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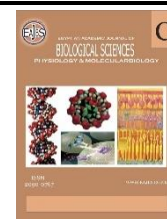
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Emerging Role of Folate-Mediated One Carbon Metabolism in Leukemia: A Review

Jinan A. Thabit¹ and Anwar J. Almzaiel²

¹Department of Chemistry, College of Science, University of AL-Qadisiyah, Al-Diywaniyah, Iraq.

²Branch of Medical Chemistry, College of Medicine, University of AL-Qadisiyah, Al-Diywaniyah, Iraq.

*E-mail: sci.chem.ph.20.3@qu.edu.iq

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ABSTRACT

Leukemia is a type of cancer that develops in the bone marrow and blood cells. When the DNA of a cell undergoes one mutation or a series of mutations results in the formation of leukemia cells. These leukemia cells, "blasts," cannot mature into fully functioning WBCs. Leukemia increases WBCs which compete for space with RBCs and platelets that the body requires to function normally; each may be classified as acute or chronic, myeloid or lymphoid, depending on the originating cell and the growth rate. Nearly 2.5% of all new cancer cases and 3.1% of cancer-related deaths were attributable to Leukemia. Leukemia can strike at any age geographical and ethnic differences in leukemia rates are also present.

Folate's significance in cancer was exclusively tied to antifolate-based cancer treatment. After discovering Antifolate associated based chemotherapy, contrary to the inhibitory action of antifolate on tumors, epidemiologic, clinical, and experimental data show that folate deficiency in normal tissues may predispose them to neoplastic transformation and that folate supplementation may suppress the development of tumors in normal tissues. Epidemiologic research reveals a negative connection between folate levels and numerous cancers, including Leukemia. The exact association between folate status and certain cancers is unknown. Vitamin B control one-carbon metabolism and human health. Folate-mediated one carbon (1C) metabolism supports a of processes that are essential for the cell. Through a number of interlinked reactions happening in the cytosol and mitochondria of the cell, folate metabolism contributes to de novo purine and thymidylate synthesis, to the methionine cycle and redox defense. Targeting the folate metabolism gave rise to modern chemotherapy. The folate and methionine cycles complete the one-carbon route with vitamin B9 or folate. Vitamin B6 regulates carbon cycling. Vitamin B dysregulation alters biochemical signaling and causes several illnesses.

INTRODUCTION

Cancer is a condition in which some cells in the body develop uncontrolled and spread to other regions of the body (Zureigat *et al.*, 2023); therefore, blood cancer is a kind of cancer that begins in blood-forming tissue, such as the bone marrow or the cells of the immune system and may be lead to damage. Examples of blood cancer are leukemia, lymphoma, and multiple myeloma. Also, it is called hematologic cancer. Changes in the DNA of only one lymphoid or blood stem cell may be enough to cause these diseases to manifest.

Cancers of the blood develop when aberrant cells multiply and survive without the normal controls that normally maintain cellular health (Andrades *et al.*, 2023).

The bone marrow, blood cells, lymph nodes, and other lymphatic organs may be attacked by blood cancers; abnormalities in the DNA of a single lymph or blood-forming stem cell lead to originate several diseases; these aberrant cells may multiply and persist in blood cancers due to a lack of regular checks and balances that keep healthy cells in check, buildup these substances in the bone marrow, blood, and lymphatic tissue disrupts the maturation and function of red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs), This process may lead to severe anemia, bleeding, a weakened immune system, and death (The Leukemia & Lymphoma Society 2018).

When a person has Leukemia, their bone marrow produces abnormal white blood cells, known as leukemia cells. Leukemia cells do not die when they should, unlike normal blood cells. They might compete with healthy RBCs, PLTs, and WBCs for space. This makes it difficult for healthy blood cells to perform their functions when symptoms appear (Mohamed *et al.*, 2018).

The malignancies known as Leukemia, originating in the bone marrow, do not produce solid tumors. Leukemic blast cells, which increase in vast numbers and crowd out healthy white blood cells in the bone marrow and blood, cause Leukemia. When fewer normal blood cells are present, the body may be more difficult to prevent bleed, fight infections, or deliver oxygen to its tissues. Four common varieties of Leukemia are categorized into acute and chronic forms depending on how quickly the condition worsens and the type of blood cell in which the malignancy first appears (lymphoblastic or myeloid). Leukemia grows more slowly in chronic kinds than in acute forms (Mohamed *et al.*, 2018). The types of cell involved are myeloid, myelogenous, lymphoid, lymphocytic, or

lymphoblastic. Uncontrolled blood cell accumulation is the main feature of Leukemia. However, the treatments utilized to treat each type's patient and their natural history differ (The Leukemia & Lymphoma Society 2018).

