The Pathways Related to Glutamine Metabolism, Glutamine Inhibitors and their Implication for Improving the Efficiency of Chemotherapy in Triple-Negative Breast Cancer

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Review

The pathways related to glutamine metabolism, glutamine inhibitors and their implication for improving the efficiency of chemotherapy in triple-negative breast cancer

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ABSTRACT

Breast cancer (BC) is a heterogeneous cancer with multiple subtypes affecting women worldwide. Triple-negative breast cancer (TNBC) is a prominent subtype of BC with poor prognosis and an aggressive phenotype. Recent understanding of metabolic reprogramming supports its role in the growth of cancer cells and their adaptation to their microenvironment. The Warburg effect is characterized by the shift from oxidative to reductive metabolism and external secretion of lactate. The Warburg effect prevents the use of the required pyruvate in the tricarboxylic acid (TCA) cycle progressing through pyruvate dehydrogenase inactivation. Therefore, it is a major regulatory mechanism to promote glycolysis and disrupt the TCA cycle. Glutamine (Gln) can supply the complementary energy for cancer cells. Additionally, it is the main substrate to support bioenergetics and biosynthetic activities in cancer cells and plays a vital role in a wide array of other processes such as ferroptosis. Thus, the switching of glucose to Gln in the TCA cycle toward reductive Gln metabolism is carried out by hypoxia-inducible factors (HIFs) conducted through the Warburg effect. The literature suggests that the addiction of TNBC to Gln could facilitate the proliferation and invasiveness of these cancers. Thus, Gln metabolism inhibitors, such as CB-839, could be applied to manage the carcinogenic properties of TNBC. Such inhibitors, along with conventional chemotherapy agents, can potentially improve the efficiency and efficacy of TNBC treatment. In this review, we discuss the associations between glucose and Gln metabolism and control of cancer cell growth from the perspective that Gln metabolism inhibitors could improve the current chemotherapy drug effects.

Contents

Abbreviations: BC, breast cancer; TNBC, triple-negative breast cancer; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid; Gln, glutamine; GLS, glutaminase; PR, progesterone receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor2; TME, tumor microenvironment; ROS, reactive oxygen species; GSH, glutathione; NADPH, nicotinamide adenine dinucleotide phosphate; PFK1, phosphofructokinase1; PFK2, phosphofructolinase2; PDK1, pyruvate dehydrogenase kinase 1; HIF-1, hypoxia-inducible factor-1; PGK1, phosphoglycerate kinase1; GLUT, glucose transporters; HK, hexokinases; ENO, enolases; ALDO, aldolases; PKM, pyruvate kinase M; LDHA, lactate dehydrogenase A; PHD, prolyl-hydroxylase; MCT4, monocarboxylate transporter4; VHL, Von Hippel Lindau; ACLY, ATP citrate lyase; PCD, programmed cell death; LOX, lipid peroxidase; GPX4, glutathione peroxidase; α-KG, α-ketoglutarate; GLUD1, glutamate dehydrogenase 1; GOT2, glutamate oxaloacetate transaminase 2; GPT2, glutamate-pyruvate transaminase 2; ETC, electron transport chain; OAA, oxaloacetate; SMAC, second mitochondria-derived activator of caspases; BPTES, Bis-2-5-phenylacetamido-13,4-thiadiazol-2-yl)ethyl sulfide; PAC, paclitaxel; mTOR, mammalian target of rapamycin; M, mesenchymal; MSL, mesenchymal stem-like; IM, immunomodulatory; LAR, luminal androgen receptor; FH, fumarate hydratase; SDH, succinate dehydrogenase; IDH, isocitrate dehydrogenase; MPC, mitochondria pyruvate carrier; OXPHOS, oxidative phosphorylation; HGFR, hepatocyte growth factor receptor; TfR, transferrin Receptor; GOT, glutamate oxaloacetate transferase; PARP, poly (ADP-Ribose) polymerase; MET, Mesenchymal to Epithelial Transition.

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1. Introduction

Breast cancer (BC) is the most common public health problem among women worldwide with multiple subtypes [1], of which the triple-negative breast cancer (TNBC) has a high rate of mortality and early relapse because of poor prognosis and severe clinical outcomes [2]. Furthermore, its prevalence is increasing among young women, with various clinical symptoms [3], and the aggressiveness of TNBC is often associated with the absence of expression of the progesterone receptor (PR), the estrogen receptor (ER) and the human epidermal growth factor receptor 2 (HER2) when compared with the other BC subtypes [4]. Metabolic pathways are common properties of cancer cells that can characterize tumor context and the microenvironment of cancers. Accordingly, they are considered the main therapeutic targets and potent biomarkers in the TNBC subtype because of the lack of the afore-mentioned hormonal receptors controlling this subtype [5].

Metabolic shifting leads to the unique metabolic profiling of cancer, which is a hallmark for cancer development [6]. Based on previous studies, the mutual association of cancer metabolism mediators and tumor microenvironment (TME) factors is a significant factor involved in metabolism-related signaling of cancer cells [7]. The energy of normal cells is primarily supplied by oxidative phosphorylation of pyruvate in the mitochondria, whereas cancer cells obtain energy via re-programming glucose metabolism in the cytosol through severe glycolysis and lactate pathways, the so-called the "Warburg Effect," which occurs even in the presence of high oxygen levels [8]. The output of the TCA cycle in cancer cells are some mediators, such as α-ketoglutarate and citrate and others, that contribute to cancer development [9]. The Warburg effect prevents the pyruvate progression in the TCA cycle by inactivation of pyruvate dehydrogenase (PDH), and the redirection to the lactate pathway leads to the proliferation and invasiveness of cancer. Furthermore, the Warburg effect might prevent the mitochondrial overproduction of citrate and ATP, leading to the obstruction of glycolysis at various levels (PFK1 and PK), whereas cancer cells are subjected to high levels of glucose as an adaptive metabolism. Glucose and Gln are the main nutrients utilized by tumor cells for their growth and proliferation, while the glycolytic route produces metabolic intermediates and ATP for biosynthesis, and Gln metabolism can supply the necessary nucleic acids, glutathione and amino acids for cell proliferation [10,11]. Therefore, Gln can play a vital role in the proliferation and growth of many cancer cells, such as TNBC [6,12]. TNBC has been characterized by elevated glutaminase (GLS) expression, leading to increased Gln metabolism [13]. Recent studies have shown that GLS, as a critical and rate-limiting step in the Gln metabolism, is an attractive anticancer therapeutic target [14].

