

Serine, glycine and one-carbon metabolism in cancer (Review)

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Abstract. Serine/glycine biosynthesis and one-carbon metabolism are crucial in sustaining cancer cell survival and rapid proliferation, and of high clinical relevance. Excessive activation of serine/glycine biosynthesis drives tumorigenesis and provides a single carbon unit for one-carbon metabolism. One-carbon metabolism, which is a complex cyclic metabolic network based on the chemical reaction of folate compounds, provides the necessary proteins, nucleic acids, lipids and other biological macromolecules to support tumor growth. Moreover, one-carbon metabolism also maintains the redox homeostasis of the tumor microenvironment and provides substrates for the methylation reaction. The present study reviews the role of key enzymes with tumor-promoting functions and important intermediates that are physiologically relevant to tumorigenesis in serine/glycine/one-carbon metabolism pathways. The related regulatory mechanisms of action of the key enzymes and important intermediates in tumors are also discussed. It is hoped that investigations into these pathways will provide new translational opportunities for human cancer drug development, dietary interventions, and biomarker identification.

Contents

1. Introduction
2. SSP
3. Association of the SSP with cancer cell proliferation and regulation
4. Inputs and outputs of one-carbon metabolism

5. Association of one-carbon metabolism with cancer cell proliferation and regulation
6. Cancer treatment and potential new opportunities
7. Conclusions

1. Introduction

Metabolic reprogramming is an important feature of cancer (1-3). Cancer cells maintain their survival and rapid proliferation through metabolic reprogramming, which can provide a large amount of energy and macromolecular substances required for metabolic conversion (4). Under varying stress conditions, cancer cells quickly obtain the necessary components for cell proliferation, including nucleotides, proteins and lipids, as well as important cofactors, which maintain the cancer cell redox state (5-7). The Warburg effect suggests that tumor cells produce energy in a unique manner (8). Normally, cells rely on mitochondria to oxidize carbohydrate molecules to release energy, whereas most tumor cells provide energy for themselves through glycolysis, which has a relatively low productivity (3,9). In addition to upregulating glucose consumption, many tumors also increase the absorption of amino acids, such as glutamine, which is converted to α -ketoglutarate (α -KG) to supplement the tricarboxylic acid (TCA) cycle (4,10). Interestingly, it has been found that in proliferating cells, including cancer cells, even a high consumption of glucose and glutamine is insufficient to support the accumulation of biomass (4,10). Instead, non-glutamine amino acids provide the majority of the carbon and nitrogen units (10), such as serine, which is essential for cancer cell survival (10). The glycolysis and glutaminolysis pathways provide the precursors 3-phosphoglycerate (3-PG) and glutamate, respectively, thereby fueling serine biosynthesis (11-13). The serine synthesis pathway (SSP) represents a critical turning point for glucose conversion. Serine derived from the glycolysis branch of synthesis and exogenous uptake can be converted to glycine and provide one-carbon unit for one-carbon metabolism (14). One-carbon metabolism includes the folate cycle, methionine cycle and trans-sulfuration pathway, which support porphyrin, thymidine, purine, glutathione (GSH) and S-adenosylmethionine (SAM) synthesis (15). These intermediate metabolites can be used as important precursors for the synthesis of proteins, lipids, nucleic acids and

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other cofactors, interlocking to form a complex metabolic network (15,16).

The SSP and one-carbon pathway create an upregulated metabolic network in tumors and are of high clinical relevance (15,17-19). In the present review, the significance of the SSP in cancer and its related regulatory mechanisms of action are outlined, as well as the contribution that one-carbon metabolism provide for cancer metabolic reprogramming pathways. These findings may help in the development of targeted antimetabolite treatments by highlighting new translational opportunities for dietary interventions, drug development and biomarker identification.

2. SSP

Cancer cells generally use glycolysis to maintain their energy supply and serine biosynthesis is an important branch of glycolysis (11). 3-PG, an intermediate product of glycolysis, is a precursor of serine biosynthesis (9). Overall, ~10% of 3-PG is converted into serine after a three-step enzymatic reaction (Fig. 1): In the first step, 3-PG is oxidized to 3-phosphate hydroxypyruvate by phosphoglycerate dehydrogenase (PHGDH) (18). It is then catalyzed to 3-phosphoserine and α -KG by phosphoserine aminotransferase (PSAT1), and finally dephosphorylated to serine by 1-3-phosphoserine phosphatase (PSPH) (18). The mutual conversion of serine and glycine can then be achieved by serine hydroxymethyl transferase (SHMT1/2) (20). It has been reported that the gene encoding PHGDH, located on chromosome 1p12, is upregulated in most types of human tumors, such as breast cancer and melanoma (21,22). In addition, short hairpin RNA screening results reveal that breast cancer cell lines and melanoma cell lines require PHGDH amplification to support tumorigenesis (21-23). Similarly, high levels of PHGDH and SHMT2 have been found in a subgroup of patients with lung cancer who have a particularly poor prognosis (24). PHGDH inhibition can reduce tumor growth and differentiation of neuroendocrine prostate cancer *in vivo* (25). All of these studies indicate that PHGDH is very important for the proliferation and survival of tumor cells. Other studies have revealed that PSAT1 is upregulated in colorectal cancer (CRC), esophageal squamous cell carcinoma and non-small cell lung cancer, and that PSAT1 overexpression leads to a poor prognosis by enhancing cancer cell proliferation, metastasis and chemoresistance (26-28). Additionally, in patients with hepatocellular carcinoma (HCC), the expression levels of PSPH gradually increase with HCC progression and the abnormal expression of PSPH is highly correlated with patient mortality, indicating that the PSPH protein is a probable prognostic biomarker for HCC (11). Taken together, high expression levels of metabolic enzymes in the SSP may be necessary and sufficient to maintain cancer growth and oncogenic transformation.

