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Mini-review

How folate metabolism affects colorectal cancer development and treatment; a story of heterogeneity and pleiotropy

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ABSTRACT

Folate was identified as an essential micronutrient early in the twentieth century and anti-folate chemotherapy such as 5-fluorouracil (5-FU) has been central to the medical management of solid tumours including colorectal cancer for more than five decades.

In the intervening years, evidence has been gathered which shows that folate deficiency leads to many human diseases throughout the life-course. However, we still do not know all of the mechanisms behind functional folate deficiency, or indeed its rescue through supplementation with natural and particularly synthetic folates. There is growing evidence that one adverse effect of folic acid fortification programmes is an increased risk of colorectal cancer within populations.

The complexity of folate-dependent, one-carbon metabolism and the heterogeneity that exists between individuals with respect to the enzymes involved in the anabolic pathways, and the catabolism of 5-FU, are explored in this review. The enzyme products of some genes such as *MTHFR* exert multiple and perhaps unrelated effects on many phenotypes, including cancer development. We describe this pleiotropy and the common genetic variants that affect folate metabolism; and discuss some of the studies that have investigated their potential as predictive biomarkers.

1. Introduction

Folates are a group of B vitamins that are particularly abundant in green leafy vegetables, yeast, legumes, fruit and animal protein. We are unable to produce this micronutrient *de novo* and the body's reserves are short-lived but if dietary sources of folate are inadequate deficiency can be circumvented by supplementation with synthetic forms of folate, such as folic acid. The successful use of folic acid supplementation through inexpensive public health initiatives to prevent neural tube defects illustrates that there is functional folate deficiency within populations that can be corrected [12,19,34].

Folate-dependent, one-carbon metabolism is essential for DNA biosynthesis, DNA repair and DNA methylation. The maintenance of stability within the genome and epigenome depends on both adequate bioavailability of folate and complex protein networks. The biosynthesis of purines, thymidylate and methylation-intermediates occur in three linked anabolic pathways that involve multi-enzyme complexes and the circulation of folates [55]. The cyclic metabolism of folate depends on another micronutrient, vitamin B12, because of an intrinsic metabolic bottleneck, referred to as the *folate trap* [26,51]. The reaction catalysed at a key branch point by methylene tetrahydrofolate (MTHFR) converts 5,10-methylenetetrahydrofolate (5′,10′methyleneTHF) to 5-methyltetrahydrofolate (5-methylTHF) and is irreversible. To maintain the circulation of folate, THF is produced in the re-methylation of homocysteine to methionine. This reaction is mediated by methionine synthase (encoded by the *MTR* gene) and the essential co-factor vitamin-B12, the deficiency of this micronutrient leads to decreased methionine synthase activity, the accumulation of 5-methylTHF, and therefore a metabolic block (see Fig. 1).

Altered folate metabolism, through folate and/or vitamin-B12 deficiency, rare in-born errors of metabolism, and even common enzyme polymorphisms, may lead to inadequate purine and pyrimidine synthesis and changes in methylation, with a concomitant impact on DNA replication and cell division. Cancer is a disease characterised by the accumulation of somatic mutations; by aberrant gene expression; and the result is uncontrolled cell growth. There is also evidence that DNA hypomethylation may drive tissue-specific mutagenesis in cancer [14]. It is therefore unsurprising that perturbed folate metabolism is associated with

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aspects of the malignant phenotype (see Fig. 2) or that this critical pathway should be a target for cancer therapies.

Colorectal cancer (CRC) is a significant worldwide cause of morbidity and mortality and comprises a heterogeneous collection of diseases. The traditional histological classification of CRC has been extended by molecular methods and the first sub-groups to be identified were based on chromosome instability (CSI), microsatellite instability (MSI) and CPG island methylator phenotype (CIMP). Recent studies using gene expression signatures have identified between 3 and 6 subgroups, and in one study these subgroups were correlated with crypt cell phenotype ranging from stem cell-like to enterocyte-like [49]. Furthermore, individual phenotypes coupled with particular somatic mutations have been reported to have prognostic potential [16,41].

In this review we consider how variations in folate metabolism, through folate deficiency or genetic heterogeneity, could influence the development or medical management of CRC.

2. The influence of folate metabolism on colorectal cancer development

In the 1930s, Lucy Wills described an essential dietary factor in yeast and liver extracts that proved to be folate. The nutrient was identified as a treatment for macrocytic anaemia of pregnancy [65], and this finding soon led to the synthesis of folic acid [4]. Since then, natural and synthetic forms of folate have been described as nutritional cure-alls throughout the life-course, from the earliest stages of development *in utero* to a healthy old age [7,12,63], and an inverse relationship has been reported for folate intake or status and the risk of CRC [50].

