Serine, glycine and one-carbon units: cancer metabolism in full circle

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Abstract | One-carbon metabolism involving the folate and methionine cycles integrates nutritional status from amino acids, glucose and vitamins, 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 recently been shown to have additional complexity. Genetic and functional evidence suggests that hyperactivation of this pathway is a driver of oncogenesis and establishes a link 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.

Cell growth and proliferation require the construction of building blocks for new cellular components, including proteins, lipids and nucleic acids, as well the maintenance of cellular redox status, and genetic and epigenetic status[1–6]. Amino acid metabolism involving serine and glycine, and the carbon units that they provide, satisfies many of these requirements[7,8]. 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 during which a carbon unit is transferred to other metabolic pathways and is eventually replenished by several sources. Modern cancer therapy partly 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 more than 60 years[14–17] (FIG. 1 (TIMELINE)).

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]. The serine and glycine metabolic pathway expands the compendium of metabolic pathways essential to cancer biology[8,22]. Recent developments in our understanding of this pathway have led to the recognition of new relationships between metabolism and cancer biology. 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 that aspects of serine, glycine and one-carbon metabolism can function as drivers of cancer pathogenesis. Additional roles for one-carbon metabolism, including in genome integrity and epigenetic alterations, also imply a function in tumour maintenance. Ultimately, these findings may result in new translational opportunities for drug development, dietary intervention in cancer prevention and biomarkers that indicate in which tumours antimetabolic chemotherapeutic drugs are likely to produce an efficacious response. Together, these findings alter our approach to the study of cancer pathogenesis[23].

One-carbon metabolism and nutrient integration

A way to conceptualize the role of one-carbon metabolism in cellular physiology is to consider its function as an 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 mTOR signal transduction pathway, but has been used less frequently to describe metabolic pathways per se[24]. In the case of mTOR signalling, 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

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Key points

- 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 to be directly linked to one-carbon metabolism through protein and nucleic acid methylation.
- One-carbon metabolism has long been the focus of antimetabolite-based chemotherapy that 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 in 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.

donation of carbon units from specific amino acids. These carbon units 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 the trans-sulphuration pathway (FIG. 2). Loss-of-function mutations in enzymes that are involved in these pathways can lead to growth defects both in animals and in humans, underscoring the role of one-carbon metabolism in modulating cell growth[7,25–27].

One-carbon metabolism and the trans-sulphuration pathway. Folic acid is a B vitamin that is found in and is 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 number 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 trans-sulphuration pathway is connected to the methionine cycle through the intermediate homocysteine. Serine can be directly metabolized through trans-sulphuration, eventually resulting in the generation of glutathione, one of the major redox-regulating metabolic systems in cells (discussed below).

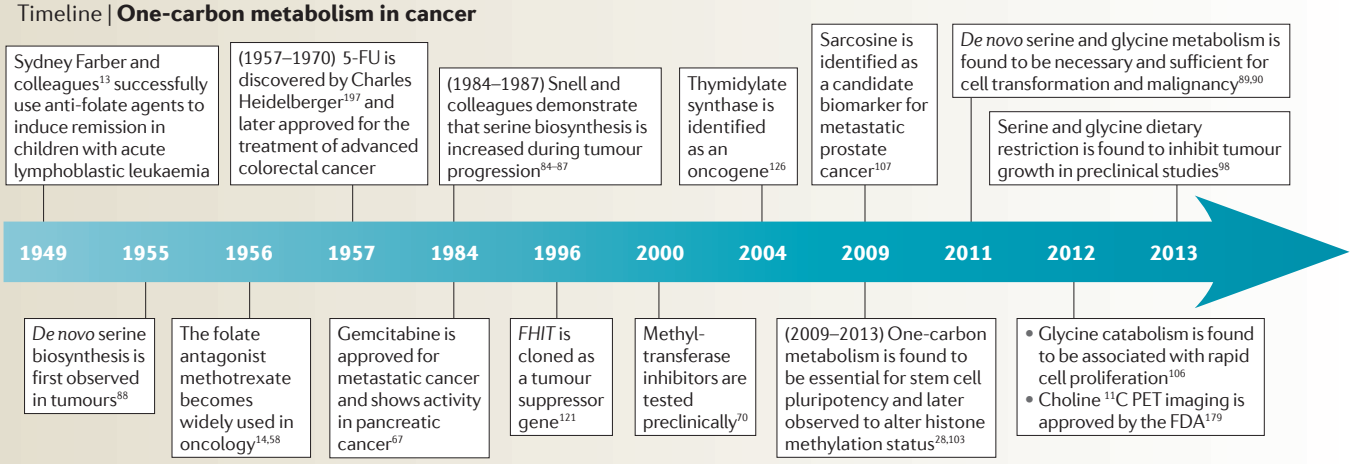
Inputs to one-carbon metabolism. The carbon units that feed into one-carbon metabolism can be synthesized *de novo* (FIG. 4). For example, an intermediate metabolite involved in glycolysis, 3-phosphoglycerate (3PG), can be converted into serine. Serine donates the carbon atom from its side chain to folate, converting serine to glycine and converting 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 in which the enzymatic cleavage of glycine produces ammonia, carbon dioxide and a carbon unit for the methylation of THF. 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 demethylation.