Glutamine metabolism plays a crucial role in supporting rapid cancer growth. The high requirement of cancer cells for Gln triggers a Gln addiction phenotype; therefore, targeting the enzymes of this metabolic pathway can be a hopeful aim for the design of new therapeutic strategies [15]. Accordingly, GLS is a target in this pathway. Recently, several small synthetic inhibitors of GLS have been reported [16,17]. Ongoing basic and clinical studies on GLS inhibitors have evaluated their toxicity and synergistic effects on improving the efficiency of the current anticancer drugs [18]. In this review, we evaluated the literature on Gln-related pathways, such as the Warburg effect, tricarboxylic acid cycle (TCA), ferroptosis, ROS (reactive oxygen species) and apoptosis. We also evaluated complementary clinical studies showing that the application of metabolism modification to control cancer development has led to identification of some inhibitors to improve the efficiency of conventional chemotherapy in combination therapy.

2. The pathways related to glutamine metabolism in cancers

Although circulating blood is rich in Gln, its transportation to cells depends on the presence of Gln-related transporter channels, which are overexpressed in cancer cells compared with normal cells [19]. The indispensability of Gln is related to the supply of an energy source and synthesis of various macromolecules such as nucleic acids, lipids, and proteins in tumor cells. Additionally, it exerts a precursor role in providing nicotinamide adenine dinucleotide phosphate (NADPH) and Glutathione (GSH) for controlling redox balance [20]. According to various studies, the glutaminolysis pathway is one of the major metabolism-shifting phenomena related to Gln, which is severely activated in many aggressive forms of different cancers [21]. The addiction of malignant cells to Gln leads to the growth and proliferation of cancer cells. Therefore, the Gln pathway may be targeted for controlling cancer cells, and some of these inhibitors are in clinical trial phases to evaluate their efficacy [19].

2.1. Glutamine, hypoxia and Warburg effect

Metabolism shifting from the glucose to Gln pathways leads to the supply of energy for cancer cells under harsh conditions, such as nutrition shortage and hypoxia, contributing to the proliferation, growth, and survival of cancer cells [22,23]. Hence, it is a well-known hallmark in the metabolism of cancer cells. Anaerobic glycolysis, characterized by shifting from oxidative to reductive metabolism, leads to the secretory lactate pathway termed the Warburg effect [24]. In this metabolic mode, overexpression of phosphofructokinase 1 (PFK1) leads to the enrichment of the glycolysis pathway in cancer cells through limiting ATP and citrate

production by the mitochondria, and by increasing the concentration of phosphofructokinase2 (PFK2) [11,25]. Increasing the consumption of glucose through the presence of many glucose channels, and shifting oxidative to reductive metabolism, leads to the inhibition of PDH by phosphorylation and activation of pyruvate dehydrogenase kinase 1 (PDK1) [11,26]. PDH inhibition disrupts the TCA cycle of glycolysis. Therefore, it decreases the activity of the TCA cycle and reduces mitochondria-derived ATP. Further studies demonstrated that PDK1 could also be activated by hypoxia-inducible factor-1 (HIF-1), as well as by two mitochondria-translocated kinases, Akt (Protein kinase B) and Phosphoglycerate kinase 1 (PGK1) [11,27]. Additionally, the disconnection of glycolysis from the TCA cycle is related to the downregulation of mitochondrial pyruvate carrier channels [11]. So, HIF-1-targeted genes, such as Glucose transporters 1 and 3 (GLUT1 and GLUT3, respectively), and transactivation genes encoding glycolytic enzymes, are involved in the conversion of glucose to pyruvate with related enzymes, including phosphofructokinases (PFKL and PFKP), hexokinases (HK1 and HK2), enolases (ENO1 and ENO2), PGK1, aldolases (ALDOA and ALDOC), and pyruvate kinase M (PKM) [28,29]. On the other hand, lactate dehydrogenase A (LDHA) involved in the conversion of pyruvate to lactate, and monocarboxylate transporter 4 (MCT4) for lactate transportation, are trans-activated by HIF-1 when subjected to the Warburg effect, such that production and transportation of lactate to the outside of cancer cells promotes their survival under these conditions (Fig. 1) [11,30]. To the best of our knowledge, HIF-1 is stabilized, upregulated, and activated in the presence of O_2 through some intra- and extra-cellular pathways such as inhibition of prolyl-hydroxylase (PHD) via succinate, fumarate, and lactate; inactivates the mitochondrial tumor suppressor deacetylase, sirtuin3 (SIRT-3), because of mitochondrial NAD^+ shortage; increases the production of reactive oxygen species (ROS) and mutations in the tumor suppressor Von Hippel Lindau (VHL); and upregulates pyruvate kinase M2 (PKM2) and NF-kB pathways. The above-mentioned processes in the activation and/or stabilization of HIF-1 lead to glucose consumption and lactate production as a positive feedback loop [11,31].

Apart from the Warburg effect with its long-term metabolic reprogramming of cancer cells, there is another phenomenon called the “Crabtree effect”, which exhibits short-term adaptation properties [32]. The engagement of the Crabtree and Warburg effects contributes to adapting the development of solid tumors in TME. In this event, fructose 1,6-biphosphate, a PFK1 product, triggers inactivation of the complex IV of oxidation phosphorylation (OXPHOS) in mitochondria. Owing to this fact, the proliferation of cancer cells is restricted by mitochondrial production of citrate and ATP. It seems that both the Warburg and the Crabtree effects collaborate together in this process [11,32].

