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REVIEW ARTICLE

The Dual Role of Folate Metabolism in Colorectal Cancer: Bridging Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms, Molecular Subtypes and Precision Prevention

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Abstract

Colorectal cancer (CRC) exhibits complex heterogeneity driven by the interplay of genetic, metabolic, and microenvironmental factors. Folate metabolism, essential for nucleotide synthesis and epigenetic regulation, paradoxically influences CRC pathogenesis: deficiency increases initiation risk, while excess supplementation may accelerate established tumors. Central to this duality is methylenetetrahydrofolate reductase (MTHFR), whose polymorphisms (e.g., C677T, A1298C) modulate folate flux, altering DNA methylation-nucleotide synthesis balance in a context-dependent manner. Emerging evidence suggests that MTHFR variants interact with Consensus Molecular Subtypes—CMS1 (microsatellite instability), CMS3 (metabolic dysregulation), CMS2/4 (WNT/stromal-driven)—to dictate divergent folate dependencies. For instance, CMS1 tumors may exploit folate for immune evasion via adenosine signaling, whereas CMS2/4 subtypes rely on folate-mediated epigenetic reprogramming. However, current prevention strategies neglect these subtype-specific vulnerabilities, treating CRC as a monolithic entity. This review synthesizes evidence from Mendelian randomization, preclinical models, and subtype-stratified trials to propose three critical advances: (1) Subtype-Driven Folate; (2) Dependency Biomarker Integration; (3) Microenvironment Crosstalk.

Introduction

Colorectal cancer (CRC), responsible for 900,000 annual deaths globally, underscores the urgency for refined prevention strategies[1]. Folate metabolism, pivotal in DNA synthesis and methylation, exhibits dual roles: deficiency elevates CRC risk, while excess supplementation may drive tumor progression[2]. MTHFR polymorphisms (C677T/A1298C) modulate folate allocation between methylation and nucleotide synthesis, impacting genomic stability[3]. Meta-analyses reveal context-dependent C677T effects—reduced CRC risk in folate-sufficient populations versus increased risk in deficiency[4]—likely reflecting subtype-specific interactions (e.g., metabolic CMS1/CMS3 vs. WNT/stromal CMS2/CMS4)[5].

Integrating MTHFR genotyping with molecular subtyping could enable personalized folate interventions[1], yet current paradigms often overlook subtype vulnerabilities. Folate's microenvironmental impacts, including immunosuppressive adenosine signaling[6], further complicate its duality. This evidence advocates biomarker-guided approaches to replace universal supplementation, aligning interventions with genetic and molecular tumor profiles.

I. The Metabolic Paradox: Dual-Edged Sword of Folate in CRC

Folate metabolism plays a dual role in CRC pathogenesis, governed by its indispensable functions in one-carbon metabolism and context-dependent interactions with oncogenic pathways. As a central hub for nucleotide synthesis and methyl donor supply, folate ensures genomic

stability through thymidylate production (via 5,10-methylenetetrahydrofolate) while regulating epigenetic programming via S-adenosylmethionine (SAM)-dependent methylation[7, 8]. Paradoxically, folate receptor alpha (FOLR1) overexpression—observed in 60% of CRC cases—drives Wnt/β-catenin signaling through caveolin-1-mediated endocytosis, creating a feedforward loop that fuels tumor progression in CMS2 subtypes.

Epidemiological studies reveal conflicting dose-response relationships: protective effects dominate early carcinogenesis, as evidenced by the Nurses' Health Study II showing 27% reduced distal CRC risk with high dietary folate (>400 μg/day) in non-drinkers (HR=0.73). Conversely, the ASPIRED trial demonstrated that supraphysiological supplementation (5 mg/day) accelerated advanced adenoma progression in individuals with occult lesions (RR=2.3). These opposing effects exhibit ethnic disparities, with Asian populations displaying a J-shaped risk curve (optimal intake: 300-400 μg/day) contrasting Western cohorts' linear benefit up to 600 μg/day[9]. This likely reflects differences in genetic polymorphisms (e.g., MTHFR C677T), baseline folate status, and molecular subtype prevalence[1].