Aldol cleavage
A chemical reaction that can be catalysed enzymatically resulting in the splitting of a β-hydroxy ketone.

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[29,30]. Amino acids, such as methionine, can be generated from one-carbon metabolism and used to generate proteins[31]. Nucleotides required for DNA and RNA are also constructed through

Timeline | **One-carbon metabolism in cancer**



5-FU, 5-fluorouracil; FDA, US Food and Drug administration; *FHIT*, Fragile histidine triad gene; PET, positron emission tomography.

reactions that involve the folate cycle[32]. Deoxythymidine monophosphate is synthesized through the methylation of deoxyuridine 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]. Phospholipids can also be generated partly through the methionine cycle[34]. Phosphatidylcholine is a major component of the cell membrane that can account for up to 50% of lipid membrane content[35]. The head group of phosphatidylcholine 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[37,38].

Redox balance. The metabolism of carbon atoms through one-carbon metabolism is linked to changes in redox status. These changes mostly occur through the reduction of NADPH and the 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* on the basis of known *in vitro* activities[39]. Glutathione, which is one output of the trans-sulphuration pathway, is also important for the maintenance of the ratio of NADPH to $NADP^+$. Glutathione is a tripeptide that is comprised of cysteine, glycine and glutamate, and it is one of the most abundant metabolites in cells, often reaching concentrations as high as 5 mM[40,41]. Thus, it is 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 desulphhydration products in the trans-sulphuration pathway also lead to the sulphhydration of proteins. These post-translational modifications are considered to be an important and currently under-explored 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 substrates 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].

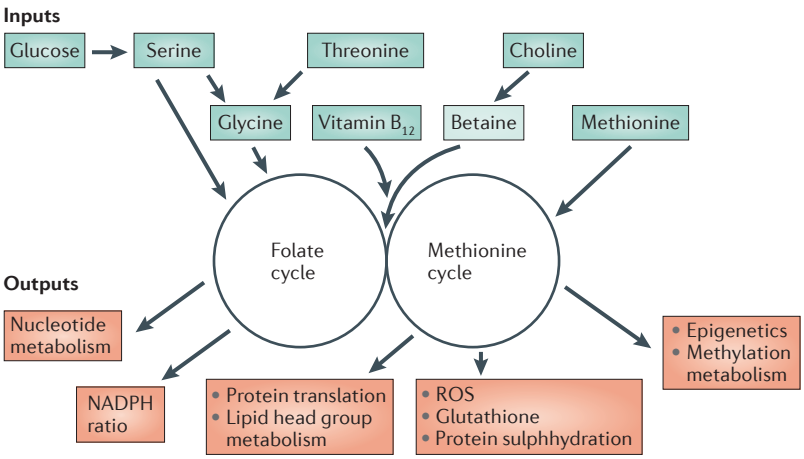


Figure 2 | **One-carbon metabolism is an integrator of nutrient status.** Nutrient sources involving amino acids are either imported (shown in green) or synthesized *de novo* (such as betaine; shown in light green) and enter one-carbon metabolism. One-carbon metabolism can be viewed as composed of two modular units (that is, as two pathways that exist separately from one another) that comprise the folate cycle and the methionine cycle. Nutrients are processed through these metabolic cycles. Multiple outputs can be generated (shown in red boxes), including nucleotides, proteins, lipids, reducing power and substrates for methylation reactions. ROS, reactive oxygen species.

