Mitochondrial Secrets of Youthfulness

Keshav K. Singh, PhD
Birmingham, AL

Summary: The genetic basis of youthfulness is poorly understood. The aging of skin depends on both intrinsic factors and extrinsic factors. Intrinsic factors include personal genetics, and extrinsic factors include environmental exposure to solar radiation and pollution. We recently reported the critical role of the mitochondria in skin aging phenotypes: wrinkle formation, hair graying, hair loss, and uneven skin pigmentation. This article focuses on molecular mechanisms, specifically mitochondrial mechanisms underlying skin aging. This contribution describes the development of an mitochondrial DNA depleter-repleter mouse model and its usefulness in developing strategies and identifying potential agents that can either prevent, slow, or mitigate skin aging, lentigines, and hair loss. The ongoing research efforts include the transplantation of young mitochondria to rejuvenate aging skin and hair to provide youthfulness in humans. (Plast. Reconstr. Surg. 147: 33S, 2021.)

Aging is a natural, multifactorial process characterized by a progressive decline in cellular functions and regenerative abilities, resulting in increased susceptibility to morbidity and mortality.\(^1\) Mechanistically, mitochondrial dysfunction is considered one of the aging hallmarks that contributes significantly to the physiology of aging and the pathophysiology of age-related disorders, such as cardiovascular, metabolic, and neurodegenerative diseases.\(^2\)

The skin is the most perceptible organ to show initial signs of aging. These include developing wrinkles, sagging skin, loss of elasticity, loss of smoothness, and skin pigmentation.\(^3\) Being the outermost protective layer of the body, the skin is exposed to both intrinsic (genetic, metabolic, and endocrine factors) and extrinsic (ultraviolet radiation, environmental pollution, and smoking) stimuli that drive the aging process.\(^4\) Skin aging caused by intrinsic factors is mostly characterized by reduced basal cell proliferation and reduced lipid levels in the stratum corneum and abnormal epidermis.\(^5,6\) The changes include reduced blood vessels and cell density and reduced collagen and elastin fibers in the dermis. In extrinsic aging, ultraviolet (UV), in particular, UVA radiation from sunlight, causes a reduction in collagen content and denaturation of elastic fibers, leading to atrophy of the extracellular matrix. Also, UVB radiation further causes elastic fiber denaturation by inducing the expression of elastase and matrix metalloproteinases (MMPs) 1, 3, and 9.\(^7\) UV radiation-induced mitochondrial DNA (mtDNA) damage and oxidative stress cause photoaging. Like photoaging, other extrinsic factors like environmental pollution and smoking contribute to skin aging by inducing MMP expression, melanocyte activity, and oxidative stress.\(^8\)

Besides the modulators, as mentioned above, the amount of melanin in the epidermal melanosomes plays a significant role in determining the rate and degree of skin aging.\(^27\) The primary difference between people with skin of color and fair-skinned people is attributed to the epidermal melanin content and the pattern of melanosome dispersion. Also, it is well documented that people with darker skin with high melanin content and singly dispersed large melanosomes, such as in African Americans, are significantly less susceptible to UV-induced photoaging than their fair-skinned counterparts with low melanin content and small, aggregated melanosomes, such as whites.\(^8,28\) Unlike whites with a thinner stratum corneum, a thicker stratum corneum, a thicker stratum corneum and increased activity of large, multinucleated dermal fibroblasts help provide photoprotection and preserve skin elasticity in African Americans. Although genetic and environmental factors play a significant role...
in skin aging predisposition among various ethnicities, studies have identified other factors that may influence the skin aging manifestation. For instance, Japanese women show significantly less facial wrinkles than German women. However, the development of lentigines is faster in Japanese women than age-matched German women. This is attributed to a specific genotype distribution of SNP rs26722 in the SLC45A2 gene.

