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# Serine, glycine and the one-carbon cycle: cancer metabolism in full circle

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## Abstract

One carbon metabolism involving the folate and methionine cycle integrates carbon units from amino acids, including serine and glycine, and generates diverse outputs, such as the biosynthesis of lipids, nucleotides and proteins, the maintenance of redox status, and the substrates for methylation reactions. Long considered a 'housekeeping' process, this pathway has been recently shown to have additional complexity. Recent genetic and functional evidence also suggests that hyperactivation of this pathway is a possible driver of oncogenesis and establishes links to cellular epigenetic status. Given the wealth of clinically available agents that target one carbon metabolism, these new findings could present opportunities for translation into precision cancer medicine.

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**Links**

**Database of the human metabolic network:humanmetabolism.org**
**Status of Cancer clinical trials in the United States: cancer.gov/clinicaltrials**
Encyclopedia of metabolic pathways: metacyc.org
Human Metabolite Database: hmdb.ca
Jason Locasale's website: Jlocasale.human.cornell.edu
Metabolomics resources on behalf of Dr. Gary Siuzdak's lab: metlin.scripps.edu
National Institutes of Health initiative on metabolomics: commonfund.nih.gov/Metabolomics/

**Timeline**

1944 – Soldiers in the Pacific Islands with tropical anemia are treated with B vitamins.
1949 – Sydney Farber successfully uses anti-folate agents to induce remission in children with Acute Lymphoblastic Leukemia.
1955 – De novo serine biosynthesis is observed in tumors.
1956 - The folate antagonoist, Methotrexate becomes widely used in oncology
1957–1970 - 5-FU is discovered and later approved for the treatment of advanced colorectal cancer.
1984 - Gemcitabine is approved for metastatic cancer and shows activity in pancreatic cancer.
1987 – Snell and colleagues demonstrate that serine biosynthesis is increased during tumor progression in rat models.
1996 – *FHIT* is cloned as a tumor suppressor gene.
2000 - Methyltransferase inhbitors are tested pre-clinically.
2004 – Thymidylate synthase is identified as an oncogene.
2009 - Sarcosine identified as candidate biomarker for metastatic prostate cancer.
2009–2013 – One carbon metabolism found to be essential for stem cell pluripotency and later observed to alter histone methylation status.
2011 – De novo serine and glycine metabolism is found to be necessary and sufficient for cell transformation and malignancy.
2012 – Glycine catabolism is found to be associated with rapid cell proliferation.
2012 - Choline C11 PET imaging is FDA-approved.
2013 – Serine and glycine dietary restriction found to inhibit tumor growth in preclinical studies

## Introduction

Cell growth and proliferation requires the construction of building blocks for new cellular components including proteins, lipids and nucleic acids, as well the maintenance of cellular redox, genetic and epigenetic status[1–6]. Amino acid metabolism involving serine, glycine and threonine and the carbon units they provide satisfies many of these requirements[7,818,19]. One-carbon metabolism encompasses a complex metabolic network that is based on the chemical reactions of folate compounds[9–11]. These reactions proceed in a cyclical nature where a carbon unit is transferred to other metabolic pathways and eventually replenished by several sources. Modern cancer therapy in part arose from the hypothesis that antagonists of folates could reduce the proliferation of malignant blood cells[12,13]. The antagonism of folate metabolism and its downstream effectors, such as nucleotide metabolism, has been used in chemotherapy for over sixty years[14–17] (see the Timeline in Fig 1).

Recently, there has been a surge of interest in the study of the metabolic processes associated with cancer[18]. Much of this focus has been on the role of two nutrients, glucose and glutamine, in supporting energy metabolism and anabolic processes[19–21]. Expanding the compendium of metabolic pathways essential to cancer biology is that of serine and glycine metabolism[8,22]. Developments in our understanding have led to new biology related to the function of this pathway in cancer. These advances include roles for epigenetics, redox status, genome maintenance, protein translation, and biosynthesis for cell proliferation. Genetic and functional evidence also points to the activity of this pathway as having a role as a driver of oncogenesis.

This Review discusses recent developments that are transforming our understanding of serine, glycine, and one-carbon metabolism in cancer pathogenesis. These advances include genetic and functional evidence for its role as a driver of cancer pathogenesis and new roles for one carbon metabolism in tumor maintenance, including genome integrity and epigenetic maintenance. Ultimately these findings may result in new translational opportunities for drug development, dietary intervention in cancer prevention, biomarkers that indicate in which tumors antimetabolic chemotherapeutic drugs are likely to produce an efficacious response, and new frameworks for approaching the study of the pathogenesis of cancer[23].

## One-carbon metabolism: an integrator of nutrient status

A way to conceptualize the role of one-carbon metabolism in cellular physiology is that it functions as a metabolic integrator of nutrient status (Fig 2). Inputs in the form of glucose and amino acids enter the pathway, are processed through chemical reactions, and are then output for diverse biological functions. This analogy has been used extensively to describe growth control by the mammalian target of rapamycin (mTOR) signal transduction pathway, but has been used less for metabolic pathways per se[24]. In the case of mTOR signaling, inputs in the form of amino acids and growth factors are integrated to generate outputs such as protein translation, autophagy inhibition, and anabolic metabolism. For one-carbon metabolism, the integration is carried out through the donation of carbon units from specific amino acids. These carbon units are the substrates for one carbon metabolism and are distributed via a series of chemical reactions for use in diverse cellular processes that include cellular biosynthesis, regulation of redox status, regulation of epigenetics through nucleic acid and protein methylation, and genome maintenance through the regulation of nucleotide pools. The partitioning of carbon units into these different cellular outputs involves three pathways: the folate cycle, the methionine cycle and transsulfuration pathway (Fig 2). Loss of function mutations in enzymes involved in these pathways lead to growth defects both in animals and humans, underscoring the role of one carbon metabolism in modulating cell growth[7,25–27].

### One carbon metabolism and the transsulfuration pathway

Folic acid is a B vitamin found in and added to foods that are widely available in Western diets. In cells, folic acid is reduced by a series of enzymes leading to the generation of tetrahydrofolate (THF) (Fig 3). THF participates as a scaffold in a set of metabolic reactions that involve the movement of carbon atoms to different positions along the THF-moiety (Fig 3). The folate cycle is coupled to the methionine cycle through the generation of methyl-THF (mTHF). mTHF donates a carbon through methylation of homocysteine and this generates methionine. Thus, the folate cycle coupled to the methionine cycle constitutes a bi-cyclic metabolic pathway that circulates carbon units. These metabolic cycles are collectively referred to as one-carbon metabolism. The transsulfuration pathway is connected to the methionine cycle through the intermediate homocysteine. Serine can be directly metabolized through transsulfuration eventually resulting in the generation of glutathione, one of the major redox-regulating metabolic systems in cells (discussed below).