Folic acid and vitamin B12 help hematopoietic stem cells (HSCs) produce and matured blood cells, maintaining genetic health. Folic acid and folates are chemoprotective micronutrients that support health and genomic integrity due to it contributes to nucleotide biosynthesis and methylation activities in the cell, which are necessary for DNA synthesis, repair, and methylation, also folic acid maintains mitochondrial DNA and mitochondrial activity (mtDNA), and folate deficiency may impair DNA synthesis, repair, and methylation, compromising genomic stability and gene expression. These pathways are disrupted in chronic disorders, including cancer and cardiovascular disease. Folate metabolism may be influenced by food and other factors, therefore insufficient folic acid may potentially cause cancer since it maintains genetic integrity, and Folic acid deficiency during pregnancy may increase the risk of infantile Leukemia. In particular, a shortage of folates may cause DNA damage, which can cause chromosomal abnormalities, a hallmark of cancer and Leukemia. Homocysteine is a metabolite closer to DNA methylation in the one-carbon cycle, and higher levels have been linked to several malignancies in adults. This is because of the inverse connection between homocysteine concentration and folate status (Metayer *et al.* 2023). The link between folate metabolism and cancer was established for the first time when it was discovered that folic acid promoted cancer growth. This discovery led to the use of the folate antagonist 4-aminopropyl glutamic acid (aminopterin) in the treatment of childhood acute lymphoblastic leukemia (ALL) (Moulik, Kumar, and Agrawal 2017).

Folate's significance in cancer was exclusively tied to antifolate-based cancer

treatment. After discovering folic acid in the 1940s, Sidney Farber and colleagues gave children with leukemia folic acid polyglutamate conjugates because "folic acid concentrate" promoted breast tumor regression in mice. Still, folic acid increased Leukemia, surprising them. Sidney Farber showed that aminopterin, a folic acid antagonist, caused complete remissions in children with acute Leukemia. Antifolate-based chemotherapy began with this finding. Contrary to the inhibitory action of antifolate on tumors, epidemiologic, clinical, and experimental data show that folate deficiency in normal tissues may predispose them to neoplastic transformation and that folate supplementation may suppress the development of tumors in normal tissues. Epidemiologic research reveals a negative connection between folate levels and numerous cancers, including Leukemia. The exact association between folate status and certain cancers is unknown. Patients with lymphomas or Leukemia have had their urine excretion of FALG and serum folic acid levels examined. The findings show that a prevalent side effect of these disorders is folic acid deficiency. Vitamin B control one-carbon metabolism and human health. The folate and methionine cycles complete the one-carbon route with vitamin B9 or folate. Vitamin B6 regulates carbon cycling. Vitamin B dysregulation alters biochemical signaling and causes several illnesses (Franco *et al.*, 2022).

1. Folic Acid (Folate):

The term "folate" refers to all water-soluble B vitamin compounds that share folic acid's parent structure and demonstrate a shared vitamin action (pteroyl-L-mono glutamic acid). Folate, often known as vitamin B9 or folic acid, comes in various forms, including folic acid (synthetic paper), the B vitamins (including pteroyl-L-glutamate), and their precursors (including folic acid, methyl tetrahydrofolate, folacin, tetrahydrofolic acid, and folacin). Folate has the chemical formula $C_{19}H_{19}N_7O_6$ and a molecular weight of 441.39746 g/mol. It has low solubility in water, alkaline hydroxides,

and carbonates and high insolubility in alcohol. Crystalline folic acid appears yellow to orange in colour. Pteridine is a B vitamin connected to para-aminobenzoic acid through a methylene bridge and to glutamic acid through a peptide bond. Pteroyl-glutamic acid is made up of L (+) glutamic acid, p-aminobenzoic acid, and the 6-methyl pteridine system. The oxidation state of the pteridine ring and the amount of glutamic acid residues set folic acid and its derivatives apart (1 to 11). The human body may make the pteridine ring. However, it cannot be mixed with other compounds (Cieřlik and Cieřlik 2018).

Microbes and plants could produce folates. While humans cannot synthesize folates and rely on dietary intake, plant foods are a primary source of folate for humans (Liang *et al.*, 2020). Kinetic studies using labeled folate estimated the overall amount in the human body to be between 20 and 70 mg. The cytosol and the mitochondria in the liver store around half of the total amount. Yeast, grains, meat, vegetables, dairy, fruits, and nuts are excellent sources of folate in the diet. Even in industrialized countries, folate intake may not be optimal despite having plenty of calories and appearing to be a balanced and sufficient diet (Cieřlik and Cieřlik 2018). Folate is a coenzyme to transfer necessary one-carbon units to form deoxy thymidylate, pureness, and other methylation processes. Ingested folate becomes useful through intestinal absorption, circulation, cell transfer, and structural alterations. Chronic disorders like cancer, cardiovascular disease, and cognitive dysfunction have been linked to nutritional folate status. Pathological effects of a folate deficit include anemia, reproductive health impairments, and embryonic development defects. FA impacts global DNA methylation at low and high levels (Alnabbat *et al.*, 2022). (Pattnaik *et al.*, 2020) observed a correlation between FA levels and clinical outcomes in hospitalized patients with leukemia. An inadequate diet is a possible contributor to these cases. Folate deficiency results in chromosomal instability due to

increased DNA strand breakage, uracil misincorporation, and faulty repair. Cancers, including leukemia, carcinomas, and lymphomas, may raise the body's folate needs to dangerous levels without proper supplementation. Multiple factors may contribute to deficiencies in FA, including insufficient dietary intake and illnesses that induce poor folate absorption. Drugs like methotrexate and trimethoprim may limit folate's absorption or conversion to its active form, which can cause a folate deficit, in addition lack of folate may result from congenital disabilities in the enzymes needed for its metabolism(Wu *et al.*, 2021).