SKIN PIGMENTATION

Genome-wide association studies have been conducted to identify single nucleotide polymorphisms (SNPs) associated with skin aging. The most widely documented genotypes are multiple SNPs of the melanocortin 1 receptor (MC1R) gene. These are found to be significantly associated with both overall and particular characteristics of skin aging, such as pigmented lesions. In epidermal melanocytes, MC1R plays a vital role in regulating melanin synthesis. Melanocortin-induced activation of MC1R is also associated with antioxidant defense mechanisms. However, these studies have revealed that skin aging induced by different allelic variants of MC1R is not related to the melanin synthesis. Instead, inflammation appears to be the causative factor for MC1R SNP-induced skin aging. Another important mechanism is considered to be the oxidative stress induced by different melanin compounds. For example, α-melanocyte-stimulating hormone (α-MSH)-induced activation of MC1R causes increased eumelanin synthesis and reduced pheomelanin synthesis to help prevent skin aging as eumelanin is darker and can protect the skin from UVB radiation. Moreover, eumelanin can prevent oxidative damage to the skin by scavenging reactive oxygen species (ROS), whereas pheomelanin can increase UV sensitivity by increasing ROS production. Thus, an induction in the level of pheomelanin over eumelanin due to loss-of-function mutations of the MC1R gene can potentially increase the risk of photoaging, most likely by ROS-induced oxidative stress.

SKIN WRINKLES

Besides MC1R, SNP rs322458, located on chromosome 3, is reported to be associated with skin wrinkles. The study revealed that SNP rs322458 is in linkage disequilibrium with STXBP5L gene SNPs and another SNP that causes increased expression of the FBXO40 gene in the skin. The functional connection of the FBXO40 gene with inflammation and myogenesis may partially explain the involvement of SNP rs322458 information of skin wrinkles.

MITOCHONDRIAL DYSFUNCTION INDUCES SKIN WRINKLES

Mitochondria are the cellular hub of ROS production. Age-related mutations in mtDNA occur faster than nuclear DNA because of the persistent oxidative environment within mitochondria. Interestingly, human fibroblasts obtained from old individuals show accumulation of point mutations in D-loop controlling mtDNA replication. The depletion of mtDNA is also reported during aging. The synthesis of mtDNA requires synergistic interactions between several intricate processes, such as synthesis of nucleotides through mitochondrial nucleotide salvage pathways; translocation of nucleotides from the cytosol to mitochondria; and mitochondrial dynamics (fission and fusion). Thus, disruption of any of these processes can impair mtDNA synthesis and cause mtDNA depletion.

Given the importance of mtDNA in aging and age-related disorders, we evaluated aging outcomes in mtDNA depletor mice. To induce mtDNA depletion, a doxycycline-inducible mtDNA-depleter mouse was developed that expresses a dominant-negative mutation in the polymerase domain of mitochondrial DNA polymerase γ (Fig. 1). Our study showed that induction of mtDNA depletion and ensuing mitochondrial dysfunction results in gray hair and profound hair loss.

Besides the mitochondrial impact on hair, mtDNA dysfunction-induced skin wrinkles. Histological analysis of the skin revealed that mtDNA depletion induces pathological changes resulting in wrinkles in the skin. Electron microscopic images showed that the skin changes were accompanied by severe mitochondrial degeneration in the skin. Another critical observation included infiltration of many inflammatory cells, such as mast cells, granulocytes, macrophages, B-lymphocytes, and T-lymphocytes, in the epidermis dermis of mtDNA-depleted mice. Besides, the expression of inflammatory genes, such as IFNB1, IL28a, and CCL5 and NFKB and COX2, was increased. These findings reveal the inflammation as an underlying cause of skin aging due to mitochondrial dysfunction. Furthermore, an increased expression of MMP2 and MMP9 and reduced expression of TIMP1 suggests disruption of collagen homeostasis. Finally, increased expression of skin aging markers (IGF1R, VEGF, and MRPS5) depicts mitochondrial dysfunction in skin aging.

Surprisingly, we observed that restoration of mtDNA content (therefore restoration of ensuing mitochondrial function) led to a reversal of skin aging phenotypes (wrinkles and hair loss);
restoration of normal skin morphology; reduction in the inflammatory infiltrate in the skin layers; and reversal of inflammation- and skin aging-related gene expressions to wild type. These studies suggest that mitochondrial dysfunction induces skin aging phenotypes that can be reversed by merely restoring mitochondrial homeostasis. Notably, observations in mtDNA-depleter mice and MC1R SNP-associated skin aging in humans show inflammation as an underlying common cause of skin aging.\(^{25,26}\) The mtDNA-depleter mouse model can be a good model for studying in-depth mechanisms underlying skin aging and other skin-related disorders.