Therefore, the Warburg effect, as a major regulatory mechanism, leads to the avoidance of glycolysis arrest through decreasing the production of both ATP and citrate, and by inhibition of PFK1 and pyruvate kinase (PK) expression levels. In addition, ATP citrate lyase (ACLY) is upregulated in cancer cells to maintain the low levels of ATP and citrate, contributing to the development of tumor aggressiveness, dedifferentiation, invasion, and resistance to apoptosis. Therefore, the metabolism shifting in cancer cells triggers a decrease to half of the activity attainable via oxidative phosphorylation [11]. The frequent destruction of the respiratory chain, occurring primarily at the ATPase (complex V), leads to a diminished production of ATP by the mitochondria of cancer cells [33]. Based on the current studies, there is evidence of high levels

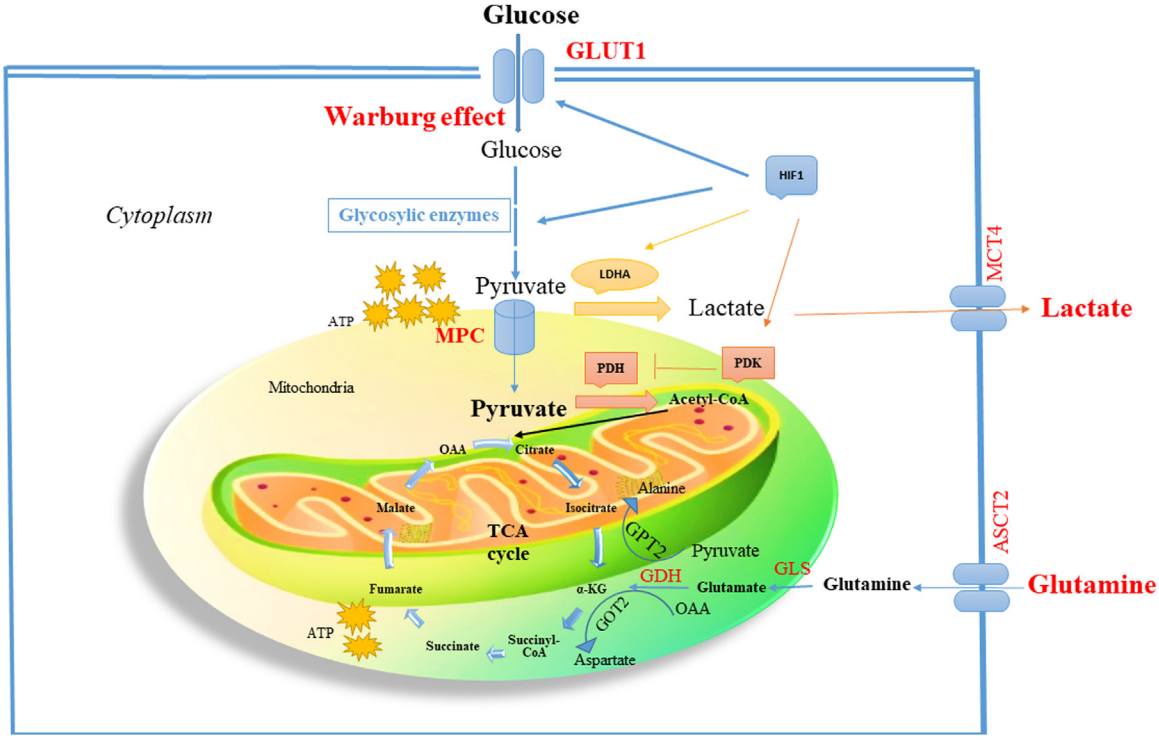


Fig. 1. The Warburg effect in cancer cells. Activated HIF-1α upregulates glucose transporter GLUT1 to increase the intake of glucose into cancer cells. HIF-1α accelerates the glycolysis step by upregulating the genes related to glucose and pyruvate metabolism. Pyruvate is not converted to Acetyl-CoA because HIF-1α upregulates PDK-1, which inhibits PDH. On the other hand, pyruvate is metabolized to lactate and transported out of cancer cells by the HIF-1α target genes LDHA and MCT4. Although the Warburg effect enables cancer cells to uptake and use mainly glucose for obtaining abundant ATP. The complementary energy supply can be provided by other main sources such as Gln because cancer cells suppress oxidative phosphorylation pathway. In glutaminolysis pathway, the conversion of Gln to glutamate and then α-ketoglutarate (α-KG) is mediated via glutaminase and glutamate dehydrogenase 1 (GLUD1) or transaminases such as glutamate-pyruvate transaminase 2 (GPT2) or glutamate oxaloacetate transaminase 2 (GOT2), respectively.

of metabolic heterogeneity and plasticity in cancer cells, which can be applied to glucose, Gln, and lactate pathways to supply the biosynthetic and bioenergetics demands of cancer cells [22]. Indeed, lactate, as a factor involved in the pseudohypoxia response, activates intracellular signaling pathways, such as c-MYC in oxygenated cancer cells through stabilization and activation of hypoxia-inducible factor-2a (HIF-2a). Subsequently, c-MYC upregulates glutaminase 1 (GLS1) and Gln transporter (ASCT2), resulting in changes in Gln metabolism. Compared to HIF-1α, it seems that HIF-2a activity plays a vital role in c-MYC induction [34].

Interestingly, the severe glycolytic activity of cancer cells leads to the enhancement of cancerous features accompanied by some advantages, including changes in metabolism. Although it enables cancer cells to uptake and use the majority of glucose for obtaining abundant ATP, the complementary energy supply can be derived from other main sources, such as Gln, because of a decrease in oxidative phosphorylation pathway in cancer cells [35,36]. Glucose catabolism is mostly used in biosynthetic pathways for supplying the intermediate products and Gln metabolism to synthesize various macromolecules for proliferation and also to supply required agents for making cancer properties such as ribose sugars for nucleotides, non-essential amino acids, citrate and glycerol for lipids, and production of NADPH through the oxidative pentose phosphate pathway [34]. Furthermore, a significant increase in glycolytic flux rapidly produces ATP in the cytoplasm of proliferating cells, while reducing the cytoplasmic NAD^+/NADH ratio. Lactate dehydrogenase A (LDH-A) catalyzes the reduction of pyruvate to lactate by oxidizing NADH to NAD^+. NAD^+ allows frequent and severe glycolysis, and thus lactate is externally secreted from the cancer cells [34,35,37].