### Inputs to one-carbon metabolism

The carbon units that feed into one-carbon metabolism can be synthesized de novo (Fig 4). For example, an intermediate metabolite involved in glycolysis, 3-phosphoglycerate (3PG), can be converted into serine. Serine donates the carbon atom from its side-chain to folate, converting serine to glycine and THF to mTHF, which starts the folate cycle. Serine can also be directly imported from the extracellular environment by facilitated transport through amino acid transporters. In addition to the side chain of serine, there are other routes of entry into one-carbon metabolism. A glycine cleavage system is active in some cells where the enzymatic cleavage of glycine produces ammonia, carbon dioxide and a carbon unit for the methylation of THF, which also charges the folate cycle. In some cells, threonine can also be converted to glycine through an aldol cleavage[28]. Glycine can also be generated from many other sources including choline, betaine, dimethylglycine and sarcosine (also known as N-methylglycine) through a series of reactions that involve demethlyation.

## Outputs of one-carbon metabolism

### Biosynthesis

All cells require the synthesis of macromolecules, such as proteins, lipids and nucleic acids for cellular renewal and proliferation[2930]. Amino acids, such a methionine, can be generated from one-carbon metabolism and used to generate proteins[31]. Nucleotides required for DNA and RNA are also constructed through reactions that involve the folate cycle [32]. Deoxy-thymidine monophosphate is synthesized through the methylation of deoxy-uridine monophosphate by thymidylate synthase. This methylation reaction generates THF from mTHF. Purine nucleotide bases are also generated from the folate pool through the intermediate 10-formyltetrahydrofolate (F-THF), which is derived from 5,10-methylene-THF (me-THF). The ribose moiety of RNA and DNA is derived from the pentose phosphate pathway[33]. Lipids can also be generated in part through the methionine cycle[34]. Phosphatidylcholine (PC) is a major component of the cell membrane that can account for up to half of lipid membrane content[35]. The head group of PC is synthesized from choline[36] through the adenylation of methionine to S-adenosylmethionine (SAM). SAM functions as a methyl donor for the three subsequent methylation reactions that generate the lipid head group[3738].

### Redox balance

The metabolism of carbon atoms through one carbon metabolism is linked with changes in redox status. These changes largely occur through the reduction of NADPH and oxidation of $NADP^+$. Tetrahydrofolate reductase reduces THF and this reaction consumes one molecule of NADPH for each turn of the folate cycle. Although these reactions are thought to proceed

in a reductive manner, it is conceivable that the reverse of these reactions could occur *in vivo* based on known *in vitro* activities[39]. Glutathione, one output of the transsulfuration pathway, is also important for the maintenance of the ratio of NADPH and $NADP^+$. A tripeptide comprised of cysteine, glycine and glutamate, glutathione is one of the most abundant metabolites in cells, often reaching concentrations as high as five millimolar[40,41]. Thus it serves as a major contributor to the redox balance in cells through its ability to scavenge and reduce reactive oxygen species (ROS) and to maintain the appropriate NADPH/ $NADP^+$ ratio, which is required for anabolic metabolism[2]. The desulfhydration products in the transsulfuration pathway also lead to the sulfhydration of proteins. These post-translational modifications are considered to be an important and underexplored signal transduction mechanism[42].

### Methylation reactions

Cells also require substrates derived from metabolism to carry out signal transduction through post-translational modifications. Methyl groups derived from one carbon metabolism provide a major source of substrate for post-translation modifications[43–46]. As the major methyl donor in cells, SAM is involved in histone, DNA and RNA methylation, and all general protein lysine and arginine methylation[47–49]. SAM is also involved in other metabolic pathways that require methyl moieties,[50] including polyamine synthesis[51–53].

## Cancer therapy and one carbon metabolism

One of the first modern chemotherapies resulted from the observation that anemic patients could be treated with B vitamins to stimulate red blood cell production[12]. Sydney Farber also noted that folic acid could stimulate the proliferation of acute lymphoblastic leukaemia (ALL) cells and therefore investigated whether intermediates of the chemical synthesis of B vitamins might act to antagonize cell proliferation. In a landmark study by Farber and colleagues one of these molecules, aminopterin, was shown to induce remissions in children with acute lymphoblastic leukemia (ALL)[13,54]. To this day, chemical variants, such as methotrexate and pemetrexed, of these initial folate antagonists constitute a major class of cancer chemotherapy agents and are used as frontline chemotherapy for diverse cancers including ALL, breast cancer, bladder cancer and lymphomas[14,55–59]. These agents inhibit di- and tetrahydrofolate reductase activity in humans resulting in a disruption of one carbon metabolism[60,61]. It is interesting to note however that disruption of one carbon metabolism by these agents is not efficacious in all cancer types. More recent findings (discussed below) might help in understanding why this variation exists and potentially enable patients who will benefit most from these drugs to be identified.

In addition, multiple pathways downstream of one-carbon metabolism are the targets of numerous cytotoxic chemotherapies. 5-fluorouracil (5-FU) targets nucleotide metabolism that is derived from the folate cycle and is a standard of care agent for many cancers including advanced stage colorectal cancer[62,63]. 5-FU is an analog of the DNA base pyrimidine. It is a potent inhibitor of thymidine synthase thus blocking the methylation dUMP to dTMP and disrupting the folate cycle[64]. Gemcitabine, which is used to treat patients with pancreatic cancer[65], is another inhibitor of nucleotide metabolism[66,67]. Gemcitabine is a nucleoside analog that interferes with the biosynthesis of cytidine[68] and it inhibits ribonucleotide reductase (RNR), preventing the formation of deoxynucleotides[66]. Gemcitabine efficacy in pancreatic cancer is variable, but whether this stems from resistance because of alterations in one carbon metabolism, or from differences in drug delivery is yet to be fully resolved.

Other pathways downstream of one-carbon metabolism are also the target of cancer therapy. Targeting the epigenetic status of tumors is a hotly pursued area[69–73]. Several drugs that

target the enzymes that are involved in the post-translational modifications of histones and DNA are being evaluated pre-clinically and in early-stage clinical trials[74–76]. These include inhibitors of methyltransferases that interfere with SAM-mediated methylation of histones and DNA[77,78]. Changes in the levels of histone and DNA methylation would be predicted to alter cellular epigenetics through the activation and repression of many genes[79,80]. Examples of specific enzyme targets include DNA methyltransferases that are targeted by azanucleosides[81]. Inhibitors of histone methyltransferases have been developed and are being considered pre-clinically[76,82]. Polyamine metabolism, which involves the decarboxylation of SAM and results in the generation of spermidine, has also been heavily explored as targets for anti-cancer therapy, reaching clinical trials in some cases[83]. These agents include 2-difluoromethyl ornithine (DMFO), an inhibitor of ornithine decarboxylase and methylglyoxal bis(guanylhydrazone) (MGBG) and 4-amidinoindydrazone-1-(SAM486A), both of which are competitive inhibitors of S-adenosylmethionine decarboxylase. Both enzymes are required for spermidine synthesis.