**MITOCHONDRIAL MECHANISMS UNDERLYING SKIN YOUTHFULNESS**

Multiple mechanisms and pathways may be involved in controlling the skin and hair phenotypes and youthfulness. In addition to mitochondrial dysfunction, another hallmark of aging is inflammation termed inflammaging.\(^{23}\) Inflammation is involved in several age-related diseases such as arthritis, atherosclerosis, cancer, diabetes, or neurodegeneration.\(^{34}\) Our study provides a direct and causal link between mitochondrial dysfunction and inflammation underlying skin aging in mice. Consistent with our findings, a study also described mtDNA deletions’ accumulation and concomitant increase in inflammation in humans during skin aging. More than 170 genes are involved in the regulation of pigmentation in humans\(^{35}\); our recent study indicates that mtDNA encoded genes also play a role in skin pigmentation, particularly in lentigines.\(^{36}\) This lentigo study brings up questions of (1) whether the growth of lentigines is also attributable to inflammation-induced due to mitochondrial dysfunction in mtDNA depleter mice and (2) whether mitochondrial dysfunction and inflammation also underlie senile and environmental agent (such as UV)-induced lentigines in humans.\(^{37}\) Accumulated senescent cells during aging acquire the senescence-associated secretory phenotype, which contributes to inflammation.\(^{36,39}\) We have described the upregulation of inflammatory genes in the skin of mtDNA-depleter mice. More importantly, our studies suggest that restoring mitochondrial function can downregulate inflammatory genes to a normal level. Therefore, it is likely that epigenetic mechanisms such as DNA methylation play a significant role in the restoration of inflammatory gene expression to a healthy level. However, more studies are needed to identify mechanisms related to reversing or mitigating skin and hair phenotypes due to mitochondrial dysfunction.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Healthy skin and hair represent the overall perception of health and youthfulness in humans. Skin aging and hair loss involves multiple complex processes that are influenced by both intrinsic and extrinsic factors. In the past decade, several therapeutic strategies have been developed to provide skin youthfulness. These approaches include both noninvasive and invasive strategies. The noninvasive method utilizes lasers for rejuvenation. The invasive process removes and resurfaces the damaged epidermis and formation of new collagen. Chemical ablation remodels the skin by regeneration and repair of the epidermis and dermis. Injectable dermal fillers are also used by a plastic surgeon.\(^{40}\) Mitochondria have essential roles in skin function. The decline in mitochondrial function increases with age in skin cells and in response to solar light and pollution, resulting in accelerated extrinsic aging of the skin. Aging skin shows reduced
wound healing capacity and increased susceptibility to infection. Hair loss and graying is another phenotype associated with aging. Current antiaging approaches to treat skin aging mainly include antioxidants and retinoids in antiaging creams. The antioxidants, such as vitamins, polyphenols, and flavonoids, reduce oxidative stress, and reduce collagen degradation. Retinoids directly affect collagen metabolism and enhance collagen production. Polypeptides or oligopeptides mimicking a peptide sequence of collagen or elastin are also used. 3

Although extrinsic skin aging can be prevented by avoiding exposure to environmental factors and the use of topical cream, intrinsic skin aging is genetically determined. Our recent studies unraveled a direct causal link between a decline in mtDNA content, subsequent mitochondrial dysfunction and skin aging, wrinkle formation, lentigines, hair graying, and loss. 24 Our study also provided proof of the concept that restoring mitochondrial function by repletion of mtDNA can reverse aging-associated skin phenotypes. 24 We also demonstrated that mitochondrial dysfunction induced inflammation plays a pivotal role as a mechanism underlying the aging-related skin phenotypes. 24 The development of mtDNA depletoreleaser mouse provides a unique opportunity for modulating mitochondrial function at will in the whole animal or in desired tissue to study the aging of specific organs. This mouse model will facilitate novel strategies and serve as a preclinical tool for screening agents to prevent, slow, and treat aging as a whole and specific tissue such as skin and hair. The agents may either reduce oxidative stress, inhibit inflammation, induce or turn down novel cellular pathways to restore or rescue mitochondrial function. Isolated mitochondria could also serve as an attractive therapeutic agent of choice. 43 Isolated autologous mitochondria can be transferred into the skin to enrich the endogenous pool with healthy mitochondria to develop alternative therapies to treat not only aging of skin but a range of skin diseases associated with mitochondrial dysfunction. 45 Together, these approaches should result in the development of novel strategies that can prevent and delay skin aging and provide youthfulness in humans.