Cancer therapy and one-carbon metabolism

One of the first modern chemotherapies resulted from the observation that patients with anaemia could be treated with B vitamins to stimulate red blood cell production[12]. Sydney Farber[12] 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 antagonize cell proliferation. In a landmark study by Farber and colleagues[13] one of these molecules, aminopterin, was shown to induce remissions in children with acute lymphoblastic leukaemia (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 a diverse range of cancers, including ALL, breast cancer, bladder cancer and lymphomas[14,55–59]. These agents inhibit dihydrofolate reductase and tetrahydrofolate reductase activity in humans, resulting in the 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 could potentially enable the identification of patients who will benefit most from these drugs.

In addition, multiple pathways downstream of one-carbon metabolism are the targets of numerous cytotoxic chemotherapies. 5-fluorouracil (5-FU) is a standard-of-care agent for many cancers, including advanced stage colorectal cancer, and it targets nucleotide metabolism, which relies on metabolites produced from the folate cycle[62,63]. 5-FU is an analogue of the DNA base uracil. It is a potent inhibitor of thymidine synthase, thus blocking the methylation of 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 analogue that interferes with the biosynthesis

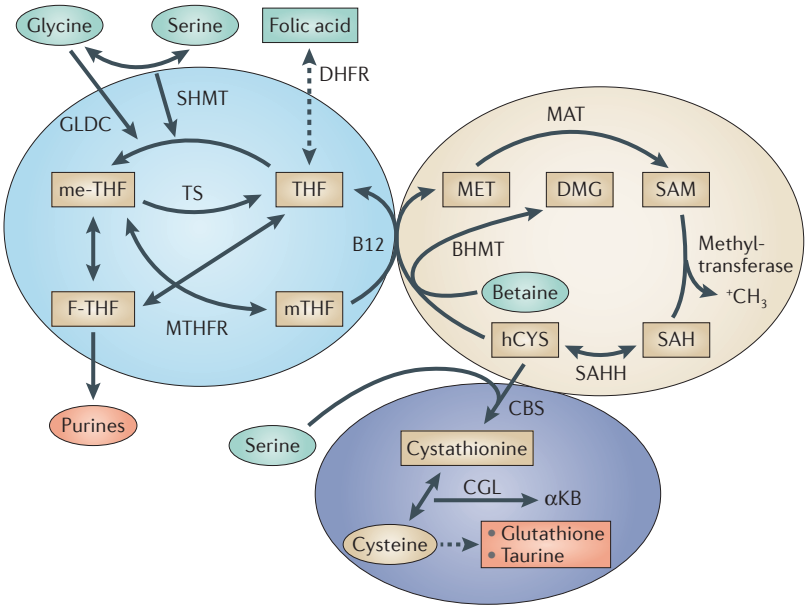


Figure 3 | **Folate and methionine metabolism constitute one-carbon metabolism.** The folate cycle and the methionine cycle are two metabolic pathways that exist independently and thus in modules. In the folate cycle, folic acid is imported into cells and reduced to tetrahydrofolate (THF). THF is converted to 5,10-methylene-THF (me-THF) by serine hydroxymethyl transferase (SHMT). Vitamin B_6 seems to have an influence on this reaction but the interaction is probably indirect. me-THF 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 (hCYS) by methionine synthase and its cofactor vitamin B_{12} (B12). The methionine cycle begins with homocysteine that accepts the carbon from the folate pool through mTHF to generate methionine (MET). 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 trans-sulphuration pathway. This pathway is connected to the methionine cycle through the intermediate homocysteine. Serine can condense enzymatically with homocysteine to generate cystathionine by cystathionine synthase (CBS). Cystathionine is then cleaved by cystathionine lyase (CGL) to generate α-ketobutyrate (αKB) and cysteine, which can be shunted into glutathione production and taurine metabolism. The metabolism of cysteine can also lead to its desulphhydration and the production of hydrogen sulphide through CBS and CGL. Bi-directional arrows denote reversible steps. Dashed arrows denote multiple biochemical steps. BHMT, betaine hydroxymethyltransferase; DHFR, dihydrofolate reductase; DMG, dimethylglycine; GLDC, glycine decarboxylase; TS, thymidylate synthase.