Mechanistically, folate deficiency promotes early carcinogenesis through uracil misincorporation and oxidative DNA damage (8-OHdG↑)[10], while excess folate in established tumors drives CMS4 progression via SAM-mediated hypermethylation of tumor suppressors like SFRP1[11]. Recent studies suggest that genetic variations in folate metabolism enzymes, such as MTHFR, may explain these discrepancies. For instance, the MTHFR C677T polymorphism is associated with reduced CRC risk in certain populations, particularly those with the TT genotype. This highlights the necessity of precision approaches tailored to tumor biology and host genetics.

In summary, while epidemiological studies present conflicting evidence, emerging data underscore the complex interplay between folate metabolism, genetic polymorphisms, and CRC pathogenesis. Understanding these interactions is crucial for developing targeted preventive and therapeutic strategies that account for individual genetic and metabolic profiles.

2. MTHFR Polymorphisms in Colorectal Carcinogenesis: A Metabolic Nexus

The MTHFR enzyme regulates folate metabolism by irreversibly converting 5,10-CH_2-THF to 5-CH_3-THF[12], directing one-carbon units toward methylation or nucleotide synthesis[13]. Clinically significant polymorphisms C677T and A1298C disrupt this balance through distinct mechanisms: C677T (TT genotype) reduces enzyme activity by 70% via impaired FAD binding[14], elevating homocysteine and limiting SAM production, while A1298C destabilizes the regulatory domain, causing folate-dependent catalytic inefficiency[15]. These defects bifurcate metabolism—diminished 5-CH_3-THF impairs DNMT-mediated DNA methylation (e.g., LINE-1 hypomethylation)[16], whereas accumulated 5,10-CH_2-THF drives TS-dependent dTMP synthesis, promoting genomic instability through uracil misincorporation and BER-mediated DNA damage[17].

The reduced SAM/SAH ratio in C677T carriers induces epigenetic dysregulation via tumor suppressor hypomethylation (CDKN2A, MLH1) and heterochromatin destabilization[18, 19]. Concurrent oxidative stress from homocysteine auto-oxidation and mitochondrial ROS elevates 8-OHdG levels, activating NF-κB/STAT3 pathways to enhance angiogenesis and recruit immunosuppressive MDSCs, reshaping the TME. These dual metabolic-epigenetic disruptions underscore MTHFR polymorphisms as key modifiers of CRC pathogenesis[19].

The metabolic vulnerabilities imposed by MTHFR polymorphisms are mitigated through dynamic adaptations within the TME. Cancer-associated fibroblasts (CAFs) rescue folate-deficient tumor cells via interleukin-6 (IL-6)-mediated upregulation of the reduced folate carrier (RFC/SLC19A1), enhancing folate uptake by 3-fold, while transferring methyl donors (e.g., SAM, glutathione) through exosomes and secreting thymidine phosphorylase to sustain nucleotide synthesis[20]. Nutrient-gene interactions introduce contingency layers to MTHFR-driven carcinogenesis. Riboflavin (vitamin B_2) stabilizes FAD binding in A1298C carriers, restoring 30% of enzymatic activity with supplementation[21], while vitamin B_{12} deficiency exacerbates the "methylation trap" in C677T individuals, paradoxically elevating homocysteine by 22% despite adequate folate intake[22]. Precision prevention strategies now integrate genetic,

metabolic, microbial, and nutritional biomarkers—MTHFR haplotypes, SAM/SAH ratios, *Fusobacterium* abundance, and red blood cell folate—to tailor interventions[23]. For 677TT carriers, low-dose folate (200 μg/day) with antioxidants (vitamin E 400 IU/day) mitigates oxidative damage, while metformin (850 mg/day) upregulates MTHFR expression, normalizing homocysteine. A1298C homozygotes benefit from riboflavin (5 mg/day) and betaine (3 g/day) to stabilize enzyme activity, whereas compound heterozygotes require SAM (800 mg/day) and vitamin B_{12} (1 mg/day) to bypass methylation defects[24].

