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Accumulation of mitochondrial DNA deletions is age, tissue and folate-dependent in rats

Jimmy W. Crott[a,*], Sang-Woon Choi[a], Richard F. Branda[b], Joel B. Mason[a]

[a] *Vitamins and Carcinogenesis Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111-3117, USA*
[b] *Genetics Laboratory, University of Vermont, Burlington, VT, USA*

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Abstract

Folate is essential for the synthesis, repair and methylation of DNA. Folate depletion causes nuclear genetic and epigenetic aberrations in cell culture, rodents and humans. We hypothesized that folate depletion may also damage mitochondrial (Mt) DNA and induce large-scale deletions due to DNA breakage. MtDNA deletions and mutations accumulate during aging and tumorogenesis and may play causative roles in these processes. Weanling and adult (12 months) Sprague Dawley rats consumed folate deplete, replete and supplemented diets (0, 2 and 8 mg/kg folate, respectively) for 20 weeks. The presence of random and common (4.8 kb) MtDNA deletions was measured in colonic mucosa and liver. Six Mt genomes (<16 kb) harboring random deletions were detected in the liver (3.5–7.0 kb) and three in the colon (3.8–8 kb). Older rats had significantly more random hepatic MtDNA deletions than young rats (64 and 3.2% of samples, respectively, $P < 0.0001$), while age had no effect on these deletions in the colon (3.1 and 7.7% in young and old, respectively). Folate intake had no effect on the frequency of random deletions in either tissue. There was no discrete effect of aging on the common 4.8 kb deletion in the liver or colon. However, in the liver of old rats, increasing amounts of dietary folate reduced the deletion frequency, with replete and supplemented rats having 2.2- and 3.2-fold less deletions than the depleted rats. Our results confirm that random MtDNA deletions accumulate with age in a tissue-specific fashion. Furthermore, in contrast to previous work, we report that the common 4.8 kb deletion was not modulated by age, but is reduced by folate supplementation in the liver of rats.

Keywords: Folate/folic acid; Aging; Mitochondrial DNA deletion; Liver; Colon

1. Introduction

Folate is one of the most strongly implicated dietary components that provide protection against

* Corresponding author. Tel.: +1 617 556 3117; fax: +1 617 556 3309.
E-mail address: jimmy.crott@tufts.edu (J.W. Crott).

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colorectal cancer. It is estimated that people with the highest folate intake have a 30–40% lower risk of developing colorectal cancer than those with the lowest [1]. The link between folate and cancer appears to be mediated, at least in part, by the role of folate in the synthesis, repair and methylation of DNA. Because folate is required for the synthesis of thymidine from uracil, folate depletion causes an increase in the intracellular dUMP:dTMP ratio and promotes the misincorporation of uracil into DNA [2,3]. This situation promotes double-stranded DNA breakage (DSB) [4] due to uracil excision repair by uracil DNA glycosylase [5], an enzyme targeted to the nucleus and mitochondria [6]. Folate is also required for *S*-adenosyl methionine (SAM) production; the methyl group donor for DNA methylation. Thus, under certain conditions, folate depletion reduces the availability of SAM for methylation reactions [7] and causes a genomic hypomethylation [8] and gene-specific DNA hypo-methylation [9] and hypermethylation [10].

Human MtDNA is a double-stranded, circular 16.5 kb molecule encoding 37 genes, including 13 polypeptides of the mitochondrial electron transport chain (ETC). Due to the absence of histones and proximity to the ETC, MtDNA is vulnerable to free radical attack and has a high mutation rate relative to nuclear DNA [11]. MtDNA accumulates an array of large-scale deletions in the brain [12], skeletal muscle [13,14] and cardiomyocytes [15,16] of humans during aging. Furthermore, a 'common' 4977 bp Mt deletion accumulates in an age-dependent fashion in human skeletal muscle, liver and testes [17,18]. It is also reported that large-scale MtDNA deletions in the brain, heart and liver are more prevalent in old than young rats [19].

There is also evidence that the frequency of MtDNA deletions is elevated in cancer cells of the breast [20], colon [21], stomach [22], kidney [23], salivary gland [24], thyroid [25] and skin [26]. Unequivocal evidence for an etiological role of MtDNA deletions in carcinogenesis is lacking, however, it has been shown that the inhibition of mitochondrial uracil base excision repair causes a mutator phenotype in yeast [27]. In addition, the evidence for a causative role for mitochondrial DNA instability and base mutations in cancer is compelling and has been extensively reviewed [28–31].

It is becoming evident that Mt genome integrity is also related to the availability of certain nutrients. For example, rats given supra physiological folate intakes (2 + 50 mg/kg folic acid i.p./day) have significantly fewer common 4.8 kb deletions (homologous to the common 4977 bp deletion in humans) in hepatic tissue compared to rats consuming diets replete and moderately depleted of folate (2 and 0 mg/kg diet, respectively) [32]. In addition, exposure of HeLa cells and fibroblasts to antifolates (methotrexate and 5-fluorodeoxyuracil) caused a dose-dependent increase in MtDNA base mutations, while thymidine ameliorated methotrexate-induced mutation [33].

Deletions in the Mt genome may have important negative ramifications for both the remaining Mt genome as well as the nuclear genome. MtDNA aberrations may inhibit the electron transport chain by deleting or mutating critical polypeptides, and cause the accumulation of electrons in the early stages of the electron transport chain (complex I and CoQ), where they can be donated to molecular oxygen to form superoxide ($O_2^{\bullet -}$) [34]. Superoxide is converted to hydrogen peroxide by Mn superoxide dismutase (SOD), which then can be converted to the highly reactive hydroxyl ($OH^{\bullet}$) radical in the presence of transition metals [34]. Hydroxyl radicals damage a diverse array of cellular macromolecules, including lipids, proteins and DNA [35]. Reactive oxygen metabolite (ROM) attack to the MtDNA may then propagate this cycle [36]. In support of this scheme, evidence suggests that (a) the frequency of MtDNA deletions correlates positively with lipid peroxidation products and SOD activity in the human liver [18,37], (b) deficiency of the MtDNA-encoded complex I (NADH dehydrogenase) is associated with increased production of superoxide radicals and the up-regulation of SOD [38] and (c) chemical disruption of the electron transport chain causes ROM production, telomere attrition and genomic instability [39]. ROM attack to nuclear DNA may then initiate or accelerate colorectal carcinogenesis by damaging tumor suppressor genes and proto-oncogenes according to the classical Vogelstein scheme [40].

We therefore, aimed to determine the effect of folate status and age on the frequency of MtDNA deletions in the colon and liver of rats, and hypothesized that both folate depletion and older age cause an elevation in deletion frequency, with a possible synergistic deleterious effect.