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 to one-carbon metabolism, or from differences in drug delivery, is not yet fully resolved.

Other pathways downstream of one-carbon metabolism are also targets of cancer therapy. Targeting the epigenetic status of tumours 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 preclinically 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 are predicted to alter cellular epigenetics through the activation and the repression of many genes[79,80]. DNA methyltransferases, which are targeted by azanucleosides, are specific enzyme targets, for example[81]. Inhibitors of histone methyltransferases have been developed and are being considered preclinically[76,82]. Polyamine metabolism, which involves the decarboxylation of SAM and which results in the generation of spermidine, has also been heavily explored as a source of targets for anticancer therapy. Some of these drugs have entered clinical trials[83]. These agents include 2-difluoromethyl ornithine (DMFO), an inhibitor of ornithine decarboxylase, as well as methylglyoxal bis(guanylhydrazone) (MGBG) and (E)-2-(4-carbamimidoyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinecarboximidamide (SAM486A), both of which are competitive inhibitors of *S*-adenosylmethionine decarboxylase. Both ornithine decarboxylase and *S*-adenosylmethionine decarboxylase 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 then 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 tumours[88]. Further extensive work by Snell and colleagues[84,86,87] showed that flux through this branch point in glycolysis correlated with cancer progression in rat carcinoma models. Recent studies using isotope tracing with ^{13}C-labelled 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; also known as 3PGDH)[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 analysing 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, but this region of the genome harbours no known oncogenes. These data suggested that tumours containing a *PHGDH* amplification may have gained a selective advantage by expressing many copies of the gene. A short 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

