ELSEVIER

Journal of Nutritional Biochemistry 14 (2003) 568–575

THE JOURNAL OF Nutritional Biochemistry

Folate deficiency, mismatch repair-dependent apoptosis, and human disease

Guo-Min Li*, Steven R. Presnell, Liya Gu

Department of Pathology and Laboratory Medicine, Markey Cancer Center, University of Kentucky Medical Center, Lexington, KY, 40536

Received 10 November 2002; received in revised form 30 March 2003; accepted 4 June 2003

Abstract

The vitamin that is most commonly deficient in the American diet is folate. Severe folate deficiency in humans is known to cause megaloblastic anemia and developmental defects, and is associated with an increased incidence of several forms of human cancer. Although the exact mechanisms by which this vitamin deficiency may cause these diseases are not known at the present time, recent work has shown that folate deficiency also causes genomic instability and programmed cell death (or apoptosis). Additionally, it is known that the DNA mismatch repair pathway mediates folate deficiency-induced apoptosis. This review will first describe work suggesting that folate deficiency causes genomic instability and apoptosis, then discuss possible mechanisms by which the mismatch repair pathway could trigger folate deficiency-induced apoptosis, which has either protective or destructive effects on tissue.

Keywords: Folate deficiency; Mismatch repair; Apoptosis; Genomic instability; Megaloblastic anemia; Cancer

1. Introduction

Folate, one of the water-soluble B vitamins, is an essential carrier of methyl groups within the cell. In this role, folate acts as either a donor or receiver of one-carbon moieties in numerous enzyme-catalyzed reactions [1]. Highlighting the importance of folate in nutrition is the observation that a deficiency of this vitamin in the diet is associated with an increased risk of certain types of cancer and many other diseases, including megaloblastic anemia, neural tube defects, and heart disease [2,3]. Although the mechanisms by which folate deficiency may cause these diseases remains to be elucidated, it has been demonstrated that folate deficiency is associated with genomic instability, defective DNA repair, and programmed cell death (also called apoptosis) (for reviews see [3–7]). Very recently, a functional DNA mismatch repair (MMR) pathway has been shown to be required for folate deficiency induced apoptosis [8]. As one of the primary maintenance systems for the genome, MMR guards genomic stability by correcting biosynthetic errors and by promoting DNA damage-induced apoptosis [9,10]. This review will discuss the molecular link between folate deficiency and MMR-dependent apoptosis and its implications to human diseases.

2. Folate deficiency causes genomic instability

Folic acid has an important role in DNA metabolism. As depicted in Fig. 1, folate metabolites are cofactors essential for the biosynthesis of nucleotides and for DNA modification. Dietary folate is metabolized into several reduced and methylated forms in the cell, and one of these derivatives, 5,10-methylenetetrahydrofolate (5,10-MTHF), is a required precursor for the *de novo* biosynthesis of thymidylate. In a reaction catalyzed by thymidylate synthase, 5,10-MTHF donates a methyl group to the uracil moiety of deoxyuridine monophosphate (dUMP) to form deoxythymine monophosphate (dTMP). A deficiency of folate decreases the production of dTMP, resulting in an increased cellular dUMP/dTMP ratio [11]. Because DNA polymerases cannot easily discriminate between dTTP and dUTP, an increased ratio of dUMP/dTMP results in an increased amount of DNA polymerase-mediated dUTP incorporation into DNA [12,13].

The presence of uracil in DNA promotes genomic instability in a variety of ways. Uracil, if left uncorrected, can mispair with guanine, producing a point mutation [14,15]. Additionally, there are at least four DNA glycosylases

* Corresponding author. Tel.: +1-859-257-7053; fax: +1-859-323-2094.

E-mail address: gmli@uky.edu (G.-M. Li).

