

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.

Contents

1. Introduction
2. Cystine/cysteine and TNBC
3. Cystine/cysteine transporters and breast cancer
4. Cystine/cysteine metabolism and TNBC progression
5. Cystine/cysteine metabolism and TNBC therapy
6. Conclusion and future perspectives

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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

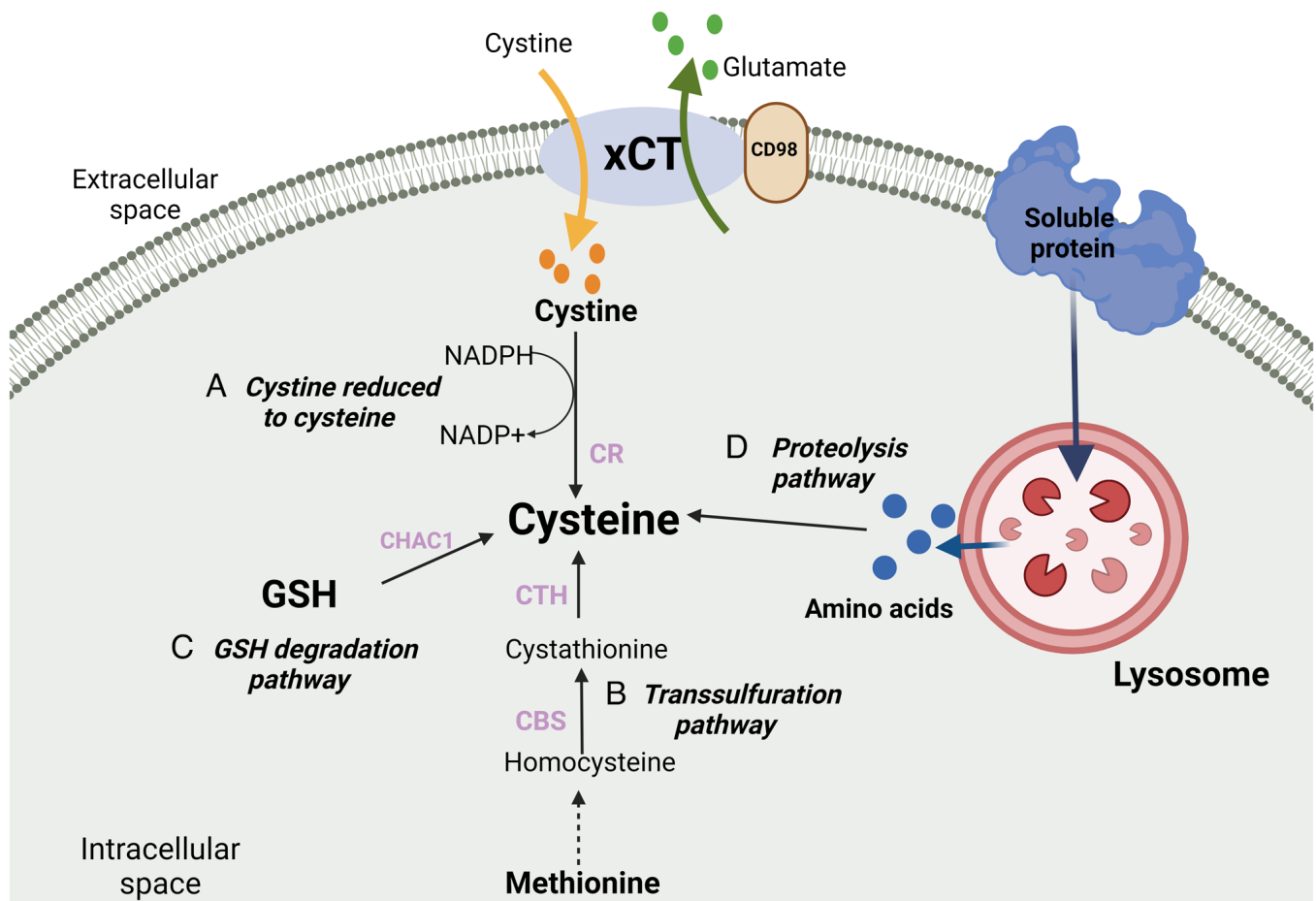


Figure 1. Cysteine synthesis pathways. (A) Cysteine synthesis through the system x_c^- , which consists of the chaperone SLC3A2 and the SLC7A11 transporter. The extracellular oxidized cystine is imported by xCT in exchange for intracellular glutamate. xCT is associated with the stem-like cancer cell 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 7 member 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 SLC3A1 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 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 IC₅₀ values 3-fold lower compared with those of sulfasalazine (SASP),