## One-carbon metabolism and cancer pathogenesis

### De novo serine and glycine metabolism

An intermediate in glycolysis, 3PG, can be oxidized to form 3-phosphohydroxypyruvate (pPYR). This reaction is the initial and committed step for de novo (that is originating from glucose) serine biosynthesis[84,85]. Thus, carbon derived from glucose can be shunted from glycolysis into de novo serine metabolism and from here into the folate cycle. It has been known for many years that this pathway correlates with tumorigenesis[84,86,87]. Initial studies that provided the biochemical characterization of this pathway also showed that it was active in tumors[88]. Further extensive work by Snell and colleagues showed that flux through this branch point in glycolysis correlated with cancer progression in rat carcinoma models[84,86,87]. Recent studies using isotope tracing with $^{13}C$ labeled glucose showed that a subset of cancer cells diverted a substantial amount (approximately 10%) of 3PG away from glycolysis and into one carbon metabolism through phosphoglycerate dehydrogenase (PHGDH)[89,90]. This resulted in large amounts of de novo serine biosynthesis[90,91]. PHGDH was also found to be overexpressed in the triple negative subtype of breast cancer[90–92].

Despite these observations, it was not known whether the activity of this pathway had any causative role in cancer development or maintenance. One context in which this pathway might be relevant to cancer came from data analyzing the copy number variations in human cancers[93]. It was observed that the genomic locus encoding PHGDH was the subject of a focal, recurrent gene amplification and this region of the genome harbors no known oncogenes. These data suggested that tumors containing a PHGDH amplification may have gained a selective advantage in expressing many copies of the gene. A small hairpin RNA (shRNA) screen revealed that a breast cancer cell line required PHGDH for *in vivo* tumorigenesis[91]. It was also confirmed that breast cancer and melanoma cell lines containing the amplification required PHGDH for proliferation[90,91]. Furthermore, expression of PHGDH in cells that exhibited no detectable flux into de novo serine metabolism was shown to increase serine biosynthesis and induce phenotypic properties that predispose cells to malignancy. These properties include loss of polarity and proliferation in the absence of extracellular matrix contact [90]. Together these findings have provided evidence that de novo serine metabolism could be both necessary and sufficient for tumour maintenance and promotion of oncogenesis[22].

Additional studies have identified further roles for this pathway in tumour cells[94–99]. In a series of studies, it was shown that the activity of an isoform of pyruvate kinase (PKM2), the enzyme that catalyzes the final step in glycolysis, can regulate the flow of glucose into serine metabolism[94,97]. These studies have claimed that this regulation occurs through the

allosteric activation of PKM2. However, serine appears to be a weak activator of PKM2, with activity occurring at concentrations greater than one millimolar[94,97]. The regulation is likely more complex. Other enzymes appear to also be involved directly in the activity of PHGDH including PKCζ, which has been shown to phosphorylate PHGDH and inhibit its activity. PKCζ's tumor suppressor activity is thought to occur through this mechanism[99]. Furthermore, a study revealed that 2-phosphoglycerate, the product of phosphoglycerate mutase that uses 3PG as a substrate, can activate PHGDH, providing additional points of regulation in glycolysis[95].

## Glycine uptake, cleavage and entry into one-carbon metabolism

Recent work has also implicated the glycine cleavage system in cell transformation and tumorigenesis[28,100–103]. This pathway has been studied extensively in plants and lower eukaryotes but its role in mammalian physiology and pathology has been less well explored. Studies in mouse embryonic stem cells (ESCs) showed that these cells uniquely required threonine for stem cell maintenance and self-renewal[28,101,102,104]. Withdrawal of threonine from the culture media, but not any of the other nineteen amino acids, induced rapid cell death. Dramatic reductions in methylation on specific histone residues, including lysine 4 on histone 3 (H3), were also observed[103,105]. Intriguingly, the absence of methionine induced a similar, albeit milder phenotype. Using isotope tracing of the metabolic flux emanating from threonine, it was shown that threonine entered one-carbon metabolism through glycine cleavage. Threonine is converted to glycine by the enzymes threonine dehydrogenase (TDH) and glycine C-acetyltransferase (GCAT). The activity of glycine dehydrogenase (decarboxylating) (GLDC) mediates glycine cleavage and the charging of the folate cycle. Additional studies also confirmed that this pathway was essential for the maintenance of stem cell pluripotency in mice[103].

Recent work on cancer metabolomics has shown that glycine metabolism is associated with cancer cell proliferation. In a survey of the NCI-60 cell line panel, the uptake and release rates of over two hundred metabolites was measured[106]. Surprisingly, glucose uptake and lactate production (i.e. the Warburg Effect) were not associated with cell proliferation. By correlating individual metabolic fluxes with cell proliferation, it was found that glycine uptake was most strongly associated with cancer cell proliferation[106]. Isotope tracing revealed that glycine cleavage was involved in the catabolism of the glycine taken up from the media. This pathway was also shown to be required in rapidly dividing cells[106]. GLDC activity has also been implicated as having a causal role in tumorigenesis. One study found that a subpopulation of tumor-promoting cells expressed high levels of GLDC and that ectopic expression of GLDC was sufficient to induce tumor formation in xenografts of NIH 3T3 cells[100]. Interestingly, this study also reported that ectopic expression of two other enzymes involved in serine and glycine catabolism phosphoserine aminotransferase (PSAT) and serine hydroxymethyltransferase (SHMT) in NIH 3T3 cells could induce tumor formation *in vivo*. Importantly, the induction of tumorigenesis depended on the enhanced enzyme activity of GLDC. Another study also reported that increased availability of glycine or sarcosine could increase the invasiveness of prostate cancer cells[107]. Together, these findings provide evidence that glycine uptake and catabolism promotes tumorigenesis and malignancy.

In addition to the newly appreciated functional roles of one carbon metabolism in cancer progression and maintenance, several biological pathways that are involved in tumorigenesis, including those related to protein translation, genome maintenance, epigenetic status and cellular redox status, are linked to one carbon metabolism.

## Protein translation

Recently, an *in vivo* screen for new tumor suppressor genes in lymphoma identified two genes, S-adenosylmethionine decarboxylase (*AMD1*) and eukaryotic initiating factor 5α (*EIF5A*), which are associated with methionine metabolism and hypusine biosynthesis[108]. AMD1 decarboxylates SAM, diverting SAM into a pathway involving the biosynthesis of spermine and spermidine[109]. This pathway eventually leads to the production hypusine, which is required for the hypusination of proteins. So far, only two eukaryotic initiating factors, including EIF5α, have been shown to be modified by hypusination [110–113]. Subsequent work demonstrated that other genes involved in the production of hypusine were also sufficient to induce lymphomas. The hypusination of EIF5α is thought to be required for its physical association with ribosomes and is essential for translation elongation[111,114]. Together these findings link tumor suppression with methionine metabolism through its ability to directly modify protein translation.