2. Folate Bioavailability:

Bioavailability is a multi-stage process involving several stages: liberation, absorption, distribution, metabolism, and elimination phases (LADME). Absorptive and postabsorptive processes determine bioavailability, controlled by dietary factors, individual differences, and intricate diet-host interactions. Folic acid is essential for humans and other animals since it serves as one of the fundamental building blocks and a catalyst for several critical metabolic processes. Due to its stability, greater bioavailability, and oxidized form, folic acid is frequently found in dietary supplements and foods fortified with folate. While naturally occurring folate is commonly found in foods such as leafy green vegetables, oranges, beans, and legumes(Williams *et al.*, 2021). The biological effects of folate are carried out by tetrahydrofolate and other derivatives. The liver's dihydrofolate reductase is what drives biological activity. In humans, this movement happens remarkably slowly(Seelan *et al.*, 2021).

One-carbon units from folate can produce methionine from homocysteine, interconvert serine and glycine, or nucleotides (thymidine and purines). Foliates are necessary for the production of nucleotides, the maintenance of methylation processes, and the intermediate metabolism of amino acids. They also have a connection to neurotransmitter synthesis. It was

discovered that the proton-coupled folate transporter (PCFT) is essential for moving folate across the proximal small intestine's apical brush-border membrane. After transport, reductase enzymes convert mono glutamates to di- and tetrahydrofolate (THF). The parent substance of every biologically active form of folate is THF. The THF is then changed into 5,10-methylenetetrahydrofolate and 5-MTHF in the following phase. They are then sent to the hepatic portal vein, which links to the liver, systemic blood flow, and body tissues(Menezo *et al.*,2022).

3. Folate Storage:

The liver is the principal organ for folate metabolism and storage, crucial to maintaining the proper folate level; the liver serves as the body's primary repository for folates stored as polyglutamates. The two main folate transporters that are in charge of folate uptake in the basolateral membrane of hepatocytes are the (PCFT) and the reduced folate carrier (RFC). Folate and folic acid (FA), the synthetic form of folate, are essential for renewing S-adenosyl methionine molecules and maintaining proper cellular methylation. Deregulating DNA methylation contributes to cancer development since genomic methylation changes may aid stem cell reprogramming and dedifferentiation processes that result in a cancer stem cell phenotype(Sid *et al.*, 2018).

4. The Folate Intermediates:

"Folate" refers to various compounds containing a pteroyl group. (THF) is the active form of naturally occurring folate (Vitamin B9), and methyltetrahydrofolate (MTHF) is the most common form of folic acid in the blood; however, two other forms of this molecule are also water-soluble. Folic acid is an artificial chemical used as a nutritional supplement. In food fortification, it is entirely oxidized and cannot reach circulation when not ingested in the form of food or supplements. The liver's enzyme dihydrofolate reductase (DHFR) is essential to its biological function, although its

activity is very sluggish in humans; first, FA must be reduced by DHFR to Dihydrofolate (DHF), and then to (THF) before it can participate in the folate cycles and carry out its physiological functions (THF), and finally to the physiologically active 5-MTHF(Menezo *et al.*, 2022; Sobczyn and Harrington 2018).

5. Folate and One-carbon Metabolism:

Folate metabolism is a universal metabolic activity that allows biosynthetic processes, including the production of pyrimidines and thymidines and the remethylation of homocysteine, to proceed by activating and transferring 1C units. Folate metabolism supports a broader range of transformations known as one-carbon (1C) metabolism. One-carbon (1C) metabolism, governed by the cofactor folate, supports many physiological functions. These include redox defence, amino acid balance, epigenetic maintenance, and biosynthesis (purines and thymidine). 1C metabolic processes are compartmentalized inside and between eukaryotic cells and organs. The process of serine and glycine interconversion, de novo purine synthesis, de novo thymidylate synthesis, and homocysteine remethylation to methionine is known as folate-mediated one-carbon metabolism (FOCM), which is made up of a network of linked folate-dependent metabolic pathways. These routes have separate locations in the mitochondria, cytosol, and nucleus. The FOCM network's enzymes compete with one another to limit intracellular folate concentrations. Although feedback mechanisms control how the folate cofactors are distributed across the folate-dependent pathways, the effect of cell cycle regulation on FOCM is less well-known (Lan, Field, and Stover 2018).

Regulation of FOCM enzyme levels at transcription, translation, post-translational modification, and regulation of FOCM enzyme subcellular localization might result in temporal changes in FOCM metabolic inputs and outputs. Mammalian cells' de novo thymidylate biosynthesis pathway includes the enzymes serine hydroxymethyltransferases 1 and 2 (SHMT1 and SHMT2), thymidylate synthase, and dihydrofolate reductase translocate to the nucleus for DNA replication and repair(Anderson *et al.*, 2012).