0955-2863/03/$ – see front matter
doi:10.1016/S0955-2863(03)00115-3

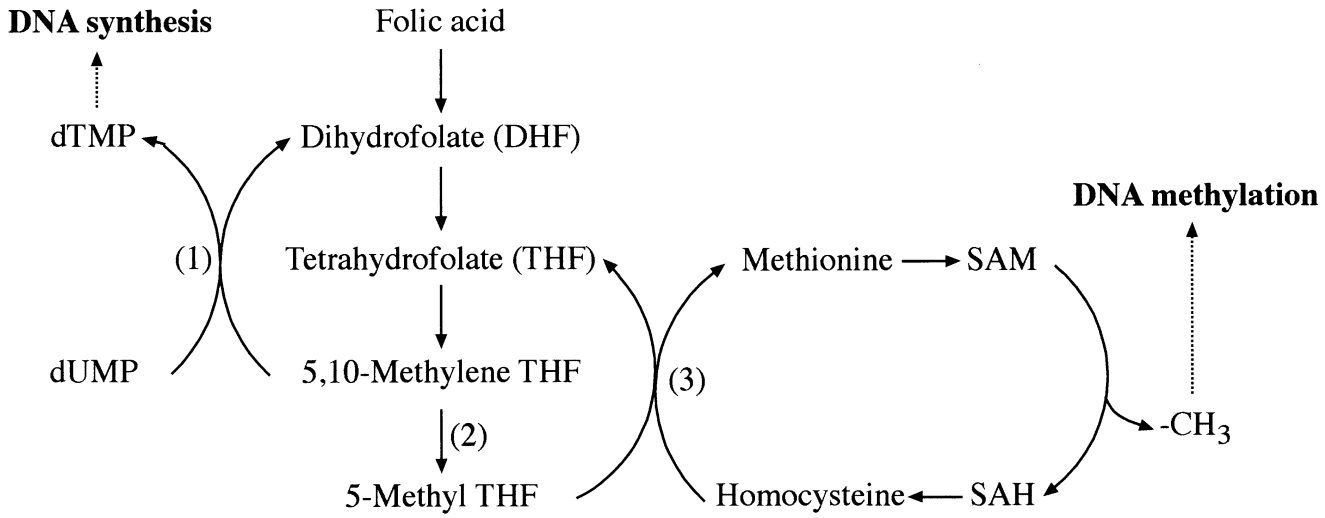

Fig. 1. Role of folate coenzymes in DNA synthesis and modification. Arrows indicate natural biosynthetic pathways. dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; THF, tetrahydrofolate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine. Enzymes: [1], thymidylate synthase; [2], 5,10-methylene-THF reductase; [3], methionine synthase.

(UNG, TDG, hSMUG1, and MBD4) in human cells that can remove uracil from DNA in concert with an apyrimidinic endonuclease [15,16]. This process creates transient single-strand breaks that could lead to double-strand breaks if two uracil residues that are closely located on the opposite strands are excised by this base excision repair mechanism [17]. The double-strand breaks generated by this mechanism have been shown to be associated with gene deletions and could lead to chromosomal breakage [17,18].

In additional to single base modifications and chromosomal breaks, folate deficiency has recently been shown to cause insertion or deletion of small repetitive sequences in the genomic DNA of colon tissue [19]. Changes in the number of simple repetitive sequences is known as microsatellite instability (MSI), and was initially identified in patients with colorectal cancer about ten years ago [20,21]. Cravo et al. [19] have demonstrated that intracellular folate levels influence microsatellite status in the non-neoplastic mucosa of patients with ulcerative colitis, a condition known to be associated with both folate deficiency and an increased risk of colorectal neoplasia. In this study, folate levels and MSI statuses in the colonic mucosa of 26 patients with chronic ulcerative colitis and 10 patients with Crohn's colitis were determined. MSI was found in 3 patients with ulcerative colitis and in none of the patients with Crohn's colitis. Interestingly, all three patients with MSI exhibited lower levels (30–50% lower) of folate concentrations in their serum, whole blood, and colonic mucosa. However, after one of the patients with MSI received folate supplementation for 6 months, three of six microsatellite markers tested became stable, implicating a role of folate in stabilizing microsatellite sequences. Since defects in DNA mismatch repair (MMR) are known to cause MSI [22–24], this study suggests that intracellular folate concentrations may regulate the MMR function [7].

While folate is indispensable for maintaining the integrity of the base sequence of genomic DNA, it is also important for DNA methylation, a modification that controls gene expression. This is because 5,10-MTHF, a precursor for dTTP synthesis (see Fig. 1), is also the primary source of methyl groups for cytosine methylation (through S-adenosylmethionine, or SAM, see Fig. 1). In human cells, DNA methylation usually occurs in cytosines of palindromic CpG sequences, particularly in the promoter region. Whether or not this epigenetic modification can initiate repression of gene expression is still a controversial issue [25,26]. However, the amount of cytosine methylation in the promoter region of a gene is, in general, inversely correlated with its transcription level. The pattern of cytosine methylation in genome is tightly controlled through the cell cycle [27], but the amount of methylation is directly affected by the intracellular folate level. *In vivo* studies have shown that feeding rats a severely methyl deficient diet causes DNA hypomethylation and leads to elevated expression of several oncogenes, including *c-myc*, *c-fos*, and *c*-Ha-*ras* [28]. DNA hypomethylation caused by a diet deficient only in folate has also been shown to be associated with instability and decreased expression of tumor suppressor genes. For example, Kim and co-workers showed that, in rats, a folic acid deficient diet can cause hypomethylation and strand breaks specific to the p53 gene in the absence of genome-wide alterations [29], and a decreased level of its transcription [30]. It is not known for certain whether hypomethylation can promote mutagenesis, but hypomethylated DNA is known to be more susceptible to the action by nucleases compared to methylated DNA [31]. The increased expression of oncogenes and impaired expression of tumor suppressor genes in folate deficient cells suggest at least one way in which folate deficiency may promote tumorigenesis.

3. Folate deficiency induces apoptosis

Although folate deficiency is associated with increased cancer risk, historically the principal marker of folate deficiency has been megaloblastic anemia. This association can be traced back to the 1930's, when a macrocytic anemia in Indian women with pregnancy could be cured by the treatment of a product derived from folate-rich autolyzed yeast [32]. Megaloblastic anemia is a disorder characterized by dramatic changes in the morphology of both developing and mature blood cells. Under conditions of folate deficiency, hematopoietic cells in the marrow, such as proerythroblasts and metamyelocytes (the progenitors of erythrocytes and neutrophils, respectively), appear to have enlarged cytoplasm and disfigured nuclei. This morphology is likely caused by the uncoupling of cell growth from division; hence, the cytoplasm in these cells is relatively "mature" (due to normal RNA and protein synthesis) while the nuclei are "immature" (due to inhibited DNA synthesis). As a result, these cells either die in the marrow, or are arrested (as megaloblastic cells) at the S and G_2 phases of the cell cycle. A large fraction of the mature erythrocytes and neutrophils that are released into the circulation under these conditions also have a distorted morphology (reviewed in [33]). Studies utilizing *in vitro* cell culture suggest that cells other than blood cells have similar morphology when grown under conditions of folate depletion (see below for details), and either survive with serious genomic abnormalities (discussed above), or undergo programmed cell death (or apoptosis).

