How to breakthrough mitochondrial DNA methylation-associated networks

William Wang · Xiaoxia Liu · Xiangdong Wang

Received: 17 May 2020 / Accepted: 2 June 2020 / Published online: 8 June 2020
© Springer Nature B.V. 2020

Abstract Mitochondrial DNA (mtDNA) plays an important role in regulating mitochondrial homeostasis, transcription, cell metabolism, and drug sensitivity. Patterns and regulations of mtDNA methylation vary among cell types, species, functions, and diseases. High-resolution mtDNA methylation maps of human and animal mitochondrial genomes addressed that the light (L)-strand non-CpG methylation of mtDNA varied among species, developing stages, and ages. Of DNA methyltransferases, DNMT3A was a critical enzyme in the dynamic regulation of mtDNA regional methylation patterns and strand bias. Altered mtDNA methylations may regulate dynamic occurrences of pathogenic mtDNA mutations. The number and sites of control regions, involved enzymes, and regulators during mtDNA methylation may vary among cell types and diseases. Specific regulatory and functional networks associated with mtDNA methylation mainly include mtDNA, regulatory factors, methyltransferases, nucleotides, mt-rRNAs, and other epigenetic modifications. Those carry out precise functions and regulations of mtDNA methylation-associated network, interactions

with genome DNA and other signal pathways, and decisive roles in patient phenomes. A breakthrough in mtDNA methylation-associated networks will be a crucial milestone in the journey of understanding mitochondrial function at a higher level and discovering new mitochondria-based biomarkers and therapeutic targets.

Keywords Mitochondria · Mitochondrial DNA · Methylation · Networks · Epigenetics

Mitochondrial DNA (mtDNA) is a key component responsible for mitochondrial dysfunction to contribute to the development of cytotoxicity, organ injury, and acute and chronic diseases by regulating the signaling in immune responses, cell differentiation, and energy metabolism (Hasnat et al. 2019; Fang et al. 2019; Sun et al. 2017). The stability, methylation, and homeostasis in mtDNA are regulated by many factors such as USP30 deubiquitylation, mitochondrial ribosomes, or oxygen free radicals. Intra- and extra-cellular factors such as caveolins can influence mtDNA expression, sequence, and function by altering mitochondrial fatty acid catabolism and respiration, stimulating peroxisome proliferator-activated receptor α -dependent fatty acid oxidation, and enhancing ketogenesis production (Yan et al. 2020). Sun and Wang (2017) published a book with a special focus on the association between mtDNA and diseases, highlighting the effect of mtDNA sequencing, methylation, stability, and mutation in the initiation and progression of disease as well as the

W. Wang · X. Liu · X. Wang
Zhongshan Hospital Institute for Clinical Science, Shanghai
Institute of Clinical Bioinformatics, Shanghai Engineering
Research for AI Technology for Cardiopulmonary Diseases,
Fudan University, Shanghai, China

W. Wang · X. Liu · X. Wang (✉)
Zhongshan Hospital Institute of Clinical Science, Jinshan Hospital
Centre for Tumor Diagnosis and Therapy, Fudan University
Shanghai Medical College, Shanghai, China
e-mail: xdwang@fuccb.com

molecular mechanisms by which mtDNA regulates mitochondrial homeostasis, drug resistance, and mitochondrial dysfunction in lung disease. Here, we call special attentions and address urgent needs on developing an in-depth understanding of the patterns, changes, and regulations of mtDNA methylation to understand its roles in developing cytotoxicity and diseases.

There is a growing evidence demonstrating the importance of mtDNA methylation, e.g., modifying mtDNA methylation with DNA methyltransferase (DNMTs or DNA MTases) by adding a methyl group from S-adenosyl-L-methionine (AdoMet) to the fifth carbon position of cytosine (cytosine-5 or C5) within the mitochondria. Dou et al. (2019) developed high-resolution mtDNA methylation maps of human and animal mitochondrial genomes and pointed out that the light (L)-strand non-CpG methylation of mtDNA varied among species, developing stages, and ages. DNMT3A was suggested as a critical and dominant enzyme in the dynamic regulation of mtDNA regional methylation patterns and strand biases, by which mtDNA methylation contributes to mitochondrial gene expression, copy number, and oxygen respiration. Mitochondria play a central role in cell metabolism and sensitivity in response to intracellular and extracellular challenges, especially to drugs. It is questioned whether such high-resolution mtDNA methylation maps can be changed and monitored during the development of diseases with the specificity for disease category, severity, stage, duration, and prognosis. The patterns and strand biases of mtDNA methylation can be detected dynamically and diagnostically in diseases and during therapy. Preclinical studies showed that mtDNA methylation was associated with cancer development and metabolism, fibrogenesis and organ fibrosis, and processing of chronic cytopathy.

