Perturbations in folate metabolism are associated with risk for colon cancer, although the underlying mechanisms remain to be established. It is not known if the associations between disruptions in folate metabolism and cancer result from altered S-adenosylmethionine (SAM; also known/abbreviated as AdoMet) synthesis and/or deoxythymidylic acid (dTMP) synthesis. Cytoplasmic serine hydroxymethyltransferase (cSHMT) is a metabolic switch that directs the partitioning of folate-activated one-carbon units between dTMP and SAM biosynthesis. cSHMT is expressed in tissues associated with folate-related pathologies, including the colon. Therefore, gain-of-function and loss-of-function cSHMT mouse models can be used to elucidate the contributions of the dTMP and SAM biosynthetic pathways to colon cancer.

Nutrition and genetics interact to contribute to the initiation and progression of carcinogenesis. Molecular antecedents that promote the development of sporadic colon cancer include DNA damage (single point mutations, the loss of heterozygosity and microsatellite instability), and epigenetic alterations including chromatin methylation, which affect both genome stability and gene expression. Implicated in the development of cancer is the loss of function of the tumor suppressor genes adenomatous polyposis coli (APC) and p53, as well as epigenetic alterations, such as methylation of DNA that acts to silence tumor suppressor genes. Dietary deficiency of critical nutrients, including folate, and the generation of oxidative stress, increase rates of DNA damage and thereby have the potential to induce genetic mutations and instability that are responsible for the initiation and progression of carcinogenesis.

Tetrahydrofolate (THF) serves as a cofactor that chemically activates and carries one-carbon units at three different oxidation levels for a network of reactions known as one-carbon metabolism, which occurs in the mitochondria and the cytoplasm (Figure 1). The primary role of mitochondrial one-carbon metabolism is to generate glycine and formate from serine. Mitochondrial-derived formate traverses to the cytoplasm where it is a major source of one-carbon units for cytoplasmic one-carbon metabolism. One-carbon metabolism in the cytoplasm is necessary for the de novo synthesis of purines (supplies carbon 2 and 8 of the purine ring) and thymidylate (methylation of dUMP (deoxyuridylic acid) to dTMP, and for the remethylation of homocysteine to methionine. Methionine can be adenylylated to form SAM, which is a cofactor and one-carbon donor for numerous other methylation reactions.

**PERTURBATIONS IN FOLATE METABOLISM AND COLON CANCER**

Epidemiological studies implicate impaired folate metabolism in several pathologies including neural tube defects and cancer, notably colon cancer. Folate metabolism can be impaired by folate and other B-vitamin deficiencies and/or genetic mutations and polymorphisms. Biomarkers for impaired folate metabolism include increased uracil content in DNA and elevations in serum and tissue homocysteine. Increased homocysteine leads to elevations in serum and cellular S-adenosylhomocysteine, a potent inhibitor of SAM-dependent methylation reactions. For example, S-adenosylhomocysteine inhibits DNA and protein methyltransferases, leading to hypomethylated DNA and protein (including histones). Folate deficiency also depresses deoxynucleotide triphosphate (dTTP) synthesis in humans, which results in the misincorporation of uracil into DNA during replication and repair. However, little is known about the biochemical aspects of folate metabolism in the context of colon cancer.
mechanisms that detail the role of altered folate metabolism in the initiation or progression of folate-associated pathologies. Do the associations between disruptions in folate metabolism and cancer result from altered S-adenosylmethionine synthesis and/or from dTMP synthesis? Without detailed knowledge of mechanism, intervention and prevention strategies lack predictive value with respect to overall benefit and risk, and cannot be designed rationally.

REGULATION OF THE FOLATE METABOLIC NETWORK AND CANCER

The cellular concentration of folate-binding proteins exceeds that of folate derivatives, and therefore the concentration of free folate in the cell is negligible. This indicates that folate-dependent biosynthetic pathways must compete for a limiting pool of folate cofactors. The competition is most pronounced for reactions that utilize methyleneTHF (Figure 1). MethyleneTHF is required for several key reactions: 1) the conversion of dUMP to dTMP, catalyzed by thymidylate synthase (TS); 2) the conversion of glycine to serine, catalyzed by cSHMT, a metabolic switch that directs the partitioning of folate-activated one-carbon units between dTMP and SAM biosynthesis; and 3) the synthesis of 5-methylTHF, catalyzed by methylenetetrahydrofolate reductase (MTHFR), a reaction that commits one-carbon units to the methionine cycle. A number of studies have investigated the competition for methyleneTHF among folate-dependent enzymes. Scott et al. proposed that limited methyl group availability shifts the flux of one-carbon units such that folate cofactors are preferentially shuttled to the methionine cycle to protect methylation reactions and thereby suppress DNA synthesis. Similarly, based on the known affinities of several relevant enzymes that utilize methyleneTHF, Green et al. predicted that folate coenzymes are preferentially directed towards SAM-dependent methylation reactions at low cellular folate concentrations. Additionally, these authors predicted that the MTHFR enzyme would be insensitive to changes in methyleneTHF availability, whereas TS activity would be highly dependent on them, assuming that these two enzymes directly compete for a common cellular pool of methyleneTHF. These studies demonstrate that thymidylate and methionine (SAM) synthesis are competitive reactions that coordinately regulate each other and suggest that SAM synthesis has priority over dTMP synthesis.