2.2. Ferroptosis and glutaminolysis

Regulated and programmed cell death (PCD) is essential for fundamental physiological processes such as development, maintaining tissue homeostasis, proliferation, growth, and immunity [38]. Recently, studies have demonstrated that multiple cancers are associated with dysregulation, imbalance and disequilibrium of cell death [39]. One type of regulated death in terms of morphological, genetic, and biochemical distinction is known as ferroptosis [40], which is characterized by lipid peroxidation and dependence on iron and ROS [36]. Recently, many studies have shown a close interaction between ferroptosis, cellular metabolism and redox balance [41,42]. The ferroptosis mechanism is accomplished by lipid peroxidase (LOX) and inhibited by Glutathione Peroxidase 4 (GPX4) [43]. Additional genes such as P53, and metabolism pathways including lipid metabolism, iron metabolism, and amino-acid metabolism, have been found to regulate ferroptosis [39,44]. As mentioned, some of the crucial pathways associated with ferroptosis modulation are the amino acid metabolism pathways [45]. Glutamine and cysteine amino acids, and transferrin, an iron-carrier protein, are associated with the regulation of ferroptosis [42]. An amino acid transporter, called system Xc (cystine/glutamate antiporter), mediates the exchange of the imported cystine with the exported glutamate. Therefore, the concentration of cystine is limited, and its entry into the cell can be prevented by some factors, such as the high concentration of glutamate and Gln, thereby reducing the synthesis of glutathione. This leads to an increase in ROS, and is one of the factors that induce ferroptosis (Fig. 2) [42,46]. Although ferroptosis functions through high levels of Gln and deprivation of cysteine, it cannot be induced in the presence of the high concentrations of Gln alone. According to previous studies, ferroptosis can be derived through glutaminolysis and the related enzymes (GLS1 and GLS2) [47]. On the other hand, it has been demonstrated that the metabolic process glutaminolysis is essential for ferroptosis, and deprivation and obstruction of cystine's entry into the cell to decrease its concentration cannot solely lead to ferroptosis. Therefore, it seems ferroptosis is carried out through the presence of high levels of Gln and the absence of cysteine, acting in synergic roles in cancer cells; subsequently, both processes are needed for this phenomenon [47]. These processes causing ferroptosis result in decreased levels of glutathione and increased ROS levels [45]. They further induce and activate lipid peroxidation leading to ferroptosis. Notably,

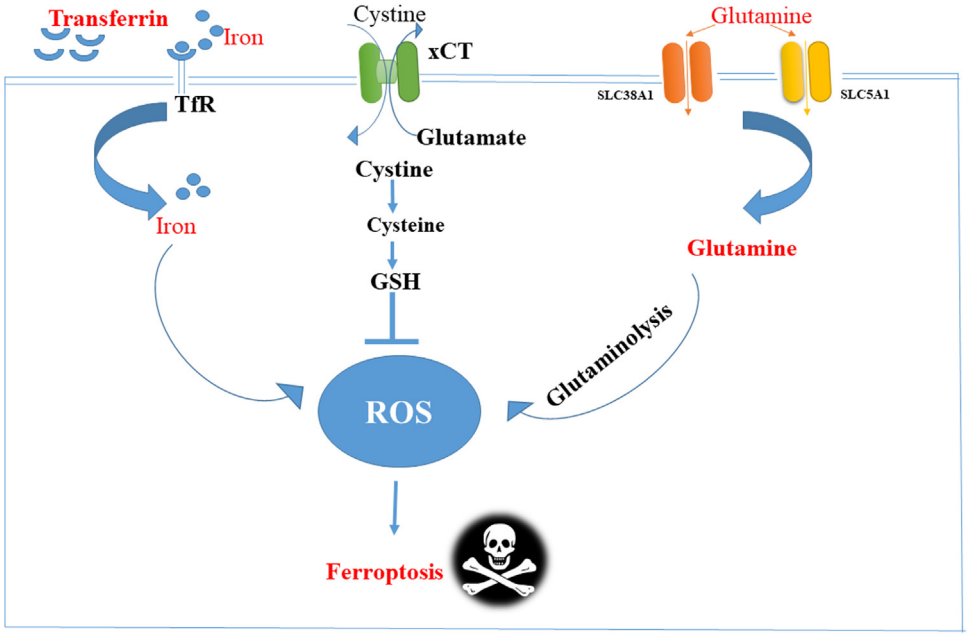


Fig. 2. Transferrin and the intracellular metabolic process of glutaminolysis are required for the execution of a form of programmed cell death known as ferroptosis. On the other hand, high concentration of Gln prevents cystine entry into the cell. A lack of cysteine, an important precursor of glutathione (GSH) synthesis, results in reduced levels of GSH meanwhile ROS accumulation. The observation that cysteine deprivation (or equivalently, Xc inhibition) can immediately trigger ferroptosis suggested the role of ROS in ferroptosis.

although conversion of Gln to glutamate is catalyzed by the GLS1 and GLS2, only GLS2 as a transcriptional target of p53 is needed for ferroptosis [44]. Furthermore, the susceptibility to glutathione discharge and the regulated cell death via ferroptosis might be observed in OXPHOS-dependent cancer cells and several cancer types, such as TNBC, that require glutaminolysis for proliferation [43,48].