There have been suggestions that mtDNA methylation could act as a biomarker and diagnostic tool for detecting disease at an early stage, although it needs to be furthermore identified and validated. It is important to consider the association between mtDNA methylation and cell metabolism and regulation or clinical phenomes, the direction of mtDNA signaling, and the roles in epigenetics. Liu et al. (2020) described that hypermethylation of mitochondrial D-loop regions by DNMT1 could cause mitochondrial gene expression, dysfunction, and loss of contractile phenotype of vascular smooth muscle cells in arterial stenotic-occlusive diseases. It seems that the activity and amount of

DNMT1 play a decisive and dependent role in the process of mtDNA methylation to maintain the balance of mitochondrial epigenetics. The implant of DNMT1-deleted mitochondria into cells with mtDNA hypermethylation could improve hypermethylation-caused impairments of mitochondrial respiration and cell function. The mitochondrial DNA copy number is cell- and tissue-specific and critical in the control of gene expression during differentiation and development, by regulating the methylation status of a CpG island within exon 2 of the nuclear-encoded mtDNA polymerase γ catalytic subunit during differentiation and cell specialization. The altered mtDNA methylations may regulate dynamic occurrences of pathogenic mtDNA mutations which is heteroplasmic and co-exists with wild-type molecules. The mtDNA pathogenic mutations can be modified/corrected indirectly by the epigenetic regulation of mtDNA methylation or directly by mitochondria-targeted gene editing.

Of major sites, C-phosphate-G (CpG) and non-CpG nucleotides, in the L-strand of the D-loop for DNA methylation, non-CpG is the main mtDNA control region for epigenetic modification, although CpG and other unusual regions are also involved in mtDNA methylation. DNA methyltransferases within the mitochondria could play a critical role in the maintenance of CpG and non-CpG region balance mainly in the promoter region of the heavy strand, rather than the inactivation of Dnmt1, Dnmt3a, and Dnmt3b in mouse embryonic stem cells. Liu et al. (2016) discovered 83 CpG sites across mtDNA in human samples and concluded that human mtDNA CpG methylation might be a very rare event at most DNA regions. The methylation occurs more frequently in circular mtDNA rather than in linear mtDNA. The number and sites of control regions, involved enzymes, and regulators during mtDNA methylation vary among cell types, tissues, species, and diseases. Hao et al. (2020) recently evidenced that N⁶-Methyldeoxyadenosine (6 mA) presented in mammalian mtDNA and the amount of 6 mA in mtDNA was 1300-fold higher than that in genome DNA in HepG2 cells under normal growth conditions, while 6 mA changed with challenge. Of methyltransferases-like (METTLs), METTL4 controlled mtDNA 6 mA methylation and down-regulated mtDNA transcription, copy number, or DNA binding and bending by mitochondrial transcription factor. This study delivers an important message that the regulatory mechanisms of mtDNA methylation may be more

comprehensive and precise than what was previously predicted. It is crucial that we define the specificity of 6 mA in response to intra- and extra-cellular environmental changes, pathogens, and diseases in regulation of enzymes, bindings, and signals, as well as in metabolism of lipids, oxygen, and energies.

There are increased number of studies on molecular mechanisms of mtDNA methylation-regulated alternations of gene transcription, protein synthesis, epigenetic inheritance, chromatin structure, and cell function. For example, the cytosine methylation of nuclear DNA at CpG sequences in the heavy strand promoter could influence the primary DNA compaction and transcription-initiation by up-regulating the binding affinity and multimerization of mitochondrial transcription factor A and factor-DNA recognition (Dostal and Churchill 2019). It is questioned whether regulatory mechanisms and factors vary between genome and mtDNA CpG methylations, how DNMTs and METTLs function between nuclear and mitochondria, and whether the modification of mitochondrial ribosomal RNA (mt-rRNAs) influences mtDNA methylation processes. The structure, amount, location, function, and regulation of those mtDNA methylation-associated enzymes and subtypes vary among organelles, cells, and species in response to different challenges. For example, 6 mA can be modified by METTL3, METTL4, METTL5, and others, highly dependent upon DNA or RNA, genome or mitochondria, and cell phenotypes. The mtDNA specificity of METTLs should be further clarified, especially in pathophysiological conditions. METTL15 could regulate cytosine N4-methylation at position 839 (m4C839) of the 12S small subunit in mt-rRNA to control the translation of mitochondrial protein-coding mRNAs and influence the capacity mitochondrial respiration (Chen et al. 2020). Importantly, mtDNA methylation contributes to transcription, processing, and decay of mitochondrial RNA, although roles of methyltransferase substrate specificities and modification patterns of mt-rRNAs in mtDNA methylation need to be defined. The interaction between mtDNA and mt-rRNA can be further understood during the reconstruction of whole mitochondrial genome through RNA sequencing.