## Nucleotide metabolism and genome maintenance

Genome maintenance requires the successful incorporation of the appropriate nucleotides during both DNA replication and repair[115,116]. Nucleotide metabolism has been directly implicated in tumorigenesis[117,118]. Recent studies have demonstrated that a decrease in nucleotide levels is sufficient to induce genome instability and increase mutagenesis rates[119,120]. The mechanism involves the incorporation of uracil into DNA due to its increased relative abundance to thymidine. Several enzymes involved in nucleotide metabolism are considered to be *bona fide* tumor suppressor genes. *FHIT*, a gene that codes for an enzyme with dinucleotide hydrolase activity involved in purine metabolism, is one of the most frequently deleted genes in human cancer — almost half of all human colon cancers contain a focal deletion of this gene[121–123]. *Fhit* deletion in mice has been shown to induce genome instability and spontaneous tumor formation, which can be rescued by the introduction of an *FHIT* transgene[124,125]. The mechanism through which this phenomenon occurs remains unclear but probably has some connection to genome maintenance and DNA repair through the ability of repair enzymes to appropriately incorporate nucleotide bases into DNA.

Other cancer-associated genes involved in nucleotide metabolism include thymidylate synthase and RNR[126–128]. Overexpression of RNR induces lung tumors in mice through alterations in DNA repair, indicating that it can function as an oncogene[129]. Additionally, thymidylate synthase can also function as an oncogene[126,130]. The ability of its overexpression to induce cell transformation and the growth of cells as tumour xenografts in mice is dependent on its catalytic activity. More studies are needed to define the mechanistic principles of these phenomena but a possible mechanism lies in the maintenance of genome integrity.

## Epigenetic alterations

The methylation reactions mediated by SAM involve the transfer of methyl groups onto the lysine and arginine residues of proteins, DNA, RNA and intermediary metabolites[131–133]. These modifications have long been observed to affect gene regulation, but the extent to which they are reversible has been less clear[73]. Much of the recent interest in this area of cancer biology has come from multiple genetic and functional studies that have identified methyltransferases and demethylases as being recurrently mutated and having causal roles in cancer development[134–139].

Recent pulse-chase experiments using isotopically labeled SAM have revealed that methylation modifications are dynamic[140,141] and tightly regulated [73,80,142,143]. As SAM

concentrations fluctuate in cells and affect the activity of methyltransferases, the levels of SAM influence the levels of histone methylation[3,20,45,47,105].

## Translational opportunities for cancer therapy

### Drug development

The surge of work carried out in the study of cancer metabolism has created the expectation that the mechanistic understanding will lead to the development of new therapeutics that target key nodes within the cancer metabolic network. Metabolic enzymes, having evolved to carry out chemical catalysis, are thought to be druggable[56,144–146]. In addition to targeting the catalytic site of metabolic enzymes, it is also possible to design small molecules that target allosteric binding sites that naturally fit endogenous metabolites. Pre-clinical studies are underway that are currently evaluating the promise of targeting multiple nodes in one carbon metabolism[22,147]. These targets include, but are not limited to, PHGDH, PSAT, PSPH, GNMT, GLDC and GCAT. Drugs that target the generation of ROS, an indirect product of metabolism, are also actively being pursued[148]. One question that is often raised is what improvements would these new targets offer over currently existing drugs, such as methotrexate and pemetrexed, that already target one carbon metabolism? One possible advantage to targeting these nodes lies in the potential for an improved therapeutic window. For example, since some tumors and most tissues appear not to require PHGDH and thus serine synthesis from glucose, targeting this pathway might prove less toxic in some contexts. Furthermore, some tumors display hyperactivation of this pathway and thus, since other pathways in addition to de novo serine biosynthesis enter into folate metabolism, these tumors might be more susceptible to PHGDH inhibition than inhibition of MTHFR by methotrexate and pemetrexed. Nevertheless more data are needed to resolve the contexts in which directly targeting enzymes involved in one carbon metabolism would provide greater efficacy than the administration of anti-folate chemotherapy.

### Metformin, cancer and one carbon metabolism

Metformin has recently come into focus as a promising agent for cancer therapy. Metformin is the most commonly used treatment for type II diabetes[149] and also exhibits other activities, such as an anti-aging effect in *Caenorhabditis elegans* through inhibition of one carbon metabolism in the gut microbiota[150]. Epidemiological evidence has suggested that metformin may have anti-cancer effects[151]. These data have been augmented with pre-clinical studies showing an anti-tumor activity for metformin[152] and so this drug has advanced to clinical trials for both cancer treatment and prevention. The mechanism of action of metformin has proven controversial with numerous mechanisms having been proposed[153]. Recent work has provided evidence that metformin acts on one carbon metabolism in patients. A metabolomics study of a cohort of patients treated with metformin revealed that the signature of metformin response was remarkably similar to that obtained from patients treated with anti-metabolite chemotherapies[154]. These findings suggest that metformin confers some and maybe many of its effects through the alteration of folate and one carbon metabolism. Additional support for this hypothesis would be useful to complement current clinical trials of metformin in cancer patients.

### Dietary intervention in cancer treatment and serine and glycine metabolism

A complementary strategy to targeting cancer metabolism with pharmacological agents is to affect the same nodes through changes in nutrient uptake. This intervention could occur through changes in diet, especially as high carbohydrate intake is positively correlated with cancer incidence[155–158]. Pre-clinical [159] and clinical[160,161] studies have shown that reducing carbohydrate (glucose) intake can have negative effects on tumour biology.

Pre-clinical work has also explored the possibility of restricting serine and glycine metabolism for cancer intervention. Isogenic colon cancer cells containing wild-type or null alleles of *Trp53* were studied *in vitro* and grafted into mice fed diets containing no serine and no glycine[98,162]. The removal of serine and glycine dramatically affected cell proliferation and tumor growth. In the absence of p53, serine and glycine withdrawal had an even greater effect, suggesting an epistatic interaction between p53 and the availability of serine and glycine. Mechanistically, the absence of serine and glycine increased de novo serine and glycine metabolism and this was found to decrease glutathione synthesis and increase ROS levels suggesting that this effect could contribute to tumor growth. Strikingly, it was observed that removal of serine and glycine had an even larger effect on the reduction of *in vivo* tumor growth than the re-introduction of wild-type p53 alleles into the tumour cells. Other mechanisms are likely to also be relevant, such as a reduced if not disrupted rate of biosynthesis, nucleotide metabolism that may affect AMPK activity[163], and possibly changes in epigenetic status. Removing serine and glycine from a natural diet seems difficult. However, specific diets might be constructed that could circumvent this problem, as is the case for dietary intervention for several diseases, such as the ketogenic diet which is used to prevent seizures for epileptic patients or diets such as a gluten-free diet that are used to alleviate autoimmune disorders.