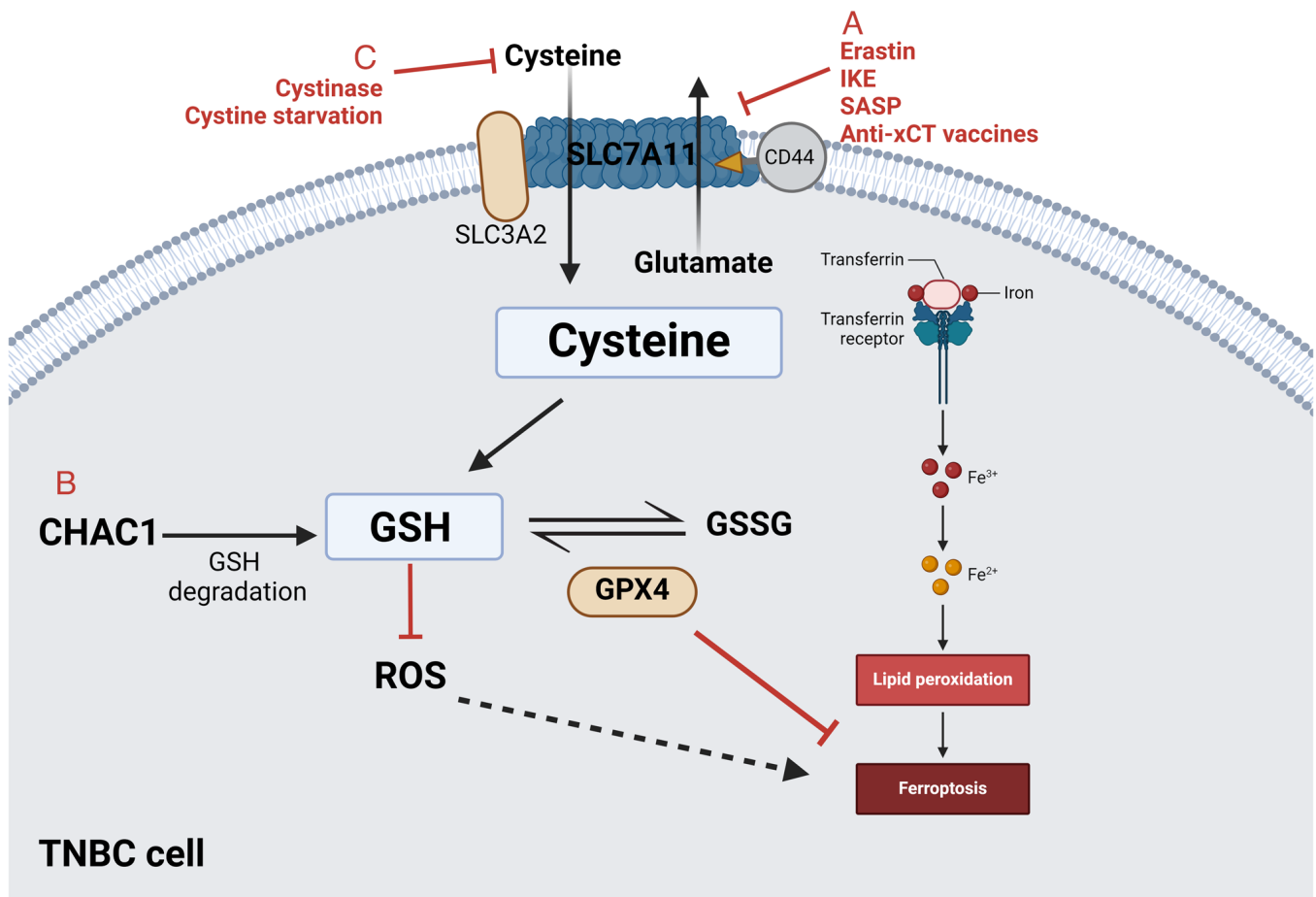


Figure 2. Prospective therapeutic strategies for TNBC. (A) Direct inhibition of SLC7A11 cystine transporter activity using erastin, IKE and DNA inhibitors. These drugs inhibit cystine uptake through SLC7A11 to induce lipid peroxidation and ferroptotic cell death. (B) CHAC1 degrades GSH by stimulating 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- κ B, 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 CD44⁺ 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)
Anti-xCT vaccines	Imidazole ketone erastin	i) Anticancer activity in a xenograft model of diffuse large B cell lymphoma; and ii) induces ferroptosis in cancer.	(73)
	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)
Ferroptosis	Viral vectors vaccine	Inhibits growth and metastasis of TNBC and HER2 ⁺ breast cancer in preclinical models.	(91)
	-	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 through 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.

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