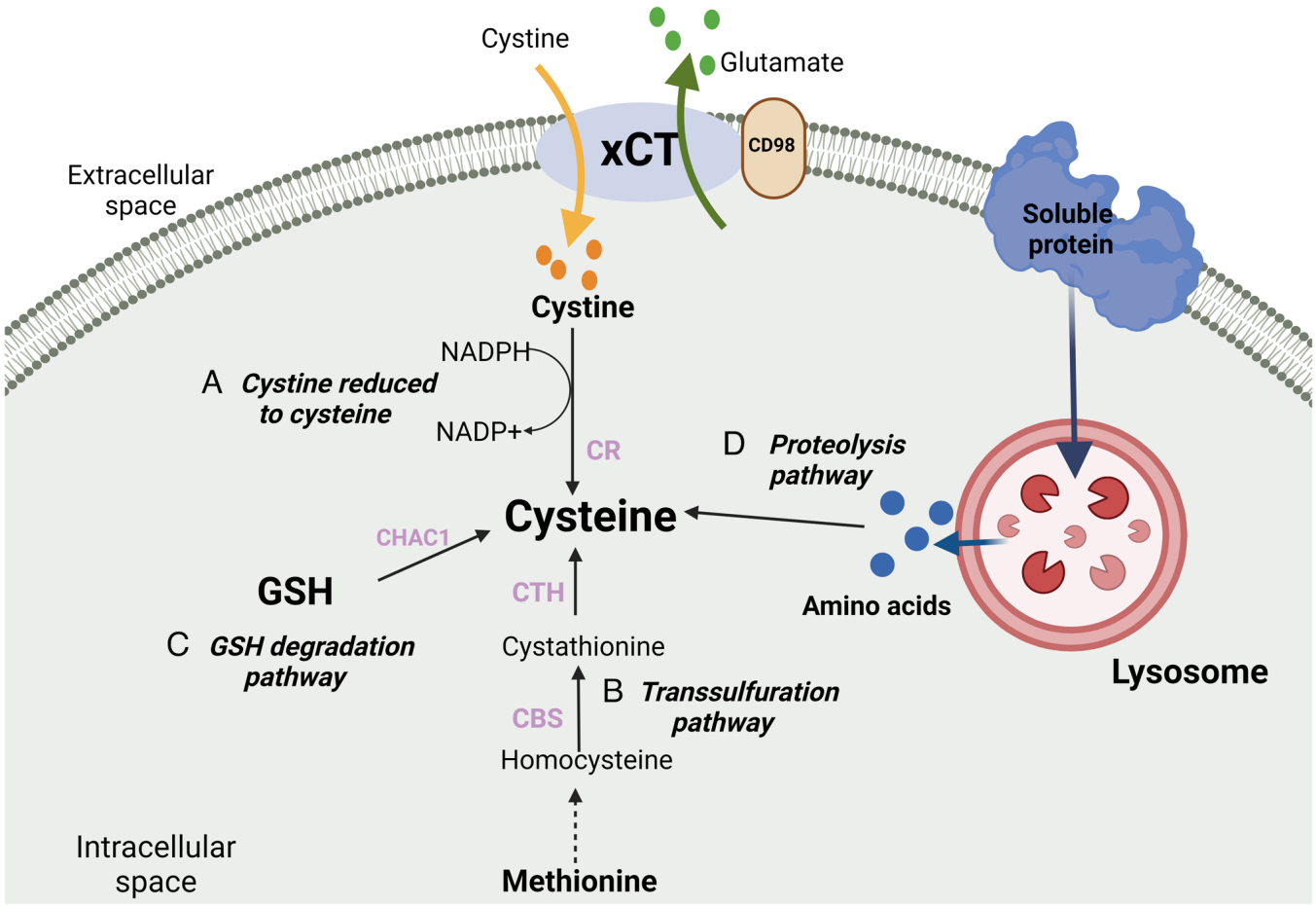

Figure 1. Cysteine synthesis pathways. (A) Cysteine synthesis through the system xc^-, which consists of the chaperone SLC3A2 and the SLC7A11 transporter. The extracellular oxidized cystine is imported by xCT in exchange for intracellular glutamate. xCT is associated with the stem-like cancer cell marker CD44v. Cystine is then converted into cysteine via cysteine reductase. (B) Cysteine synthesis via methionine conversion through the transsulfuration pathway. The two crucial steps include: i) The enzyme cystathionine-synthase, which converts homocysteine into cystathionine; and ii) the enzyme cystathionase, which produces cysteine from cystathionine. (C) Cysteine synthesis through intracellular degradation of glutathione via CHAC1. (D) Cysteine synthesis through soluble proteins in the extracellular fluid via macropinocytosis. The macropinosome fuses with the lysosome before hydrolysis with proteins to amino acids, including cystine. Created with BioRender.com. SLC3A2, solute carrier family 3 member 2; SLC7A11, solute carrier family 7member 11; GSH, glutathione; CR, cysteine reductase; CBS, cystathionine-synthase; CTH, cystathionase.

tissue samples and across all subtypes of breast cancer (43,44). Notably, xCT expression levels were significantly increased in TNBC compared with Luminal A, Luminal B and HER2-enriched breast cancer and correlated with poor patient prognosis. Timmerman *et al* (45) identified a subtype of TNBC by evaluating the functional metabolic profiles of breast cell lines, which demonstrated that xCT expression levels are upregulated in one-third of TNBC cases. Mucin 1 (MUC1)-C and CD44 variants (CD44v) could also regulate xCT expression levels and thereby control GSH levels to protect TNBC cells from ROS-induced damage and thus reduce the rate of cancer cell death (46,47).

Cysteine transporters and breast cancer. Cysteine uptake primarily relies on heterodimeric amino acid transporters (HATs) located on the cell membrane. HATs consist of the heavy chain solute carrier family (SLC) 3 and light chain SLC7 (48). The heavy chain SLC3 is essential for plasma membrane localization and light chain stabilization, and comprises of two subunits, SLC3A1 and SLC3A2 (49). Most light chain SLC7s interact with SLC3A2, while SLC3A1 forms heterodimers with SLC7A9, which are associated with cystinuria (50). A large proportion of cancer cells experience high levels of oxidative stress (51,52), which indicates that increasing cysteine through *de novo* biosynthesis or protein metabolism cannot satisfy the high demand for antioxidant defense in cancer cells. Instead, cancer cells typically acquire cystine from the extracellular environment primarily through the aforementioned cystine transporter, system x_c^-, which is then converted to cysteine (37). However, certain types of cancer cells, including chronic lymphocytic leukemia cells, can preferentially obtain extracellular cysteine via cysteine transporters (17). SLC3A1 has been indicated to promote the proliferation of stem cells in hepatocellular carcinoma cells (53) and was evidenced to be an effective therapeutic target in metastatic colorectal cancer through the recognition of metabolic signatures specific to metastatic cell lines (54). In addition, SLC3A1 may be used to assess the prognosis of renal clear cell carcinoma and is a prognostic indicator (55). Furthermore, SLC3A1 upregulation has been demonstrated to promote breast cancer growth through cysteine uptake (49).

Previous research has shown that SLC3A1 is highly upregulated in various breast cancer cell lines compared with the upregulation of the light chains SLC7A5, SLC7A7 and SLC7A9 (49). Furthermore, the expression levels of SLC3A1 correlates with the clinical stage of breast cancer (49). These findings indicate that intracellular cysteine in breast cancer cells may also be acquired from extracellular uptake via cysteine transporter proteins. However, it is unclear whether the expression of SCL3A1 in TNBC is distinct from other subtypes.

4. Cystine/cysteine metabolism and TNBC progression

Cystine/cysteine metabolism and TNBC proliferation. Glutamate serves a crucial role in the growth and development of malignant breast cancer cells, with its levels primarily regulated by amino acid transporters, notably SLC1A5 and SLC7A5 (56). SLC1A5 serves as a standalone prognostic marker in breast cancer and is implicated in drug resistance and breast cancer growth through numerous pathways (57-59). Similarly, SLC7A11 participates in the growth of TNBC (45,46). Although most breast cancer cells are resistant to glutamine deprivation, occasionally TNBC cells are sensitive to glutamine deprivation effects, necessitating the influx of cysteine via SLC7A11 (45).