Koury and colleagues have developed an *in vitro* mouse model of megaloblastic anemia, and have used it to demonstrate that folate deficiency-induced anemia causes erythroblasts to undergo apoptosis [34–36]. A hematopoietic disease with characteristics similar to megaloblastic anemia can be induced in mice by first feeding them a folate-deficient diet and then infecting them with a particular strain of Friend virus that causes erythroblasts to proliferate rapidly. Proerythroblasts isolated from the spleens of the infected mice and cultured in a folate-deficient medium were found to accumulate in the S-phase of the cell cycle and underwent apoptosis, whereas control proerythroblasts grown in normal medium survived and differentiated into reticulocytes over a period of two days [34,36]. These observations suggest that apoptosis of erythroblasts during differentiation leads to decreased erythrocyte production and to anemia. Additionally, the apoptotic cell death of the folate-deficient erythroblasts can be prevented by the addition of folic acid or thymidine to the folate-deficient medium, implicating decreased thymidylate synthesis and/or uracil misincorporation into DNA as the main cause of apoptosis in the folate-deficient erythroblasts [34,35]. Although a slight accumulation of p53 was associated with folate deficiency-induced apoptosis [35], more recent work has shown that folate-induced apoptosis occurs in a p53-independent manner. Proerythroblasts also undergo apoptosis when harvested from folate-deficient, Friend virus-infected p53 knockout mice and cultured in a folate-deficient medium [36].

A methyl deficient diet has also been shown to induce apoptosis in tissues other than blood tissue. James et al. [37] investigated the effect of a methyl deficiency on rat liver and observed increased apoptotic bodies in rat livers after two days on a methyl deficient diet, and that this increase continued over the 9-week feeding period. Additionally, folate deficiency induces apoptosis not only in experimental animals, but also in established cell lines. When a human hepatoma cell line was cultured in folate-deficient media for one week, an accumulation of cells in the S- and G2/M-phases was observed [38]. Like mouse proerythroblast cells, these folate-deficient hepatoma cells underwent nucleosomal DNA fragmentation, a classical characteristic of apoptosis. Folate supplementation in the medium, however, was able to normalize the cell cycle and diminish DNA fragmentation in these cells [38]. Similar observations have also been made in Chinese hamster ovary cells [37,39] and human cytotrophoblastic cells [40] under conditions of folate deficiency. Taken together, these *in vitro* data clearly indicate that folate deficiency induces cell cycle arrest and programmed cell death.

4. Involvement of mismatch repair proteins in folate deficiency-induced apoptosis

It is well established that folate deficiency induces apoptosis, but the molecular mechanism of the process is less understood. Given that the addition of thymidine to cell culture prevents folate deficiency-induced cell death, DNA damage (*i.e.*, uracil misincorporation) seems to play a major role in the initiation of apoptosis caused by folate deficiency. Interestingly, recent work from several groups has suggested that DNA mismatch repair (MMR) proteins not only recognize base-base mismatches, but also recognize many forms of DNA damage and are required to trigger DNA damage-induced apoptosis (reviewed in [9]).

MMR is best known for its role in correcting DNA mispairs formed during DNA replication and recombination (for reviews see [41–44]). In *Escherichia coli*, MMR is carried out by eleven activities including MutS and MutL [45,46]. In eukaryotic cells, an MMR system homologous to the *E. coli* MutS- and MutL-dependent pathway has been characterized. Both the *E. coli* and the eukaryotic pathways involve mismatch recognition (by the proteins MutS and MutL or their homologues), removal of the mismatch by excision (by exonucleases), and DNA resynthesis (by replicative DNA polymerases). While only a single form of MutS or MutL has been identified in *E. coli*, there are multiple forms of MutS and MutL homologs in eukaryotic cells, each of which is a heterodimer. The MutS homolog MSH2 interacts with two other MutS homologs, MSH6 and MSH3, to form MutSα and MutSβ, respectively [47–50].

Like the *E. coli* MutS protein, MutSα and MutSβ are mismatch recognition proteins in eukaryotes. The MutL homolog MLH1 interacts with three other MutL homologs, PMS1, PMS2, and MLH3, to constitute three MutL heterodimers in eukaryotic cells [51–55]. It has been demonstrated that defects in MMR cause genome-wide instability, particularly in microsatellite sequences, and are the genetic basis for certain types of cancer, including hereditary nonpolyposis colon carcinoma (HNPCC) [56–62].