2.3. Glutamine and tricarboxylic acid cycle (TCA)

In tumor cells, the TCA cycle is the metabolic hub and the main source of biomass building blocks, including nucleotides, amino acids, and lipids for synthesizing macromolecules [49]. It is also the gateway for the metabolism of many macromolecules, such as glucose, Gln, and fatty acids that can be converted to an acetyl group [50]. The Warburg effect prevents the use of pyruvate to drive the TCA cycle by inactivation of PDH phosphorylation. According to previous studies, there is significant relationship between dysfunction of the TCA cycle and mutations of TCA cycle-related enzymes such as isocitrate dehydrogenase (IDH), fumarate hydratase (FH), succinate dehydrogenase (SDH), and familial cancers [51]. However, continuous use of intermediate TCA-derived compounds in further pathways leads to a decrease in mitochondrial function. According to several studies, Gln provides a carbon source for restoring this route [19,37]. Therefore, Gln, as a precursor of intermediate compounds, is preferred by many cancer cells for their growth, especially in Gln-addicted cells. The conversion of Gln to glutamate and then α-ketoglutarate (α-KG) is mediated via glutaminase and glutamate dehydrogenase 1 (GLUD1) or transaminases, such as glutamate-pyruvate transaminase 2 (GPT2) or glutamate oxaloacetate transaminase 2 (GOT2), respectively [36]. Accordingly, when the function of the electron transport chain (ETC) or TCA cycle is changed by mutations or hypoxia, the source of citrate/isocitrate in mitochondria is provided by carboxylation of Gln-derived α-KG, which is cleaved to produce Acetyl-CoA and oxaloacetate (OAA) [36]. Therefore, these compounds that fuel the TCA cycle can be supplied by Gln via glutamate dehydrogenase. The upregulation of hypoxia-inducible factors (HIF1a and HIF2a) [11] and the loss of HIF regulation by VHL mutation and citrate concentration, as a sensor of this switching, are among the factors that support the switching of TCA cycle (from glucose) to reductive Gln metabolism. Additionally, the entry of pyruvate into the mitochondria is prevented by mitochondrial pyruvate carrier 1 and 2 (MPC1 and MPC2) blocking, resulting in the enhancement of the reliance on glutamate dehydrogenase and glutaminase for survival and proliferation [36]. GPT2 is a critical enzyme that links the glycolytic pathway to the TCA cycle by transferring nitrogen from glutamate to an acceptor molecule, such as pyruvate, to form α-KG for the proliferation of cancer cells [52]. Altogether, these observations suggest a central role for Gln in multiple intermediate metabolism pathways to produce glutamate. For this purpose, α-KG is a convenient molecule for cells to utilize as a source of carbon for the TCA cycle [53].

2.4. Glutamine and apoptosis

Since reprogrammed metabolism and resistance to apoptosis are recognized as hallmarks of cancer, they provide opportunities for therapeutic strategies against cancer [54]. In this regard, Gln, as a key source of energy for many tumor cells, can be used as a potent target in order to eliminate cancer cells [55]. The effects of Gln deprivation on cell death depends on various criteria such as the type of drug used, the cancer type, and cell growth conditions [56,57]. Individually, the cancer cells' vulnerability to apoptosis depends on the extracellular Gln concentration. Therefore, deprivation of Gln triggers sensitization of the cells to tumor necrosis factor-alpha (TNF-α), Fas ligand (CD95), and heat shock-mediated apoptosis. Recent studies have demonstrated that Gln deprivation stimulates the cleavage of Poly (ADP-Ribose) polymerase (PARP) and activates caspase-2,3, and cytochrome C release to induce apoptosis in cancer cells. In some cancer cells, such as Sp/0 murine hybridoma cells, some factors that induce apoptosis include the second mitochondria-derived activator of caspases (SMAC), BAX translocation, caspase-9 activation, and cytochrome C release following Gln deprivation [56]. However, the activation of caspase-8,9 in apoptosis pathways does not take place in some cancer cells. Observations of such specificity in cancerous cell types have shown that the induction of apoptosis is affected by the deprivation of Gln [56].

Furthermore, Gln plays crucial roles in the efficacy of chemotherapy drugs. Valter et al. used Etoposide and Cisplatin to induce apoptosis in cancer cell lines and showed that Cisplatin and Etoposide alone induced apoptosis in cancer cells with upregulation of P53. In this regard, Gln interferes with the mechanism of the above-mentioned drugs to control cancer cells. The deprivation of Gln increases the induction of apoptosis in Cisplatin-treated cells by increasing the level of P53 and ROS, while the induction of apoptosis is suppressed in Etoposide-treated cells via downregulation of P53. Therefore, Gln can be used as a promising target with respect to its effects on chemotherapy drug processes (Fig. 3) [57].

2.5. Glutamine and reactive oxygen species (ROS)

Despite the availability of multiple defense mechanisms in cancer cells, they cannot avoid exposure to ROS because they are so ubiquitous. In this regard, GSH, a tripeptide of glutamate, glycine, and cysteine, is a well-known antioxidant substrate that promotes the ability of cancer cells to protect themselves against ROS. Glutathione peroxidase, as a critical enzyme of the protection system, helps eliminate ROS by the production of hydrogen peroxide [15]. Glutamine is a signaling molecule that regulates required metabolic pathway for the growth and antioxidant defense against oxidative stress by supporting GSH synthesis through NADPH and intracellular glutamate supply. Further, the required cysteine for glutathione synthesis is provided through its import into the cell and glutamate export via the xCT system. Therefore, Gln is a crucial precursor of GSH [58,59], such that Gln deprivation reduces GSH concentrations, leading to an overproduction of ROS and enhancement of the vulnerability of cells to ROS-generating agents. Based on previous studies, the withdrawal of Gln can contribute to the apoptotic response of cancer cells and improve some chemotherapy drug effects, such as Cisplatin, by increasing the level of ROS [22,57].