The survival of breast cancer cells, particularly TNBC cells, is cystine-dependent. Therefore, inhibition of cystine uptake can rapidly induce breast cancer cell death, particularly affecting TNBC cells, which inhibits tumor progression and growth (28). SLC3A1 is highly upregulated in breast cancers. Patients with breast cancer with high expression levels of SLC3A1 expression tend to have worse prognoses across all histological grades compared with those with low SLC3A1 expression levels (49). In addition, overexpression of SLC3A1 was indicated to accelerate breast cancer growth in an *in vitro* study (49). Furthermore, inhibition of SLC3A1 was shown to suppress the malignancy-promoting effects of NAC in an animal model of TNBC (49).

Dietary supplementation with the antioxidant n-acetylcysteine (NAC) significantly accelerated tumor growth and reduced the survival of an animal model of lung carcinoma (60).

Cystine/cysteine metabolism and TNBC metastasis. With the exception of BLBC, metastasis of all subtypes of breast cancer predominantly occurs in the bone (61). However, BLBC has notably decreased rates of liver and bone metastasis compared with brain, lung and lymph node metastasis (61). TNBC is classified as a subtype of BLBC based on gene expression profiling, that demonstrates an overlap of 60-90% between TNBC and BLBC, compared with 11.5% for non-TNBC and BLBC (62). SLC7A11 contributes to the distant metastasis of breast cancer, particularly in cases of TNBC (44). Moreover, RNA sequencing analysis has demonstrated that SLC7A11 expression levels were significantly increased in mouse models of breast cancer with brain metastases (63,64). In addition, increased SLC7A11 expression levels in breast cancer cells have been associated with lung metastasis (65). The basal breast cancer marker CD44 binds to SLC7A11, which stabilizes its expression levels and consequently increases cystine intake. CD44 has also been also associated with increased lung metastasis in patients with TNBC (66). The aberrant upregulation of the transmembrane glycoprotein MUC1 has been demonstrated in TNBC; MUC1 directly binds to the intracellular domain of CD44, which further stabilizes the expression levels of SLC7A11 (7,46). Further characterization of these genes related to distant metastasis in breast cancer could provide a foundation for the development of novel therapeutic strategies to improve the prognosis of patients with cancer.

5. Cystine/cysteine metabolism and TNBC therapy

Cystine/cysteine metabolism and TNBC therapy. In previous years, the concept of metabolic reprogramming has garnered attention in the field of cancer therapy. In addition to glucose metabolism in cancer cells, amino acid metabolism has also become a research hotspot, notably treatments related to the restriction of amino acid metabolism that can selectively target highly proliferative cancer cells (Fig. 2; Table I).

Targeted xCT therapy. xCT and other amino acid transporters, such as L-type amino acid transporter 1 (LAT1) and LAT2, are potential therapeutic targets due to their availability and pharmacological properties. Particularly, xCT is essential for cell survival and the maintenance of glutathione (GSH) homeostasis and has thus been identified as key target for anticancer therapy. Increased expression levels of xCT signify that cancer cells rely on extracellular cystine, as it is upregulated in CSCs in several solid tumors, including breast cancer (43). Moreover, high xCT expression levels are associated with poor prognosis of patients with breast cancer (44,45). CSCs are a subset of cancer cells with the ability to self-renew, differentiate and possess unlimited self-renewal potential. Furthermore, the presence of EMT markers in CSCs confers a high metastatic potential in breast cancer (67). CSCs exhibit a resistance to radiation and chemotherapy due to the upregulation of numerous detoxifying enzymes, such as superoxide dismutase 2, glutathione peroxidases and heme oxygenase 1, that increase drug efflux and DNA repair capabilities (44,67,68). The development of therapies that effectively reduce tumor size through the eradication of CSCs is challenging. The identification of optimal CSC-associated targets is difficult as CSCs can switch between a more quiescent state and a proliferative state (69). Conventional anticancer methods primarily target developed tumors but are ineffective against CSCs. However, therapies targeting xCT, such as xCT inhibitors and targeted xCT vaccines, have been utilized in the treatment of exogenous cystine-dependent cancer cells (65).

xCT inhibitors

Erastin and imidazole ketone erastin (IKE). Erastin is an inhibitor of system xc^- *in vitro*. Chemical screening has shown that erastin induces ferroptosis in RAS-mutated variation cell lines (70). Erastin can inactivate SLC3A2 and block cysteine import (71). Furthermore, *in vitro* studies have shown that erastin can trigger ferroptosis in various cancer types, including brain, ovarian, renal, breast and lymphoma malignancies (72). Erastin induces considerable apoptosis and reduces the growth capacity of metastatic colorectal cancer, with IC_{50} values 3-fold lower compared with those of sulfasalazine (SASP),