Recently, a novel genome maintenance function of MMR, *i.e.*, mediating DNA damage-induced apoptosis, has been identified. Cells proficient in MMR are much more sensitive to killing than their isogenic MMR-deficient cells by many physical and chemical agents, including *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) [63–65], cisplatin [66,67], ionizing radiation [68], and environmental chemical carcinogens [69]. All these agents are known to directly react with DNA bases to from DNA adducts [14]. Upon treating MMR proficient cells with these DNA damaging agents, cells were found to undergo apoptosis. The requirement for both MutS and MutL homologs in this process was proven by showing that cell lines deficient in either *MSH2* or *MLH1* did not undergo significant amounts of apoptosis in response to treatment with DNA-damaging agents [64,69–71]. Additionally, it has also been documented that MutS and its eukaryotic homologs (*e.g.*, MutSα) specifically recognize many DNA adducts. Among these DNA adducts are O^6-methyl guanine [72–74], cisplatin [72,75], 2-aminofluorene [76], *N*-acetyl-2-aminofluorene [76], benzo[a]pyrene dihydrodiol epoxide [69], cyclobutane pyrimidine dimers [77,78], 8-oxo-guanine [79,80], and exocyclic DNA adducts [81]. The recognition of DNA adducts by MMR proteins has led to a hypothesis that MMR proteins function as a DNA damage sensor to initiate apoptosis [9,82,83].

Based on these previous studies, Gu *et. al.* [8] hypothesized that apoptosis caused by folate deficiency is initiated in a manner similar to that of apoptosis caused by DNA damage agents; *i.e.*, mismatch recognition proteins recognize folate deficiency-induced DNA damage (uracil-containing base pairs), and then signal damaged cells to switch on the apoptotic pathway. In fact, this hypothesis was found to be correct. First, MutSα, the major mismatch recognition protein in human cells, was found to specifically bind DNA containing U:A or U:G pairs [8]. These investigators then compared the cellular responses of MMR-proficient and deficient cells to folate deficiency under conditions of *in vitro* culture. Cells proficient in MMR indeed underwent apoptosis after 4 days in folate-free medium, as evidenced by the observation of DNA fragmentation in these cells. However, the apoptotic response was not detected in cells deficient in either MutLα (a heterodimer of MLH1 and PMS2) or MutSα [8], suggesting that folate deficiency does require a functional MMR system to induce apoptosis.

5. Mechanism and implications of MMR-dependent apoptosis induced by folate deficiency

The involvement of MMR proteins in folate deficiency-induced apoptosis underscores the importance of the MMR system in maintaining genomic stability. However, the molecular events involved in this response have not yet been established. This apoptotic response probably involves a signaling cascade, possibly with the recognition of uracil by MutSα/MutLα as the upstream initiating event and with cell death as the downstream terminal event. Two possible mechanisms for this signaling pathway are depicted in Fig. 2, and are based on models previously proposed on how chemically-induced DNA damage may trigger apoptosis [9,82,83]. One model proposes that massive misincorporation of uracil into newly synthesized DNA causes a futile repair cycle, which triggers apoptosis. As diagrammed in Fig. 2A, uracils are incorporated into DNA in the newly synthesized strand during DNA replication under conditions of folate deficiency. Because uracil-adenine base pairs are recognized by the MMR proteins MutSα and MutLα [8], strand-specific excision to remove uracils from DNA is provoked. However, given an unbalanced nucleotide pool under conditions of folate deficiency, uracils are immediately added back into the DNA during the resynthesis step of the MMR reaction, which will lead to another round of attempted repair by the MMR system. This futile repair cycle may signal cells to switch on apoptotic machinery. A "futile cycle" model has also been suggested to explain the triggering of apoptosis induced by chemical/physical agents [9,82,83]; it is worth mentioning, however, that the locations of DNA damage in these two cases are different. While uracils are expected to be added only to the newly synthesized strand, DNA adducts caused by chemical and physical agents are located in the template DNA strand. Thus, the futile repair cycle is provoked in the later case by an un-removable offending adduct in the template strand (because MMR only occurs in the newly synthesized strand), but in the former case by the reoccurrence of uracil incorporations in the newly synthesized strand. Alternatively, another model (Fig. 2B) hypothesizes that the death signal could come from the binding of MutSα/MutLα to uracil in the replication fork and may be unrelated to the repair process. These protein/uracil complexes may block DNA transactions such as replication, transcription, and repair, and could be recognized as a signal for cell cycle arrest and cell death [9,83].

What is the mechanism by which the MMR system activates the apoptotic pathway? Both p53 and p73 are known to be mediators of programmed cell death in response to DNA damage [84–87], and previous studies have implicated p53 and/or p73 in MMR-mediated apoptosis [9,67,88,89]. Additionally, phosphorylation of these two proteins is associated with MMR-dependent apoptosis [67,88,89]. It has been postulated that the MMR-processing of DNA damage activates certain protein kinases to phos-