3. Glutamine-related enzymes and their inhibitors

The first and critical step of the Gln pathway in the mitochondria is initiated by the production of glutamate from Gln, catalyzed by glutaminase [60]. There are two main glutaminase isoforms with different expression patterns and functions in humans: liver-type glutaminase (GLS2, LGA or GAB) and kidney-type glutaminase (GLS, KGA or GAC) encoded by GLS2 and GLS1, respectively [61]. The GLS2 has been characterized as a tumor suppressor agent, whereas GLS has oncogenic properties [62]. Various studies have shown that GLS1 expression level is higher than GLS2 in many cancer cells such as TNBC because of higher consumption, and owing to its increase in the dependence on Gln for proliferation and growth [60]. Recently, several small-molecules such as BPTES, DON, UPGL00004, and CB-839, have been reported as inhibitors of glutaminase, with attractive features such as permeability, microsomal instability, and oral bioavailability (e.g. CB-839) compared to other glutaminase inhibitors [16,63]

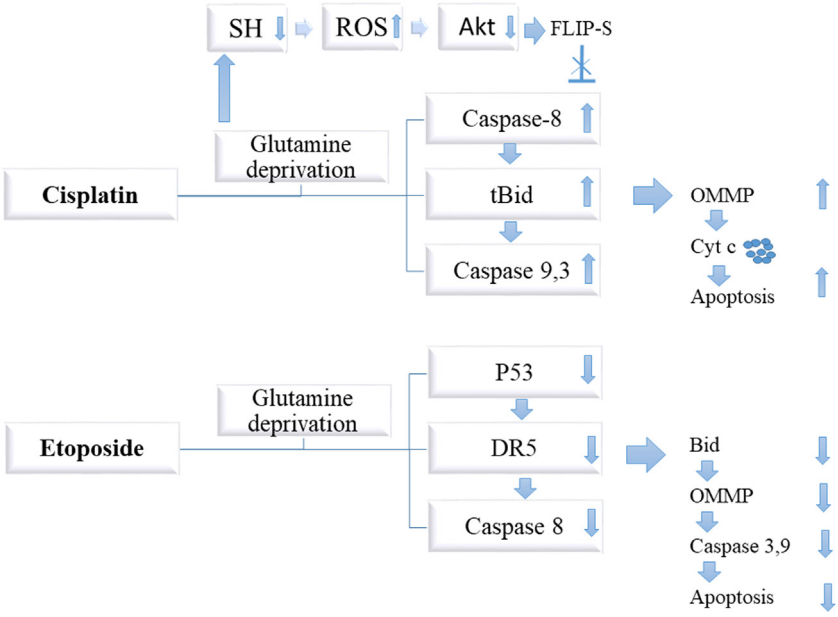


Fig. 3. Schematic representation of modulations in the apoptotic response following Gln deprivation in cells treated with Etoposide or Cisplatin. When the Gln deprivation stimulates apoptosis induced by Cisplatin, ROS production suppresses Akt pathway following GSH depletion, which results in the attenuation of c-FLIP-S level followed by the activation of caspase-8 and cleavage of Bid and stimulation of caspase-3. This results in OMM permeabilization (OMMP), release of cytochrome C, processing of caspase-9 and activation of caspase-3 and cell death in the end (A). The Gln deprivation prevents stimulation of p53 expression following the treatment with Etoposide. This results in the attenuation of DR5 level, processing of caspase-8, and cleavage of Bid, leading to the stabilization of OMM toward permeabilization. As a result, the processing of caspase-9 and activity of caspase-3 are suppressed and cell death declines (B).

(Fig. 4, Table 1). In this regard, CB-839, is a potent and selective inhibitor, as indicated by significant anti-proliferative activity in TNBC, which might have synergistic effects on chemotherapy drugs such as Paclitaxel (Pac) [13,16,64]. Signaling pathways, such as the mammalian target of rapamycin (mTOR), regulate cell growth and metabolism. It has been observed further that CB-839 can have synergistic inhibitory effects in combination with mTOR inhibitors, such as Everolimus by alteration of the mTOR pathway [65]. Previous studies have shown that the crosstalk between Gln metabolism and the growth of cancer cells results in some therapeutic benefits for glutaminase inhibitors [60,63]; therefore, the recent clinical trial with CB-839 is shown in Table 2 because of its importance and potential impact.

4. TNBC subtype and glutaminase inhibitor

Triple-negative breast cancer is a highly heterogeneous kind of BC with variable prognosis in terms of clinical, pathologic, and genetic factors. This molecular heterogeneity of TNBC leads to diverse biological behaviors and differential responses in