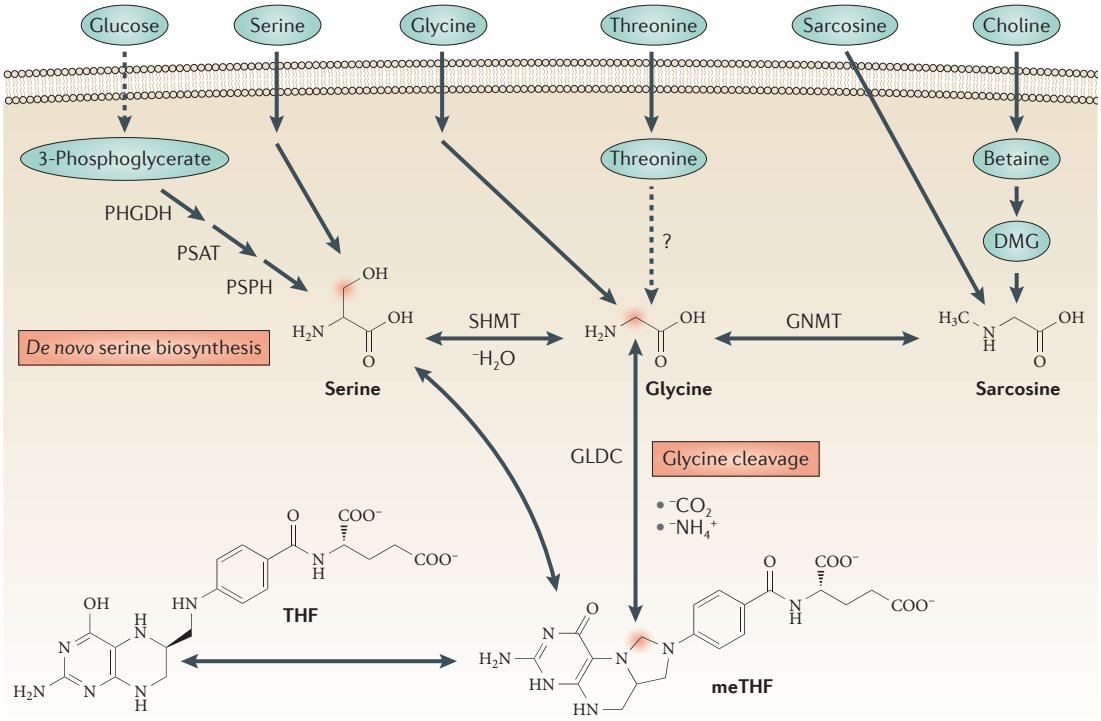


Figure 4 | **Nutrients that fuel one-carbon metabolism.** Serine and glycine can be generated *de novo* from glycolysis through the oxidation of the metabolic intermediate 3-phosphoglycerate. Serine and glycine are also transported into cells. Sarcosine and possibly threonine can also enter cells and be converted to glycine. The question mark indicates that, although threonine catabolism has been found to be used in mammalian cells, including in mice, an analogous pathway in humans has not been identified. Bi-directional arrows denote reversible steps. Dashed arrows denote multiple biochemical steps. GLDC, glycine decarboxylase; GNMT, glycine *N*-methyltransferase; me-THF, 5,10-methylenetetrahydrofolate; PHGDH, phosphoglycerate dehydrogenase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

amplification required PHGDH for proliferation[90,91]. Furthermore, expression of PHGDH in cells that exhibited no detectable flux into *de novo* serine metabolism increased serine biosynthesis and induced 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] (TABLE 1).

Additional studies have identified other metabolic processes that regulate the flux of glycolysis metabolites into serine metabolism in tumour cells[94–99]. A series of studies showed that the activity of an isoform of pyruvate kinase (PKM2), the enzyme that catalyses the final step in glycolysis, can regulate the flow of glucose into serine metabolism[94,97]. These studies reported that this regulation occurs through the allosteric activation of PKM2. However, serine seems to be a weak activator of PKM2, with activity occurring at concentrations greater than 1 mM[94,97]. The regulation is probably more complex. Other enzymes also seem to be directly involved in the activity of PHGDH, including protein kinase Cζ (PKCζ), which has been shown to phosphorylate PHGDH and to inhibit its activity. The tumour suppressive activity of PKCζ is thought to occur through this mechanism[99]. Furthermore, a study revealed that 2-phosphoglycerate (2PG), 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] (TABLE 1). This pathway has been extensively studied 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 of any of the other 19 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. Isotope tracing of the metabolic flux emanating from threonine showed 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; also known as GCSP) mediates glycine cleavage and the

Table 1 | **Candidate oncogenes and tumour suppressor genes in one-carbon metabolism**

Pathway	Genes	Evidence
De novo serine biosynthesis	*PHGDH*, *PSAT*, *PSPH* and *SHMT1*	Genetic aberrations and functional data[90,91,100]
Glycine cleavage	*GLDC* and *GCAT*	Overexpression and functional data[100,106]
Polyamine synthesis	*AMD1*, *EIF5A*, *SRM* and *DHPS*	Genetic aberrations and functional data[108]
Methylation metabolism	*IDH1*, *EZH2*, *SET9*, *DNMT1*, *ARID1A*, *JARID1C*, *UTX* and *SETD2*	Genetic aberrations and functional data[73]

AMD1, *S*-adenosylmethionine decarboxylase 1; *ARID1A*, AT-rich interactive domain-containing protein 1A; *DNMT1*, DNA (cytosine-5)-methyltransferase 1; *EIF5A*, eukaryotic-initiating factor 5α; *GCAT*, glycine C-acetyltransferase; *GLDC*, glycine dehydrogenase (decarboxylating); *PHGDG*, phosphoglycerate dehydrogenase; *PSAT*, phosphoserine aminotransferase; *SHMT*, serine hydroxymethyltransferase.

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 more than 200 metabolites were measured[106]. Surprisingly, glucose uptake and lactate production (that is, 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 in tumorigenesis, in which it may have a causal role. One study found that a subpopulation of tumour-promoting cells expressed high levels of GLDC and that ectopic expression of GLDC was sufficient to induce tumour formation in xenografts of NIH3T3 cells[100]. Interestingly, this study also reported that the ectopic expression of two other enzymes involved in serine and glycine catabolism, phosphoserine aminotransferase (PSAT) and serine hydroxymethyltransferase (SHMT) in NIH3T3 cells could induce tumour formation *in vivo*. Importantly, the induction of tumorigenesis depended on the enhanced enzymatic activity of GLDC. Another study also reported that an 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 promote 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 (FIG. 1).