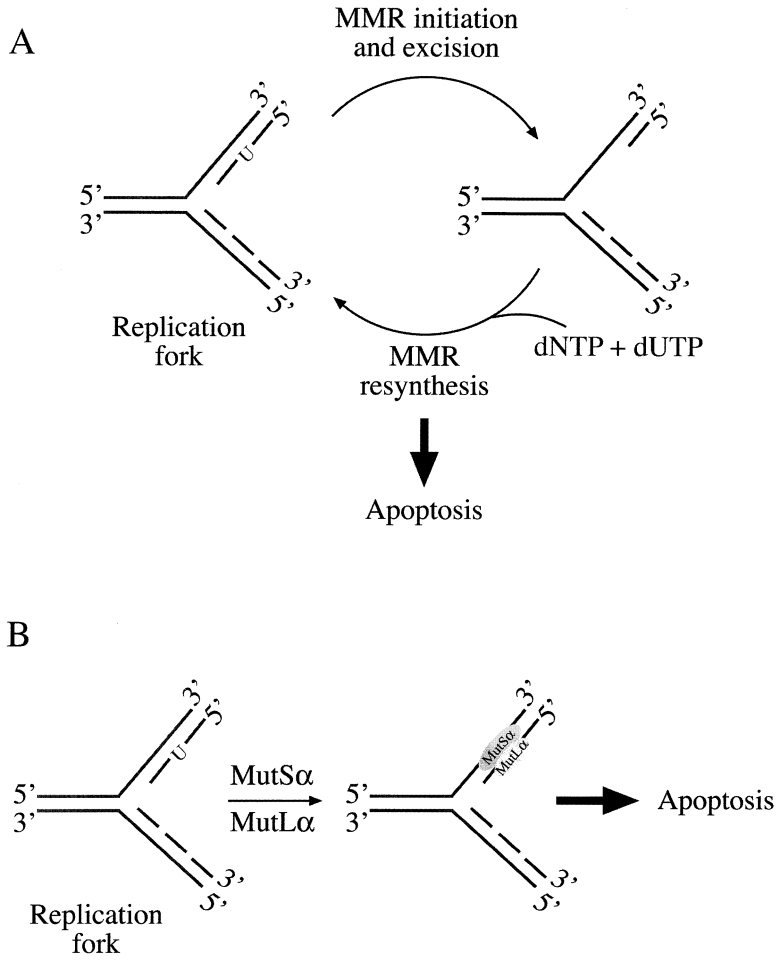

Fig. 2. Possible mechanisms for MMR-mediated apoptosis induced by folate deficiency. A, The futile repair model. Uracil misincorporation into DNA provokes strand-specific MMR excision to remove uracil (*upper arrow*). However, because of a high ratio of dUTP/dTTP under conditions of folate deficiency, uracil is immediately inserted into DNA by polymerases during the MMR resynthesis step (*lower arrow*). This leads to repeated cycles of repair initiation, excision, and resynthesis, triggering apoptosis. B, Alternative model. MMR proteins (*e.g.*, MutSα and MutLα) bind to uracil-containing base pairs at locations in the replication fork where they cannot be displaced. The complexes block other DNA transactions (*e.g.*, replication), which triggers apoptosis. In both models, the MMR processing of uracil may activate protein kinase(s) that phosphorylate an apoptotic mediator (*i.e.*, p73) to execute cell death.

phorylate p53 and/or p73, which in turn activate the apoptotic machinery [9]. Folate deficiency-induced apoptosis, however, seems to be independent of p53 since cells deficient in p53 undergo apoptotic cell death under conditions of folate depletion [36,38]. It remains to be established whether the folate deficiency-induced apoptotic process uses p73 or other proteins to execute apoptosis.

The MMR pathway is well known for its function of promoting genomic stability by correcting base-base mispairs [41–44,57,59–62]. The newly identified apoptotic function of MMR, however, is thought to be as important as its repair function for maintaining genomic stability. Normally, base excision repair and nucleotide excision repair pathways are responsible for repair of DNA damage induced by physical and chemical agents [14,15]. However, when excision repair pathways are not available or there is too much damage to be repaired, genomic DNA is in danger of accumulating a large number of mutations, which are believed to be the cause of cancer. Therefore, eliminating these damaged cells from the body would be beneficial. It is the MMR system that eliminates these pre-tumorigenic cells by promoting apoptosis, and the inability of this system to commit damaged cells for apoptosis is thought to be the molecular basis for HNPCC and other cancers [9,10].

It is generally accepted that MMR-dependent apoptosis is a mutation avoidance system necessary for cancer prevention. However, in the case of folate deficiency, the apoptotic function of MMR appears to have both positive and negative effects on human health. The positive effect of folate deficiency induced, MMR-mediated apoptosis is to prevent cells with severe DNA damage from growing, reducing cancer risk. On the other hand, several folate deficiency-induced diseases including megaloblastic anemia and developmental disorders appear to be caused by MMR-mediated apoptosis (see above). Interestingly, these diseases occur in vigorously growing tissues or organs. For example,

megaloblastic anemia occurs in the proliferating bone marrow and developmental diseases occur during embryonic development. The exact reasons for the disparate effects of apoptosis on different tissues are not clear. It is likely, however, that at least a part of the pathology caused by folate deficiency on rapidly proliferating tissue is due to the large scale destruction of it by apoptosis, so that it can no longer create cells to form developing tissues, or to replace ones lost during the normal processes of cellular maturation. Hence, while folate deficiency induced apoptosis is likely beneficial to slowly proliferating tissue, it is apparently destructive to rapidly proliferating tissue.

