Ekowati AL, Siswanto FM. *Journal of Urban Health Research* (2024) 2:3, p 25-41

e-ISSN 2964-4194

REVIEW ARTICLE Open Access

Epigenetic Alterations in Aging: A Brief Review

Ana Lucia Ekowati[1], Ferbian Milas Siswanto[2*]

[1]Departement of Medical Biology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia

[2]Departement of Chemistry and Biochemistry, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia

*Corresponding author: Ferbian Milas Siswanto, ferbian.siswanto@atmajaya.ac.id

Abstract

Background: Extensive studies have reported the involvement of epigenetic dynamics in aging. Common epigenetic modifications in aging include the methylation of DNA, rearrangement of chromatin, and regulation of gene expression by non-coding RNA (ncRNA). Some important conclusions that have emerged from various studies in the past few decades are that the lifespan of living organisms is largely determined by epigenetics instead of genetics, where environmental and lifestyle influences that change epigenetic information have a dominant effect.

Purpose: This study aims to review current understanding of the mechanisms involved in epigenetic regulation during aging that would provide new insights for the development of strategies to prevent aging.

Methods: A search for literature regarding epigenetic regulations in biological aging was carried out in Web of Science and Scopus on "epigenetics" [AND] "aging". In this study, we used a total of 73 articles published between 2010 and 2024.

Results: Aging is accompanied by various alterations in epigenetic marks, including DNA methylation (global hypomethylation in non-CpG regions and hypermethylation OF CpG islands), rearrangement of chromatin (global reduction of histones and redistribution of histone modifications), and ncRNA (particularly miRNA). Epigenetic is a reversible molecular mechanism that allow therapeutic interventions to improve or reverse aging-related pathogenesis. Chemical-based epigenetic manipulation and lifestyle-based epigenetic reprogramming strategies can be developed to improve or reverse aging-related conditions.

Conclusion: Based on extensive literature review, we found that epigenetic changes are potential biomarkers for early detection of aging and age-related diseases. Drugs that target key epigenetic signatures are therefore promising to intervene aging.

Keywords: aging - age-related disease - biomarker - epigenetic regulation

INTRODUCTION

Globally, the process of population demographics shifts towards an aging population in the world, including in Indonesia, cannot be avoided. Studies showed that the percentage of people aged over 60 years has increased globally from 9% in 1994 to 12% in 2014, and is projected to reach 21% in 2050. Indonesia has recently become aging

population, where around 10% residents are older people.[1] The aging population in urban areas, in particular, requires attention regarding public facilities that support the activities and health.[2] Improving clinician's understanding on cellular and molecular changes is important.[3,4] Continuous study in the field of gerontology to uncover the underlying process of natural aging and the pathobiology of aging-related diseases, especially at molecular levels, has not yet been completely successful.[5]

Previous research has formulated hallmarks of aging to facilitate research in the field of gerontology. The most recent hallmarks of aging are genomic instability, impaired macroautophagy, telomere shortening, epigenetic modification, loss of proteostasis, chronic inflammation, impaired nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, and dysbiosis.[6,7,8] The epigenetic mechanism is one of the hallmark of aging that is less popular. Epigenetics are defined as heritable modification in the level of gene expression without changing the sequence of DNA. It acts as a connecting hub between the genetic factor and environmental cues by influencing the gene expression and individual phenotype.[9,10] These aging-related epigenetic modifications include alteration in methylation signature of DNA, variation in chromatin rearrangement, and the regulation by non-coding RNA (ncRNA) such as microRNA (miRNA) regulation, which can change genome function under the influence of environmental or exogenous factors.[11–13]

Understanding of epigenetic changes occurred during physiological aging would serve as basis for developing clinical strategies to delay aging. By reviewing the recent development of research on aging-related epigenetic changes, we would be able to design novel preventive and curative strategies for aging and age-related diseases. A deeper knowledge on the critical role of epigenetics in the progression of aging would advance clinical prevention and treatment of aging and aging-related diseases, respectively.[14]

DEFINITION AND ROLE OF EPIGENETICS IN AGING

Epigenetics comes from the words "epi" and "genetic" which mean above and gene, respectively. It studies the interactions between environmental cues, the activity of gene, and the levels of gene expression, which then determine the organismal phenotype.[15] As time progressed, researchers defined epigenetics as the study of heritable alteration marks of gene expression regulators that do not involving DNA sequence modification, changes in phenotype without changing the genotype, but can influence how cells read genes. Based on this, gene expression refers to the event that a protein is formed as a

result of the instructions of a gene in a person's body. In contrast to genetic changes in general, epigenetic changes influence gene expression to easily "activate" and "deactivate" genes due to the environment and daily behavior, such as healthy lifestyle habits, smoking, and others. Epigenetic changes are a normal and natural event but are affected by various factors including biological age, environment, and lifestyle.[16,17] There are various cellular epigenetic systems including methylation of DNA, chromatin rearrangement, and noncoding RNA (ncRNA).[18]

In the following review, we sum up our current understanding of the role of epigenetic regulation in general in aging, which includes DNA methylation, chromatin remodeling, and ncRNA regulation (Figure 1). Furthermore, we also discuss the potential use of epigenetic regulation as a biomarker of aging.