NCI-60
A panel of tumour-derived cell lines originating from diverse tissue types. Extensive genomic, biochemical and pharmacological data have been obtained on these cell lines.

Protein translation. Recently, an *in vivo* screen for new tumour suppressor genes in lymphoma identified two genes, *S*-adenosylmethionine decarboxylase 1 (*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 that involves the biosynthesis of spermine and spermidine[109]. This pathway eventually leads to the production of hypusine, which is required for the hypusination of proteins. So far, 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 tumour suppression to methionine metabolism through the ability of methionine metabolism 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 owing to its increased abundance relative to thymidine. Several enzymes involved in nucleotide metabolism are considered to be bona fide tumour suppressor genes. Fragile histidine triad (*FHIT*), a gene that encodes an enzyme with dinucleotide hydrolase activity that is involved in purine metabolism, is one of the most frequently deleted genes in human cancer — almost 50% 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 tumour 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 the various subunits of RNR[126–128]. Overexpression of RNR induces lung tumours in mice through alterations in DNA repair, indicating that it can function as

Therapeutic window
A term used in drug development and medical practice that refers to ranges of drug concentrations that satisfy the trade-off between an efficacious clinical response and unwanted toxicities.

an oncogene[129]. Additionally, thymidylate synthase can also function as an oncogene[126,130], and 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 as having causal roles in cancer development[134–139].

Recent pulse-chase experiments using isotopically labelled 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 mechanistic understanding will lead to the development of new therapeutics that target key nodes in the cancer metabolic network. Metabolic enzymes, having evolved to carry out chemical catalysis, are thought to be druggable targets[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. Preclinical studies are currently underway that are evaluating the promise of targeting multiple nodes in one-carbon metabolism[22,147] (TABLE 2). These targets include 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]. There is one question that is often raised: what improvements would these new targets offer compared with currently existing drugs, such as methotrexate and pemetrexed, which already target one-carbon metabolism? One possible advantage of targeting these nodes lies in the potential for an improved therapeutic window. For example, as some tumours and most tissues do not seem to require PHGDH, and thus serine synthesis from glucose, targeting this pathway might prove less toxic in some contexts. Furthermore, some tumours display hyperactivation of this pathway and thus, as other pathways, in addition to *de novo* serine biosynthesis, enter into folate metabolism, these tumours might be more susceptible to PHGDH inhibition than to 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 2 diabetes[149] and also exhibits other activities, such as an anti-ageing effect in *Caenorhabditis elegans* through the inhibition of one-carbon metabolism in the gut microbiota[150]. Epidemiological evidence has suggested that metformin may have anticancer effects[151]. These data have been augmented with preclinical studies showing an antitumour 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 proved controversial, with numerous mechanisms having been proposed[153]. Recent work has provided evidence that metformin may act on one-carbon metabolism in patients. A metabolomics study that considered metformin revealed that the signature of metformin

Table 2 | **Drug targets in one-carbon metabolism**

Enzymes	Compounds	Status
Methylenetetrahydrofolate reductase	Methotrexate and pemetrexed	Approved for multiple cancers[14]
Thymidylate synthase	5-FU	Approved for multiple cancers, most notably colorectal cancer[14]
Ribonucleotide reductase	Gemcitabine	Approved for multiple cancers, most notably pancreatic cancer[14]
Polyamine synthesis enzymes	Various	Clinical trials ongoing[83]
DNA methyltransferases	Azanucleosides	Approved for myeloid leukaemias[72]
Histone methyltransferases	Various (SAM analogues)	Clinical trials ongoing[72]
Histone demethylases	Various	Preclinical studies[72]
Ornithine decarboxylase	DMFO	Clinical trials ongoing[83]
S-adenosyl decarboxylase	MGBG and SAM486A	Preclinical studies[83]

5-FU, 5-fluorouracil; DMFO, 2-difluoromethyl ornithine; MGBG, methylglyoxal bis(guanylhydrazone); SAM, S-adenosylmethionine; SAM486A, (E)-2-(4-carbamimidoyl-2,3-dihydro-1H-inden-1-ylidene)hydrazinecarboximidamide.

