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One-Carbon Vitamins, Epigenetic/Genetic Integrity and Colon Cancer: Research is Needed to Understand the Effect on Tumorigenic Signaling Pathways

Zhenhua Liu*

Jean Mayer USDA Human Nutrition Research Center on Aging and Friedman School of Nutrition Science and Policy, Tufts University, 711 Washington Street, Boston, MA, 02111, USA

Evidence has established the protective effect of folic acid fortification and periconceptional supplementation on neural tube defects. A large and growing body of compelling evidence from both epidemiological and pre-clinical studies also indicates that adequate folate status, and possibly in combination with other one-carbon nutrients, is associated with a decreased risk of colorectal carcinogenesis [1,2]. Evidence is still accumulating in support of these claims, but some recent studies raise warnings in regard to the safety for use folate as a chemopreventive component. Not to mention some null observations [3,4], it is important to point out that some studies suggest that overly abundant intake of folate may paradoxically promote tumor progression if administered in an inopportune time when an individual already has harbored foci of precancerous or cancerous cells [5-8]. To fully take advantage of this chemopreventive agent and avoid its cancer-promoting effect (though more studies are needed to confirm), research is urgently needed to understand specific molecular mechanisms by which these paradoxical effects are mediated.

A major biochemical function of folate is to serve as an essential conveyor of one-carbon units necessary for biological methylation and nucleotide syntheses via one-carbon metabolism [9]. Three other B-vitamins (B2, B6 and B12) are also involved in this metabolic network and therefore together with folate were called "One-carbon Vitamins" here. Vitamin B12 is a co-factor for methionine synthase. Vitamin B6 is a necessary co-factor for the inter-conversion between different co-enzymatic forms of folate. Vitamin B2 is the precursor of the co-factor for methylenetetrahydrofolate reductase [10]. This one-carbon metabolism network is the universal source for most, if not all, biological reactions which request methyl group. On one hand, the methyl donor for DNA methylation is derived from *S*-adenosyl methionine, which in turn obtains the moiety from 5-methyltetrahydrofolate via methionine as the intermediate. Not surprisingly, inadequate delivery of one-carbon vitamins has been reported to produce low levels of methylation in various experimental settings [10,11]. On the other hand, folate, in the form of 5,10-methylenetetrahydrofolate, is required for the synthesis of thymidine from uracil. Therefore, limited availability of folate promotes the misincorporation of uracil into DNA, as has been reproducibly shown in cell culture, animal models, and humans [12-14].

It is well established that folate, in conjunction with other one-carbon nutrients, lies at the intersection of metabolic pathways that are involved in DNA methylation and nucleotide synthesis, but it falls short to explain various and even paradoxical functions of folate observed in epidemiological and pre-clinical studies. To properly explore the benefits of this agent, research on specific cellular signaling pathways is needed to elucidate the dual cancer-protective and potential deleterious effect shown in recent publications [5,15]. Most colorectal carcinomas arise from adenomas, which, via multiple steps, gradually progress into carcinomas. Different cellular signaling pathways drive the progression along the gradually progressing cascade [16,17]. Clear understanding the effect of one-carbon vitamins on those pathways which controls the progression at different stages should significantly contribute the development of intelligent dietary strategies for the prevention of colorectal cancer, but surprisingly, research in this setting is just at the early stage with limited experimental support.