Keshav K. Singh, PhD  
Department of Genetics  
University of Alabama at Birmingham  
720 20th Street South  
620 Hugh Kaul Building  
Birmingham, AL 35294  
ksingh@uab.edu  
Twitter: @mitoscientist

ACKNOWLEDGMENTS
National Institutes of Health grant R01CA204430 supports Dr. Singh. The author thanks members of his laboratory.

REFERENCES
1. López-Otín C, Blasco MA, Partridge L, et al. The hallmarks of aging. Cell. 2013;153:1194–1217.
2. Jang JY, Blum A, Liu J, et al. The role of mitochondria in aging. J Clin Invest. 2018;128:3662–3670.
3. Zhang S, Duan E. Fighting against skin aging. Cell Transplant. 2018;27:729–738.
4. Kruutmann J, Boulac A, Sore G, et al. The skin aging exosome. J Dermatol Sci. 2017;85:152–161.
5. Farage MA, Miller KW, Ehner P, et al. Structural characteristics of the aging skin: a review. Cutan Ocul Toxicol. 2007;26:343–357.
6. Makrantonaki E, Zouboulis CC, William J. Unlilife Scientific Awards. characteristics and pathomechanisms of endogenously aged skin. Dermatology. 2007;214:352–360.
7. Ichihashi M, Ando H, Yoshida M, et al. Photoaging of the skin. Anti-Aging Med. 2009;6:46–59.
8. Venkatesh S, Maymone MBC, Vashi NA. Aging in skin of color. Clin Dermatol. 2019;37:351–357.
9. Flood KS, Houston NA. Savage KE, et al. Genetic basis for skin youthfulness. Clin Dermatol. 2019;37:312–319.
10. Liu F, Hamer MA, Deelen J, et al. The MC1R gene and youthful looks. Curr Biol. 2016;26:1213–1220.
11. Elafik A, Ezedine K, Latrèille J, et al. Functional MC1R-gene variants are associated with increased risk for severe photoaging of facial skin. J Invest Dermatol. 2010;130:1107–1115.
12. Jacobs LC, Hamer MA, Gunn DA, et al. A Genome-Wide Association Study identifies the skin color genes IRF4, MC1R, ASIP, and BNC2 influencing facial pigmented spots. J Invest Dermatol. 2015;135:1735–1742.
13. Herrera C, Garcia-Borrón JC, Jiménez-Cervantes C, et al. MC1R signaling. Intracellular partners and pathophysiological implications. Biochim Biophys Acta Mol Basis Dis. 2017;1863(10 Pt A):2448–2461.
14. García-Borrón JC, Sánchez-Laorden BL, Jiménez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res. 2011;18:393–410.
15. Clerc SL, Taing L, Ezedine K, et al. A Genome-Wide Association Study in Caucasian Women points out a putative role of the STXB5L1 gene in facial photoaging. J Invest Dermatol. 2013;133:929–935.
16. Kauppila TES, Kauppila JHK, Larsson NG. Mammalian mitochondria and aging: an update. Cell Metab. 2017;25:57–71.
17. Linnane AW, Marzuki S, Ozawa T, et al. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet. 1989;1:642–645.
18. Michikawa Y, Mazzucchelli F, Bresolin N, et al. Aging-dependent large accumulation of point mutations in the human mitochondrial DNA control region for replication. Science. 1999;286:774–779.
19. Stout R, Birch-Machin M. Mitochondria’s role in skin ageing. Biology. 2019;8:29.
20. Visconi C, Zeviani M. MdDNA-maintenance defects: syndromes and genes. J Inherit Metab Dis. 2017;40:587–599.
21. Ballout RA, Al Alam C, Bonnen PE, et al. FBXL4-related mitochondrial DNA depletion syndrome 13 (MTPDS13): a case report with a comprehensive mutation review. Front Genet. 2019;10:39.
22. El-Hattab AW, Craigen WJ, Scaglia F. Mitochondrial DNA maintenance defects. Biochim Biophys Acta Mol Basis Dis. 2017;1863:1539–1555.
23. Schroeder P, Gremmel T, Berneburg M, et al. Partial depletion of mitochondrial DNA from human skin fibroblasts induces a gene expression profile reminiscent of photoaged skin. *J Invest Dermatol.* 2008;128:2297–2305.