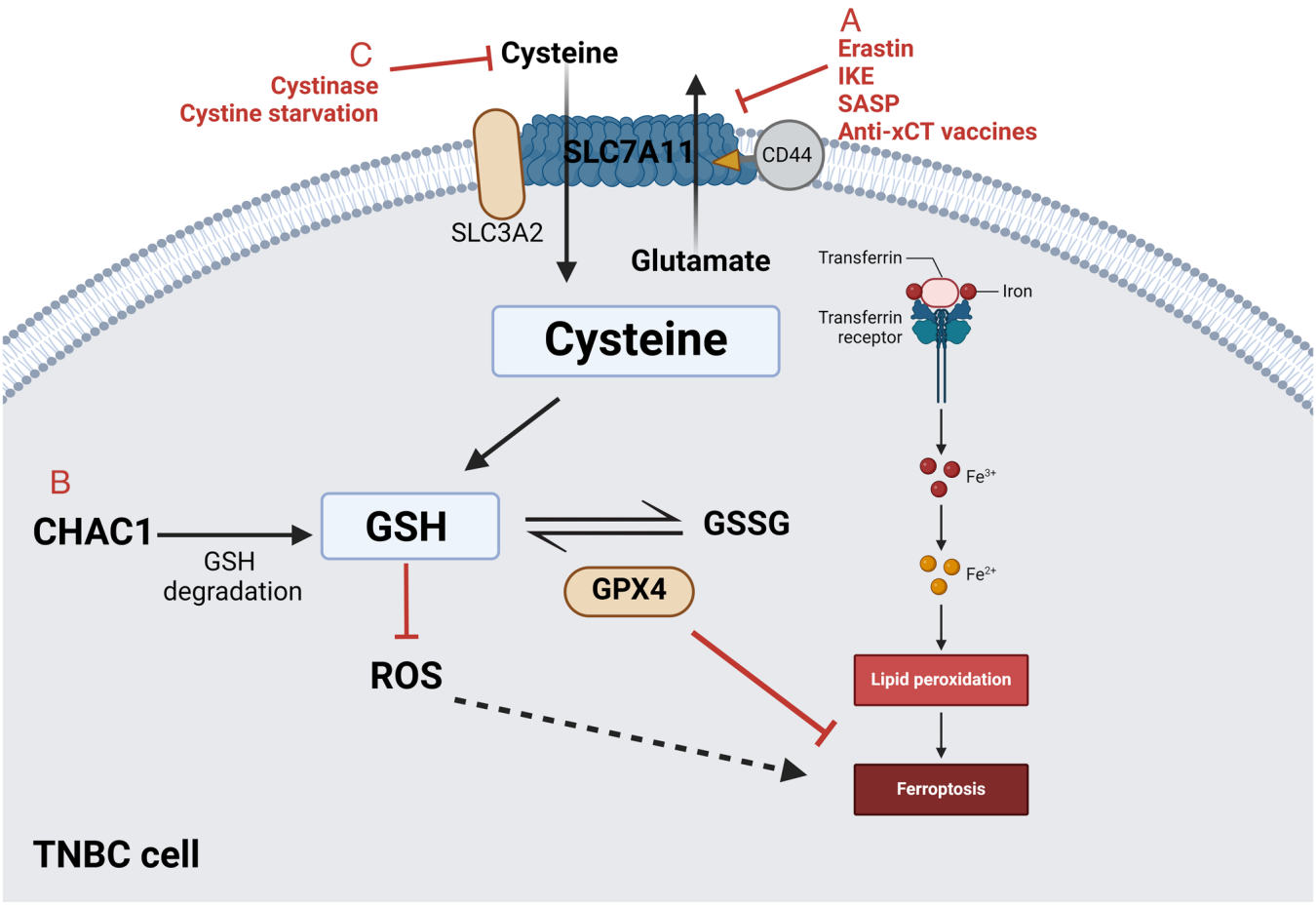

Figure 2. Prospective therapeutic strategies for TNBC. (A) Direct inhibition of SLC7A11 cystine transporter activity using erastin, IKE and DNA inhibitors. These drugs inhibit cystine uptake through SLC7A11 to induce lipid peroxidation and ferroptotic cell death. (B) CHAC1 degrades GSH by stimulating the GCN2-eIF2-ATF4-CHAC1 pathway. GSH depletion increases ROS levels, thereby promoting ferroptosis in TNBC. GPX4 reduces lipid hydroperoxides to lipid alcohols through GSH, thereby suppressing ferroptosis in TNBC. (C) Cystine starvation and cystinase can restrict tumor progression by decreasing the level of cystine. Created with BioRender.com. TNBC, triple-negative breast cancer; SLC7A11, solute carrier family 7 member 11; eIF2, eukaryotic translation initiation factor 2; ATF4, activating transcription factor 4; IKE, imidazole ketone erastin; SASP, sulfasalazine; GSSG, GSH disulfide; GPX4, GSH peroxidase 4; GSH, glutathione.

which suggested that erastin is more effective compared with SASP (54). IKE is an erastin analog containing isopropoxy, ketone, and imidazole moieties, with stronger water solubility and higher metabolic stability compared to erastin. A previous study found that an increased stable form of erastin, IKE, has antitumor activity in a mouse model of diffuse large B-cell lymphoma (DLBCL), which suggests that IKE is a viable therapeutic option for DLBCL. Specifically, IKE exerts anti-cancer activity by inducing lipid peroxidation and upregulating ferroptosis genes, including SLC7A11 (73).

SASP. SASP is an anti-inflammatory drug that is commonly used to treat ulcerative colitis and rheumatoid arthritis (74). Additionally, SASP inhibits the amino acid transporter xCT, which reduced intracellular GSH levels, diminishes cellular antioxidant defense and induces ferroptosis in cancer cells (74). In addition, SASP can inhibit TNBC growth through the suppression of the expression levels of inflammation-related genes such as NF-κB, TNF, RELA and IL-6 and MMP-related genes such as MMP1, MMP2 and MMP9 (75). SASP also demonstrates significant inhibitory activity against lymphoma, small cell lung cancer and prostate cancer (76-78). Clinical trials have explored the use of SASP alone or in combination with chemotherapy such as cisplatin for the treatment of gastric cancer (79,80) and lung cancer (81). SAS monotherapy reduced the number of $CD44v^+$ CSCs, while combination therapy significantly improved progression-free survival of patients with breast cancer (64). The National Cancer Institute conducted chemoinformatics analysis of 60 cell lines and showed a negative correlation between xCT expression levels and sensitivity to compounds associated with GSH-mediated resistance, which indicated that xCT expression induces chemoresistance via GSH-mediated ROS detoxification activity (82). Several studies have demonstrated that the combination of xCT inhibition with chemotherapy can effectively counteract this chemoresistance. For example, SASP reduces GSH expression levels at the cellular level, which induces growth arrest in breast cancer cells and increase the efficacy of anticancer drugs, such as doxorubicin (83), similar to preclinical investigations *in vitro* and *in vivo* (84).

Recent preclinical mouse models have shown that immune-targeting the xCT antigen can enhance the activity of a viral vectors-based vaccine against HER2, and reduces the growth of $HER2^+$ breast cancer, frequency of CSCs and

Table I. Summary of potential treatments for TNBC related to cystine/cysteine metabolism.

Therapy	Name	Functions	(Refs.)
xCT inhibitors	Sulfasalazine	i) Treatment for ulcerative colitis; and ii) suppresses TNBC growth.	(75)
	Erastin	i) Inhibits cysteine uptake and induces ferroptosis in certain types of cancer; and ii) reduced the growth of metastatic colorectal cancer.	(54,72)
	Imidazole ketone erastin	i) Anticancer activity in a xenograft model of diffuse large B cell lymphoma; and ii) induces ferroptosis in cancer.	(73)
Anti-xCT vaccines	DNA vaccine	i) Prevents breast cancer metastasis in mice; ii) inhibits subcutaneous tumor growth and lung metastasis in mice; and ii) increases the sensitivity of breast cancer stem cells to doxorubicin.	(44,83,92)
	Virus-like particle-based vaccine	Reduces lung metastasis of aggressive TNBC models.	(95)
	Viral vectors vaccine	Inhibits growth and metastasis of TNBC and $HER2^+$ breast cancer in preclinical models.	(91)
Ferroptosis	-	Clinical medications such as sorafenib and artesunate can induce ferroptosis in numerous types of cancer.	(111,112)
Cystine/cysteine starvation therapy	Dietary regulation	Suppresses the growth of glioma cells in mice.	(115)
	Cyst(e)inase	i) Suppresses the growth of prostate and breast cancer; and ii) doubles the median survival time of chronic lymphocytic leukemia.	(121)
Cysteine-related protein	-	Reduces the biological aggressiveness of TNBC or basal-like breast cancer.	(123)
Cysteine modification	-	Antitumor and targeting potentiality in TNBC.	(107)

-, not applicable; TNBC, triple-negative breast cancer.

metastatic events (85). Furthermore, SASP can inhibit xCT activity and TNBC growth (45,75). Notably, TNBC cell lines are more sensitive to SASP treatment compared with other breast cancer cell types (28). Also, SASP induces a more pronounced growth-inhibiting phenotype in TNBC cells compared to other breast cancer cell types (86). The combination of xCT immune-targeting treatments with traditional or novel medicines could activate the immune response and target differentiated cancer cells or CSCs. For example, the glioma-toxic impact of temozolomide (TMZ) can be potentiated by xCT inhibitors, such as erastin, thus enhancing the efficacy of TMZ (87). Furthermore, high vitamin E doses combined with SASP have synergistic antitumor effects on breast cancer cells (88).

Nevertheless, SASP can increase mortality in mice and also cause adverse effects such as weight loss and hypothermia, regardless of the impact of intact SASP or its metabolites on the system (89). However, clinical trials have shown that xCT inhibitors are ineffective in patients with glioma and have numerous SASP-related side effects, which highlights the need for further clinical trials in patients with high tumor burdens (90). Therefore, future clinical trials should ascertain whether SASP can be used in the treatment of breast cancer and particularly TNBC. Also, further studies should assess whether SASP may be used for the prevention or the treatment of metastatic breast cancer.

Targeted xCT vaccines. Several vaccines have been developed using plasmid DNA, virus-like particles (VLP) and viral vectors. A DNA vaccine targeting SLC7A11 has been developed to prevent breast cancer metastasis in mice. This vaccine induces a humoral immune response and delays the growth of initial tumors (43,91). The injection of a DNA vaccine expressing xCT proteins and immune targeting of xCT antigens on the cell surface effectively inhibited subcutaneous tumor growth and lung metastasis in mice and increased the chemosensitivity of breast CSCs to doxorubicin (44,83,92). Although numerous clinical trials are underway with promising results, DNA vaccines have not been formally utilized in patients with cancer (49,93). A novel VLP-based immunotherapy that targets xCT could significantly decrease lung metastases in a treatment model of aggressive TNBC (49,94,95).

