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Minireview

Mitochondrial DNA methylation as a next-generation biomarker and diagnostic tool

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ABSTRACT

Recent expansion of our knowledge on epigenetic changes strongly suggests that not only nuclear DNA (nDNA), but also mitochondrial DNA (mtDNA) may be subjected to epigenetic modifications related to disease development, environmental exposure, drug treatment and aging. Thus, mtDNA methylation is attracting increasing attention as a potential biomarker for the detection and diagnosis of diseases and the understanding of cellular behavior in particular conditions.

In this paper we review the current advances in mtDNA methylation studies with particular attention to the evidences of mtDNA methylation changes in diseases and physiological conditions so far investigated. Technological advances for the analysis of epigenetic variations are promising tools to provide insights into methylation of mtDNA with similar resolution levels as those reached for nDNA. However, many aspects related to mtDNA methylation are still unclear. More studies are needed to understand whether and how changes in mtDNA methylation patterns, global and gene specific, are associated to diseases or risk factors.

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Abbreviations: AD, Alzheimer disease; AIMs, amplification of inter-methylated sites; ALS, amyotrophic lateral sclerosis; ChIP, chromatin immunoprecipitation; CTH, cystathionine γ-lyase; CHARM, comprehensive high-throughput arrays for relative methylation; CBS, cystathionine β-synthase; DNMT, DNA methyltransferase; DS, Down's syndrome; Hcy, homocysteine; 5hmC, 5-hydroxymethylcytosine; MAT, methionine adenosyltransferase; MS, methionine synthase; MCA, methylated CpG island amplification; Ms-AP-PCR, methylation-sensitive arbitrarily primed PCR; MsCC, methylation-sensitive cut counting; 5mC, 5-methylcytosine; MTHFR, methylenetetrahydrofolate reductase; 5-MTHF, 5-methyl-tetrahydrofolate; MT, methyltransferase; MMAss, microarray-based methylation assessment of single samples; MBE, mitochondrial bifunctional enzyme; mtDNA, mitochondrial DNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; nDNA, nuclear DNA; PD, Parkinson disease; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAHH, SAH hydrolase; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; TET, ten–eleven translocation; THF, tetrahydrofolate; TFA, transcription factor A; TFB, transcription factor B; MeDIP and mDIP, methylated DNA immunoprecipitation; WGsBs, whole-genome shotgun bisulfite sequencing.

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1. Introduction

Among the epigenetic processes, DNA methylation is perhaps the best understood epigenetic adaption and the most common DNA modification [1]. Two methylated cytosine-derived bases, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), have been detected in the DNA. 5mC is derived from incorporation of a methyl group at position 5 of cytosine (Fig. 1). 5hmC is produced from 5mC through a hydroxymethylation reaction catalyzed by TET (ten–eleven-translocation) (Fig. 1) [2]. While nDNA methylation is a well established feature, mtDNA methylation has been controversial and a matter of debate [3–9]. Therefore, very little attention has been devoted to mitochondrial epigenetics. The prevailing opinion was that mtDNA cannot be methylated for two main reasons: (i) methylase does not or cannot access mitochondria in vertebrates, and (ii) mtDNA is devoid of histones and is arranged in clusters, called nucleoids, which are bound to the mitochondrial membrane [10]. Only recently, methodological and functional approaches unequivocally have identified mtDNA methylation as part of mammalian mitochondria physiology. Using mass spectrometry, technological tool that overcomes the sensitivity problems of the bisulfite method, our laboratory demonstrated for the first time the presence of methylated bases (5-methyl-2′-deoxycytidine) in human mtDNA [11]. Then, Shock et al. [12] demonstrated the presence of a methyltransferase, DNMT1, inside mitochondria. Through a mitochondrial targeting pre-peptide DNMT1 translocates into mitochondria sequence, where it binds to mtDNA and modifies transcription of the mitochondrial genome. In addition to DNMT1 Chestnut et al. [13] found also the methyltransferase DNMT3a in mitochondrial fractions of mouse and human CNS.