In light of these findings, it is tempting to speculate that similar activities could be obtained with the restriction of B vitamins that are readily available in food. This seems particularly relevant given the initial evidence that the effects of metformin might work by targeting folate metabolism. However, numerous longitudinal studies have associated folate and vitamin B12 intake with alterations in DNA methylation and cancer risk. Lack of adequate intake of folate during pregnancy is associated with improper germline transmission of methylation patterns. Folate consumption has also been shown to affect DNA methylation during development[26,27,184,185]. Decreased folate intake is associated with cancer, most notably colorectal cancer[186–194]. In breast cancer, reduced folate intake is associated with cancer development and global hypomethylation[195,196]. Given data on serine and glycine deprivation and its anti-tumor effects, and the metformin data, the relationship between diets related to one carbon metabolism is apparently complex with more work needed to define the mechanisms related to one-carbon metabolism activity and cancer susceptibility.

## Biomarkers for precision medicine and diagnosis

Many of the intermediary metabolites in serine, glycine and one-carbon metabolism are water soluble and detectable in biological fluids such as serum and urine. These properties allow for the possibility of innovations in diagnostics. For example, increased homocysteine levels in serum are used as a biomarker for folate deficiency[164]. The buildup of homocysteine results from the lack of available methyl donors to complete a turn of the folate cycle. The use of anti-metabolite chemotherapies has identified biomarkers, mostly in the form of the enzymes, which these drugs target, that predict response or resistance. For example, expression of thymidylate synthase has been shown to predict response to 5-FU[165–169]. Biomarkers of response to methotrexate have been found in serum metabolites[170,171] and a recent meta-analysis reported that expression of folate-metabolizing enzymes and those involved in serine and glycine metabolism could predict tumor response to methotrexate in diverse tumor types[172].

A metabolomics study of urine from patients with benign prostatic disease, localized prostate cancer and metastatic prostate cancer revealed that glycine metabolismis a predictor of metastatic cancer[107]. Glycine and sarcosine were identified in the metastatic urine samples[107]. Subsequent studies have found conflicting results and the probability that sarcosine is a biomarker of metastatic disease is likely to depend on additional variables[173–175]. Nevertheless, given the non-invasiveness of this metabolomics assay, it is

possible that these results could eventually be used clinically. In some contexts, choline metabolism has been found to be increased during tumor progression[176,177]. Positron emission tomography (PET) imaging of C11 choline has been approved by the US Food and Drug administration (FDA) as a biomarker for advanced malignancy[178,179]. Gene expression levels of glutathione-metabolizing enzymes is also an FDA-approved biomarker for treatment decisions in node-negative, estrogen receptor-positive breast cancers[180]. Together, these findings currently have and will probably continue to result in clinical impact. With the new found molecular mechanisms connecting one carbon metabolism to cancer pathogenesis that have been recently characterized, additional advances in biomarker discovery for precision medicine surrounding anti-folate agents could be obtained.

## Summary and future directions

Once thought to be the subject of mundane biochemistry lectures and the target of non-specific cytotoxic chemotherapies, amino acid and one carbon metabolism has reemerged as a core feature in the biology of cancer (Figure 5). These findings have occurred alongside the discovery of an ‘oncometabolite’ the R enantiomer of 2-hydroxyglutarate (2HG), a product of mutant isocitrate dehydrogenase (IDH) enzymes[181]. IDH1 and IDH2 are recurrently mutated in leukemia and gliomas, as well as other cancer types[182]. 2HG has been shown to have numerous functions, including the alteration of epigenetic marks through the inhibition of histone demethylases[183]. Perhaps the intermediates in one carbon metabolism are also oncometabolites, whose aberrant activity promotes cancer pathogenesis.

Many other questions also remain. For example, how one carbon metabolism is integrated with signals from diverse nutrient inputs to generate the appropriate downstream carbon portioning is not understood. The extent to and context under which this pathway modulates epigenetics, genome maintenance, redox status and anabolic metabolism are only just beginning to be understood. Whether any of these newly appreciated roles in cancer pathogenesis will lead to further clinical benefit is a subject for further exploration. Nevertheless, with technological advances, it is expected that we will uncover many other unknown angles that connect epigenetics, nucleic acid metabolism and redox biology with one carbon metabolism and the pathology of cancer. Metabolomics, computational models and integrative bioinformatics approaches will hopefully allow for rapid progress in this area.

Another appealing aspect of studying serine, glycine and one-carbon metabolism in cancer pathogenesis is the wealth of drugs already clinically available and dietary options that may be further available. For example, chemotherapies such as methotrexate, 5-FU and gemcitabine have a dramatic response in subsets of patients with cancer, but our ability to predict these responses remains poor. If any of this new found knowledge could be harnessed for advances in precision medicine, dramatic inroads would be made.

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## Glossary

**Aldol Cleavage** A chemical reaction that can be catalyzed enzymatically resulting in the splitting of a betahydroxy ketone

| | |
|---|---|
| **NCI-60** | A panel of tumor-derived cell lines originating from diverse tissue types. Extensive genomic, biochemical, and pharmacological data have been obtained on these cell lines |
| **Therapeutic Window** | A term used in drug development and medical practice that refers to range of drug concentrations that satisfy the tradeoff between an efficacious clinical response and unwanted toxicities |

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## Biography

**Jason W. Locasale, Ph.D**. is an Assistant Professor at Cornell University. He graduated from Rutgers University with degrees in Chemistry and Physics. He received his Ph.D. at MIT in Biological Engineering. He then studied cancer metabolism at Harvard Medical School as a postdoctoral fellow with Lew Cantley. Dr. Locasale's research focuses on the roles of metabolism in cell growth and cancer. At the core of this effort lies the use of computational modeling and mass spectrometry-based metabolomics. This systems biology approach combines these tools with an integration of genetics, biochemistry, and cell biology.

### Online 'at-a-glance' summary

- One carbon metabolism integrates cellular nutrient status by cycling carbon units from amino acid inputs to generate diverse outputs including redox maintenance and cellular biosynthesis.
- The epigenetic status of cells also seems directly linked with one carbon metabolism through protein and nucleic acid methylation.
- One carbon metabolism has long been the focus of anti-metabolite based chemotherapy and includes the agents methotrexate and 5-Fluorouracil – two of the most widely used chemotherapies. Additional therapies are currently being explored.
- Recent findings have provided genetic and functional evidence that multiple nodes within the pathway contain candidate driver genes for oncogenesis.
- Additional research in one carbon metabolism may provide biomarkers that would enable advances in patient selection for antimetabolite chemotherapy.