Table 3 | **Oncology biomarkers in one-carbon metabolism**

Biomarker	Source	Use
Choline	^{11}C PET imaging	Detection of advanced malignancies[187]
Sarcosine	Urine metabolite	Possible predictor of metastatic prostate cancer[107]
Thymidylate synthase	Tumour mRNA expression	Expression correlates with response to 5-fluorouracil[181]
Glutathione sulphur transferase	Tumour mRNA expression	FDA-approved as part of assay for decision to use chemotherapy in oestrogen receptor-positive breast cancer[193]

FDA, US Food and Drug Administration; PET, positron emission tomography.

response was remarkably similar to that obtained with antimetabolite chemotherapies[154]. These findings suggest that metformin may confer 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 diet or nutrient modification. High carbohydrate intake is positively correlated with cancer incidence[155–158], and preclinical[159] and clinical[160,161] studies have shown that reducing carbohydrate (glucose) intake can have negative effects on tumour biology.

Preclinical 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 that were fed diets containing no serine and no glycine[98,162]. The removal of serine and glycine dramatically affected cell proliferation and tumour 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 tumour growth. Strikingly, it was observed that the removal of serine and glycine had an even greater effect on the reduction of *in vivo* tumour growth than did the re-introduction of wild-type *Trp53* alleles into the tumour cells. Other mechanisms are also likely to be relevant, such as a reduced, if not disrupted, rate of biosynthesis, altered 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 in patients with epilepsy 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 anticancer effects of metformin might work by targeting folate metabolism. However, numerous longitudinal studies have associated folate and vitamin B_{12} intake with alterations in DNA methylation and cancer risk. Lack of an 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[26,27,164,165]. Decreased folate intake is associated with cancer, most notably colorectal cancer[166–174]. In breast cancer, reduced folate intake is associated with cancer development and global hypomethylation[175,176]. Given data on serine and glycine deprivation and its antitumour effects, as well as the metformin data, the relationship between diet and one-carbon metabolism is apparently complex, and more work is 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 are detectable in biological fluids such as serum and urine. These properties allow for the possibility of innovations in diagnostics (TABLE 3). For example, increased homocysteine levels in serum are used as a biomarker for folate deficiency[177]. The build-up of homocysteine results from the lack of available methyl donors to complete a turn of the folate cycle. The use of antimetabolite chemotherapies has identified biomarkers, mostly in the form of the enzymes, which these chemotherapies target, that predict response or resistance. For example, expression of thymidylate synthase has been shown to predict response to 5-FU treatment[178–182]. Biomarkers of response to methotrexate treatment have been found in serum metabolites[183,184], and a recent meta-analysis reported that the expression of folate-metabolizing enzymes and of those involved in serine and glycine metabolism could predict tumour response to methotrexate in diverse tumour types[185].

A metabolomics study of urine from patients with benign prostatic disease, localized prostate cancer and metastatic prostate cancer revealed that glycine metabolism is 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[186–188]. 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 tumour progression[189,190]. Positron emission tomography (PET) imaging of ^{11}C choline has been approved by the US Food and Drug Administration (FDA) as a biomarker for advanced malignancy[191,192]. Gene expression levels of glutathione-metabolizing enzymes are also an FDA-approved biomarker for treatment decisions in node-negative, oestrogen receptor-positive breast cancers[193]. With the initial molecular mechanisms connecting one-carbon metabolism to cancer pathogenesis recently characterized, additional advances in biomarker discovery for precision medicine with anti-folate agents could be realized.

Summary and future directions

Once thought to be the subject of mundane biochemistry lectures and the target of nonspecific cytotoxic chemotherapies, amino acid and one-carbon metabolism has re-emerged as a core feature of the biology of cancer (FIG. 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[194]. IDH1 and IDH2 are recurrently mutated in leukaemia and gliomas, as well as in other cancer types[195]. 2HG has been shown to have numerous functions, including the alteration of epigenetic marks through the inhibition of histone demethylases[196]. Perhaps the intermediates in one-carbon metabolism are also oncometabolites, the aberrant activity of which promotes cancer pathogenesis.