Acknowledgments

Studies in authors' laboratories are supported in part by grants from the National Cancer Institute and American Cancer Society.

References

[1] Wagner C. Biochemical role of folate in cellular metabolism. In: In: Bailey LB, editor. Folate in health and disease. New York: Marcel Dekker, 1995. pp. 23–42.
[2] Ames BN. Cancer prevention and diet: help from single nucleotide polymorphisms. Proc Natl Acad Sci USA 1999;96:12216–8.
[3] Green R, Miller JW. Folate deficiency beyond megaloblastic anemia: hyperhomocysteinemia and other manifestations of dysfunctional folate status. Semin Hematol 1999;36:47–64.
[4] Fenech M. The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res 2001;475:57–67.
[5] Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 1999;55:578–92.
[6] Mason JB. Folate, colitis, dysplasia, and cancer. Nutr Rev 1989;47: 314–7.
[7] Choi SW, Mason JB. Folate and colorectal carcinogenesis: is DNA repair the missing link? Am J Gastroenterol 1998;93:2013–6.
[8] Gu L, Wu J, Qiu L, Jennings CD, Li GM. Involvement of DNA mismatch repair in folate deficiency-induced apoptosis small star, filled. J Nutr Biochem 2002;13:355–63.
[9] Li G-M. The role of mismatch repair in DNA damage-induced apoptosis. Oncol Res 1999;11:393–400.
[10] Fishel R. The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. Cancer Res 2001;61:7369–74.
[11] Das KC, Herbert V. In vitro DNA synthesis by megaloblastic bone marrow: effect of folates and cobalamins on thymidine incorporation and de novo thymidylate synthesis. Am J Hematol 1989;31:11–20.
[12] Goulian M, Bleile B, Tseng BY. Methotrexate-induced misincorporation of uracil into DNA. Proc Natl Acad Sci USA 1980;77:1956–60.
[13] Wickramasinghe SN, Fida S. Bone marrow cells from vitamin B12- and folate-deficient patients misincorporate uracil into DNA. Blood 1994;83:1656–61.
[14] Friedberg EC, Walker GC, Siede W. DNA Repair and Mutagenesis. Washington, DC: ASM Press, 1995.
[15] Lindahl T, Wood RD. Quality control by DNA repair. Science 1999; 286:1897–905.
[16] Kavli B, Sundheim O, Akbari M, Otterlei M, Nilsen H, Skorpen F, Aas PA, Hagen L, Krokan HE, Slupphaug G. hUNG2 Is the Major Repair Enzyme for Removal of Uracil from U: A Matches, U:G Mismatches, and U in Single-stranded DNA, with hSMUG1 as a Broad Specificity Backup. J Biol Chem 2002;277:39926–36.
[17] Dianov GL, Timchenko TV, Sinitsina OI, Kuzminov AV, Medvedev OA, Salganik RI. Repair of uracil residues closely spaced on the opposite strands of plasmid DNA results in double-strand break and deletion formation. Mol Gen Genet 1991;225:448–52.
[18] Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 1997;94:3290–5.
[19] Cravo ML, Albuquerque CM, Salazar de Sousa L, Gloria LM, Chaves P, Dias Pereira A, Nobre Leitao C, Quina MG, Costa Mira F. Microsatellite instability in non-neoplastic mucosa of patients with ulcerative colitis: effect of folate supplementation. Am J Gastroenterol 1998;93:2060–4.
[20] Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993;260:816–19.
[21] Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, Lynch HT, Watson P, Tallqvist G, Juhola M, Sistonen P, Hamilton SR, Kinzler KW, Vogelstein B, de la Chapelle A. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994;54:1645–8.
[22] Strand M, Prolla TA, Liskay RM, Petes TD. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 1993;365:274–6.
[23] Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, Jen J, de la Chapelle A, Kinzler KW, Vogelstein B, Modrich P. Hypermutability and mismatch repair deficiency in RER^+ tumor cells. Cell 1993; 75:1227–36.
[24] Umar A, Boyer JC, Thomas DC, Nguyen DC, Risinger JI, Boyd J, Ionov Y, Perucho M, Kunkel TA. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem 1994;269:14367–70.
[25] Jones PA. DNA methylation, and cancer. Oncogene 2002;21:5358–60.
[26] Jubb AM, Bell SM, Quirke P. Methylation and colorectal cancer. J Pathol 2001;195:111–34.
[27] Zingg JM, Jones PA. Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. Carcinogenesis 1997;18:869–82.
[28] Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer Res 1992;52:2071s–77s.
[29] Kim YI, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, Mason JB. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Am J Clin Nutr 1997;65:46–52.
[30] Kim YI, Shirwadkar S, Choi SW, Puchyr M, Wang Y, Mason JB. Effects of dietary folate on DNA strand breaks within mutation-prone exons of the p53 gene in rat colon. Gastroenterology 2000;119:151–61.
[31] Hansen RS, Ellis NA, Gartler SM. Demethylation of specific sites in the 5′ region of the inactive X-linked human phosphoglycerate kinase gene correlates with the appearance of nuclease sensitivity and gene expression. Mol Cell Biol 1988;8:4692–9.
[32] Wills L, Clutterbuch P, Evans BDF. A new factor in the production and cure of macrocytic anaemias and its retion to other haemopoietic principles curative in pernicious anaemia. Biochem J 1937;31:2136–47.
[33] Antony AC. Megaloblastic anemias. In: In: Hoffman R, editor. Hematology: basic basic principles and practice. New York: Churchill Livingstone, 2000. pp. 446–85.
[34] Koury MJ, Horne DW. Apoptosis mediates and thymidine prevents erythroblast destruction in folate deficiency anemia. Proc Natl Acad Sci USA 1994;91:4067–71.