3. Association of the SSP with cancer cell proliferation and regulation

Patients with malignant tumors are at high risk of malnutrition, with 40-80% afflicted by this condition. Under nutritional deprivation, cancer cells are adept at obtaining any required energy during the opportunistic mode to support their own survival

and growth, which means metabolic reprogramming (4). It has long been known that both exogenously ingested serine and endogenously synthesized serine are associated with cancer and functionally support cancer development (12,29,30). As aforementioned, the high expression levels of the metabolic enzymes PHGDH, PSAT1 and PSPH in the SSP may be indispensable for maintaining cancer growth and oncogenic transformation (21,23,25-27). Moreover, metabolic enzymes in the SSP are subject to transcriptional regulation by various transcription factors after stress response or oncogene activation, to cope with various types of stress, including nutritional deficiencies (11). The present review subsequently discusses the ways in which the transcriptional factors activating transcription factor 4 (ATF4) and c-MYC, as well as the oncogene p53, activate the SSP and perform genomic modification of the metabolic enzymes in the SSP, to assist tumor metabolic reprogramming under nutritional deficiency and/or serine deprivation (11,31,32).

Activating transcription factor 4 (ATF4) is a member of the cyclic adenosine monophosphate responsive element-binding (CREB) protein family. According to previous reports, the gene encoding the CREB protein family is not only expressed in a variety of tumors, but also is a potent stress-response gene in tumors (33,34). Many ATF4 target genes are involved in the maintenance of amino acids homeostasis (35-37). By regulating the adverse environment, ATF4 can protect tumor cells from nutritional stress and a series of cancer therapeutic agents (37-40). PHGDH, PSAT1 and PSPH inhibition by ATF4 small interfering RNA was first reported by Adams (41). In addition, under amino acid starvation, high expression levels of PHGDH, PSAT1 and PSPH can be induced through the general control nonderepressible 2-ATF4-dependent pathway (42). Gao *et al* (43) were the first to reveal that the expression levels of PSAT1 in ER-negative breast cancer were significantly upregulated. ATF4 was also found to directly enhance the expression of PSAT1 in ER-negative breast cancer, which upregulated cyclin D1 through the GSK3 β / β -catenin pathway, and finally promoted the proliferation of ER-negative breast cancer cells *in vitro* and *in vivo* (43). DeNicola *et al* (31) integrated metabolite tracing with gene expression analysis revealing that NF-E2-related factor 2 positively regulated the expression levels of PHGDH, PSAT1 and SHMT2 in SSP by targeting ATF4, which controlled the metabolic flux of glycolysis to serine, thereby supporting the production of GSH and nucleotides. Epigenetic modifiers also regulate the expression of key enzymes in the SSP (44-46). Histone H3 lysine 9 (H3K9) methyltransferase G9A is required for the transcriptional activation of key enzymes in the SSP during an active state, marked by H3K9 monomethylation, which is dependent on ATF4 (47). Coincidentally, Zhao *et al* (45) speculated that the H3K9 demethylase lysine demethylases 4 (KDM4) may also play a role in the transcriptional regulation of SSP. KDM4C specifically epigenetically activates the metabolic enzyme genes PHGDH, PSAT1 and SHMT2, by removing the restrictive modification of H3K9me3. This action requires ATF4 and interacts with ATF4 to target the metabolic enzyme genes and enhance the expression of their mRNA and protein, suggesting that KDM4C exerts a role in coordinating amino acid metabolism through a series of regulatory mechanisms (45). These studies indicate that as an upstream regulator of the SSP, targeting ATF4 is an effective

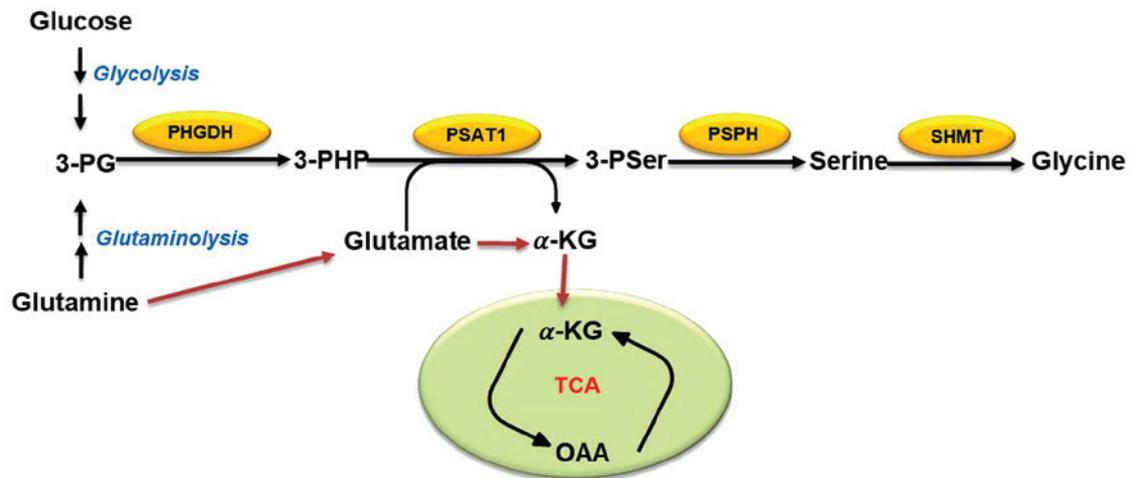


Figure 1. The Serine-glycine biosynthesis pathway. The glycolysis pathway and glutamine catabolism provide an intermediate metabolite, 3-PG, which is gradually catalyzed into serine by PHGDH, PSAT1 and PSPH. Finally, serine is converted into glycine by SHMT1/2. Yellow ovals represent metabolic enzymes. 3-PG, 3-phosphoglycerate; 3-PHP, 3-phosphate hydroxypyruvate; 3-Pser, 3-phosphoserine; α -KG, α -ketoglutarate; OAA, oxaloacetate; PHGDH, phosphoglycerate dehydrogenase; PSAT, phosphoserine aminotransferase; PSPH, phosphoserine phosphatase; SHMT, serine hydroxymethyltransferase; TCA, tricarboxylic acid.

mechanism for blocking the SSP in a coordinated fashion. As such, ATF4 may be a promising therapeutic target.