Furthermore, the bovine herpesvirus 4 vector that expresses the full-length murine xCT protein could induce T-lymphocyte activation and generation of anti-xCT antibodies in mice, through the production of antibody-dependent cytotoxicity. Preclinical models of TNBC and $HER2^+$ breast cancer have shown that this immune response can suppresses the development and spread of the malignancy (91). These findings suggest that xCT immunotargeting could inhibit cancer growth and reduce the formation of metastases, although it may not represent a curative treatment method for the disease. Anti-xCT immunization may be utilized as an adjuvant therapy

in patients with breast cancer who are resistant to conventional therapies.

In addition to the ongoing clinical trials for the DNA targeting of SLC7A11, two anti-xCT vaccines are in the preclinical testing phase, at the time of writing (44,96,97). The development of effective vaccines for treating TNBC is anticipated to improve disease management. Future research to discover additional vaccines that target xCT and its associated pathways is warranted in order to broaden the spectrum of available treatment options and the clinical utility of such vaccines for patients with breast cancer. Furthermore, clinical studies are warranted to evaluate whether the anti-xCT vaccine could serve as an adjuvant therapy for patients with breast cancer who have developed resistance to standard therapy.

Ferroptosis. Ferroptosis is a form of cell death independent of apoptosis, which relies on iron ions and ROS to induce lipid peroxidation (70). Numerous pathways, including the GTP cyclohydrolase 1/tetrahydrobiopterin-phospholipid axis (98), the cystine/cysteine-GSH-peroxidase 4 (GPX4) axis (99,100), the ferroptosis suppressor protein 1-coenzyme Q (CoQ) 10 axis on the plasma membrane (101,102) and the mitochondrial dihydroorotate dehydrogenase/CoQ system, serve to counteract ferroptosis and maintain basal lipid peroxidation (103). As a result, ferroptosis is mainly induced trough the disruption of the aforementioned endogenous ferroptosis inhibitory pathways. Notably, the cystine/cysteine-involved ferroptosis pathway has been extensively studied. Depletion of cystine/cysteine reduces intracellular GSH levels and GPX4 activity within the cystine/cysteine-GSH-GPX4 axis, thus causing ferroptosis (18,99). Furthermore, cysteine depletion triggers extensive ferroptosis reactions compared with GSH deletion (104). Additionally, limiting cystine availability effectively induces ferroptosis in pancreatic cancer and head and neck cancer (105,106). Previous studies have demonstrated that it may be a promising strategy for treating certain types of tumors, including TNBC (107,108).

While TNBC may exhibit increased susceptibility to ferroptosis compared with other breast cancer subtypes, the precise mechanisms are unknown (28). A recent study demonstrated that cystine deprivation can reduce the expression of GPX4 by preventing mTORC1/eukaryotic translation initiation factor 4E-binding protein 1-mediated protein repression (109). Chemical and genetic inhibition of mTORC1 signaling induced ferroptosis in cancer cells under cystine starvation conditions (109). Furthermore, it was reported that the combination of mTORC1 inhibitors with IKE exhibits synergistic tumor suppression in lung cancer models (109). Moreover, SLC7A11 overexpression inhibited ROS-induced ferroptosis and counteracted p53-mediated tumor growth inhibition (110).

Previous research has demonstrated that xCT serves a crucial role in ferroptosis in certain cell types such as F98, 143B, BjeHLT, BJeLR, Calu-1 and HT-1080 cells (87,111). The aforementioned small molecule inhibitors targeting xCT could induce ferroptosis in tumor cells through inhibition of xCT activity. Moreover, several US Food and Drug Administration-approved clinical medications, including sorafenib and artesunate, demonstrated the ability to induce ferroptosis in numerous types of cancer cells, such as renal cell carcinoma, head and neck cancer and TNBC, which indicates that ferroptosis may be used in both preclinical and clinical settings (111,112). Lei *et al* (113) reported that the proteasomal chaperone gankyrin inhibits ferroptosis through the activation of the p53/SLC7A11/GPX4 signaling axis in TNBC cells. These novel mechanisms provide valuable insights to guide further research and develop new treatments for TNBC. Decreased cystine absorption and increased expression levels of ferroptosis-inhibiting molecules, such as SLC7A11 and GPX4, inhibited the occurrence of ferroptosis in TNBC cells (109). The aforementioned studies have identified a novel mechanism by which these molecules influence the survival of cancer cells through ferroptosis-induced cell death. In summary, triggering ferroptosis is an effective therapeutic strategy for TNBC.

Cystine starvation therapy. The dietary control of non-essential amino acids (NEAA) has garnered considerable attention in recent years, owing to the increased demand of NEAA reported in cancer cells (21). Certain types of cancers demonstrate an increased ability for amino acid synthesis, occasionally necessitating *de novo* NEAA synthesis to support their growth and viability (114). *In vivo* studies have demonstrated that dietary deprivation of methionine and cystine decreases the growth of glioma cells in mice (115). Furthermore, inhibition of asparagine production or elimination of asparagine from the diet could significantly inhibit the metastasis of breast cancer (116). Cystine is indispensable in TNBC growth and progression (27). An alternative treatment to xCT inhibition involves the depletion of its substrate, cystine. Cysteine deprivation or restriction of xCT by erastin or SASP could limit GSH synthesis, increase the levels of lipid peroxidative stress and ultimately induce ferroptosis in cysteine-dependent tumor cells (70). Cystine starvation has been shown to impede TNBC growth, which affects stem cell properties and chemotherapy resistance in TNBC (27), thereby promoting TNBC cell ferroptosis through activation of the general control nonderepressible 2-eukaryotic translation initiation factor 2 subunit 1-activating transcription factor 4-CHAC1 pathway through the specific cytosolic GSH degradation enzyme CHAC1 (28).

Cysteine deprivation may induce anticancer effects through reducing the ability of cancer cells to remove ROS. Cancer cells typically exhibit elevated levels of ROS (50). While excessive levels of ROS can cause apoptosis in cancer cells, moderate ROS levels promote tumor development and progression (49). Cancer cells mitigate these ROS levels through GSH, thereby inhibiting tumor cell apoptosis. Limiting dietary intake of cysteine lowers plasma cysteine levels, and consequently increases ROS levels in cancer cells through the reduction of GSH biosynthesis (117). However, cysteine starvation may protect tumor cells by disruption of the polyamine pathway, which is used by cancer cells to defend themselves against ROS (118).

Cysteine starvation may diminish the capacity of the immune system to destroy cancer cells as cysteine is necessary for T cell activation and function (119). However, a previous study has shown that cysteine starvation could increase the anticancer immune response of T cells (120). Consequently, the precise impact of cysteine starvation on the efficacy of the immune system in combating cancer remains currently unclear. Cramer *et al* (121) reported that cystinase could eliminate cystine from the body. Cyst(e)inase prevented breast

tumor growth, prolonged the survival of mice with chronic lymphocytic leukemia and slowed the growth of prostate tumors. In contrast to SASP toxicity, long-term treatment with cysteine enzymes did not produce toxic side effects in mice.

Despite the promising outcomes associated with cystine-restricted approaches, numerous challenges remain. Research has suggested that increased activity of the endogenous transsulfuration pathway could improve cancer cell survival in environments with diminished extracellular cysteine levels (22). Future investigations should confirm the efficacy of reducing amino acid intake in patients with cancer. Another challenge is that only a subset of TNBCs exhibiting EMT, which represents ~50% of TNBC cases, are vulnerable to eradication through cysteine depletion therapy (27). This suggests that a large portion of TNBC and luminal breast carcinoma cells are not reliant on cysteine and may demonstrate a limited response to cysteine deficiency. However, a significant number of luminal breast cancers and TNBCs with epithelial characteristics are also independent of cysteine and exhibit resistance to cysteine deficiency. Research suggests that histone deacetylase (HDAC) 6 inhibitors, such as tubacin, could improve the synthetic lethality of cysteine deprivation and overcome resistance in non-mesenchymal TNBCs (122).

Overall, cysteine starvation therapy aims to diminish the availability of cystine/cysteine or enhance its elimination. The impact of cysteine deprivation on TNBC encompasses various aspects, including proliferation, resistance to chemotherapy and antitumor properties. Therefore, additional research is needed to validate the impact of a reduced amino acid intake in patients with cancer.