Aberrant *Wnt*-signaling is an early event in 90% of human colorectal cancers and is thought to play an important role in the development of colorectal cancer, particularly at the initiation stage [18]. In 2000, our lab reported that *Apc* expression is impaired by a severe degree of folate depletion in the rat colon [19]. In 2007, I demonstrated that mild depletion of folate, when present in conjunction with the mild depletion of other B-vitamins (B2, B6 and B12), alters several components of *Wnt* pathway in the colonic mucosa in a pro-transformational manner [10]. More recently, using a *Wnt*-reporter mouse model and a colorectal cancer mouse model, I further demonstrated that the combined one-carbon vitamin depletion (folate in conjunction with B2, B6 and B12 depletion) does increase the *Wnt*-signaling accompanying with elevated tumor incidence [20]. Moreover, other investigators have also observed significant changes in the expression of several modulators of the *Wnt* pathway, such as *WISP1*, *WNT5A*and *DKK-1*, as a result of folate depletion [21]. Indirect evidence that folate depletion alters the *Wnt* pathway can also be drawn from studies of the rodent model of folate-sensitive neural tube birth defects, the crooked tail mouse, the genetic basis of which is a mutation in *Lrp6*, a *Wnt* co-receptor [22]. These findings of ours and others collectively indicate dietary one-carbon vitamins modulate colorectal tumorigenesis, at least in part, via alterations in the *Wnt* pathway, providing evidence in support of the preventive effect of folate at the early stage, but further studies are requested to understand how one-carbon vitamins mediate this critical pathway, and therefore diet-based or pharmaceutical strategies targeting this pathway can be developed.

The most acceptable explanation of cancer-promoting effect once the proneoplastic lesion is developed lies on folate as a cofactor in nucleotide synthesis. Hyper-proliferation is a feature of most dysplastic neoplasms, which request abundant availability of vitamins to meet the rapidly dividing cells for DNA synthesis [23]. Evidence of this effect has been reported in both animal models [8,24-26] as well as clinical trials [5,15,27]. However, it is entirely unknown how one-carbon vitamins accelerates the progression of colorectal cancer via altering those signaling pathways in a manner which facilitates the progression in later stages, such as *KRAS*, *P53*, and *SMAD* pathways. In contrast, the integrity (including region-specific methylation and strand breaks)

***Corresponding author:** Zhenhua Liu, Ph.D, Jean Mayer USDA Human Nutrition Research Center on Aging and Friedman School of Nutrition Science and Policy, Tufts University, 711 Washington Street, Boston, MA, 02111, USA, Tel: 617-556-3234; Fax: 617-556-3234; E-mail: zhenhua.liu@tufts.edu

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of *P53*, which inhibits the transformation from large adenoma to cancer at the later stage, is better protected with folate sufficiency in both cell culture [28,29] and animal models [30-32] when compared to folate deficiency. These observations show that a protective effect of folate, rather than a deleterious effect, at the later stage during the progression from large adenomas to carcinomas. These observations at the molecular level are inconsistent with the cancer-promoting effect suggested in the recent clinical trials [5,15,27].

In addition to the abovementioned mechanisms, several other cellular pathways have been investigated, though in a lesser degree comparing the *Wnt* pathway, as the means by which folate inadequacy mediates carcinogenesis. For incidence, age-related enhancement of p16 promoter methylation occurred in a manner dependent dietary folate status, but surprisingly, this promoter hypermethylation in old animals with folate repletion and supplementation is associated with an increase of p16 expression [33], which is opposite to a general understanding that promoter hypermethylation is associated with a suppression of gene expression. Genome-wide expression microarrays have been utilized to screen for cellular pathways by which folate mediates colorectal carcinogenesis [34,35]. The expression of a quite amount of genes, falling into several functional categories, was substantially and significantly altered by folate depletion. Although a discussion of all of them is beyond the scope of this article, it is of interest that the transcriptional alteration of *apc* and *β-catenin*, the critical elements in *Wnt* pathway, as well as *p53* gene were observed under the status of folate depletion, in concordance with the observations in our animal studies described above.

In summary, it is clear that the relationship between folate and the development of colorectal cancer is complex, and the underlying mechanism(s) is particularly insufficient for understanding the paradoxical effects and for developing dietary strategies. Epidemiologic studies should expand their investigations toward molecular level. Of more urgency are mechanistic studies, which were surprisingly much lesser than observational studies, to understand the biological relevance.

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