Human cells compartmentalize (FOCM) into the cytosol, nucleus, and mitochondria(Feng *et al.*, 2021). Folate, transformed into the active form (THF) by the enzyme (DHFR), transports one-carbon groups in various processes. (SHMT) and (MTHFR) then work together to change THF into 5,10-MTHF, which is then converted to 5-methyl-THF. MTHFR is essential for one-carbon metabolism as it transforms methylene-THF into 5-methyl-THF. On the other hand, 5-methyl-THF is a transporter of the one-carbon unit involved in producing purines and pyrimidines, which provide a basic single-carbon unit for synthesizing nucleic acids. In contrast, a methyl group is taken off 5-methyl-THF, a substrate. It is progressively transferred to the vitamin B-12 coenzyme before homocysteine, resulting in methionine, which is used in DNA methylation and the production of proteins and phospholipids.

Each of the responses, as mentioned earlier, affects how cells develop, proliferate, and differentiate, all of which are essential for maintaining human health(Disorders and Huanyu 2019). Human diseases like cancer and neural tube defects (NTDs) are linked to abnormalities in folate metabolism (**Fig.1**).

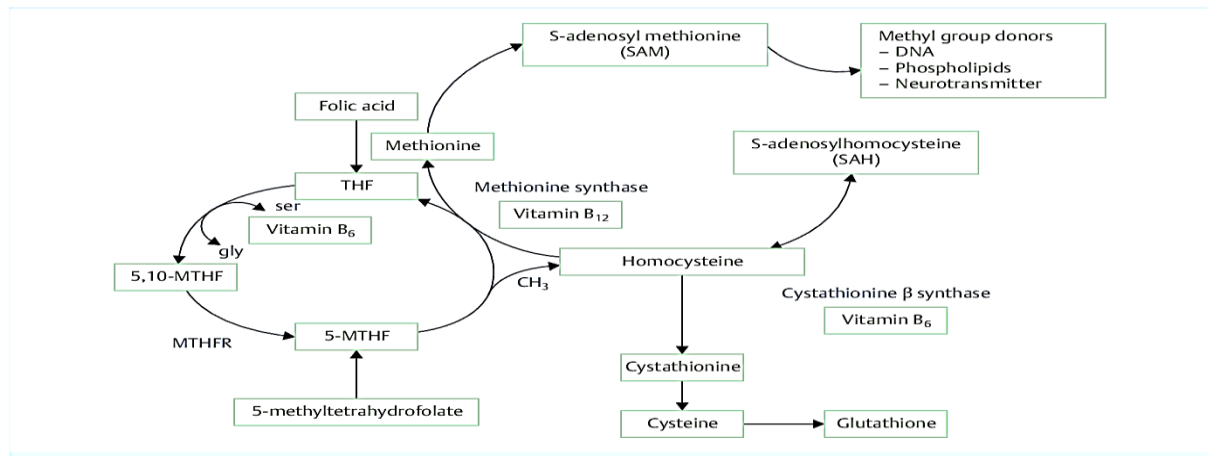


Fig. 1: Folate-mediated one-carbon metabolism(Thaler 2014)

Folic acid has no coenzyme activity and needs to be reduced via dihydrofolate (DHF) to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR). THF is metabolized via serine hydroxymethyl transferase (SHMT) to 5,10-methylene-tetrahydrofolate (me-THF). Serine, by conversion to glycine, donates a one-carbon unit during the methylation of THF. Native food folates appear reduced and are mainly methylated in 5-methyltetrahydrofolate (5-CH₃THF).

6. Folate in DNA Synthesis:

Folates serve as a means of distributing one-carbon groups. The vitamin is covalently modified by polyglutamylation once delivered to the cell. At multiple levels of oxidation, it is additionally formate (10-formylTHF), formaldehyde (5,10-MTHF), and methanol (one-carbon molecular substitution at positions N5 and N10) (5-methylTHF). In one-carbon metabolism, serine and glycine are the only direct sources of 1C groups. So, the fundamental step of the folate cycle is the conversion of serine to glycine by the SHMT1 and SHMT2 enzymes. Through the transfer of the 1C-group from serine to THF, 5,10-MTHF is formed, which serves as the first donor in the folate cycle. The enzymatic cleavage of glycine may also produce 5,10-MTHF by the mitochondrial enzyme glycine decarboxylase (GLDC)(Shuvalov *et al.*, 2017).

Metabolic alterations in cancer include heightened glycolysis, the pentose-phosphate pathway, and an acquired ability for de novo synthesis of fatty acids; these alterations are consistent with the increased rate of one-carbon metabolism and nucleotide production(Shuvalov *et al.*, 2017). The synthesis of the pyrimidine nucleotide thymidylate likewise needs one-carbon units. This specifically happens when dUMP is methylated to create dTMP, a reaction facilitated by the enzyme thymidylate synthase (TYMS) and uses methylene-THF as the methyl donor. During this reaction, methylene-THF is transformed into DHF, which DHFR then reduces to THF. Both methotrexate use and a folate deficit severely impede dTMP synthesis, which results in the incorporation of uracil in its place into DNA(Newman and Maddocks 2017).