Figure 1 Folate-mediated one-carbon metabolism. Tetrahydrofolate-mediated one-carbon metabolism is required for the synthesis of purines, thymidylate, and the remethylation of homocysteine to methionine. Serine is the major source of one-carbon units, which are generated in the mitochondria in the form of formate, or in the cytoplasm through the activity of cSHMT. Mitochondrial-derived formate enters the cytoplasm and function as a one-carbon unit for folate metabolism. The one-carbon is labeled in bold. Abbreviations: AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; cSHMT, cytoplasmic serine hydroxymethyltransferase; DHFR, dihydrofolate reductase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate; TS, thymidylate synthase.
activity, and is associated with decreased risk of developing colorectal cancer compared with carriers of the common allele. These observations indicate that appropriate partitioning of methyleneTHF is critical for cancer prevention.

**CYTOPLASMIC SERINE HYDROXYMETHYLTRANSFERASE (cSHMT) AND NUCLEAR FOLATE METABOLISM**

The cSHMT enzyme is strategically positioned to mediate competition between the synthesis of DNA precursors and methionine synthesis. The SHMT enzyme catalyzes the THF-dependent reversible conversion of serine and glycine as follows:

\[
\text{serine} + \text{THF} \leftrightarrow \text{glycine} + 5,10\text{-methyleneTHF}
\]

When catalyzing serine cleavage, this reaction generates one-carbon units for folate-dependent anabolic reactions and is the major source of one-carbon units in mammals; SHMT exists in both the mitochondria (mSHMT) and the cytoplasm (cSHMT). The two proteins are encoded by distinct genes and have different intracellular functions. The mSHMT enzyme is responsible for glycine synthesis, and the generation of one-carbon units in these cells is expressed at similar levels among human tissues, appearing to function as a housekeeping gene. The human cSHMT mRNA is enriched only in liver, skeletal muscle, the intestine, and kidney. Other tissues contain less than 5% of the levels found in liver, which is consistent with previous studies that examined the level of SHMT activity in tissues.

Recently, we have demonstrated that the cSHMT enzyme regulates the metabolic competition between TS and MTHFR in MCF-7 cells. When cSHMT protein is increased twofold or greater in MCF-7 cells, cSHMT preferentially shuttles methyleneTHF to dTMP synthesis, increasing the efficiency of this pathway and simultaneously inhibiting SAM biosynthesis by sequestering 5-methylTHF. The metabolic changes observed in cSHMT overexpressing MCF-7 cells are associated with dramatic alterations in the MCF-7 cell proteome. A mechanism whereby cSHMT preferentially partitions one-carbon units was recently established. The cSHMT, TS, and dihydrofolate reductase enzymes were shown to be the target of SUMO (small ubiquitin-related modifier) modification leading to nuclear import. The concept of nuclear thymidylate synthesis was first proposed by Reddy and Pardee, who provided evidence for the existence of a nuclear “replicase”, a multienzyme complex that synthesized nucleotides de novo at the replication fork during S phase. Ribonucleotide reductase was among the six nuclear enzymes identified by Pardee; no folate-dependent enzyme was identified. However, in 1976, Shin and Stokstad determined that 10% of total liver folate was in the nuclear compartment. We propose that SUMO-dependent nuclear thymidylate synthesis, which occurs into the nucleus during S-phase, limits the misincorporation of uracil into DNA. Overall, the data suggest that cSHMT is strategically positioned within the folate metabolic network to affect cancer risk.

The expression of cSHMT is regulated by several nutrients including zinc and retinoic acid at the level of transcription, and recently we have found that cSHMT protein levels are affected markedly by folate/choline status in the colon of mice (unpublished results). Its expression is markedly upregulated by ferritin, which binds to an internal ribosome entry sequence (IRES) on the cSHMT 5′UTR and increases rates of cSHMT translation. Collectively, these studies suggest that cSHMT may serve as a sensor for several nutrients that regulate its expression, and that changes in its expression and activity affect genome methylation and genome stability (through changes in SAM and dTMP synthesis). A few studies have investigated SHMT in relation to cancer. Importantly, cSHMT is expressed in the colon, a tissue characterized by folate-related pathology. An increase in SHMT activity has been observed in human and rat colonic tumors. Furthermore, a prevalent single-nucleotide polymorphism in the human cSHMT gene (L474F) is associated with protection against leukemia and lymphoma. Additional research is needed to determine if the cSHMT-mediated metabolic switch contributes to the etiology of folate-related pathologies and if this enzyme is a target for cancer prevention through diet. To this end, gain-of-function and loss-of-function cSHMT mouse models can be used to elucidate the contributions of the dTMP and SAM biosynthetic pathways to colon cancer.

**Acknowledgments**

Declaration of interest. The authors have no relevant interests to declare.

**REFERENCES**


11. Ames BN. DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat Res. 2001;475:7–20.