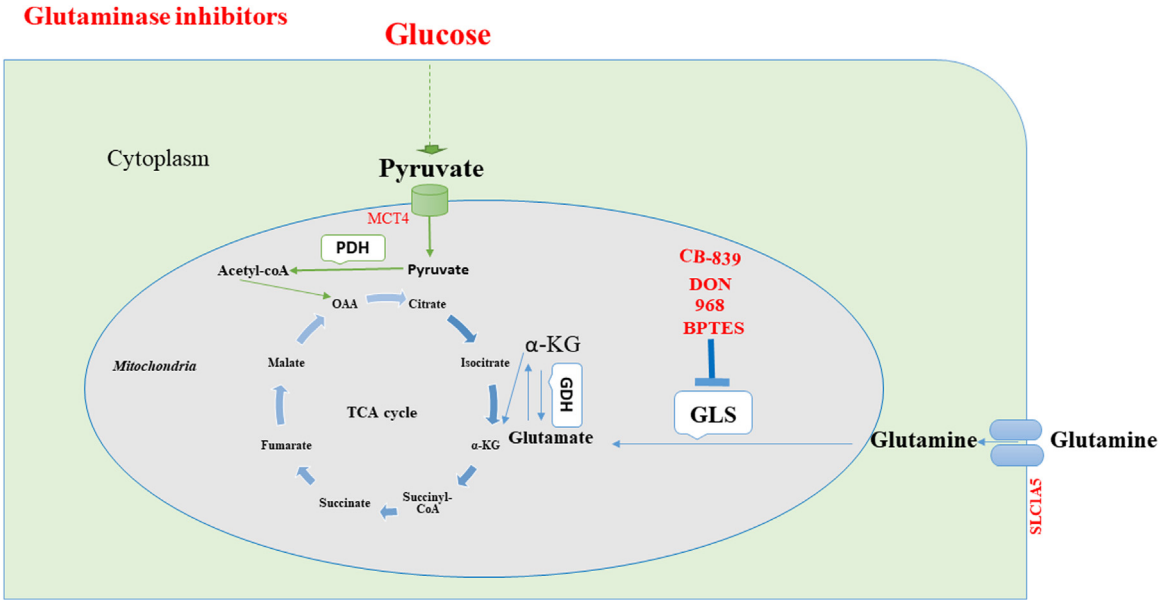
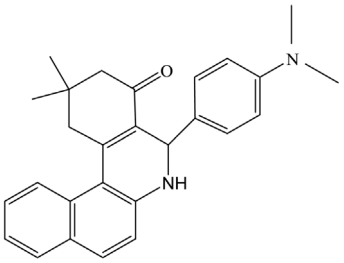
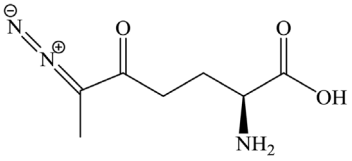
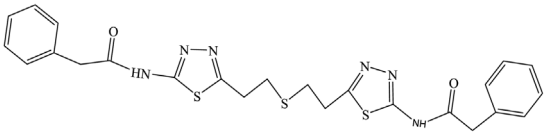
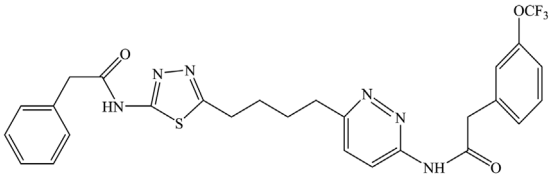
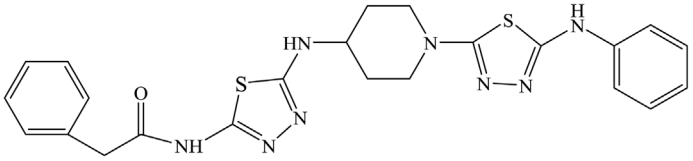


Fig. 4. Glutaminolysis in cancer cells. Glutamine is transported through transporters (i.e., SLC1A5) to enter the glutaminolysis pathway. The involved enzymes in the glutaminolysis pathway are suggested to be potential anticancer targets and inhibitors of these enzymes are listed in red.

Table 1
Glutaminase inhibitors and their structure and activity mechanisms.

Glutaminase inhibitor	Activity	Structure
968	Acts as a non-competitive allosteric inhibitor of GAC by interfering with its ability to undergo normal monomer-monomer interactions that leads to GAC dimers and ultimately to activated tetramers [84].	
DON	Binds to the enzyme active site and covalently modifies the catalytic serine (S291) [85].	
BPTES	Interacts with a flexible loop within the dimer-dimer interface of the tetrameric forms of these enzymes [85].	
CB-839	Binds at the dimer-dimer interface to promote the formation of an inactive tetramer [86].	
UPGL00004	Binds at the dimer-dimer interface to promote the formation of an inactive tetramer [85].	

treatment responses [66]. Hormone-targeted drugs such as Tamoxifen, aromatase inhibitors, and Her2-targeted drugs, including Trastuzumab, are ineffective in the treatment of TNBC due to the absence of their receptors [67]. Based on gene expression profiling, six distinct TNBC subtypes have been identified, including basal-like subclasses (BL1 and BL 2), immunomodulatory (IM), mesenchymal (M), luminal androgen receptor (LAR), and mesenchymal stem-like (MSL). Accordingly, the BL1 subtype is characterized by high expression levels of DNA damage and cell cycle markers [68]. The BL2 subtype is determined using growth factors such as EGFR and hepatocyte growth factor receptor (HGFR) or Mesenchymal to Epithelial Transition (MET) tyrosine-protein kinase. The prominent features of M subtype are trans-differentiation and growth factor signaling (e.g., upregulation of PDGFR, TGF beta, NOTCH, FGFR) [69]. The LAR subtype is identified by PIK3CA mutations and strong androgen receptor (AR) signaling [70]. The different properties of each subtype might facilitate the identification of stratifying biomarkers for selection of patients to design tailored clinical trial approaches and to predict the potential responses to each of several different treatment processes [71,72].

One therapeutic approach to control these cancer cells is the targeting of the different signaling pathways involved in the various subtypes. Pathways related to NF-κB are identified as crucial in the regulation of angiogenesis, inflammatory response, and apoptosis in TNBC. High expression and abnormal activity of NF-κB results in resistance to apoptosis in cancer cells. In this regard, Genistein, Plumbagin, and Fenofibrate have anti-proliferative effects and apoptosis-inducing roles in TNBC by interfering with gene expression and activation of NF-κB [73]. Furthermore, the JAK/STAT pathway is a key regulator in cellular functions, including cell differentiation, proliferation, migration, survival and apoptosis [103]. Higher levels of STAT3 expression in some subtypes of TNBC represents more invasive phenotypes and poor prognosis [74]. Thus, Metformin, a STAT3 inhibitor, decreases some TNBC tumor growth, and Ruxolitinib, an inhibitor of JAK1/2, in combination with Doxorubicin, Paclitaxel, and Cyclophosphamide, induces apoptosis of TNBC cells [75,76]. In addition to the above pathways, other important signaling pathways, such as hedgehog (Hh), Wnt/β-catenin, PI3K–AKT–mTOR, notch, and receptor tyrosine kinases (RTK), regulate multiple cellular functions [77–79].

Table 2
Clinical trials using glutaminase inhibitor CB-839.