[35] Koury MJ, Horne DW, Brown ZA, Pietenpol JA, Blount BC, Ames BN, Hard R, Koury ST. Apoptosis of late-stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production. Blood 1997;89:4617–23.
[36] Koury MJ, Price JO, Hicks GG. Apoptosis in megaloblastic anemia occurs during DNA synthesis by a p53-independent, nucleoside-reversible mechanism. Blood 2000;96:3249–55.
[37] James SJ, Miller BJ, Basnakian AG, Pogribny IP, Pogribna M, Muskhelishvili L. Apoptosis and proliferation under conditions of deoxynucleotide pool imbalance in liver of folate/methyl deficient rats. Carcinogenesis 1997;18:287–93.
[38] Huang RF, Ho YH, Lin HL, Wei JS, Liu TZ. Folate deficiency induces a cell cycle-specific apoptosis in HepG2 cells. J Nutr 1999; 129:25–31.
[39] James SJ, Basnakian AG, Miller BJ. In vitro folate deficiency induces deoxynucleotide pool imbalance, apoptosis, and mutagenesis in Chinese hamster ovary cells. Cancer Res 1994;54:5075–80.
[40] Steegers-Theunissen RP, Smith SC, Steegers EA, Guilbert LJ, Baker PN. Folate affects apoptosis in human trophoblastic cells. Bjog 2000; 107:1513–5.
[41] Modrich P. Mechanisms and biological effects of mismatch repair. Ann Rev Genet 1991;25:229–53.
[42] Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. Curr Opin Genet Dev 1999;9:89–96.
[43] Jiricny J. Replication errors: challenging the genome. Embo J 1998; 17:6427–36.
[44] Hsieh P. Molecular mechanisms of DNA mismatch repair. Mutat Res 2001;486:71–87.
[45] Burdett V, Baitinger C, Viswanathan M, Lovett ST, Modrich P. In vivo requirement for RecJ, ExoVII, ExoI, and ExoX in methyl-directed mismatch repair. Proc Natl Acad Sci USA 2001;98:6765–70.
[46] Lahue RS, Au KG, Modrich P. DNA mismatch correction in a defined system. Science 1989;245:160–64.
[47] Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 1995;268:1909–12.
[48] Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, D'Arrigo A, Truong O, Hsuan JJ, Jiricny J. GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. Science 1995; 268:1912–4.
[49] Palombo F, Iaccarino I, Nakajima E, Ikejima M, Shimada T, Jiricny J. hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA. Curr Biol 1996;6:1181–4.
[50] Genschel J, Littman SJ, Drummond JT, Modrich P. Isolation of MutSá from human cells and comparison of the mismatch repair specificities of MutSá and MutSα. J Biol Chem 1998;273:19895–901.
[51] Li GM, Modrich P. Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs. Proc Natl Acad Sci USA 1995;92:1950–4.
[52] Flores-Rozas H, Kolodner RD. The Saccharomyces cerevisiae MLH3 gene functions in MSH3-dependent suppression of frameshift mutations. Proc Natl Acad Sci USA 1998;95:12404–9.
[53] Raschle M, Marra G, Nystrom-Lahti M, Schar P, Jiricny J. Identification of hMutLbeta, a Heterodimer of hMLH1 and hPMS1. J Biol Chem 1999;274:32368–75.
[54] Leung WK, Kim JJ, Wu L, Sepulveda JL, Sepulveda AR. Identification of a second MutL DNA mismatch repair complex (hPMS1 and hMLH1) in human epithelial cells. J Biol Chem 2000;275:15728–32.
[55] Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, Collins FS. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 2000;24:27–35.
[56] Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–70.
[57] Fishel R, Kolodner RD. Identification of mismatch repair genes and their role in the development of cancer. Curr Opin Genet Dev 1995; 5:382–95.
[58] Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. J Med Genet 1999;36:801–18.
[59] Modrich P, Lahue R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu Rev Biochem 1996;65: 101–33.
[60] Kolodner RD. Mismatch repair: mechanisms and relationship to cancer susceptibility. Trends Biochem Sci 1995;20:397–401.
[61] Umar A, Kunkel TA. DNA-replication fidelity, mismatch repair and genome instability in cancer cells. Eur J Biochem 1996;238:297–307.
[62] Buermeyer AB, Deschenes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. Annu Rev Genet 1999;33:533–64.
[63] Kat A, Thilly WG, Fang WH, Longley MJ, Li GM, Modrich P. An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. Proc Natl Acad Sci USA 1993;90:6424–8.
[64] D'Atri S, Tentori L, Lacal PM, Graziani G, Pagani E, Benincasa E, Zambruno G, Bonmassar E, Jiricny J. Involvement of the mismatch repair system in temozolomide-induced apoptosis. Mol Pharmacol 1998;54:334–41.
[65] Hickman MJ, Samson LD. Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents. Proc Natl Acad Sci USA 1999;96:10764–9.
[66] Lin X, Kim HK, Howell SB. The role of DNA mismatch repair in cisplatin mutagenicity. J Inorg Biochem 1999;77:89–93.
[67] Gong JG, Costanzo A, Yang HQ, Melino G, Kaelin WG Jr., Levrero M, Wang JY. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature 1999;399:806–9.
[68] DeWeese TL, Shipman JM, Larrier NA, Buckley NM, Kidd LR, Groopman JD, Cutler RG, te Riele H, Nelson WG. Mouse embryonic stem cells carrying one or two defective Msh2 alleles respond abnormally to oxidative stress inflicted by low-level radiation. Proc Natl Acad Sci USA 1998;95:11915–20.
[69] Wu J, Gu L, Wang H, Geacintov NE, Li G-M. Mismatch repair processing of carcinogen-DNA adducts triggers apoptosis. Mol Cell Biol 1999;19:8292–301.
[70] Toft NJ, Winton DJ, Kelly J, Howard LA, Dekker M, te Riele H, Arends MJ, Wyllie AH, Margison GP, Clarke AR. Msh2 status modulates both apoptosis and mutation frequency in the murine small intestine. Proc Natl Acad Sci USA 1999;96:3911–5.
[71] Degtyareva NP, Greenwell P, Hofmann ER, Hengartner MO, Zhang L, Culotti JG, Petes TD. Caenorhabditis elegans DNA mismatch repair gene msh-2 is required for microsatellite stability and maintenance of genome integrity. Proc Natl Acad Sci USA 2002;99:2158–63.
[72] Duckett DR, Drummond JT, Murchie AIH, Reardon JT, Sancar A, Lilley DM, Modrich P. Human MutSα recognizes damaged DNA base pairs containing O^6-methylguanine, O^4-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci USA 1996;93:6443–47.
[73] Rasmussen LJ, Samson L. The Escherichia coli MutS DNA mismatch binding protein specifically binds O(6)-methylguanine DNA lesions. Carcinogenesis 1996;17:2085–8.
[74] Berardini M, Mazurek A, Fishel R. The effect of O6-methylguanine DNA adducts on the adenosine nucleotide switch functions of hMSH2-hMSH6 and hMSH2-hMSH3. J Biol Chem 2000;275:27851–7.
[75] Mello JA, Acharya S, Fishel R, Essigmann JM. The mismatch-repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. Chemistry & Biology 1996;3:579–89.
[76] Li G-M, Wang H, Romano LJ. Human MutSα specifically binds to DNA containing aminofluorene and acetylaminofluorene adducts. J Biol Chem 1996;271:24084–88.
[77] Wang H, Lawrence CW, Li GM, Hays JB. Specific binding of human MSH2. MSH6 mismatch-repair protein heterodimers to DNA incorporating thymine- or uracil-containing UV light photoproducts opposite mismatched bases. J Biol Chem 1999;274:16894–900.
[78] Mu D, Tursun M, Duckett DR, Drummond JT, Modrich P, Sancar A. Recognition and repair of compound DNA lesions (base damage and mismatch) by human mismatch repair and excision repair systems. Mol Cell Biol 1997;17:760–9.