However, recent data presents a paradox because whilst there is good evidence from epidemiological and nutritional studies that adequate dietary folate is protective against CRC; high doses of synthetic folate have been associated with an increase in the growth of pre-cancerous colorectal neoplasms and cancer

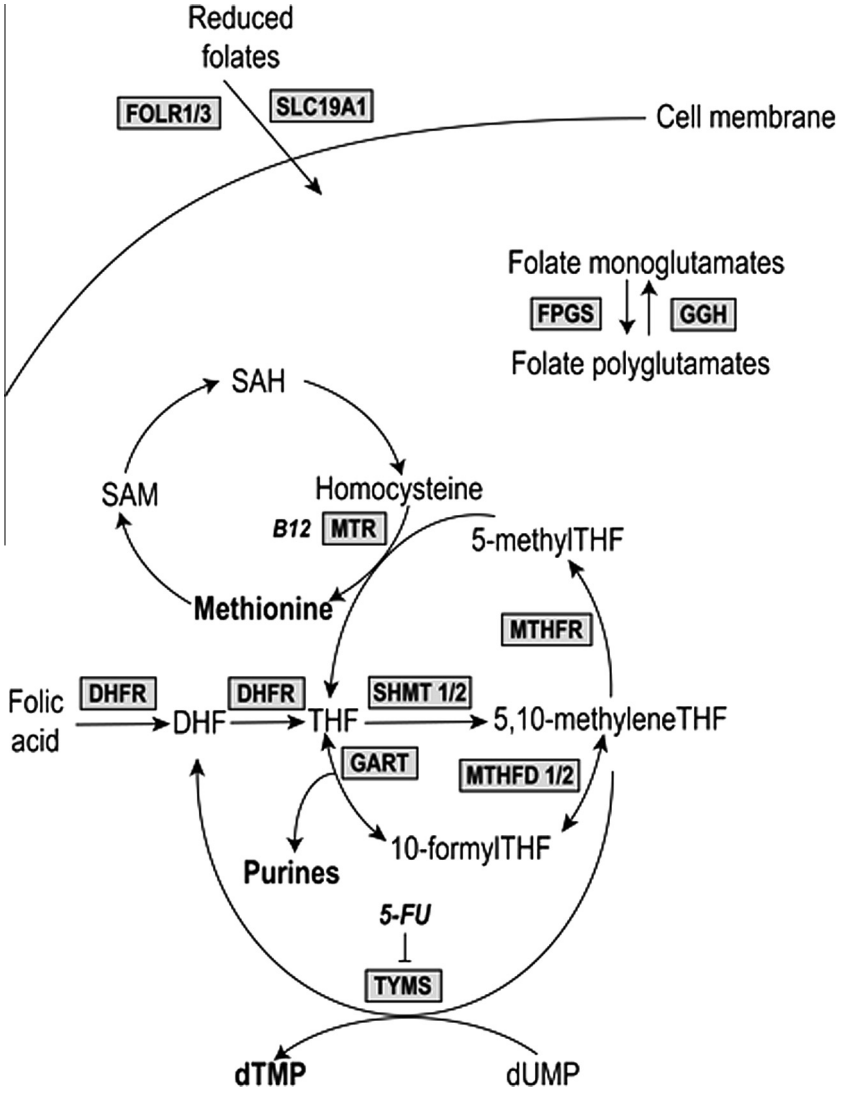

Fig. 1. A snapshot of folate uptake and intracellular metabolism. The enzymes of folate metabolism are described using protein/gene symbols in the grey shaded boxes. DHFR; dihydrofolate reductase. FPGS; folylpolyglutamate synthetase. The *GART* gene encodes a multifunctional enzyme that has the following activities phosphoribosylglycinamide formyltransferase; phosphoribosylglycinamide synthetase; and phosphoribosylaminoimidazole synthetase. GGH; gamma-glutamyl hydrolase. MTHFD1/2; methylenetetrahydrofolate dehydrogenase (NADP + dependent) 1 and 2. MTHFR; 5,10-methylenetetrahydrofolate reductase. TYMS; thymidylate synthetase which is shown as the target for the fluoropyrimidine 5-FU. MTR; 5-methyltetrahydrofolate–homocysteine methyltransferase. SLC19A1; solute carrier family 19 (folate transporter), member 1. SHMT1/2; serine hydroxymethyltransferase 1 and 2. dTMP; deoxythymidine monophosphate. dUMP; deoxyuridine monophosphate. SAH; S-adenosylhomocysteine. SAM; S-adenosylmethionine. THF; tetrahydrofolate.

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