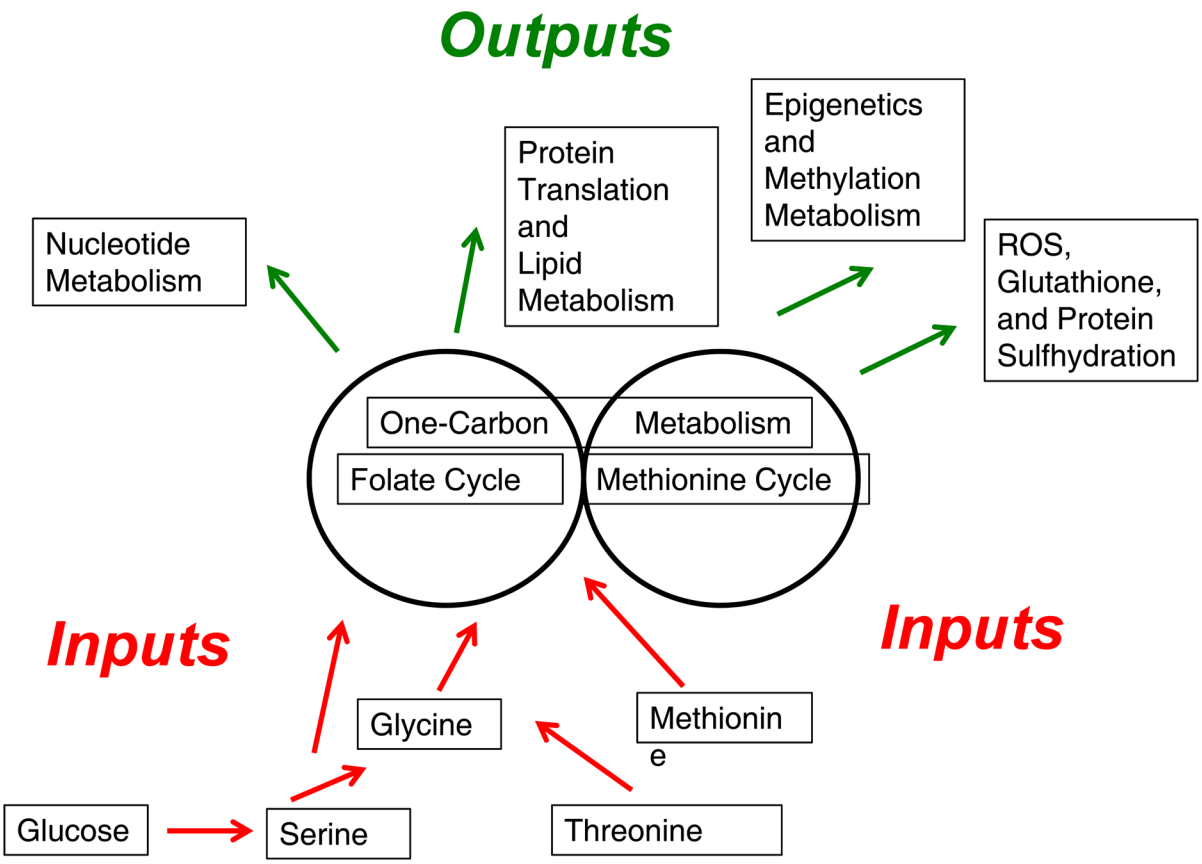


**Figure 1. One carbon metabolism is an integrator of nutrient status**

Nutrient sources involving amino acids are either imported or synthesized de novo and enter one carbon metabolism. One carbon metabolism can be viewed as a set of two modular units (i.e. two pathways existing separately from one another) involing the Folate Cycle and Methionine Cycle. Through these metabolic cycles, nutrients are processed. Upon processing, multiple outputs can be generated including nucleotides, proteins, lipids, reducing power, and substrates for methylation reactions.

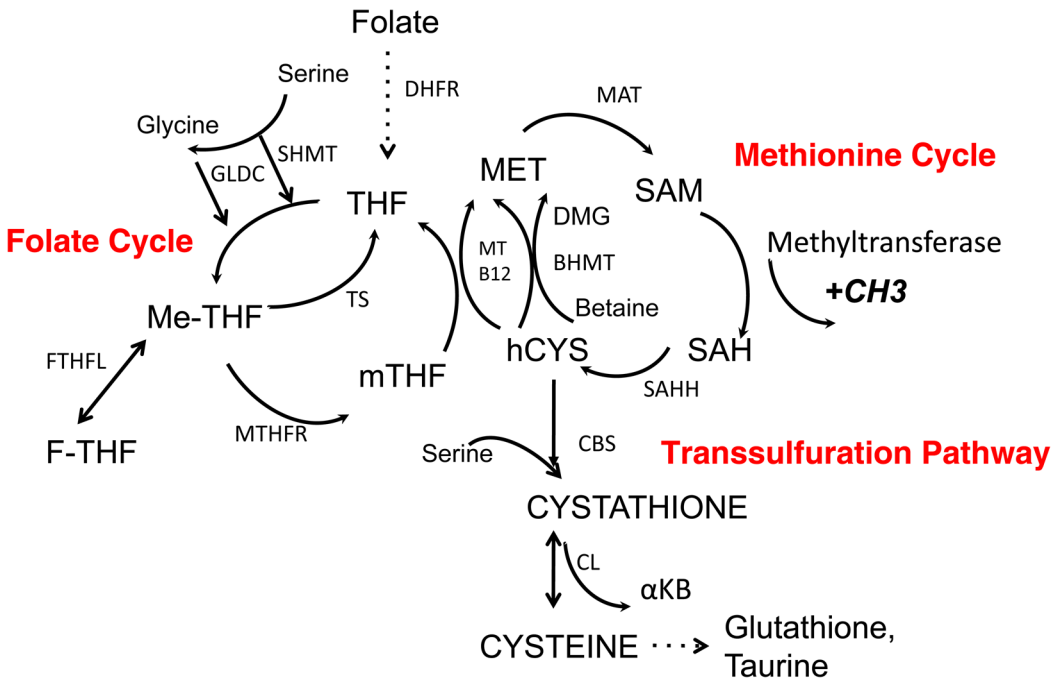


**Figure 2. Folate and methionine metabolism comprise one carbon metabolism**

The folate cycle and the methionine comprise of two metabolic pathways that exist independently and thus in modules.

The folate cycle: Folate is imported in cells and reduced to tetrahydrofolate (THF). THF is converted to 5,10-methylene-THF (me-THF) by serine hydroxymethyl transferase (SHMT). Vitamin B6 appears to have an influence on this reaction but the interaction is likely indirect. This product is then either reduced to 5-methyltetrahydrofolate (mTHF) by methylenetetrahydrofolate reductase (MTHFR) or converted to 10-Formyltetrahydrofolate (F-THF) through a sequence of steps.. mTHF is demethylated to complete the folate cycle. With the demethylation of mTHF, the carbon is donated into the methionine cycle through the methylation of homocysteine by methionine synthase and its cofactor Vitamin B12.