Studies performed in the last two years indicate that epigenetic modification of cytosine in mtDNA is much more frequent than previously believed. Furthermore, mitochondrial epigenetic can modulate nDNA and nDNA epigenetic may affect mtDNA. Thus, epigenetic events regarding mitochondria are now frequently indicated as “mitoepigenetics” [14]. Similar to nDNA methylation [15–18], occurrence of the abnormal mtDNA methylation is often depending on different factors, such as diseases, environment, drugs, and food. Thus, abnormal mtDNA methylation is attracting increasing attention as potential biomarker.

In this paper we will address recent developments in mitoepigenetic studies, particularly those aimed to investigate mtDNA methylation changes associated with diseases and other conditions, such as environmental pollution exposure, aging, drug treatment, and oxidative stress. In addition, the most recent technologies used to detect DNA methylation are reviewed.

2. The one-carbon cycle and mitochondria

The methylation of cytosine in nDNA and mtDNA is required for the maintenance of the epigenetic code and regulation of gene expression, stabilizing chromatin structure in the nDNA [19]. The universal methyl donor is S-adenosylmethionine (SAM), which is produced in the metabolic cycle of methionine (Fig. 2). Methionine is activated to SAM by methionine adenosyltransferase (MAT) transferring adenosine from ATP. Methyl transfer from SAM is catalyzed by methyltransferases (MTs) producing S-adenosylhomocysteine (SAH), which is reversibly hydrolyzed to homocysteine (Hcy) by SAH hydrolase (SAHH). In the trans-sulfuration pathway, homocysteine is irreversibly degraded to cystathionine by the vitamin B6-dependent enzyme cystathionine β-synthase (CBS) and then further catalyzed to cysteine, a precursor of glutathione, by the vitamin B6-dependent enzyme cystathionine γ-lyase (CTH). In the remethylation pathway (folate cycle), the methyl group from 5-methyl-tetrahydrofolate (5-MTHF) is transferred to homocysteine by the vitamin B12-dependent enzyme methionine synthase (MS), producing methionine and tetrahydrofolate (THF) (Fig. 2).

Mitochondria metabolism regulates production of SAM through synthesis of ATP and folate. Folate cycle reactions are duplicated in cytosol and mitochondria [20]; they are linked through exchange of serine and glycine, which are interconverted by the mitochondrial and cytosolic serine hydroxymethyltransferase (SHMT) through methylene-tetrahydrofolate (Fig. 2). Mitochondria regulate the switch between SAM and nucleotide synthesis by the action of the mitochondrial bifunctional enzyme (MBE), which is active in embryonic and cancer cells (promoting purine and pyrimidine synthesis) and is turned off in adult cells (favoring SAM synthesis and DNA methylation) [21,22]. Cytosolically synthetized SAM is transported into mitochondria by means of the specific mitochondrial carrier SAMC, where it is used for all mitochondrial methylation processes [23] (Fig. 2).

Based on the central role of one-carbon pathway in methylation reactions and nucleotide synthesis, it is not surprising that disruption of these pathways, either due to nutritional deficiencies (folate and B-vitamins) or genetic factors, has been linked to different human diseases, such as

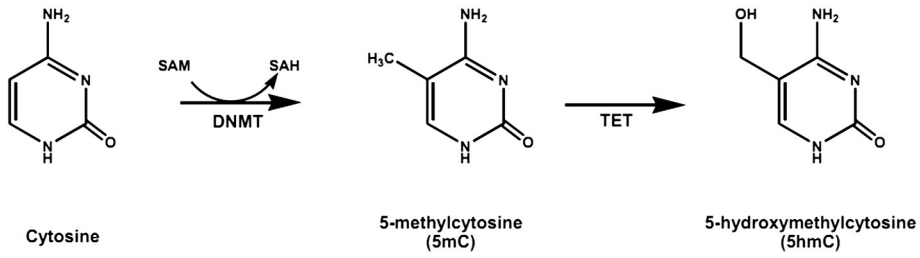

Fig. 1. Reaction of cytosine methylation and hydroxymethylation. Abbreviations: DNMT, DNA methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; TET, ten–eleven translocation.

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