As an oncogene, c-Myc drives malignant progression and induces a powerful anabolic and proliferative program, resulting in the occurrence of intrinsic stress (36,48-50). Of note, transcription factor c-Myc can regulate 10-15% of human genes and participate in the cell cycle as well as cellular development, apoptosis and metabolism (51-53). There is evidence that c-Myc selectively fine tunes the expression of various genes which are vital for cell growth and cancer progression (54-56). Not only does c-Myc regulate the metabolism of glucose, glutamate and nucleotides, but also it participates in SSP activation induced by nutritional starvation (11,57-59). Sun *et al* (11) identified the Ebox c-Myc binding site on the PHGDH, PSAT1 and PSPH loci and that knocking out c-Myc can reduce the expression of these genes. c-Myc-mediated PSPH expression and SSP activation are essential for cancer cell survival and proliferation because of their regulation of the redox levels between GSH and reactive oxygen species (ROS), nucleotide biosynthesis and cell cycle progression (11). In addition, since c-Myc activation can induce ATF4 expression by activating the integrating stress response (36,60), the induction of PHGDH by c-Myc may depend on ATF4. It is worth noting that Myc transcription induces ribonucleoprotein polypyrimidine tract binding protein, heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and hnRNPA2 to promote the production of pyruvate kinase M2 (PKM2) (61,62). Serine is the only amino acid that can act as an allosteric activator of PKM2. Serine starvation reduces the activity of PKM2 enzymes and leads to the accumulation of upstream glycolysis intermediates, including 3-PG (63). Eventually, the tumor cells develop a higher proliferation rate under metabolic stress because of the significant increase of flux into the SSP (63-66). Overall, these studies indicate that Myc may promote the SSP by implementing the above two feedback pathways, demonstrating that the overall changes in c-Myc metabolism lead to SSP activation and cancer metastasis.

The tumor suppressor p53 has become recognized as an important regulator of cell metabolism, which can affect a series of cellular metabolic processes, such as glycolysis, oxidative phosphorylation, glutaminolysis and antioxidant reactions (67-71). p53 is also a key substance in the cell response to various forms of stress, including DNA damage, hypoxia and oncogene activation (68). Under nutritional deficiencies, p53 can protect cells by supporting metabolic adaptation. p53 helps cancer cells overcome serine starvation while retaining the cellular antioxidant capacity (72). p53-deficient cells cannot respond to serine starvation due to oxidative stress, which leads to a reduced viability of cancer cells and severely impaired proliferation (72). During serine starvation, activation of the p53-p21 axis in p53^{+/+} cells results in transient p21-dependent G1 arrest and reduction of S-phase cells, thereby inducing cell cycle arrest. This pathway can facilitate cell survival by effectively depleting serine reserves for GSH synthesis (32). As both metabolic reprogramming and the Warburg effect inhibit cancer cell death through the elimination of metabolic ROS (73), Maddocks *et al* (32) emphasized that p53 can coordinate cancer metabolic reprogramming under metabolic stress. Notably, p53 is frequently mutated in various types of human cancer, such as the common mutant, R248W. Such p53 mutants lose the function of wild-type p53 to clear cellular ROS, but retain the ability of wild-type p53 to bind to p21 and MDM2 (74). Increased levels of MDM2 promote the formation of MDM2 and ATF4 complexes, which can support cancer survival and proliferation by activating the SSP and inducing antioxidant responses under serine starvation (74,75). According to related reports, p73, a p53 homolog, also plays a significant role in serine biosynthesis (76). p73 transcriptionally induces glutaminase 2 (GLS-2) to facilitate the decomposition of glutamine, which drives the SSP through glutamate to help cancer cells resist metabolic stress (76). Interestingly, in human melanoma cells, p53 induced by Nutlin-3, downregulates the expression of PHGDH to repress *de novo* serine biosynthesis (77). Moreover, under serine deprivation, Puma and Noxa can be activated by

ATF4-dependent Nutlin-3, which inhibits PHGDH and then further promotes apoptosis (77). These findings indicate that p53 promotes metabolic remodeling for cancer cell proliferation under serine starvation, although the specific regulatory role of p53 is dependent on the type of cancer cell.

4. Inputs and outputs of one-carbon metabolism

One-carbon metabolism includes a bicyclic pathway formed by the coupling of the folate cycle and the methionine cycle and the trans-sulfuration pathway (15,16). Folate is a B vitamin which occurs naturally in many foods, and dietary supplements usually contain the synthetically produced form that is defined as folic acid. In the folate cycle (Fig. 2), folic acid is reduced twice by dihydrofolate (DHF) reductase (DHFR) and finally converted to tetrahydrofolate (THF). THF accepts the one-carbon unit from the conversion of serine to glycine to form 5, 10-methylenetetrahydrofolate (me-THF). me-THF is then either converted into 10-formyltetrahydrofolate (F-THF) by methylenetetrahydrofolate dehydrogenase (MTHFD) 1/2/1L or catalyzed by methylenetetrahydrofolate reductase (MTHFR) to 5-methyltetrahydrofolate (mTHF). mTHF can then be demethylated again and converted back to THF. The demethylation of mTHF completes the folate cycle and then starts the methionine cycle. mTHF transfers carbon units to homocysteine, which is then converted to methionine by methionine adenosine transferase. Methionine is used to generate SAM. SAM is a substrate of the methylation reaction, which when demethylated forms S-adenosyl homocysteine (SAH). The latter is then catalyzed by SAH hydrolase (SAHH) and converted into homocysteine, thus completing the entire methionine cycle (16).