Other treatments. In addition to the aforementioned prospective treatments outlined for TNBC, ongoing efforts are concentrated on developing novel therapeutic approaches. A promising avenue involves the utilization of cysteine-rich protein-based tactics targeting cysteine-rich angiogenic inducer 61 (CCN1/CYR61) (123), which could potentially mitigate the biological aggressiveness of TNBC or BLBC. CCN1 is a small secreted cysteine-rich protein which mainly supports cell adhesion, migration and survival. Another focus of current research is the development of customized protein nanoparticles tailored for TNBC treatment. This involves injecting whey protein nanoparticles modified to carry chemotherapy drugs like doxorubicin into mice induced with breast cancer and observing their tumor-killing effects. The use of cysteine modification presents a novel and potentially efficacious nanoparticulate strategy for the treatment of TNBC. Overall, the group with cysteine-modified nanoparticle treatment exhibited the greatest shrinkage and damage to breast cancer cells, indicating that cysteine-modified nanoparticles have excellent anticancer and targeting capabilities (107).

6. Conclusion and future perspectives

TNBC is a subtype of breast cancer that currently lacks effective therapeutic targets. The metabolism of cystine/cysteine metabolism serves an important role in the onset and progression of TNBC and may be closely associated with patient responses to therapy. Hence, targeting the cystine/cysteine metabolic system presents a viable therapeutic approach for the treatment of TNBC, as it leverages the metabolic characteristics, biomarkers and associated signaling pathways of the disease. However, further comprehensive research into the regulatory mechanisms governing cystine/cysteine metabolism in TNBC is warranted, given the limited number of recognized regulatory pathways. The exploration of additional mechanisms may identify vital targets for the development of efficacious treatments for TNBC. Similarly, the metabolic heterogeneity and adaptive responses in TNBC poses numerous challenges to the clinical application of metabolic therapy. The metabolic heterogeneity of cancer cells arises from a diverse range of factors, such as genetic mutations and the tumor microenvironment (5,7). Therefore, future studies could combine metabolic therapy with radiotherapy, chemotherapy, targeted therapy or immunotherapy to counteract tumor heterogeneity and improve the prognosis of patients with TNBC (119).

Another notable challenge is the cellular dependence on cysteine, as demonstrated by mesenchymal TNBC cells, whereas non-mesenchymal TNBC cells frequently display resistance to cysteine deprivation (27). The development of supplementary inhibitors may significantly improve the efficacy of targeted therapies aimed at exploiting cysteine dependence to treat certain subtypes of breast cancer. In the future, the identification of specific genes or pathways could serve as direct targets, in conjunction with cysteine deprivation for cancer therapy. The search for comprehensive and combination therapies for cancer, particularly strategies that target cysteine or cysteine metabolism, has shown promising results. The cooperation observed with HDAC6 inhibitors and erastin as treatments for TNBC highlights the need for further investigation into their cellular and molecular pathways. Therefore, the identification of novel cellular molecules or pathways could potentially serve as valuable direct targets for combination treatments of TNBC.

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Authors' contributions

WX and CX were responsible for the conception and design of study. WX wrote the first draft of the manuscript. WX and CX worked on further versions of the manuscript. Both authors read and approved the final version of the manuscript. Data authentication is not applicable.

Availability of data and materials

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Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Morris GJ, Naidu S, Topham AK, Guiles F, Xu Y, McCue P, Schwartz GF, Park PK, Rosenberg AL, Brill K and Mitchell EP: Differences in breast carcinoma characteristics in newly diagnosed African-American and Caucasian patients: A single-institution compilation compared with the National Cancer Institute's Surveillance, Epidemiology, and End Results database. Cancer 110: 876-884, 2007.
3. Singh DD and Yadav DK: TNBC: Potential targeting of multiple receptors for a therapeutic breakthrough, nanomedicine, and immunotherapy. Biomedicines 9: 876, 2021.
4. Dietze EC, Sistrunk C, Miranda-Carboni G, O'Regan R and Seewaldt VL: Triple-negative breast cancer in African-American women: Disparities versus biology. Nat Rev Cancer 15: 248-254, 2015.
5. Gandhi N and Das GM: Metabolic reprogramming in breast cancer and its therapeutic implications. Cells 8: 89, 2019.
6. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, *et al*: Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 379: 753-763, 2018.
7. Wang Z, Jiang Q and Dong C: Metabolic reprogramming in triple-negative breast cancer. Cancer Biol Med 17: 44-59, 2020.
8. Nedeljković M and Damjanović A: Mechanisms of chemotherapy resistance in triple-negative breast cancer-how we can rise to the challenge. Cells 8: 957, 2019.
9. Pavlova NN, Zhu J and Thompson CB: The hallmarks of cancer metabolism: Still emerging. Cell Metab 34: 355-377, 2022.
10. DeBerardinis RJ, Lum JJ, Hatzivassiliou G and Thompson CB: The biology of cancer: Metabolic reprogramming fuels cell growth and proliferation. Cell Metab 7: 11-20, 2008.