Patients	CB-839 in combination with:	Phases of trials	References
Metastatic Renal Cell Carcinoma	cabozantinib	Phase II	[87]
Triple Negative Breast Cancer	Paclitaxel	Phase II	[13]
Melanoma, ssRCC, NSCLC	nivolumab	Phase I / II	[88]
RAS wild type colorectal cancer	• Panitumumab • irinotecan hydrochloride	phase I / II	[89]
Myelodysplastic syndrome	Azacitidine	phase I / II	[90]
Solid tumors or colorectal cancer	Capecitabine	phase I / II	[89]

These pathways play vital roles in the sensitivity and efficacy of the drugs applied to the treatment of TNBC [70]. Table 3 presents several of these signaling pathways and their related preferential targeting drugs [70]. However, "standard" or targeted chemotherapy has not been defined or approved to date in TNBC treatment because of inherent TNBC characteristics previously discussed, such as the absence of relevant receptors, poor prognosis, aggressive phenotype, and heterogeneity [80]. Furthermore, the metabolic reprogramming discussed above is a complexity of TNBC, which allows cancer cells to adapt and grow in specific microenvironments, leading to drug resistance in TNBC patients [6]. The metabolic reprogramming in the various TNBC subtypes during the cell transformation prompts continuing research to focus on targeting metabolic enzymes for cancer therapy or reversing the drug sensitivity of cancer [81]. As such, the high metastasis potency and drug resistance of TNBC characterized by elevated GLS expression, which leads to an increase in Gln metabolism [60] as discussed herein, can potentially be a targeted pathway that can be exploited in therapy against TNBC. Numerous studies have shown promise that GLS inhibitors have synergistic effects on improving the efficiency of the currently used anticancer drugs [82]. For example, CB-839, as a potent GLS inhibitor, exhibited remarkable anti-proliferative activity in combination with Paclitaxel that enhanced its efficacy and decreased its toxicity in patients with TNBC [18].

5. Future perspectives and conclusions

Triple-negative breast cancer is a very challenging disease because of numerous complexities, many of which have been reviewed here. TNBC is highly aggressive with complex genetic and metabolic heterogeneity [83]. Understanding the metabolic profile of the various TNBC subtypes may be valuable in developing targeting therapies [72]. Although the Warburg effect has been widely recognized as a common characteristic of metabolic reprogramming in cancer, recent studies have suggested other metabolic pathways that are alternatively employed in some tumor cells, including TNBC. It would be beneficial to identify new therapeutic targets and molecular biomarkers from the several metabolic pathways described herein. However, no standardized guidelines are yet available for the treatment of TNBC in its various phases. One of the notable properties of TNBC is the addiction to Gln, and its metabolism. To control TNBC, some agents have been designed to target GLS, as a critical and rate-limiting enzyme in Gln metabolism that can be applied to improving combination therapy. Some of the therapies under consideration are currently undergoing toxicity studies and clinical trials. On the other hand, the process of ferroptosis is a new candidate being targeted for therapeutic potential to control TNBC, however, more basic and clinical investigations are required, and further studies are necessary to solidify the relationship between ferroptosis and

Table 3
Signaling pathways involved with identified potential inhibitors in TNBC.

Drugs	Molecules target	Mechanism	References
Genistein	NF-κB	↑apoptosis	[73]
Plumbagin	NF-κB	↑apoptosis	[91]
Fenofibrate	NF-κB	↑apoptosis	[92]
Metformin	STAT3	↑apoptosis, tumor growth↓	[75]
Ruxolitinib	JAK1/2	↑apoptosis, tumor growth↓	[76]
BSK805	JAK2	↑apoptosis, tumor growth↓	[93]
Olaparib	PARP	DNA repair↓	[70]
Rucaparib	PARP	DNA repair↓	[77]
LY294002	PI3K	↑Chemosensitivity, BRCA1↓	[77]
Everolimus	TORC1/2	↑Chemosensitivity, BRCA1↓	[94]
BEZ235	TORC1/2	↑Chemosensitivity, BRCA1↓	[95]
ICRT-3	Wnt/β-catenin	invasion, migration, metastatic↓	[96]
Salinomycin	LRP6	Proliferation, migration↓	[97]
GSI (γ-secretase inhibitors)	Notch	Proliferation, migration↓	[98]
Erlotinib	EGFR-TK	↑apoptosis, tumor growth↓	[99]
Panitumumab	EGFR	↑apoptosis, tumor growth↓	[99]
Cetuximab	EGFR	↑apoptosis, tumor growth↓	[100]
Gefitinib	EGFR	↑apoptosis, tumor growth↓	[100]
Sunitinib	PDGF	↑apoptosis, tumor growth↓	[101]
Bevacizumab	PDGF	↑apoptosis, tumor growth↓	[102]
Cediranib	VEGF	↑apoptosis, tumor growth↓	[70]
Vismodegib	SMO (Hh pathway)	invasion, migration, metastatic↓	[70]
Niraparib	PARP	DNA repair↓	[70]
Panitumumab	EGFR	↑apoptosis, tumor growth↓	[70]
Platinum compounds	DNA repair complex	DNA repair↓	[70]

cancer. According to recent studies, Gln metabolism inhibitors provide promising effects for TNBC management. For example, some inhibitors such as CB-839 have been applied in combination with conventional anti-cancer drugs to improve their effects by decreasing their toxicity and increasing their specificity and sensitivity in the treatment of TNBC. Accordingly, CB-839 exhibits remarkable anti-proliferation activity, and in combination with chemotherapy drugs such as Paclitaxel, its efficiency is enhanced in patients with TNBC. Overall, further investigation of Gln metabolism inhibitors could potentially broaden new horizons for the management of cancers such as TNBC, whether used alone or in combination with current anti-cancer drugs. Towards those goals, further basic and clinical studies are necessary.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran with Ethics code IR. TBZMED.REC. 1398.025.

Consent for publication

Not Applicable.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author's contribution

Soheila Delgir conceived the study. Mohammad Reza Alivand wrote and supervised the first draft of the manuscript. Milad Bastami, Asma Safi, Khandan Ilkhani, contributed to searching and writing the manuscript. Farhad Seif revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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