One-carbon metabolism can circulate carbon units from various amino acids, generate a range of different outputs and integrate a variety of cellular nutritional statuses (Fig. 3) (15). The one-carbon unit is supplied by several sources. Serine is the main donor of the one-carbon unit when it is converted to glycine. Alternatively, the glycine cleavage system (GCS) can also fuel one-carbon unit in cancer cell lines with high GCS activity, such as lung tumor-initiating cells and glioblastoma-derived cells (78). Recent evidence suggests that cancer cells can alter or even rely more on these sources to maintain one-carbon metabolism for cancer cell proliferation (15). Serine derived from exogenous uptake or *de novo* SSP synthesis can be cleaved into glycine by the methyltransferases SHMT1 (in the cytoplasm) and SHMT2 (in the mitochondria), and donate one-carbon unit (18). In this pathway, the one-carbon unit cleaved from serine is transferred to THF and then converted to me-THF (15,16). This reaction can also proceed in the opposite direction, whereby the consumption of the one-carbon unit by SHMT converts glycine to serine (79,80). These reactions demonstrate that the SSP metabolic enzymes have a significant impact on the production of the one-carbon unit. By depleting the availability of the one-carbon unit, serine starvation or downregulation of SSP metabolic enzymes causes the reduction of cancer cell proliferation and xenograft growth (80-82). Additionally, glycine, similarly to serine, can also be a source of the one-carbon unit through the GCS, although this reaction only occurs within the mitochondria and fuels one-carbon metabolism (83). THF accepts a methylene group via the GCS.

The resultant methylene-THF is then used in various downstream reactions which require a one-carbon unit (83). During this pathway, NADH can be also regenerated with the release of CO₂ and ammonia (83,84). Some studies, however, have found that although the GCS can support tumorigenesis (68,85), its activity seems to be more inclined to the degradation/detoxification of glycine rather than the generation of the one-carbon unit for nucleotide synthesis (80,85). The directionality of serine/glycine conversion is a significant factor for cancer cell metabolism and evidence indicates that mitochondrial SHMT2 is the main serine-glycine converting enzyme under the above circumstances by tracing NADPH with ²H-labeled glucose (86). Choline, a vitamin from the human diet, can be metabolized into betaine and donate one-carbon unit (87,88). Moreover, one-carbon unit can also be derived from histidine and tryptophan (89). Although, these little-known pathways can theoretically contribute one-carbon unit, to the best of our knowledge, their importance for one-carbon metabolism in cancer cells has yet to be fully described.

The outputs of one-carbon metabolism include the production of ATP, NADPH and the regulation of energy balance, as well as the synthesis of biomacromolecules, such as proteins, lipids, nucleotides and substrates of methylation reactions (90-95). DNA synthesis requires nucleotides, which is a restrictive metabolic aspect of cell proliferation (19). With the methyl donor me-THF, deoxyuridine monophosphate (dUMP) can be methylated to generate deoxythymidine monophosphate (dTMP) by thymidylate synthase (TYMS), while me-THF is converted to DHF and reduced to THF by DHFR (16). In addition, purine can also be generated through the intermediate F-THF from the folate cycle (16). In the methionine cycle, not only is methionine itself necessary for protein synthesis, the SAM produced by adenylation can be used as the methyl donor for other pathways requiring methyl groups, including histone, DNA and RNA methylation; lysine and arginine methylation; polyamine synthesis; and methylation reactions that generate lipid head groups (96-99). As much as 40% of the SAM goes to phosphatidylcholine (PC) production in liver cells where the demand for PC is high, instead of through the Kennedy pathway (100). Homocysteine, the intermediate product of the methionine cycle, can produce GSH through cystathionine and then cysteine in the trans-sulfuration pathway (16).

One-carbon metabolism also plays an important role in cell redox balance. In each round of the folate cycle, a molecule of NADP⁺ is produced during the reduction of me-THF by MTHFR (16). The adjustment of the NADP⁺/NADPH ratio helps to sustain the redox state (101). In addition, GSH, a tripeptide containing cysteine, glycine and glutamic acid, contributes to the maintenance of the NADP⁺/NADPH ratio and is the main contributor to the redox balance (15,16). Therefore, cancer cells gain survival and proliferation advantages from changes in these metabolic pathways.

5. Association of one-carbon metabolism with cancer cell proliferation and regulation

In the context of disease prevention, diagnosis and treatment, the research and control of one-carbon metabolism is the basis of other medical and disease research (15-17,102). As aforementioned, the output of one-carbon metabolism is