11. Tang X, Lin CC, Spasojevic I, Iversen ES, Chi JT and Marks JR: A joint analysis of metabolomics and genetics of breast cancer. Breast Cancer Res 16: 415, 2014.
12. Wei Z, Liu X, Cheng C, Yu W and Yi P: Metabolism of amino acids in cancer. Front Cell Dev Biol 8: 603837, 2021.
13. Sheen JH, Zoncu R, Kim D and Sabatini DM: Defective regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells in vitro and in vivo. Cancer Cell 19: 613-628, 2011.
14. Tang X, Wu J, Ding CK, Lu M, Keenan MM, Lin CC, Lin CA, Wang CC, George D, Hsu DS and Chi JT: Cystine deprivation triggers programmed necrosis in VHL-Deficient renal cell carcinomas. Cancer Res 76: 1892-1903, 2016.
15. Iglehart J, York RM, Modest AP, Lazarus H and Livingston D: Cystine requirement of continuous human lymphoid cell lines of normal and leukemic origin. J Biol Chem 252: 7184-7191, 1977.
16. Edwards DN, Ngwa VM, Raybuck AL, Wang S, Hwang Y, Kim LC, Cho SH, Paik Y, Wang Q, Zhang S, *et al*: Selective glutamine metabolism inhibition in tumor cells improves antitumor T lymphocyte activity in triple-negative breast cancer. J Clin Invest 131: e140100, 2021.
17. Zhang W, Trachootham D, Liu J, Chen G, Pelicano H, Garcia-Prieto C, Lu W, Burger JA, Croce CM, Plunkett W, *et al*: Stromal control of cystine metabolism promotes cancer cell survival in chronic lymphocytic leukaemia. Nat Cell Biol 14: 276-286, 2012.
18. Daher B, Vučetić M and Pouysségur J: Cysteine depletion, a key action to challenge cancer cells to ferroptotic cell death. Front Oncol 10: 723, 2020.
19. Stipanuk MH: Sulfur amino acid metabolism: Pathways for production and removal of homocysteine and cysteine. Annu Rev Nutr 24: 539-577, 2004.
20. Zhang HF, Klein Geltink RI, Parker SJ and Sorensen PH: Transsulfuration, minor player or crucial for cysteine homeostasis in cancer. Trends Cell Biol 32: 800-814, 2022.
21. Combs JA and DeNicola GM: The non-essential amino acid cysteine becomes essential for tumor proliferation and survival. Cancers (Basel) 11: 678, 2019.
22. Zhu J, Berisa M, Schwörer S, Qin W, Cross JR and Thompson CB: Transsulfuration activity can support cell growth upon extracellular cysteine limitation. Cell Metab 30: 865-876.e5, 2019.
23. Lien EC, Ghisolfi L, Geck RC, Asara JM and Toker A: Oncogenic PI3K promotes methionine dependency in breast cancer cells through the cystine-glutamate antiporter xCT. Sci Signal 10: eaao6604, 2017.
24. Pajares MA and Perez-Sala D: Mammalian sulfur amino acid metabolism: A nexus between redox regulation, nutrition, epigenetics, and detoxification. Antioxid Redox Signal 29: 408-452, 2018.
25. Yin L, Duan JJ, Bian XW and Yu SC: Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res 22: 61, 2020.
26. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X and Perou CM: Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 12: R68, 2010.
27. Tang X, Ding CK, Wu J, Sjol J, Wardell S, Spasojevic I, George D, McDonnell DP, Hsu DS, Chang JT and Chi JT: Cystine addiction of triple-negative breast cancer associated with EMT augmented death signaling. Oncogene 36: 4235-4242, 2016.
28. Chen MS, Wang SF, Hsu CY, Yin PH, Yeh TS, Lee HC and Tseng LM: CHAC1 degradation of glutathione enhances cystine-starvation-induced necroptosis and ferroptosis in human triple negative breast cancer cells via the GCN2-eIF2α-ATF4 pathway. Oncotarget 8: 114588-114602, 2017.
29. Shibue T and Weinberg RA: EMT, CSCs, and drug resistance: The mechanistic link and clinical implications. Nat Rev Clin Oncol 14: 611-629, 2017.
30. Lin J, Lee IM, Song Y, Cook NR, Selhub J, Manson JE, Buring JE and Zhang SM: Plasma homocysteine and cysteine and risk of breast cancer in women. Cancer Res 70: 2397-2405, 2010.
31. Wu LL and Wu JT: Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clin Chim Acta 322: 21-28, 2002.
32. Sun CF, Haven TR, Wu TL, Tsao KC and Wu JT: Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: A potential new tumor marker. Clin Chim Acta 321: 55-62, 2002.
33. Xue F and Michels KB: Diabetes, metabolic syndrome, and breast cancer: A review of the current evidence. Am J Clin Nutr 86: S823-S835, 2007.
34. La Vecchia C, Giordano SH, Hortobagyi GN and Chabner B: Overweight, obesity, diabetes, and risk of breast cancer: Interlocking pieces of the puzzle. Oncologist 16: 726-729, 2011.
35. Lewerenz J, Hewett SJ, Huang Y, Lambros M, Gout PW, Kalivas PW, Massie A, Smolders I, Methner A, Pergande M, *et al*: The Cystine/Glutamate Antiporter System xc-in Health and disease: From molecular mechanisms to novel therapeutic opportunities. Antioxid Redox Signa 18: 522-555, 2013.
36. Sato H, Tamba M, Ishii T and Bannai S: Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. J Biol Chem 274: 11455-11458, 1999.
37. Koppula P, Zhuang L and Gan B: Cystine transporter SLC7A11/xCT in cancer: Ferroptosis, nutrient dependency, and cancer therapy. Protein Cell 12: 599-620, 2020.
38. Kandasamy P, Gyimesi G, Kanai Y and Hediger MA: Amino acid transporters revisited: New views in health and disease. Trends Biochem Sci 43: 752-789, 2018.
39. Miyazaki I, Murakami S, Torigoe N, Kitamura Y and Asanuma M: Neuroprotective effects of levetiracetam target xCT in astrocytes in parkinsonian mice. J Neurochem 136: 194-204, 2015.
40. Sugano K, Maeda K, Ohtani H, Nagahara H, Shibutani M and Hirakawa K: Expression of xCT as a predictor of disease recurrence in patients with colorectal cancer. Anticancer Res 35: 677-682, 2015.
41. Robert SM, Buckingham SC, Campbell SL, Robel S, Holt KT, Ogunrinu-Babarinde T, Warren PP, White DM, Reid MA, Eschbacher JM, *et al*: SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Sci Transl Med 7: 289ra86, 2015.