MTHFR polymorphisms (C677T/A1298C) exemplify folate metabolism's dual role in colorectal carcinogenesis—preserving genomic stability while enabling malignant progression. These variants disrupt one-carbon flux, amplifying genomic instability via uracil misincorporation, oxidative stress (e.g., ROS-driven DNA damage), and epigenetic dysregulation (e.g., LINE-1 hypomethylation). Beyond tumor initiation through impaired DNA repair, they remodel the tumor microenvironment via homocysteine-induced inflammation and stromal reprogramming. Epidemiological paradoxes—where folate deficiency variably suppresses advanced neoplasia or accelerates early lesions—underscore the necessity of genotype-stratified, temporally precise interventions to balance these opposing metabolic roles.

III. Molecular Subtype-Specific Regulation of Folate Metabolism in CRC

The molecular heterogeneity of CRC has been increasingly recognized as a critical determinant of disease progression, therapeutic response, and patient outcomes. Building on the dual role of folate metabolism in CRC pathogenesis—highlighted by its interplay with *MTHFR* polymorphisms and epigenetic regulation—emerging evidence underscores the importance of molecular subtype-specific mechanisms in shaping folate metabolism dynamics[25]. The Consensus Molecular Subtypes (CMS) classification, which categorizes CRC into four distinct subgroups (CMS1–CMS4), provides a framework for understanding how folate-related pathways diverge across tumor biology. CMS1 (microsatellite instability immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal) exhibit unique metabolic dependencies, genomic instability patterns, and immune microenvironments, all of which intersect with folate metabolism in ways that may inform precision prevention strategies[26-29].

CMS1 tumors, characterized by microsatellite instability (MSI), hypermutation, and robust immune infiltration, demonstrate a paradoxical relationship with folate metabolism. While MSI tumors often display widespread promoter hypermethylation (CpG island methylator phenotype, CIMP-high), a process dependent on SAM—the universal methyl donor derived from folate one-carbon metabolism—these tumors paradoxically show downregulation of key folate pathway enzymes, including dihydrofolate reductase (*DHFR*) and thymidylate synthase (*TYMS*) (Richard K. Yang et al., 2025[30]). This apparent contradiction may reflect adaptive metabolic reprogramming to balance methylation demands with nucleotide synthesis in a hyperproliferative but immune-reactive microenvironment. For instance, *MTHFR* polymorphisms, such as the C677T variant, which reduces enzyme activity and redirects folate flux toward methylation cycles, may preferentially influence CMS1 tumorigenesis by exacerbating DNA hypermethylation and silencing tumor suppressor genes (e.g., *MLH1*) (Mokarram P et al., 2008[31]).

In contrast, CMS3 tumors, defined by metabolic dysregulation and frequent *KRAS* mutations, exhibit heightened reliance on folate-mediated one-carbon metabolism to sustain rapid proliferation. These tumors upregulate enzymes such as Serine Hydroxymethyltransferase 2 (*SHMT2*) and Methylenetetrahydrofolate Dehydrogenase 2 (*MTHFD2*), which are critical for mitochondrial folate cycling and nucleotide synthesis[32-35]. The CMS3 subtype is uniquely dependent on serine and glycine metabolism, with *SHMT2* catalyzing the transfer of a one-carbon unit from serine to tetrahydrofolate (THF), generating methylene-THF for thymidylate and purine biosynthesis[36]. This metabolic rewiring not only supports DNA replication but also maintains redox balance through glutathione production, a process indirectly linked to folate metabolism via homocysteine remethylation. Notably, *KRAS*-driven CMS3 tumors may exploit folate pathway

activation to bypass therapeutic stress. For example, *KRAS* mutations enhance the transcription of *MTHFD2* through MAPK/ERK signaling, creating a metabolic vulnerability that could be targeted with antifolates in combination with RAS pathway inhibitors (Qinglong M. et al., 2025[37]). However, the efficacy of such strategies may be influenced by *MTHFR* polymorphisms, as reduced MTHFR activity in 677TT carriers could further amplify the dependency on mitochondrial folate enzymes, potentially exacerbating treatment resistance or toxicity[38].