There are mtDNA methylation-specific and associated regulatory and functional networks, including mtDNAs, regulatory factors, methyltransferases, nucleotides, mt-rRNAs, and other epigenetic modifications. The exact function and regulation within the mtDNA methylation-associated networks, interaction of

networks with genome DNA and other signal pathways, and decisive roles of networks in patient phenomes can be better explored using clinical trans-omics (Wang 2018). Breakthrough of mtDNA methylation-associated networks will be one of milestones in the journey of understanding mitochondrial function at a high level and discovering mitochondria-based biomarkers and therapeutic target, although the methodology to dynamically monitor mtDNA methylation and network function remains to be standardized, applicable, and repeatable (Gao et al. 2017). It is also important to define the molecular intercommunication between mtDNA methylation-associated networks with transcriptional and chromatin networks through single cell transcriptomes and target gene-specific editing. The mtDNA methylation-associated network should be a critical part of the epigenetic modification network in mitochondria and the part of genome DNA methylation-associated networks. Further studies are needed to address the intercommunication between mtDNA methylation and histone post-translational and chromatin modifications. Thus, we should pay special attention to mtDNA methylation-associated networks and target it as a breakthrough point in order to visualize the relationship among multi-networks and identify function-specific biomarkers and therapies.

References

- Chen H, Shi Z, Guo J, Chang KJ, Chen Q, Yao CH, et al. The human mitochondrial 12S rRNA m4C methyltransferase METTL15 is required for mitochondrial function. *J Biol Chem*. 2020;jbc.RA119.012127. <https://doi.org/10.1074/jbc.RA119.012127>.
- Dostal V, Churchill MEA. Cytosine methylation of mitochondrial DNA at CpG sequences impacts transcription factor a DNA binding and transcription. *Biochim Biophys Acta Gene Regul Mech*. 2019;1862(5):598–607. <https://doi.org/10.1016/j.bbaggm.2019.01.006>.
- Dou X, Boyd-Kirkup JD, McDermott J, Zhang X, Li F, Rong B, et al. The strand-biased mitochondrial DNA methylome and its regulation by DNMT3A. *Genome Res*. 2019;29(10):1622–34. <https://doi.org/10.1101/gr.234021.117>.
- Fang T, Wang M, Xiao H, Wei X. Mitochondrial dysfunction and chronic lung disease. *Cell Biol Toxicol*. 2019;35(6):493–502. <https://doi.org/10.1007/s10565-019-09473-9>.
- Gao D, Zhu B, Sun H, Wang X. Mitochondrial DNA methylation and related disease. *Adv Exp Med Biol*. 2017;1038:117–32. https://doi.org/10.1007/978-981-10-6674-0_9.
- Hao Z, Wu T, Cui X, Zhu P, Tan C, Dou X, et al. N⁶-Deoxyadenosine methylation in mammalian mitochondrial

- DNA. *Mol Cell*. 2020;78(3):382–395.e8. <https://doi.org/10.1016/j.molcel.2020.02.018>.
- Hasnat M, Yuan Z, Naveed M, Khan A, Raza F, Xu D, et al. Drp1-associated mitochondrial dysfunction and mitochondrial autophagy: a novel mechanism in triptolide-induced hepatotoxicity. *Cell Biol Toxicol*. 2019;35(3):267–80. <https://doi.org/10.1007/s10565-018-9447-8>.
- Liu B, Du Q, Chen L, Fu G, Li S, Fu L, et al. CpG methylation patterns of human mitochondrial DNA. *Sci Rep*. 2016;6:23421. <https://doi.org/10.1038/srep23421>.
- Liu YF, Zhu JJ, Tian XY, Liu H, Zhang T, Zhang YP, et al. Hypermethylation of mitochondrial DNA in vascular smooth muscle cells impairs cell contractility. *Cell Death Dis*. 2020;11(1):35. <https://doi.org/10.1038/s41419-020-2240-7>.
- Sun H, Wang X. Mitochondrial DNA and diseases. *Adv Exp Med Biol*. 2017;1038. <https://doi.org/10.1007/978-981-10-6674-0>, eBook Print ISBN: 978–981–10–6673-3, Online ISBN: 978–981–10–6674-0, Series Print ISSN 0065–2598, Series Online ISSN: 2214–8019, Published by Springer Nature Singapore Pte Ltd.
- Sun H, Shi W, Wang X. How far can mitochondrial DNA drive the disease? *Adv Exp Med Biol*. 2017;1038:1–8. https://doi.org/10.1007/978-981-10-6674-0_1.
- Wang X. Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. *Cell Biol Toxicol*. 2018 Jun;34(3):163–6. <https://doi.org/10.1007/s10565-018-9431-3>.
- Yan F, Su L, Chen X, Wang X, Gao H, Zeng Y. Molecular regulation and clinical significance of caveolin-1 methylation in chronic lung diseases. *Clin Transl Med*. 2020;10:151–60. <https://doi.org/10.1002/ctm2.2>.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.