42. Ji X, Qian J, Rahman SMJ, Siska PJ, Zou Y, Harris BK, Hoeksema MD, Trenary IA, Heidi C, Eisenberg R, *et al*: xCT (SLC7A11)-mediated metabolic reprogramming promotes non-small cell lung cancer progression. Oncogene 37: 5007-5019, 2018.
43. Ruiu R, Rolih V, Bolli E, Barutello G, Riccardo F, Quaglino E, Merighi IF, Pericle F, Donofrio G, Cavallo F and Conti L: Fighting breast cancer stem cells through the immune-targeting of the xCT cystine-glutamate antiporter. Cancer Immunol Immunother 68: 131-141, 2019.
44. Lanzardo S, Conti L, Rooke R, Ruiu R, Accart N, Bolli E, Arigoni M, Macagno M, Barrera G, Pizzimenti S, *et al*: Immunotargeting of antigen xCT attenuates Stem-like cell behavior and metastatic progression in breast cancer. Cancer Res 76: 62-72, 2016.
45. Timmerman LA, Holton T, Yuneva M, Louie RJ, Padró M, Daemen A, Hu M, Chan DA, Ethier SP, van 't Veer LJ, *et al*: Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. Cancer Cell 24: 450-465, 2013.
46. Hasegawa M, Takahashi H, Rajabi H, Alam M, Suzuki Y, Yin L, Tagde A, Maeda T, Hiraki M, Sukhatme VP, *et al*: Functional interactions of the cystine/glutamate antiporter, CD44v and MUC1-C oncoprotein in triple-negative breast cancer cells. Oncotarget 7: 11756-11769, 2016.
47. Ishimoto T, Nagano O, Yae T, Tamada M, Motohara T, Oshima H, Oshima M, Ikeda T, Asaba R, Yagi H, *et al*: CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc- and thereby promotes tumor growth. Cancer Cell 19: 387-400, 2011.
48. Fotiadis D, Kanai Y and Palacín M: The SLC3 and SLC7 families of amino acid transporters. Mol Aspects Med 34: 139-158, 2013.
49. Jiang Y, Cao Y, Wang Y, Li W, Liu X, Lv Y, Li X and Mi J: Cysteine transporter SLC3A1 promotes breast cancer tumorigenesis. Theranostics 7: 1036-1046, 2017.
50. Jeong JY, Oh KJ, Sohn JS, Jun DY, Shin JI, Lee KH and Lee JY: Clinical course and mutational analysis of patients with cystine stone: A Single-Center experience. Biomedicines 11: 2747, 2023.
51. Trachootham D, Alexandre J and Huang P: Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nat Rev Drug Discov 8: 579-591, 2009.
52. Chio C II and Tuveson DA: ROS in cancer: The burning question. Trends Mol Med 23: 411-429, 2017.
53. Haraguchi N, Inoue H, Tanaka F, Mimori K, Utsunomiya T, Sasaki A and Mori M: Cancer stem cells in human gastrointestinal cancers. Hum Cell 19: 24-29, 2006.
54. Tarragó-Celada J, Foguet C, Tarrado-Castellarnau M, Marin S, Hernández-Alias X, Perarnau J, Morrish F, Hockenbery D, Gomis RR, Ruppin E, *et al*: Cysteine and folate metabolism are targetable vulnerabilities of metastatic colorectal cancer. Cancers (Basel) 13: 425, 2021.
55. Bao WEI, Han Q, Guan X, Wang Z and Gu MIN: Solute carrier-related signature for assessing prognosis and immunity in patients with clear-cell renal cell carcinoma. Oncol Res 31: 181-192, 2023.
56. Cha YJ, Kim ES and Koo JS: Amino acid transporters and glutamine metabolism in breast cancer. Int J Mol Sci 19: 907, 2018.
57. Todorova VK, Kaufmann Y, Luo S and Klimberg VS: Tamoxifen and raloxifene suppress the proliferation of estrogen receptor-negative cells through inhibition of glutamine uptake. Cancer Chemother Pharmacol 67: 285-291, 2011.
58. Bernhardt S, Bayerlová M, Vetter M, Wachter A, Mitra D, Hanf V, Lantzsch T, Uleer C, Peschel S, John J, *et al*: Proteomic profiling of breast cancer metabolism identifies SHMT2 and ASCT2 as prognostic factors. Breast Cancer Res 19: 112, 2017.
59. Jeon YJ, Khelifa S, Ratnikov B, Scott DA, Feng Y, Parisi F, Ruller C, Lau E, Kim H, Brill LM, *et al*: Regulation of glutamine carrier proteins by RNF5 determines breast cancer response to ER Stress-Inducing chemotherapies. Cancer Cell 27: 354-369, 2015.
60. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P and Bergo MO: Antioxidants accelerate lung cancer progression in mice. Sci Transl Med 6: 221ra15, 2014.
61. Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH, Nielsen TO and Gelmon K: Metastatic behavior of breast cancer subtypes. J Clin Oncol 28: 3271-3277, 2010.
62. Weigelt B and Reis-Filho JS: Histological and molecular types of breast cancer: Is there a unifying taxonomy? Nat Rev Clin Oncol 6: 718-730, 2009.
63. Sato R, Nakano T, Hosonaga M, Sampetrean O, Harigai R, Sasaki T, Koya I, Okano H, Kudoh J, Saya H and Arima Y: RNA sequencing analysis reveals interactions between breast cancer or melanoma cells and the tissue microenvironment during brain metastasis. Biomed Res Int 2017: 8032910, 2017.
64. Hosonaga M, Saya H and Arima Y: Molecular and cellular mechanisms underlying brain metastasis of breast cancer. Cancer Metastasis Rev 39: 711-720, 2020.
65. Ruiu R, Cossu C, Iacoviello A, Conti L, Bolli E, Ponzone L, Magri J, Rumandla A, Calautti E and Cavallo F: Cystine/glutamate antiporter xCT deficiency reduces metastasis without impairing immune system function in breast cancer mouse models. J Exp Clin Cancer Res 42: 254, 2023.
66. Hu J, Li G, Zhang P, Zhuang X and Hu G: A CD44v+ subpopulation of breast cancer stem-like cells with enhanced lung metastasis capacity. Cell Death Dis 8: e2679, 2017.
67. Lollini PL, Cavallo F, Giovanni CD and Nanni P: Preclinical vaccines against mammary carcinoma. Expert Rev Vaccines 12: 1449-1463, 2013.
68. Nagano O, Okazaki S and Saya H: Redox regulation in stem-like cancer cells by CD44 variant isoforms. Oncogene 32: 5191-5198, 2013.
69. Kinoshita H, Okabe H, Beppu T, Chikamoto A, Hayashi H, Imai K, Mima K, Nakagawa S, Ishimoto T, Miyake K, *et al*: Cystine/glutamic acid transporter is a novel marker for predicting poor survival in patients with hepatocellular carcinoma. Oncol Rep 29: 685-689, 2013.
70. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, *et al*: Ferroptosis: An Iron-dependent form of nonapoptotic cell death. Cell 149: 1060-1072, 2012.
71. Hassannia B, Vandenabeele P and Vanden Berghe T: Targeting ferroptosis to iron out cancer. Cancer Cell 35: 830-849, 2019.
72. Xu T, Ding W, Ji X, Ao X, Liu Y, Yu W and Wang J: Molecular mechanisms of ferroptosis and its role in cancer therapy. J Cell Mol Med 23: 4900-4912, 2019.
73. Zhang Y, Tan H, Daniels JD, Zandkarimi F, Liu H, Brown LM, Uchida K, O'Connor OA and Stockwell BR: Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. Cell Chem Biol 26: 623-633.e9, 2019.
74. Zheng YW, Miao XY, Xiong L, Chen B, Kong FH, Zhou JJ, Liu ZT, Wen Y, Zhang ZJ and Zou H: Sulfasalazine sensitizes polyhematoporphyrin-mediated photodynamic therapy in cholangiocarcinoma by targeting xCT. Front Pharmacol 12: 723488, 2021.
75. Yu H, Hu K, Zhang T and Ren H: Identification of target genes related to sulfasalazine in triple-negative breast cancer through Network pharmacology. Med Sci Monit 26: e926550, 2020.
76. Gout P, Buckley A, Simms C and Bruchovsky N: Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x(c)-cystine transporter: A new action for an old drug. Leukemia 15: 1633-1640, 2001.
77. Guan J, Lo M, Dockery P, Mahon S, Karp CM, Buckley AR, Lam S, Gout PW and Wang YZ: The x c-cystine/glutamate antiporter as a potential therapeutic target for small-cell lung cancer: Use of sulfasalazine. Cancer Chemother Pharmacol 64: 463-472, 2009.
78. Doxsee DW, Gout PW, Kurita T, Lo M, Buckley AR, Wang Y, Xue H, Karp CM, Cutz JC, Cunha GR and Wang YZ: Sulfasalazine-induced cystine starvation: Potential use for prostate cancer therapy. Prostate 67: 162-171, 2007.
79. Shitara K, Doi T, Nagano O, Imamura CK, Ozeki T, Ishii Y, Tsuchihashi K, Takahashi S, Nakajima TE, Hironaka S, *et al*: Dose-escalation study for the targeting of CD44v+ cancer stem cells by sulfasalazine in patients with advanced gastric cancer (EPOC1205). Gastric Cancer 20: 341-349, 2017.
80. Shitara K, Doi T, Nagano O, Fukutani M, Hasegawa H, Nomura S, Sato A, Kuwata T, Asai K, Einaga Y, *et al*: Phase 1 study of sulfasalazine and cisplatin for patients with CD44v-positive gastric cancer refractory to cisplatin (EPOC1407). Gastric Cancer 20: 1004-1009, 2017.
81. Otsubo K, Nosaki K, Imamura CK, Ogata H, Fujita A, Sakata S, Hirai F, Toyokawa G, Iwama E, Harada T, *et al*: Phase I study of salazosulfapyridine in combination with cisplatin and pemetrexed for advanced non-small-cell lung cancer. Cancer Sci 108: 1843-189, 2017.
82. Dai Z, Huang Y, Sadee W and Blower P: Chemoinformatics analysis identifies cytotoxic compounds susceptible to chemoresistance mediated by glutathione and cystine/glutamate transport system xc-. J Med Chem 50: 1896-1906, 2007.
83. Narang VS, Pauletti GM, Gout PW, Buckley DJ and Buckley AR: Sulfasalazine-induced reduction of glutathione levels in breast cancer cells: Enhancement of growth-inhibitory activity of doxorubicin. Chemotherapy 53: 210-217, 2007.
84. Barutello G, Di Lorenzo A, Gasparetto A, Galiazzi C, Bolli E, Conti L and Cavallo F: Immunotherapy against the Cystine/Glutamate Antiporter xCT improves the efficacy of APR-246 in preclinical breast cancer models. Biomedicines 10: 2843, 2022.