The methionine cycle: The methionine cycle begins with homocysteine that accepts the carbon from the folate pool through mTHF to generate methionine. Methionine, through methionine adenyltransferase (MAT) is used to generate S-adenosylmethionine (SAM), which is demethylated to form S-adenosylhomocysteine (SAH). After deadenylation by S-adenosyl homocysteine hydrolase (SAHH), SAH is converted back to homocysteine resulting in a full turn of the methionine cycle.

Another modular unit of one carbon metabolism is the transsulfuration pathway. This pathway is connected to the methionine cycle through the intermediate homocysteine. Serine can condense enzymatically with homocysteine to generate cystathione by cystathionine synthase (CBS). Cystathionine is then cleaved by cystathione lyase (CGL) to generate alpha-ketobutyrate (αKB) and cysteine, which can be shunted into glutathione production and taurine metabolism. The metabolism of cysteine can also lead to its desulfhydration and production of hydrogen sulfide through CBS and CGL.

Abbreviations: SAM – S-adenosylmethionine, SAH – S-adenosylhomomocysteine, hCYS – homocysteine, THF – Tetrahydrofolate, mTHF – 5-methyltetrahydrofolate, Me-THF – 5,10 Methylenetetrahydrofolate, F-THF – 10 Formyltetrahydrofolate, DMG – Dimethylglycine, GLDC – Glycine Decarboxylase, TS – Thymidylate Synthase, MT – Methionine Synthase, B12 – Vitamin B12, MAT – Methionine adenyltransferase, SAHH – S-adenosyl homocysteine hydrolase, GNMT – Glycine N-methyltransferase, BHMT – Betaine

hydroxymethyltransferase, SHMT – Serine hydroxymethyltransferase MTHFR – Methyltetrahydrofolate Reductase, DHFR –Dihydrofolate reductase, CDO – Cysteine Dioxygenase. Bi-directional arrows denote reversible steps. Dotted arrows denote multiple biochemical steps.

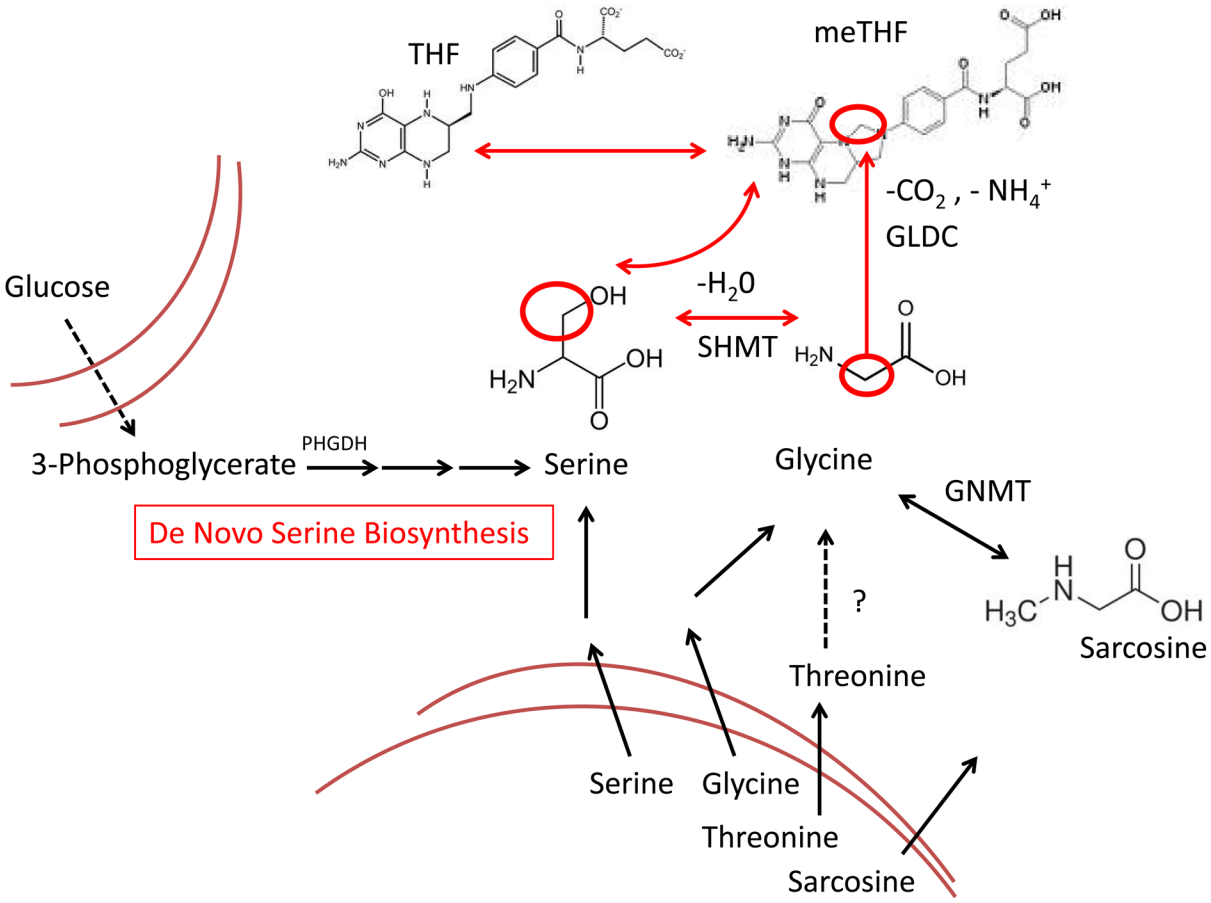


**Figure 3. Nutrients that fuel one carbon metabolism**

Serine and glycine metabolism is generated de novo from glycolysis through the oxidation of intermediate 3-phosphoglycerate. Serine and glycine are also transported into cells. Sarcosine and possibly threonine can also enter cells and be converted to glycine. The question mark denotes that threonine catabolism has been found to be utizilized in mammals including mice. Its analog in humans has not been identified. Dashed arrows denote multiple biochemical steps. Bi-directional arrows denote reversible steps. Abbreviations: PHGDH – phosphoglycerate dehydrogenase, GLDC – Glycine Decarboxylase, SHMT – Serine hydroxymethyltransferase, GNMT – Glycine N-methyltransferase, THF – Tetrahydrofolate, Me-THF – 5,10 Methylenetetrahydrofolate.