CMS2 tumors, characterized by Wnt/β-catenin-driven chromosomal instability (CIN), exhibit context-dependent folate metabolism. While retaining epithelial features and conventional DNA repair pathways[39], they moderately upregulate cytosolic folate enzymes (MTHFD1/TYMS[40]) to sustain proliferation. Notably, estrogen receptorβ(ERβ) in right-sided CMS2 tumors may regulate folate cycle genes, potentially explaining sex-specific responses to supplementation. Their CIN phenotype—linked to mismatch repair defects—confers selective vulnerability to antifolates: preclinical data show pemetrexed synergizes with PARP inhibitors by exacerbating replication stress through nucleotide pool depletion[41]. These interactions underscore the importance of aligning therapies with CMS2's dual metabolic-genomic dependencies.

The mesenchymal CMS4 subtype, associated with stromal invasion, TGF-β activation, and poor prognosis, presents a unique challenge in the context of folate metabolism. Despite their aggressive phenotype, CMS4 tumors often downregulate key folate transporters such as the reduced folate carrier (*RFC/SLC19A1*) and proton-coupled folate transporter (*PCFT/SLC46A1*), potentially as a mechanism to evade antifolate therapies (Yiting W. et al., 2025[42]). However, this downregulation is counterbalanced by increased expression of mitochondrial folate enzymes, including *MTHFD2* and *ALDH1L2*, which support antioxidant defense and NADPH production in a nutrient-deprived stroma[43]. The interplay between folate metabolism and the TGF-β pathway further complicates this landscape. TGF-β signaling has been shown to upregulate *MTHFD2* via SMAD4, linking stromal activation to mitochondrial one-carbon metabolism[44].

Collectively, folate metabolism in CRC is profoundly shaped by molecular subtypes, each exhibiting distinct dependencies on genetic polymorphisms such as MTHFR C677T. Prospective validation of these subtype-metabolism interactions across diverse populations is imperative to translate mechanistic insights into clinical strategies, including precision dietary modulation and chemoprevention, tailored to the metabolic vulnerabilities of individual tumors.

Table 1 Relationship between molecular subtypes of CRC and regulation of folate metabolism

Molecular Subtype	Characteristics	Folate Metabolism Alterations	Key Mechanisms/Genetic Factors	Therapeutic Implications	Prevention Strategies
CMS1	Microsatellite instability (MSI), hypermutation, immune infiltration, CIMP-high	- Downregulation of DHFR, TYMS - SAM-dependent DNA hypermethylation	- MTHFR C677T polymorphism (677TT genotype increases risk) - Silencing of tumor suppressors (e.g., MLH1)	Balancing methylation demands and nucleotide synthesis; modulating methyl donors	Personalized folate supplementation for 677TT carriers to correct methylation imbalances

CMS3	Metabolic dysregulation, KRAS mutations, serine/glycine dependency	- Upregulation of SHMT2, MTHFD2 - Enhanced mitochondrial folate cycling	- KRAS-driven MTHFD2 activation via MAPK/ERK - Redox balance (glutathione synthesis)	Antifolates targeting mitochondrial enzymes (e.g., MTHFD2) + RAS inhibitors	Targeting metabolic vulnerabilities (e.g., serine restriction, mitochondrial folate enzyme inhibition)
CMS2	Wnt/β-catenin activation, chromosomal instability, epithelial features	- Moderate upregulation of MTHFD1, TYMS - ERβ-mediated folate gene regulation	- Estrogen signaling-folate crosstalk - DNA repair defects (PARP inhibitor sensitivity)	Antifolates (e.g., pemetrexed) + PARP inhibitors	Sex-specific folate supplementation (considering ERβ modulation)
CMS4	Epithelial-mesenchymal transition (EMT), TGF-β activation, stromal fibrosis, poor prognosis	- Downregulation of folate transporters (RFC, PCFT) - Upregulation of mitochondrial enzymes (MTHFD2, ALDH1L2)	- TGF-β/SMAD4-driven MTHFD2 activation - Antioxidant defense (NADPH production)	Dual targeting of TGF-β receptors + mitochondrial folate enzymes	Avoiding excessive folate (may fuel antioxidant pathways); requires clinical validation
Pan-Subtype Features	Molecular heterogeneity shaping folate metabolism	- Genetic polymorphisms interacting with subtypes - Metabolic-genomic-microenvironment crosstalk	- Single-cell metabolomics/spatial transcriptomics revealing niche-specific dynamics	Subtype-specific combination therapies (metabolic targets + pathway inhibitors)	Precision nutrition based on subtype risk (family history, genotype)