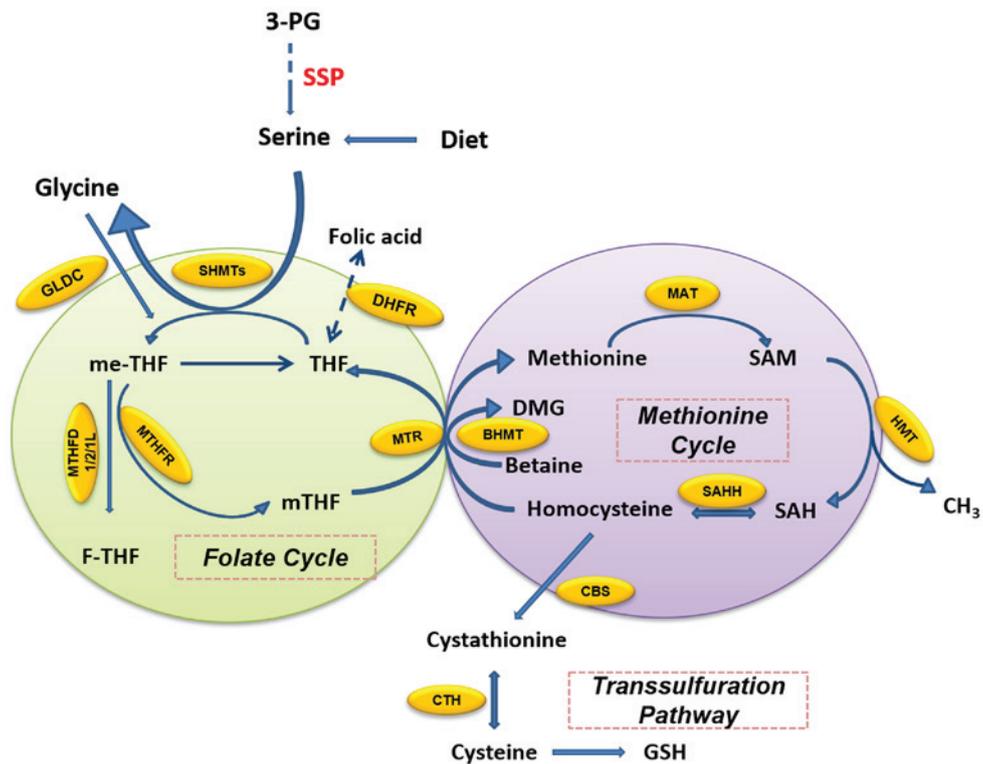


Figure 2. Overview of one-carbon metabolism around the folate cycle, methionine cycle and trans-sulfuration pathway. The folate cycle provides one carbon for the methionine cycle, as well as homocysteine, an intermediate product of the methionine cycle, which can be converted into GSH through the trans-sulfuration pathway. Yellow ovals represent metabolic enzymes. 3-PG, 3-phosphoglycerate; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; CTH, cystathionase; DHFR, dihydrofolate reductase; DMG, dimethylglycine; F-THF, 10-formyltetrahydrofolate; GLDC, glycine dehydrogenase; GSH, glutathione; HMT, histone methyl transferase; MAT, methionine adenosyltransferase; me-THF, 5,10-methylenetetrahydrofolate; mTHF, 5-methyl-tetrahydrofolate; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; SAH, S-adenosyl homocysteine; SAHH, SAH hydrolase; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; SSP, serine synthesis pathway; THF, tetrahydrofolate.

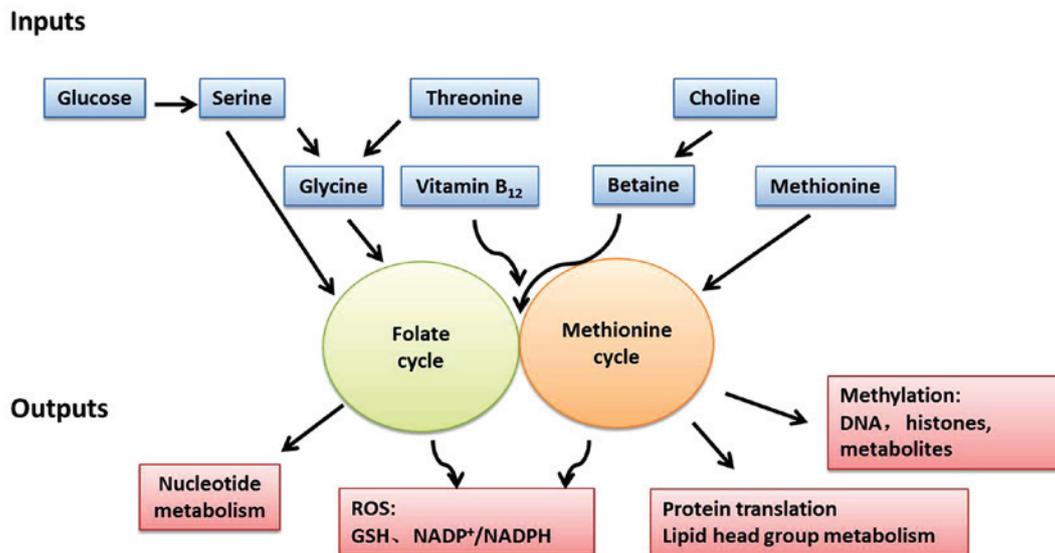


Figure 3. One-carbon metabolism integrates nutrient status and cellular functions with appropriate equilibrium in inputs and outputs. One-carbon metabolism can be viewed as a set of two modular units including the folate cycle and methionine cycle. The different nutrient sources and amino acids (serine, glycine, threonine, choline, betaine, methionine and vitamins) are inputted into the one-carbon metabolism and converted into a wide variety of outputs, such as nucleotide metabolism, redox control and post-translational modification. GSH, glutathione; ROS, reactive oxygen species.

essential for maintaining normal cell or cancer cell metabolism. For example, the methylation of DNA and histones is the most common molecular function change in cancer cells (17).

Rapidly growing cells, such as tumor cells and embryogenic cells, require the synthesis of large amounts of proteins, lipids and nucleotides to support their proliferation (94). In

addition, the redox level in the tumor microenvironment is also key to the survival of cancer cells (91). The present review subsequently aims to discuss the main products of one-carbon metabolism and their physiological relevance, in an attempt to better understand the role of one-carbon metabolism activity in tumorigenicity and tumorigenesis.

Nucleotide synthesis. The one-carbon unit is essential for the synthesis of purine and pyrimidine nucleotides, which are necessary for the synthesis of DNA and RNA (19). *De novo* purine nucleotide synthesis mainly includes two stages: i) Synthesis of the important intermediate metabolite, inosine monophosphate (IMP), a common precursor of all purine nucleotides, followed by ii) the conversion of IMP into adenosine monophosphate (AMP) and guanosine monophosphate (15). IMP synthesis requires the 5-phosphate ribose provided by the pentose phosphate pathway (PPP) to combine glycine, the one-carbon unit carried by F-THF, CO₂ and other substances during a series of reactions (15). Both glycine and the one-carbon unit must be generated from serine through folate metabolism in the cytoplasm or mitochondria (79). Restricting exogenous glycine or depleting the GCS cannot hinder cancer cell proliferation (80). Moreover, without serine, the ingestion of exogenous glycine also cannot support nucleotide synthesis (80). The above evidence indicates that folate metabolism plays an important role in nucleotide synthesis. Studies have revealed that inhibition of folate metabolism through serine starvation or the RNAi-mediated knock-down of SHMT2, leads to an accumulation of precursors upstream of IMP prior to incorporation with the one-carbon unit (80,85). Therefore, the level of one-carbon unit required for purine nucleotide synthesis can be reduced by depletion or deprivation of serine, which then inhibits cancer cell proliferation (80,81). One-carbon metabolism also provides the methyl donor for pyrimidine nucleotide synthesis. me-THF, as the methyl donor, supports the methylation reaction of dUMP to generate dTMP catalyzed by TYMS. me-THF is then converted to DHF and reduced to THF by DHFR (17). Therefore, targeting glycine dehydrogenase (GLDC), SHMT or TYMS, which promote pyrimidine synthesis, may be a potential way to suppress cancer development (24,103-105). As the key enzyme in the folate cycle, the expression of MTHFD2 is closely related to mTORC1 signaling in both normal cells and cancer cells. MTHFD2 expression is stimulated by ATF4 activated by mTORC1 independent of eukaryotic initiation factor 2 α phosphorylation and MTHFD2 enhances F-THF production to support the synthesis of purines (106,107). Interestingly, mTORC1 can also phosphorylate carbamoyl phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase with the help of its downstream target ribosomal protein, S6 kinase 1, thereby promoting pyrimidine synthesis (108,109). These relationships indicate that mTORC1 can enhance the folate cycle and nucleotide synthesis to adapt to the increased RNA and DNA synthesis required for cancer cell anabolism (19).