24. Singh B, Schoeb TR, Bajpai P, et al. Reversing wrinkled skin and hair loss in mice by restoring mitochondrial function. *Cell Death Dis.* 2018;9:735.

25. Kadekaro AL, Chen J, Yang J, et al. Alpha-melanocyte-stimulating hormone suppresses oxidative stress through a p53-mediated signaling pathway in human melanocytes. *Mol Cancer Res.* 2012;10:778–786.

26. Hill RP, MacNeil S, Haycock JW. Melanocyte-stimulating hormone peptides inhibit TNF-alpha signaling in human dermal fibroblast cells. *Peptides.* 2006;27:421–430.

27. Vashi NA, de Castro Maymone MB, Kundu RV. Aging differences in ethnic skin. *J Clin Aesthet Dermatol.* 2016;9:31–38.

28. Taylor SC. Skin of color: biology, structure, function, and implications for dermatologic disease. *J Am Acad Dermatol.* 2002;46(2 Suppl Understanding):S41–S62.

29. Vierkötter A, Krutmann J. Environmental influences on skin aging and ethnic-specific manifestations. *Dermatoendocrinol.* 2012;4:227–231.

30. Perner D, Vierkötter A, Sugiri D, et al. Association between sun-exposure, smoking behaviour and plasma antioxidant levels with the different manifestation of skin ageing signs between Japanese and German women—a pilot study. *J Dermatol Sci.* 2011;62:138–140.

31. Vierkötter A, Krämer U, Sugiri D, et al. Development of lentigines in German and Japanese women correlates with variants in the SLC45A2 gene. *J Invest Dermatol.* 2012;132(3 Pt 1):733–736.

32. Sreedhar A, Aguilera-Aguirre L, Singh KK. Mitochondria in skin health, aging, and disease. *Cell Death Dis.* 2020;11:444.

33. Monti D, Ostan R, Borelli V, Castellani G, Franceschi C. Inflammaging and human longevity in the omics era. *Mech Ageing Dev.* 2017;165(Pt B):129–138.

34. Zhuang Y, Lyga J. Inflammaging in skin and other tissues - the roles of complement system and macrophage. *Inflamm Allergy Drug Targets.* 2014;13:153–161.

35. Hudjashov G, Villes M, Kivisild T. Global patterns of diversity and selection in human tyrosinase gene. *PLoS One.* 2013;8:e74307.

36. Villavicencio KM, Ahmed N, Harris ML, et al. Mitochondrial DNA-depleter mouse as a model to study human pigmentary skin disorders. *Pigment Cell Melanoma Res.* 2020.

37. Wiley CD, Velarde MC, Lecot P, et al. Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab.* 2016;23:303–314.

38. Coppé JP, Patil CK, Rodier F, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 2008;6:2853–2868.

39. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000;908:244–254.

40. Ganceviciene R, Liakou AI, Theodoridis A, et al. Skin anti-aging strategies. *Dermatoendocrinol.* 2012;4:308–319.

41. Katrangis E, D’Souza G, Boddapati SV, et al. Xenogenic transfer of isolated murine mitochondria into human rho0 cells can improve respiratory function. *Rejuvenation Res.* 2007;10:561–570.

42. Singh B, Modica-Napolitano JS, Singh KK. Defining the momiome: promiscuous information transfer by mobile mitochondria and the mitochondrial genome. *Semin Cancer Biol.* 2017;47:1–17.