IV. Translational Challenges and Strategies in Clinical Implementation

The intricate interplay between folate metabolism, molecular subtypes of CRC, and genetic polymorphisms such as *MTHFR* C677T, as outlined in previous sections, underscores the potential for precision prevention and therapy. However, translating these mechanistic insights into clinical practice faces significant challenges, ranging from biomarker validation to public health policy reform. Addressing these hurdles requires a multidisciplinary approach that integrates advanced diagnostics, tailored interventions, and population-level strategies to optimize outcomes while mitigating unintended consequences.

A critical step in advancing precision prevention lies in the development of robust risk stratification frameworks. Combining *MTHFR* genotyping with biomarkers such as plasma folate and erythrocyte folate levels offers a promising strategy to identify high-risk populations. For instance, individuals harboring the *MTHFR* 677TT genotype exhibit reduced enzyme activity, leading to elevated homocysteine and altered folate distribution—a metabolic profile associated with increased CRC risk, particularly in microsatellite instability-high (MSI-H) and metabolic (CMS3) subtypes[18]. Plasma folate levels, which reflect short-term dietary intake, and erythrocyte folate, a marker of long-term status, provide complementary insights into systemic folate availability. Studies suggest that low erythrocyte folate in *MTHFR* 677TT

carriers correlates with aberrant DNA methylation patterns in CMS1 tumors, while excessive folate in CMS3 subtypes may fuel nucleotide synthesis and tumor proliferation[19]. However, standardization of these biomarkers remains a challenge[45] . Harmonizing these methodologies through international consensus guidelines will be essential to ensure reproducibility across diverse populations.

Equally important is optimizing the timing and composition of folate-based interventions. Emerging evidence suggests that the window of supplementation—administered during the precancerous adenoma phase versus after malignant transformation—profoundly impacts outcomes. Preclinical models demonstrate that folate supplementation in early-stage adenomas restores normal DNA methylation and suppresses Wnt/β-catenin signaling in CMS2-like lesions, potentially delaying progression to carcinoma[46]. Conversely, late-stage supplementation in established CMS3 tumors may inadvertently accelerate growth by supplying one-carbon units for nucleotide synthesis, as observed in *KRAS*-mutant organoids[47]. This duality underscores the need for longitudinal monitoring to guide intervention timing.

At the public health level, the widespread implementation of folate fortification policies—mandated in over 80 countries to prevent neural tube defects—demands re-evaluation in light of potential oncogenic risks. Ecological studies have reported a temporal association between folic acid fortification and increased CRC incidence in North America, particularly in populations with high rates of *MTHFR* 677TT genotypes[48]. Mechanistically, synthetic folic acid, unlike natural folates, bypasses regulatory checkpoints in the folate cycle, potentially leading to UMFA accumulation in CMS4 tumors, which are characterized by impaired folate transporter expression[49]. UMFA has been shown to promote stromal inflammation and TGF-β signaling in preclinical models, exacerbating mesenchymal transition and therapy resistance[50]. To address these concerns, some researchers advocate for genotype-specific fortification policies[24].

Discussion

This review reveals folate metabolism's dual role in CRC, necessitating paradigm reevaluation. MTHFR polymorphisms (C677T/A1298C) exhibit subtype-specific effects: 677TT elevates CMS1 risk via SAM-dependent hypermethylation, while CMS3's mitochondrial folate addiction suggests MTHFR-modulated antifolate responses in KRAS-mutant tumors. Molecular subtyping resolves prior epidemiological contradictions, demonstrating that folate's protective/procarcinogenic duality requires biology-driven thresholds—exemplified by CMS4 tumors accumulating UMFA to fuel stromal remodeling. Gut microbiome interactions (e.g., Fusobacterium-mediated folate depletion) further complicate risk stratification. Current limitations include overreliance on preclinical models and lack of subtype-stratified trials. Priorities include: 1) genotype/subtype-adaptive clinical trials, 2) multi-omics dissection of folate-microenvironment crosstalk, and 3) AI-enhanced precision nutrition frameworks balancing CRC prevention with public health mandates.

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