85. Conti L, Bolli E, Di Lorenzo A, Franceschi V, Macchi F, Riccardo F, Ruiu R, Russo L, Quaglino E, Donofrio G and Cavallo F: Immunotargeting of the xCT Cystine/Glutamate antiporter potentiates the efficacy of HER2-targeted immunotherapies in breast cancer. Cancer Immunol Res 8: 1039-53, 2020.
86. Yu H, Yang C, Jian L, Guo S, Chen R, Li K, Qu F, Tao K, Fu Y, Luo F and Liu S: Sulfasalazine-induced ferroptosis in breast cancer cells is reduced by the inhibitory effect of estrogen receptor on the transferrin receptor. Oncol Rep 42: 826-838, 2019.
87. Sehm T, Rauh M, Wiendieck K, Buchfelder M, Eyüpoglu IY and Savaskan NE: Temozolomide toxicity operates in a xCT/SLC7a11 dependent manner and is fostered by ferroptosis. Oncotarget 7: 74630, 2016.
88. Wei CW, Yu YL, Lu JY, Hung YT, Liu HC and Yiang GT: Anti-cancer effects of sulfasalazine and Vitamin E succinate in MDA-MB 231 Triple-negative breast cancer cells. Int J Med Sci 16: 494-500, 2019.
89. Verbruggen L, Sprimont L, Bentea E, Janssen P, Gharib A, Deneyer L, De Pauw L, Lara O, Sato H, Nicaise C and Massie A: Chronic sulfasalazine treatment in mice induces system x_c^--Independent adverse effects. Front Pharmacol 12: 625699, 2021.
90. Robe PA, Martin DH, Nguyen-Khac MT, Artesi M, Deprez M, Albert A, Vanbelle S, Califice S, Bredel M and Bours V: Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults. BMC Cancer 9: 372, 2009.
91. Donofrio G, Tebaldi G, Lanzardo S, Ruiu R, Bolli E, Ballatore A, Rolih V, Macchi F, Conti L and Cavallo F: Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells. Oncoimmunology 7: e1494108, 2018.
92. Wang F and Yang Y: Suppression of the xCT-CD44v antiporter system sensitizes triple-negative breast cancer cells to doxorubicin. Breast Cancer Res Treat 147: 203-210, 2014.
93. Marin-Acevedo JA, Soyano AE, Dholaria B, Knutson KL and Lou Y: Cancer immunotherapy beyond immune checkpoint inhibitors. J Hematol Oncol 11: 8, 2018.
94. Ruzzi F, Semprini MS, Scalambra L, Palladini A, Angelicola S, Cappello C, Pittino OM, Nanni P and Lollini PL: Virus-like particle (VLP) vaccines for cancer immunotherapy. Int J Mol Sci 24: 12963, 2023.
95. Rolih V, Caldeira J, Bolli E, Salameh A, Conti L, Barutello G, Riccardo F, Magri J, Lamolinara A, Parra K, *et al*: Development of a VLP-based vaccine displaying an xCT extracellular domain for the treatment of metastatic breast cancer. Cancers 12: 1492, 2020.
96. Bolli E, O'Rourke JP, Conti L, Lanzardo S, Rolih V, Christen JM, Barutello G, Forni M, Pericle F and Cavallo F: A Virus-Like-Particle immunotherapy targeting Epitope-specific anti-xCT expressed on cancer stem cell inhibits the progression of metastatic cancer in vivo. Oncoimmunology 7: e1408746, 2018.
97. Lopes A, Vandermeulen G and Preat V: Cancer DNA vaccines: Current preclinical and clinical developments and future perspectives. J Exp Clin Cancer Res 38: 146, 2019.
98. Kraft VAN, Bezjian CT, Pfeiffer S, Ringelstetter L, Müller C, Zandkarimi F, Merl-Pham J, Bao X, Anastasov N, Kössl J, *et al*: GTP cyclohydrolase 1/Tetrahydrobiopterin counteract ferroptosis through lipid remodeling. ACS Cent Sci 6: 41-53, 2019.
99. Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, *et al*: Regulation of ferroptotic cancer cell death by GPX4. Cell 156: 317-331, 2014.
100. Imai H, Matsuoka M, Kumagai T, Sakamoto T and Koumura T: Lipid Peroxidation-dependent cell death regulated by GPx4 and ferroptosis. Curr Top Microbiol Immunol 403: 143-170, 2017.
101. Lv Y, Liang C, Sun Q, Zhu J, Xu H, Li X, Li X, Li YY, Wang Q, Yuan H, *et al*: Structural insights into FSP1 catalysis and ferroptosis inhibition. Nat Commun 14: 5933, 2023.
102. Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, Roberts MA, Tong B, Maimone TJ, Zoncu R, *et al*: The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature 575: 688-692, 2019.
103. Mao C, Liu X, Zhang Y, Lei G, Yan Y, Lee H, Koppula P, Wu S, Zhuang L, Fang B, *et al*: DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. Nature 593: 586-590, 2021.
104. Harris IS, Endress JE, Coloff JL, Selfors LM, McBrayer SK, Rosenbluth JM, Takahashi N, Dhakal S, Koduri V, Oser MG, *et al*: Deubiquitinases maintain protein homeostasis and survival of cancer cells upon glutathione depletion. Cell Metab 29: 1166-1181.e6, 2019.
105. Shin D, Lee J, You JH, Kim D and Roh JL: Dihydrolipoamide dehydrogenase regulates cystine deprivation-induced ferroptosis in head and neck cancer. Redox Biol 30: 101418, 2020.
106. Ensink EJT, Medeiros HCD, Thurston G, Pardal A, Yu L and Lunt SY: Pyruvate kinase activity regulates cystine starvation induced ferroptosis through malic enzyme 1 in pancreatic cancer cells. bioRxiv: 2023. doi: 10.1101/2023.09.15.557984.
107. Singh S, Maurya P, Rani S, Mishra N, Nisha R, Singh P and Saraf SA: Development of doxorubicin hydrochloride-loaded whey protein nanoparticles and its surface modification with N-acetyl cysteine for triple-negative breast cancer. Drug Deliv Transl Res 12: 3047-3062, 2022.
108. Hangauer MJ, Viswanathan VS, Ryan MJ, Bole D, Eaton JK, Matov A, Galeas J, Dhruv HD, Berens ME, Schreiber SL, *et al*: Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. Nature 551: 247-250, 2017.
109. Zhang Y, Swanda RV, Nie L, Liu X, Wang C, Lee H, Lei G, Mao C, Koppula P, Cheng W, *et al*: mTORC1 couples cyst(e)ine availability with GPX4 protein synthesis and ferroptosis regulation. Nat Commun 12: 1589, 2021.
110. Jiang L, Kon N, Li T, Wang SJ, Su T, Hibshoosh H, Baer R and Gu W: Ferroptosis as a p53-mediated activity during tumour suppression. Nature 520: 57-62, 2015.
111. Dixon SJ, Patel DN, Welsch M, Skouta R, Lee ED, Hayano M, Thomas AG, Gleason CE, Tatonetti NP, Slusher BS and Stockwell BR: Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. Elife 3: e02523, 2014.
112. Lu B, Chen XB, Ying MD, He QJ, Cao J and Yang B: The role of ferroptosis in cancer development and treatment response. Front Pharmacol 8: 992, 2018.
113. Lei M, Zhang YL, Huang FY, Chen HY, Chen MH, Wu RH, Dai SZ, He GS, Tan GH and Zheng WP: Gankyrin inhibits ferroptosis through the p53/SLC7A11/GPX4 axis in triple-negative breast cancer cells. Sci Rep 13: 21916, 2023.
114. Tajan M and Vousden KH: Dietary approaches to cancer therapy. Cancer Cell 37: 767-785, 2020.
115. Liu H, Zhang W, Wang K, Wang X, Yin F, Li C, Wang C, Zhao B, Zhong C, Zhang J, *et al*: Methionine and cystine double deprivation stress suppresses glioma proliferation via inducing ROS/autophagy. Toxicol Lett 232: 349-355, 2015.
116. Knott SRV, Wagenblast E, Khan S, Kim SY, Soto M, Wagner M, Turgeon MO, Fish L, Erard N, Gable AL, *et al*: Asparagine bioavailability governs metastasis in a model of breast cancer. Nature 554: 378-381, 2018.
117. Jimenez-Alonso JJ and Lopez-Lazaro M: Dietary manipulation of amino acids for cancer therapy. Nutrients 15: 2879, 2023.
118. Zhang T, Bauer C, Newman AC, Uribe AH, Athineos D, Blyth K and Maddocks ODK: Polyamine pathway activity promotes cysteine essentiality in cancer cells. Nat Metab 2: 1062-1076, 2020.
119. Srivastava MK, Sinha P, Clements VK, Rodriguez P and Ostrand-Rosenberg S: Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res 70: 68-77, 2010.
120. Wang W, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, Liao P, Lang X, Kryczek I, Sell A, *et al*: $CD8^+$ T cells regulate tumour ferroptosis during cancer immunotherapy. Nature 569: 270-274, 2019.
121. Cramer SL, Saha A, Liu J, Tadi S, Tiziani S, Yan W, Triplett K, Lamb C, Alters SE, Rowlinson S, *et al*: Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth. Nat Med 23: 120-127, 2017.
122. Alothaim T, Charbonneau M and Tang X: HDAC6 inhibitors sensitize non-mesenchymal triple-negative breast cancer cells to cysteine deprivation. Sci Rep 11: 10956, 2021.
123. Espinoza IKC, Park CH, Vander Steen T, Kleer CG, Wiley E, Rademaker A, Cuyàs E, Verdura S, Buxó M, Reynolds C, *et al*: Depletion of CCN1/CYR61 reduces triple-negative/basal-like breast cancer aggressiveness. Am J Cancer Res 12: 839-851, 2022.