Methylation pathway. The methylation pathway is one of the tumor metabolic reprogramming pathways and all methyltransferase reactions in mammalian cells are completely dependent on the methyl donor, SAM. The levels of SAM and its derivative

SAH can directly affect the epigenetic landscape of tumor cells by regulating the activity of key epigenetic enzymes and ultimately, determine the fate of cancer cells (110). The expression of tumor-suppressor gene promoters can be suppressed through hypermethylation, which then weakens their ability to inhibit the tumorigenic transformation of cells (98,111,112). PKM2 knockdown contributes to SAM production in mouse models (113,114), suggesting that PKM2 is involved in the regulation of the SAM-mediated cancer phenotype by controlling methylation. In highly lethal prostate cancer with protein kinase C ζ (PKC ζ)/ ι deficiency, the active mTORC1-mediated ATF4-SSP/one-carbon metabolism axis upregulates SAM synthesis (25). This approach helps to increase the plasticity of cell lineages and even gives human cancer and mouse models *in vivo* resistance to targeted therapy (25). In addition, the absence of serine-threonine kinase (LKB1) in Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutant pancreatic cancer promotes tumorigenesis (115). LKB1 deletion increases the expression of SSP metabolic enzymes, which activates *de novo* serine biosynthesis and produces SAM through one-carbon metabolism, ultimately increasing the overall amount of DNA methylation and the levels of several DNA methyltransferases in LKB1-deficient KRAS mutant cells. This indicates that this type of SAM-dependent methylation pathway contributes to the metabolic reprogramming of tumors (115). Interestingly, it has generally been believed that the methionine cycle is mainly supported by the one-carbon unit cleaved from the serine/glycine synthesis pathway, but in fact, this pathway has very low activity in cancer cells (116,117). It has been reported that the metabolism of serine and glycine can support *de novo* ATP synthesis and the adenosine derived from ATP can participate in the conversion of methionine to SAM (81). Therefore, the restriction of serine can also reduce the transfer of methyl units to DNA and RNA in cancer cells by reducing *de novo* ATP synthesis (15,81).

Redox balance. NADH and NADPH are important cofactors and can provide electrons for redox reactions. These molecules can be produced by one-carbon metabolism and are essential for multiple metabolic and biosynthetic pathways (91). In the folic acid cycle, me-THF can convert to F-THF, which is catalyzed by MTHFD. NAD⁺ or NADP⁺ as the cofactor in this reaction can be reduced to NADH or NADPH, respectively. MTHFD2 and MTHFD2-Like (MTHFD2L) are two forms of mitochondrial MTHFD which can use both NAD⁺ and NADP⁺ as cofactors to generate mitochondrial NADH and NADPH (86,118,119), respectively. The cytoplasmic MTHFD1 can only utilize NADP⁺; however, the functional correlation of the aforementioned dual-specificity remains unknown (119). During the catabolism process, the MTHFD2 reaction runs at a faster rate than the one-carbon unit required for purine synthesis (79). This enables cells to increase the production of NADH. NADH is known to contribute to a respiratory chain that is coupled to oxidative phosphorylation, which circles back to ATP to maintain central energy metabolism (79). There is another pathway that produces mitochondrial NADPH, which occurs during the oxidation of F-THF to CO₂ and THF by aldehyde dehydrogenase 1 family member L2, and provides some of the energy for proline synthesis (79). Although studies have shown that NADPH is mainly produced in

mitochondria (86,90,120), cytosolic NADPH can be generated by oxidizing me-THF by MTHFD1 (79). The synthesis of fatty acids in the cytoplasm is mainly supported by NADPH formed by the action of malic enzyme. In addition, the cytoplasmic NADPH derived from folate metabolism can also specifically support fatty acid synthesis (15). Fatty acids are necessary for the production of lipid signaling molecules and membranes, and both are essential for sustaining cancer cell proliferation (121). Serine/one-carbon metabolism also depends on the cytoplasmic NADPH/NADP ratio maintained by the activity of the oxidative PPP (oxPPP). Studies have shown that the loss of glucose 6-phosphate phosphate dehydrogenase can inhibit oxPPP, leading to high NADP and impairing folate-mediated biosynthesis by inhibiting DHFR activity with high NADP in CRC cells (91). This indicates that oxPPP is crucial for maintaining normal NADPH/NADP ratios, DHFR activity and folate metabolism. SHMT2 is a direct target gene of c-Myc (122). When MYC-transformed cells are subjected to hypoxia, SHMT2 is induced and triggers the degradation of serine to CO₂ and NH₄⁺, simultaneously producing net NADPH to maintain oxidation of the tumor microenvironment (122). A study concerning human glioblastoma multiforme confirmed this was the case in this disease. SHMT2 and GLDC are highly expressed in the pseudopalisading cells around necrotic lesions (85). SHMT2 inhibits PKM2 activity and reduces oxygen consumption, which triggers a novel metabolic state, conferring a profound survival advantage to cells in tumor regions with poor vascularization (85). In addition, GSH is one of the products of the trans-sulfuration pathway and one of the most abundant metabolites in cells. It is also important for maintaining the NADPH/NADP⁺ ratio (123). GSH has the ability to scavenge and reduce ROS, as well as maintain the appropriate NADPH/NADP⁺ ratio, which greatly contributes to the redox balance in cells (123,124).