7. Folate in Methylation Reactions:

Folates are required for life as a necessary biological component and catalyst, especially in nucleotide metabolism for DNA synthesis and methylation processes (Menezo *et al.*, 2022; Moulik, Kumar, and Agrawal 2017). DNA methylation is affected comparably by low and high FA levels(Liu, Liu, and Zhang 2020). Methylation is a critical biochemical process that plays a key role in gametogenesis and ongoing embryo

development. It is also a crucial biochemical mechanism for life transmission. Cell division and the regulation of gene expression through imprinting and epigenesis are both fueled by the methylation of DNA and histones (Shaikh *et al.* 2022). Methylation, a basic epigenetic mark important for controlling the expression of genes, is typically linked to gene suppression.

One-carbon metabolism is thought to be the source of the methyl groups required for the endogenous methylation of nucleic acids and proteins, and histones. DNA methylation, noncoding RNAs, histone alterations, and chromatin remodeling are fundamental biological modifications.

DNA methylation is the epigenetic change in humans that has received the most research. In CpG dinucleotides, it refers to the insertion of a CH₃ group found on position five ' of the amino acid base cytosine, producing 5-methylcytosine. The operation of folate-dependent pathways affects the expression and stability of the genome because folate-dependent ways produce purine and thymidine nucleotides for DNA synthesis and AdoMet for methylation of DNA and chromatin. Consequently, the genome provides a source of biomarkers that provide information on the operation of folate metabolism (Field *et al.*, 2018). A methyl group donor must methylate DNA and histones in the nucleus. The primary mechanisms underlying epigenetics are Controlling how genes are expressed or how proteins function without changing their DNA sequence is called gene expression regulation (for example, DNA methylation) (Field *et al.*, 2018).

One frequent method of gene silencing is DNA methylation. A distinctive cellular event strongly associated with some activities is global DNA methylation. Gene transcription is regulated by epigenetic alterations, such as DNA methylation status, chemical modifications of histones, or RNA processes (Uramova *et al.*, 2018). DNA methylation is essential in several cellular processes. Since DNA methylation is a

frequent and important epigenetic mechanism that controls the processes involved in neoplastic transformation in eukaryotic cells, it is crucial in cancer development.

S-adenosylmethionine (AdoMet, also known as SAM) can transfer its methyl group to create a variety of crucially important methylated molecules, including substances such as sarcosine, creatine, and adrenaline; methylated proteins, RNA, and DNA; and methylated nucleic acids. De novo purine synthesis, the production of (dTMP), and a major cellular energy source through the reduction of NADP⁺ to NADPH are all dependent on folate-mediated one-carbon metabolism, which is inextricably linked to Vitamin B12 (cobalamin, Cbl) metabolism through the utilization of 5-methyltetrahydrofolate as a methyl donor by MS. SAM is extremely important since it is a methyl donor in many methylation processes, including epigenetic methyl transferases processes like DNA and histone methylation. SAM is produced in cells by a tightly controlled mechanism that pays attention to mono-carbon metabolism and vitamin intake from food. After ATP1, (SAM) is the second-most common enzyme substrate. Over 200 human methyltransferase enzymes (MTases) use it to methylate nucleic acids (DNA and RNA), phospholipids, hormones, and tiny compounds. This includes histone and non-histone proteins (Fukumoto *et al.*, 2022).

8. Folate in Amino Acid Metabolism:

The amino acids that require folate for their metabolism are histidine, methionine, serine, glycine, cysteine, and serine. As a coenzyme, folate is also involved in metabolizing methionine into homocysteine. Methionine metabolism also requires the vitamins B12 and B6 (Danchin, Sekowska, and You 2020).

Through interorgan metabolism, glycine is created and serves as a precursor for several vital metabolites. Glycine enhances human and animal growth and well-being and effectively improves health. Treatment for metabolic disorders in cancer

and other diseases can be achieved through dietary supplementation with the appropriate dose of glycine. Additionally, glycine can improve neurological and sleep quality. Glycine is essential in the nutrition and metabolism of numerous mammals, including humans. Glycine is 11.5% of the body's overall amino acid composition. Synthesis of Glycine from Serine is typically obtained through diet (Wang *et al.*, 2013). Serine/glycine biosynthesis and one-carbon metabolism play a significant role in cancer cells' survival and rapid growth and have important therapeutic significance. Serine/glycine biosynthesis is excessively activated, promoting carcinogenesis and supplying a single carbon for one-carbon metabolism. Tumor growth is supported by one-carbon metabolism, a sophisticated cyclic metabolic network based on the chemical reaction of folate molecules. Additionally, one-carbon metabolism provides substrates for the methylation reaction and preserves the redox equilibrium of the tumor microenvironment (Pan *et al.*, 2021).

Methionine is the initial amino acid residue of every polypeptide transcribed by the ribosome nanomachine. In the methionine cycle, methionine synthase catalyzes the (re)methylation of homocysteine to methionine, a crucial step in the production of this essential amino acid and the synthesis of SAM, the most important cellular methyl donor. Reduced folate compounds are essential for 1C transfer reactions because they can contain and donate one-carbon (1C) units for activities connected to folate in the human body. These processes are necessary for synthesizing the amino acid methionine and DNA nucleotides and subsequent control of homocysteine (Hcy) levels (Jones *et al.*, 2019).