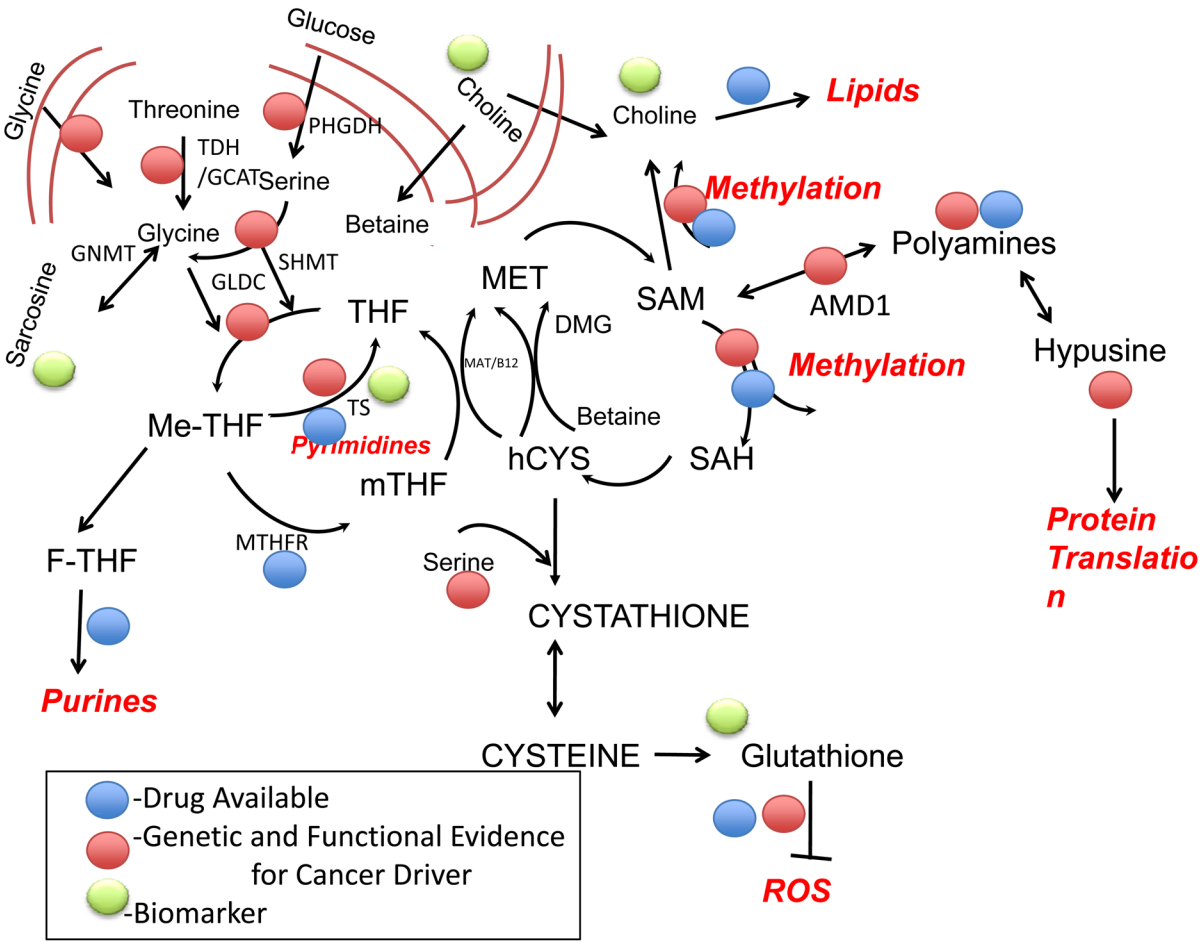


**Figure 4. One carbon metabolism and cancer pathology and intervention**

Schematic of one-carbon metabolism and the transsulfuration pathway. Recent findings have identified roles for these pathways in cancer. Genetic mutations and functional evidence for the existence of a cause of cancer (driver) at this point in the pathway (red), currently available drugs (yellow), and biomarker development (green) are highlighted. The specifics are indicated in Tables 1, 2 and 3. Causality is defined as either the presence of a genetic lesion or functional evidence (e.g. overexpression of a pathway component) that enhanced activity at this point in the pathway promotes oncogenesis. The biological outputs of the pathway are in a bold-italic red font. Abbreviations: SAM – S-adenosylmethionine, SAH – S-adenosylhomomocysteine, hCYS – homocysteine, THF – Tetrahydrofolate, mTHF – 5-methyltetrahydrofolate, Me-THF – 5,10 Methylenetetrahydrofolate, F-THF – 10 Formyltetrahydrofolate, DMG – Dimethylglycine, PC – Phosphatidylcholine; PHGDH – phosphoglycerate dehydrogenase, GLDC – Glycine Decarboxylase, TS – Thymidylate Synthase, MAT – Methionine Synthase, B12 – Vitamin B12, GNMT – Glycine N-methyltransferase, MTHFR – Methylenetetrahydrofolate Reductase, TDH/GCAT – Threonine Dehydrogenase/Glycine C-acetyltransferase, ROS – Reactive Oxygen Species. Bidirectional arrows denote reversible steps.

**Table 1**

Candidate Drivers of Cell Transformation
Candidate oncogenes and tumor suppressor genes in one carbon metabolism

| Pathway | Genes or Enzymes | Evidence |
|---|---|---|
| De Novo Serine Biosynthesis | PHGDH, PSAT, PSPH, SHMT1 | Genetic Aberrations, Functional Data |
| Glycine Cleavage | GLDC, GCAT | Overexpression, Functional Data |
| Polyamine Synthesis | AMD1, EIF5A, SRM, DHPS | Underexpression, Functional Data |
| Methylation Metabolism | IDH1, EZH2, SET9, DNMT1, ARID1A, JARID1C, UTX, SETD2 | Genetic Aberrations, Functional Data |

**Table 2**

Oncology biomarkers in one carbon metabolism

| Biomarker | Source | Use |
|---|---|---|
| Choline | C11 Pet Imaging | Detection of advanced malignancies |
| Sarcosine | Urine Metabolite | Possible predictor of metastatic prostate cancer |
| Thymidylate Synthase | Tumor mRNA expression | Expression Correlates with response to 5-Fluorouracil |
| Glutathione sulfer transferase | Tumor mRNA expression | FDA-approved as part of assay for decision to use chemotherapy in Estrogen Receptor Positive Breast Cancer |

**Table 3**

Drug targets in one carbon metabolism

| Enzymes | Compounds | Status |
|---|---|---|
| Methotetrahydrofolate Reductase | Methotrexate, Pemetrexed | Approved for multiple cancers |
| Thymidylate Synthase | 5-Fluorouracil (5-FU) | Approved for multiple cancers most notably Colorectal Cancer |
| Ribonucleotide Reductase | Gemcitabine | Approved for multiple cancers most notably Pancreatic Cancer |
| Polyamine Inhibitors | Various | Clinical trials ongoing |
| DNA Methyltransferase Inihibitors | Azanucleosides | Approved for Myeloid Leukemias |
| Histone Methyltransferase Inhibitors | Various (SAM-analogs) | Clinical trials ongoing |
| Histone Demethylase Inhibitors | Various | Preclinical studies |
| Ornithine Decarboxylase | 2-difluoromethyl ornithine (DMFO) | Preclinical studies |
| S-adenosyl decarboxylase inhibitors | methylglyoxal bis(guanylhydrazone) (MGBG), 4-amidinoindydrazone-1-(SAM486A) | Preclinical studies |