6. Cancer treatment and potential new opportunities

One of the major challenges for cancer biology is to find novel and effective therapeutic targets that can be used for interventions with chemically selective pharmaceuticals in different patients. Antimetabolite drugs (antifolates) are a landmark in cancer chemotherapy and are still the most widely used drugs in medical oncology (Table I) (125-134). Among the antifolates, methotrexate and pemetrexed are effective inhibitors of DHFR, which can reduce the THF pool and prevent cell proliferation (135,136). As such, they are a major class of cancer chemotherapeutic drugs and are currently used as a first-line chemotherapeutic agent in the treatment of various cancers, including acute lymphoblastic leukemia, breast cancer, bladder cancer and lymphoma (137,138). Studies have found that methotrexate and pemetrexed also have the ability to bind to and inhibit human SHMT *in vitro* (139). There are other drugs that target the downstream pathway of the SSP/one-carbon metabolism which have been approved for clinical use, such as gemcitabine and 5-fluorouracil (5-FU) (140,141). 5-FU, a congener of uracil and a standard drug used to treat a variety of cancers, inhibits TYMS, resulting in the reduction of the methylation of dUMP to dTMP and the interruption of the folate cycle (141). 5-FU can also be converted to 5-fluorouridine, which is incorporated into ribosomal RNA (rRNA)

molecules and inhibits rRNA processing, eventually leading to p53-dependent cell cycle arrest and/or apoptosis (142). Traditional antifolate chemotherapy drugs, such as methotrexate and 5-FU, have been used in clinical cancer chemotherapy to target one-carbon metabolic pathways for ~70 years (72). However, since the folate metabolism pathway is also important in normal cell proliferation, these drugs have many harmful side effects. Moreover, resistance to antifolates is also a common problem in cancer treatment (15). For these reasons, the development of new targets and new drugs is crucial.

Currently, other studies targeting the downstream of SSP/one-carbon metabolism are attempting to regulate the epigenetic state of the tumors and regulate the metabolic enzymes that are overactivated in the tumors (15,72,143). Epigenetic reprogramming through the regulation of the methylation pathway is essential for the malignant tumor phenotype with studies suggesting that the control of methylation is possible (144,145). As aforementioned, methotrexate has been widely used for cancer treatment since 1948, but it has only recently been found that methotrexate can decrease Wnt-induced intracellular lysosome activity and reduce typical Wnt signaling by inhibiting SAM levels and blocking arginine methylation (146). These findings indicate that methotrexate may be used to treat Wnt-driven malignant tumors. It has been found that the activation of SSP/one-carbon metabolic pathway genes during cancer metabolic control depends on the G9A epigenetic program (47,143), and the G9A inhibitor, BIX01294, can cause cell death by depriving serine *in vivo* (147). This suggests that G9A inhibition may be a therapeutic strategy for the treatment of cancer, a possibility that is contributing to the development of G9A-like drug molecules. The H3K4 demethylase jumonji AT rich interactive domain 1B (Jarid1b) (Lysine demethylase 5B/PLU-1/retinoblastoma binding protein 2-homolog 1) supports the continuous tumor growth in certain cell subsets of slow-circulating melanoma (148). These cancer cell subtypes exhibit slow DNA replication and may be resistant to chemotherapeutic agents and radiation, thereby contributing to tumor recurrence and metastasis (148). In solid cancers, histone lysine demethylase family members are associated with cancer progression. Knockdown of related genes can therefore suppress carcinogenicity and promote cell senescence (149,150). Methylation donors, ornithine decarboxylation and polyamine metabolism have been widely investigated as anti-cancer therapeutic targets, with some of these drugs entering clinical trials, such as ornithine decarboxylase inhibitor; 2-difluoromethylornithine, a competitive inhibitor of SAM decarboxylase; methylglyoxal bis (guanylhydrazone); and SAM486A (133,151). Targeting SSP metabolic enzymes also appears to be a promising method. PKC ζ not only inhibits the transcription of PHGDH and PSAT1, but also phosphorylates PHGDH to inhibit its catalytic activity (152). In addition, for certain PHGDH-dependent cancer cells, some small molecule inhibitors targeting PHGDH have been developed and successfully verified *in vitro*, which not only reduces cancer cell proliferation, but inhibits the growth of xenografts (153-156) (Table II).

Pharmacology can be used as a complementary strategy for cancers that do not upregulate the key enzymes of the SSP (21,32,157). In addition to the positive correlation

Table I. Antimetabolite drugs for the treatment of various types of cancer.

Drug name	Targets	Therapeutic uses	Refs.
Methotrexate	DHFR	Used to treat multiple cancers	(131)
Pemetrexed	DHFR, TYMS and SHMT	Used to treat multiple cancers, especially non-small cell lung carcinoma and pleural mesothelioma	(131)
Pralatrexate	DHFR	Peripheral T-cell lymphoma	(125)
Raltitrexed	DHFR and TYMS	Metastatic colorectal cancer	(126)
5-FU	TYMS	Used to treat multiple cancers, especially colorectal cancer	(127)
Gemcitabine	Ribonucleotide reductase	Used to treat multiple cancers, especially pancreatic cancer	(128)
Cytarabine	Ribonucleotide reductase	Acute leukemia	(129)
Azanucleotides	DNA methyltransferases	Myeloid leukemia	(130)
DMFO	Ornithine decarboxylase	Clinical trial	(133)
SAM analogues	Histone methyltransferases	Clinical trial	(134)
MGBG and SMA486A	S-adenosyl decarboxylase	Preclinical studies	(134)

5-FU, 5-fluorouracil; DHFR, dihydrofolate reductase; TYMS, thymidylate synthase; SHMT, serine hydroxymethyltransferase; DMFO, 2-difluoromethyl ornithine; MGBG, methylglyoxal bis (guanylhydrazone); SMA486A, (E)-2-(4-carbamimidoyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinecarboximidamide.