9. 5, 10 -Methylenetetrahydrofolate Reductase (MTHFR):

MTHFR is a cytoplasmic enzyme responsible for the irreversible conversion of 5,10-MTHF (methyleneTHF) to 5-MTHF (methylTHF), a process requiring NADPH

as an electron donor and FAD as a cofactor. Each homodimeric human MTHFR protein subunit comprises a brief linker region between the N-terminal catalytic domain, which links methyleneTHF, NADP, and FADH₂, and the C-terminal regulatory domain. The catalytic domain can complete the entire enzymatic reaction. Each subunit of the homodimeric human MTHFR protein consists of an N-terminal catalytic domain (amino acids 1-356) that binds methyleneTHF, NADPH, and FAD and a C-terminal regulatory part (amino acids 363-656) that regulates these molecules (aa 357–362) (Froese *et al.*, 2016, 2018).

The MTHFR gene polymorphism has two prevalent variations: C677T and 1298C. One of these two variations of the MTHFR gene results in a less active MTHFR enzyme, which can drop folic acid levels and raise homocysteine levels in the blood. MTHFR gene mutations result in severe 5,10-methylenetetrahydrofolate reductase (MTHFR) insufficiency, which is associated with hyperhomocysteinemia and a range of disease severity, from neonatal fatality to adult onset. MTHFR produces methionine from homocysteine by the catalysis of the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-MTHF (5-methylTHF). Methionine is the building block for SAM, the methyl group donor in over a hundred different reactions, making this reaction crucial in one-carbon metabolism. If not converted to 5-MTHF, 5,10-MTHF can take two different paths. To create dTMP, 5,10-MTHF can transfer its methylene group to dUMP or be oxidized to 5,10-MTHF, which is subsequently transformed into 10-formylTHF. Purine synthesis depends on this branch of the route. The utilization of nonmethylated folates as substrates in the de novo synthesis of nucleic acids is adversely affected by an excessive accumulation of 5-MTHF because the reduction of 5,10-MTHF by MTHFR is physiologically irreversible (Froese *et al.*, 2016, 2018).

10. 5, 10-MTHFR and Leukemia:

The enzyme (MTHFR), is a catalyst in the folate metabolism pathway, whose byproducts are involved in the remethylation of Hcy to methionine. DNA methylation and gene control depend on methionine, a precursor to a key DNA methyl donor. Rare MTHFR gene mutations have been linked to homocystinuria, an autosomal recessive MTHFR deficiency(Levin and Varga 2016).

Numerous pertinent studies have examined the relationship between MTHFR and various human illnesses, such as psychiatric disorders, cancers, cardiovascular diseases, and neurological conditions. The 5,10-MTHFR 677COT (C677T, rs1801133) is one of the polymorphisms in the MTHFR gene that have been identified. It substitutes valine for alanine at codon 222, which lowers the enzyme's activity. The frequency of the 5,10-MTHFR 677CO polymorphism varies with ethnicity. Numerous investigations have discovered a link between the 5,10-MTHF 677COT polymorphism and an elevated risk of ALL susceptibility. The MTHFR gene mutation may adversely influence enzyme activity in the normal folate metabolism pathway, raising the plasma level of homocysteine and substantially impairing normal human bodily function. ALL's etiology is heavily influenced by MTHFR polymorphisms, particularly the MTHFR C677T gene mutations. Therefore, MTHFR polymorphism may be related to acute lymphoblastic leukemia susceptibility and mitochondrial function(H. Zhang *et al.*, 2021).

A genetic variant in the methylene tetrahydrofolate reductase (MTHFR) gene has been linked to various cancers, including acute Leukemia. A crucial enzyme for the pharmacokinetics of MTX is (MTHFR) C677T and A1298C are two single-nucleotide polymorphisms influencing the MTHFR gene's activity. A study on the link between leukemia risk and MTHFR gene variants was established, particularly in pediatric ALL. According to many studies, certain MTHFR gene variants may increase the chance of getting leukemia, however,

there is not currently enough proof to utilize these mutations as reliable diagnostic or prognostic indicators. It's important to note that MTHFR blood levels are seldom considered a factor in either the diagnosis or treatment of leukemia. In reality, MTHFR gene mutations are only one of several genetic variables that may play a role in determining whether or not someone develops leukemia(Zintzaras *et al.*, 2006). A combination of environmental variables, such as dietary destitution, and genetic ones, such as polymorphism in MTHFR genes involved in Hcy metabolism, may explain why patients' MTHFR levels are low. This mutation generates a thermolabile enzyme variant in which the coenzyme FAD dissociates more quickly, causing a 50% or more loss in activity. According to the literature, 14 rare mutations in the MTHFR gene are associated with a severe enzymatic deficit, and one common mutation, C677T, is associated with a mild enzymatic deficiency. Additionally, the research done by Rim Frikha(Frikha 2020) shows that the C677T polymorphism may be a viable biomarker for ALL. This conclusion should be taken cautiously when considering other variables like folic acid consumption, gene-gene, and gene-environment interactions. In conditions of folate deprivation, increased uracil misincorporation (and leukemia susceptibility) is caused by MTHFR mutations that result in reduced enzyme activity and vice versa.