However, there are many questions that need to be addressed before we can clearly understand the links between one-carbon metabolism and cancer. For example, how is one-carbon metabolism integrated with signals from diverse nutrient inputs to generate the appropriate downstream carbon portioning? The extent and context in 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 currently unknown angles that connect epigenetics, nucleic acid metabolism and redox biology to one-carbon metabolism

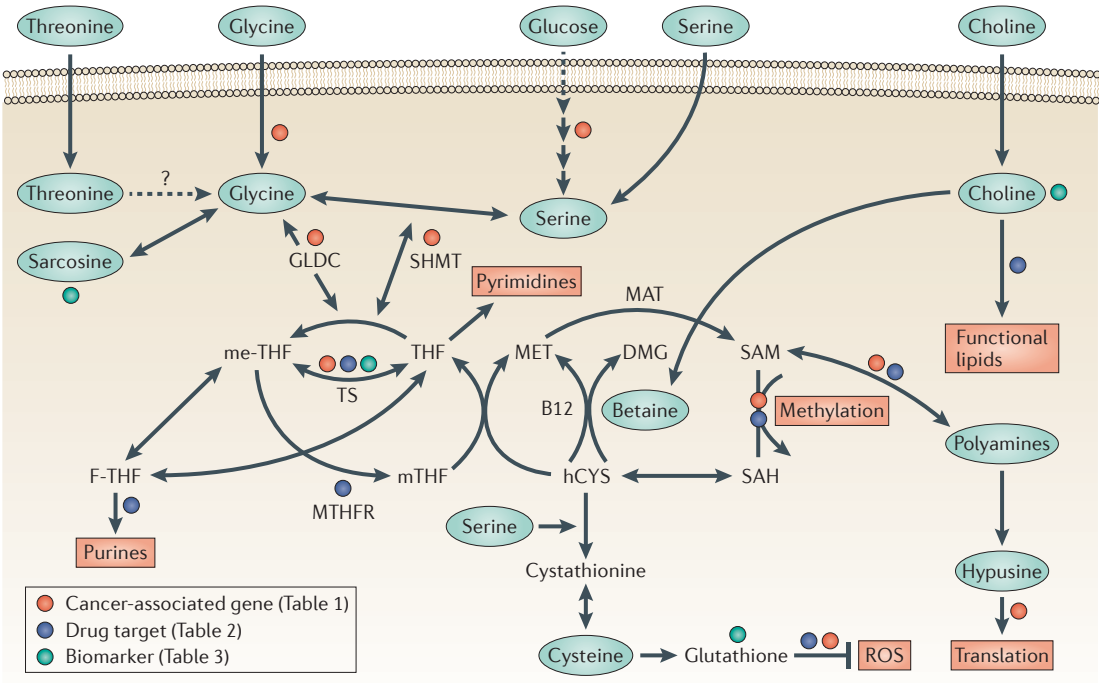


Figure 5 | **One-carbon metabolism, cancer pathology and intervention.** The schematic shows one-carbon metabolism and the trans-sulphuration pathway. Recent findings have identified roles for these pathways in cancer. Genetic mutations and functional evidence for the existence of a cause of cancer (driver) at this point in the pathway (indicated by red circles), currently available drugs (blue circles) and biomarker development (green circles) are highlighted. The specifics are indicated in TABLE 1, TABLE 2 and TABLE 3. Causality for specific cancer-associated genes has been shown by the presence of a genetic lesion or by functional evidence, such as the overexpression of a pathway component, and that this enhanced activity at this point in the pathway promotes oncogenesis. The biological outputs of one-carbon metabolism are shown in red boxes. Bi-directional arrows denote reversible steps. Dashed arrow denotes multiple biochemical steps. B12, vitamin B_{12}; DMG, dimethylglycine; F-THF, 10-formyltetrahydrofolate; GLDC, glycine decarboxylase; GNMT, glycine *N*-methyltransferase; hCYS, homocysteine; MAT, methionine synthase; me-THF, 5,10-methylenetetrahydrofolate; mTHF, 5-methyltetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; PC, phosphatidylcholine; PHGDH, phosphoglycerate dehydrogenase; ROS, reactive oxygen species; SAH, *S*-adenosylhomomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate; TS, thymidylate synthase.

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 cancer patients, but our ability to predict these responses remains poor. If any of our recent knowledge of one-carbon metabolism could be harnessed for advances in precision medicine, dramatic inroads into patient care could be made.

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Competing interests statement
The author declares no competing financial interests.

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