[79] Mazurek A, Berardini M, Fishel R. Activation of human MutS homologs by 8-oxo-guanine DNA damage. J Biol Chem 2002;277: 8260–6.
[80] Ni TT, Marsischky GT, Kolodner RD. MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in S. cerevisiae. Mol Cell 1999;4:439–44.
[81] Johnson KA, Mierzwa ML, Fink SP, Marnett LJ. MutS recognition of exocyclic DNA adducts that are endogenous products of lipid oxidation. J Biol Chem 1999;274:27112–18.
[82] Karran P, Bignami M. DNA damage tolerance, mismatch repair and genome instability. Bioessays 1994;16:833–9.
[83] Modrich P. Strand-specific mismatch repair in mammalian cells. J Biol Chem 1997;272:24727–30.
[84] Levine AJ. p53, the cellular gatekeeper for growth, and division. Cell 1997;88:323–31.
[85] Leonard CJ, Canman CE, Kastan MB. The role of p53 in cell-cycle control and apoptosis: implications for cancer. Philadelphia, PA: J.B. Lippincott, 1995.
[86] Wang JY, Ki SW. Choosing between growth arrest and apoptosis through the retinoblastoma tumour suppressor protein, Abl and p73. Biochem Soc Trans 2001;29:666–73.
[87] Jost CA, Marin MC, Kaelin WG Jr. p73 is a human p53-related protein that can induce apoptosis. Nature 1997;389:191–4.
[88] Duckett DR, Bronstein SM, Taya Y, Modrich P. hMutSα- and hMutLα-dependent phosphorylation of p53 in response to DNA methylator damage. Proc Natl Acad Sci USA 1999;96:12384–88.
[89] Shimodaira H, Yoshioka-Yamashita A, Kolodner RD, Wang JY. Interaction of mismatch repair protein PMS2 and the p53-related transcription factor p73 in apoptosis response to cisplatin. Proc Natl Acad Sci USA 2003;100:2420–5.