Table II. Inhibitors of PHGDH.

Inhibitor name	Inhibitor type	Inhibition mechanism
Indole derivative 1	Orthosteric inhibitors	Competitive inhibitors. Bind with the NAD ⁺ pocket of PHGDH, and inhibit its activity
Compound 9 (CBR-5884)	Allosteric inhibitors	Noncompetitive inhibitors. Bind to a Cys in the non-active site and disrupts its oligomeric state
(DSF)	Allosteric inhibitors	Noncompetitive inhibitors. Convert PHGDH tetramer into either an inactive dimer to inhibit PHGDH activity
Compound 14 (NCT-503)	Allosteric inhibitors	Noncompetitive inhibitors. Closely bind to the active site as a mutation of C234 in the protein's active site to reduce the inhibitory effect of PHGDH
α -ketothioamide derivatives.	Allosteric inhibitors	Decrease PHGDH activity and selectively strain the proliferation of cancer cells with elevated PHGDH expression
PKUMDL-WQ-2101	Non-NAD ⁺ competing allosteric inhibitors	Form hydrogen-bond networks with R134, K57 and T59 of site I to inhibit PHGDH activity
PKUMDL-WQ-2201	Non-NAD ⁺ competing allosteric inhibitors	Form hydrogen-bond networks with T59, T56 and K57 of site II to inhibit PHGDH activity
Azacoccone E	Natural compounds, allosteric inhibitors	Noncompetitive inhibitors. Fit at the allosteric site of PHGDH to diminish enzyme activity.
Iox A	Allosteric inhibitors	Directly coordinate at the allosteric site in the back side of the active site of PHGDH

DSF, disulfiram; Iox A, Ixocarpalactone A; PHGDH, phosphoglycerate dehydrogenase.

between high carbohydrate intake and cancer incidence (158), low glucose intake may have a negative effects on tumor growth and progression (159), making the reduction of exogenous serine intake a feasible approach. Indeed, serine and glycine starvation can successfully reduce xenograft and spontaneous tumor growth, and have been found to significantly improve survival rates in various mouse tumor

models (29,32). Particularly in the case of p53 deficiency, cancer cells are more sensitive to serine and glycine starvation (32). Metformin has recently been recognized as a promising drug for cancer treatment (160). Gravel *et al* (157) examined the anti-tumor effect of metformin combination with serine starvation. Their results showed that biguanide does not inhibit serine synthesis and that cancer cells require

serine to upregulate the glycolytic pathway to compensate for the reduction of oxidative phosphorylation induced by biguanide (159). Under a serine deficiency, biguanide activity is enhanced without relying on AMP-activated protein kinase; and serine deprivation and metformin exert joint antiproliferative effects by directly interfering with cancer cell metabolism. In addition, the deprivation of serine also changes the relative abundance of the metformin-induced TCA cycle metabolites (157). This points us to a new type of dietary manipulation that can enhance the efficacy of biguanides as antineoplastic agents.

Targeting folate metabolizing enzymes, such as MTHFD2, is another potential method for cancer treatment. MTHFD2, which is normally expressed only during embryonic development, provides the possibility of a disease-selective treatment target, through eliminating cancer cells while retaining healthy cells (161). Gustafsson *et al* (161) reported the synthesis and pre-clinical characterization of the first human MTHFD2 inhibitor, LY345899, providing a theoretical basis for the continued development of the structural framework for MTHFD2 inhibitors that can be effectively used for the treatment of various types of cancer. Recently, it has been reported that the expression of MTHFD2 and the stem-like properties can be enhanced in lung cancer cells that have acquired resistance to the targeted drug gefitinib (162). Furthermore, the overexpression of MTHFD2 makes gefitinib-sensitive lung cancer cells resistant to gefitinib. In these gefitinib-resistant cancer cells, the sensitivity to gefitinib, as well as the stem-like properties, can be restored after MTHFD2 knockdown or treatment with AICAR (162). Therefore, since cancer stem (like) cells are dependent on MTHFD2, therapies targeting MTHFD2 have been proposed as a therapeutic possibility for eradicating tumors and preventing recurrence (162). The problem with this approach; however, is that when targeting specific components of one-carbon metabolism, the tumor may reconnect with other metabolisms to compensate (163). The function of the MTHF enzyme is to convert me-THF to F-THF and mTHF for nucleotide synthesis and methionine recycling (143). The MTHFD enzyme has several forms: Cytoplasmic MTHFD1, mitochondrial MTHFD1-Like (MTHFD1L), MTHFD2 and MTHFD2L (118,143). MTHFD2 is only expressed in embryos, tumors and undifferentiated tissues, while MTHFD2L is more widely expressed (163,164). Cells primarily use mitochondrial enzymes for one-carbon metabolism, so if this effect is suppressed, cells can compensate by using cytoplasmic MTHFD1 (79). Cells primarily use mitochondrial enzymes for one-carbon metabolism, so if this effect is suppressed, cells can compensate by using cytoplasmic MTHFD1 (79).

7. Conclusions

In the past few years, researchers' interest in cancer metabolism has surged, leading to an expanding understanding of the metabolic pathways of cancer biology. Recent advances in comprehending the relationship between cancer and metabolism highlight the correlation between the SSP and one-carbon metabolism. At present; however, the molecular regulatory mechanism between SSP/one-carbon metabolism and cancer metabolism is not fully understood. To

explore its therapeutic potential, it is necessary to biochemically dissect the ways in which these metabolic pathways promote cancer biology, in the hope of solving the mystery and helping to clinically overcome the worldwide problem of cancer.

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Authors' contributions

SP, MF and ZL collected information and wrote the manuscript. HW and XL collected information and edited the manuscript. All authors read and approved the final version of this manuscript.

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Competing interests

The authors declare that they have no competing interests.

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