11. Serine Hydroxymethyltransferase (SHMT):

Serine hydroxymethyltransferase (SHMT) is an essential enzyme for nucleotide biosynthesis in prokaryotes, eukaryotes, and archaeobacteria. Pyridoxal-5'-phosphate is required. SHMT catalyzes the reversible reaction of serine cleavage to glycine, and the resulting hydroxymethyl group is transferred to tetrahydrofolate (THF), producing 5,10-MTHF and H₂O. For the de novo production of purine and pyrimidine nucleotides, the enzyme provides the main source of activated 1-carbon (1C) units. The SHMT1 and SHMT2 isoforms in

humans are found in the cytosol and mitochondria, respectively, and have a high degree of sequence identity (66%)(Scaletti *et al.*, 2019).

In one-carbon (1C) metabolism, the folate cofactor mediates the enzyme (SHMT), which has cytosolic (SHMT1) and mitochondrial (SHMT2) isoforms. SHMT catalyzes the conversion of serine and (THF) into glycine and 5,10-MTHF. Because of their critical role in supplying 1C units for DNA synthesis, the 1C/folate metabolism enzymes SHMT2 are among cancer's most frequently overexpressed metabolic enzymes. The amino acid serine is the primary 1C donor in rapidly reproducing cells, particularly cancer cells(Scaletti *et al.*, 2019).

12. SHMT and Leukemia:

More recent research has revealed that SHMT is necessary for cancer cells to proliferate and become tumorigenic to their full potential, highlighting the significance of serine catabolism in the disease. Cytoplasmic pathway's arm of One carbon atom requires SHMT1. It can also generate glycine and format intermediates that contribute to the methionine and glutathione cycles, the production of purines and pyrimidines, and other metabolic processes. The one-carbon folate pathway is inhibited by drugs that treat various malignancies, including ALL, AML, and others. Although acute leukemias are frequently very proliferative, the effect of one-carbon folate pathway inhibition utilizing a new inhibitor of SHMT1 and SHMT2 was stronger in ALL than in AML(Pikman, Ocasio-Martinez, Alexe, Dimitrov, Kitara, Diehl, Robichaud, Conway, Ross, and Su 2022).

Previous studies conducted by Angela Tramonti, Aamod S. Dekhne, Yana Pikman, and Ludovica Di Martino *et al.*(Dekhne *et al.*, 2020; Di Martino *et al.*, 2021; Pikman, Ocasio-Martinez, Alexe, Dimitrov, Kitara, Diehl, Robichaud, Conway, Ross, Su, *et al.*, 2022; Tramonti *et al.*, 2021), that demonstrated an in comparison to normal tissues, the gene for SHMT2 is one of the most consistently

overexpressed metabolic enzymes in cancer. Glycine consumption and serine catabolism, particularly SHMT2, were shown to be strongly linked with cancer cell proliferation. SHMT2 is one of the essential enzymes in metabolism, converting serine and (THF) into glycine and 5,10-MTHF. SHMT2 catalyzes the conversion of serine to glycine in mitochondria and is an essential supply of glycine for rapidly dividing cells. According to previous research(Minton *et al.*, 2018), SHMT2 encodes the mitochondrial version of a pyridoxal phosphate-dependent enzyme essential for energy metabolism. Since SHMT2 has been shown to promote cancer cell survival and tumor development in vivo, it shows promise as a diagnostic biomarker and potential therapeutic target for leukemia. SHMT2 may play a significant role in cell proliferation by influencing mitochondrial function (P. Zhang and Yang 2021).

According to research by Y. Pikman, N. Ocasio, G. Alexe, *et al.*(Pikman, Ocasio-Martinez, Alexe, Dimitrov, Kitara, Diehl, Robichaud, Conway, Ross, Su, *et al.*, 2022), leukemia progression in vivo is inhibited by SHMT2 inhibition. Additionally, it lowered the leukemia burden in vivo; this emphasizes the role of the one-carbon folate pathway in acute leukemia due one-carbon unit contributing to the biosynthesis of nucleotides and proteins involved in tumor growth and encourages the continued development of SHMT inhibitors for the treatment of leukemia and other malignancies. However, further investigation into SHMT2's significance in leukemia is required

13. 5-Methyltetrahydrofolate:

There are several forms of folate, including methyltetrahydrofolate (the most common form in circulation), methyltetrahydrofolate, folinic acid, folacin, and pteroylglutamic acid. Tetrahydrofolic acid is the active metabolite (vitamin B9)(Di Tinno, Cancelliere, and Micheli 2021). As a reminder of these pathways' importance, the one-carbon metabolic cycle uses folate as a carrier and is the source of 5-MTHF (CH₃-THF). In the blood and tissues, CH₃-THF is

the most prevalent type of folate (Froese, Fowler, and Baumgartner 2019). L-5-methyltetrahydrofolate (L-5-MTHF), a reduced synthetic version of folate, could be an alternative supplement. Vitamin B-12-dependent MS converts L-5-MTHF to THF, which is then used to synthesize purines and pyrimidines. Therefore, 5-MTHF will not be able to participate in nucleotide synthesis when there is a vitamin B-12 deficiency, and it is unlikely that it will be able to hide the hematological effects of the shortfall. Also, L-5-MTHF is a pure crystalline synthetic derivative of 5-MTHF, a naturally occurring type of folate found in the bloodstream. According to previous studies, L-5-MTHF is just as bioavailable in fortified bread as folic acid. Overall, L-5-MTHF may be more beneficial as a supplement and fortifier than folic acid. But because vitamin B-12 is essential for the metabolism of L-5-MTHF, a shortage of that vitamin leads to the accumulation of L-5-MTHF in the body, leading to a functional folate deficiency. Dietary folates and folic acid are transformed into (5-MTHF), a methyl donor for homocysteine remethylation to methionine. Methionine synthase is the enzyme responsible for catalyzing this reaction (MS), and vitamin B-12, the cofactor. Even when folate levels are sufficient, low vitamin B-12 status can cause folate to be trapped as 5-MTHF, impairing the synthesis of purines and pyrimidines (Guéant *et al.*, 2022).

At the cellular level, levomefolic acid is the most important physiologically active form of folate for DNA replication, the cysteine cycle, and homocysteine regulation. It is also known as L-5-MTHF, L-methylfolate, L-5-MTHF, and (6S)-5-MTHF. It is also the type that crosses membranes to enter tissues and pass through the blood-brain barrier. In the cell, homocysteine is methylated with L-methyl folate to produce methionine and (THF). The methionine and folate cycles are connected by the MS reaction, which uses a methyl group on 5-methyl-THF generated from the folate cycle to regenerate methionine (Korimerla and Wahl 2022). FA

deficit may develop after vitamin B-12 deficiency if methionine synthase is impaired, leading to folate being trapped as 5-methyl-THF and methylene THFA accumulation in serum. The deficiency of vitamin B12 mimics the acute leukemia, and a nutritional study shows that vitamin B12 deficiency is common in leukemia patients (Konda *et al.*, 2019). The specific relationship between serum 5-MTHF levels and leukemia has not been firmly established in scientific literature. Evidence suggests that changes in folate metabolism, notably 5-MTHF levels, may be linked to an increased risk of various cancers, including leukemia, although the nature of this association is complicated and not fully understood. 5-MTHF levels may be affected by the chemotherapy medicines used to treat leukemia, such as methotrexate since these treatments interfere with folate metabolism. However, these changes would probably be caused more by the medication than by the leukemia itself. In previous research, serum 5-MTHF levels are not measured in the clinical diagnosis or management of leukemia.

14. Tetrahydrofolic acid (THFA) and Leukemia:

Tetrahydrofolic acid, also known as tetrahydrofolate (FH4), is the active form of FA formed by adding four hydrogen atoms to the 5,6,7, and 8 positions of the pteridine ring. THFA polyglutamate is a class of cofactors that transport and chemically activate one-carbon units for biosynthesis; they are (THF) derivatives found naturally in fresh fruits, vegetables, and beans. The cytoplasm, mitochondria, and nucleus all play roles in THF-mediated one-carbon metabolism, a metabolic network of interconnected biochemical pathways (Fox and Stover 2008). Tetrahydrofolic acid (THFA), commonly referred to as folate, is an important component of DNA and RNA synthesis and is necessary for cellular replication and division. DNA and RNA production is substantially more necessary in conditions like leukemia, a kind of malignancy marked by the fast growth of aberrant white blood

cells. With such a strong need for cellular reproduction, serum THFA levels may rise. The quickly replicating leukemia cells need a higher concentration of THFA to sustain their fast growth and division. Additionally, it has been shown that certain leukemia cells overexpress folate receptors, which may lead to increased absorption of folate from the circulation and contribute to elevated serum THFA levels. Although THFA levels have been linked to an increased risk of developing leukemia, the strength of this association varies greatly amongst individuals, leukemia subtypes, and other variables (Fox and Stover 2008).

Conclusions:

The metabolic reactions that are mediated by folic acid in 1C metabolism are essential to biosynthesis. Interest in folate metabolism is growing since rapidly dividing cells, especially cancer cells, rely on it to meet their biosynthetic requirements. Recent research has attempted to illuminate the biological significance of route compartmentalization. In cancer, its implications have led to the development of cutting-edge treatments. Many hematological malignancies have benefited greatly from the use of antifolates, notably MTX, which is now widely utilized to treat a wide range of neoplastic disorders. However, new techniques are needed to target this route because of the unresolved issues of side effects and resistance to antifolates. By inhibiting enzymes involved in serine 1C metabolism, may be able to overcome the drug resistance that has been noted for conventional antifolates. What makes them attractive is that they do not require any folate transporters or intracellular polyglutamate. Thus, a deeper understanding of the way in which these inhibitors work might aid in the development of novel treatment strategies.

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