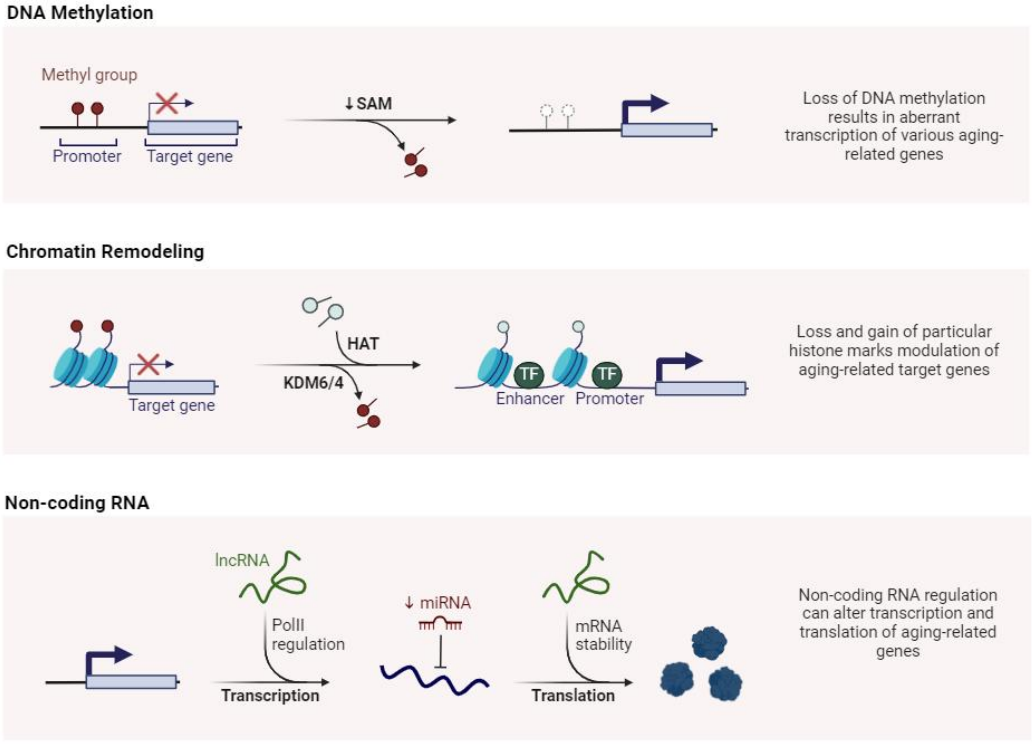

Fig. 1. Alteration in epigenetic regulations that leads to aging (created with BioRender.com)

DNA METHYLATION SIGNATURES IN AGING

Vanyushin et al. (1973) was the first to report variations in 5-methylcytosine (5mC) in mice. DNA methylation is a covalent change in cytosine residue that acts as inhibitor of gene expression.[19] This process can be passed on to descendants through DNA replication and cell division.[20] The methylation of DNA is linked with a variety of biological processes, such as chromosome stability, X chromosome inactivation, genome imprint, differentiation of stem cells, control of gene expression, autoimmune diseases, carcinogenesis, and aging.[21] Several studies have implicated that

methylation is critical for the regulation of cell proliferation and differentiation. Currently, a large body of literature has revealed genome-wide methylation modifications in response to advancing age in various living species. Epigenetic changes in aging can occur systemically or be specific to certain tissues. Aging-induced changes in DNA methylation also occur in germ cells and may be inherited.[22,23]

Methylation of DNA is a process that includes the attachment of a methyl group (CH_3) at carbon position 5 (C5) of the cytosine base of DNA and generally causes inhibition of gene expression.[24] The addition of a methyl group is catalyzed by two families of DNA methyltransferase (DNMT) enzymes, namely DNMT3A and DNMT3B to form new 5-methylcytosine (5mC) from unmethylated CpG island (dinucleotides in the proximity of promoters and are related to the levels of gene expression). Furthermore, the methylation pattern that has been formed would be maintained by DNMT1.[25] In general, DNA methylation in the promoters (generally upstream of the transcription start site). By directly interfering with transcription factors' recognition sequences or other transcriptional machinery in the regulatory region of a gene, methylation of cytosine can inhibit the transcription. Furthermore, additional studies have demonstrated that the inhibition of transcription activating factors or the activation of transcription inhibitory factors that bind to the transcription start site are involved in mechanism of DNA methylation to reduce the transcription of the gene.[26] In contrast, there are reports that methylation of the coding region of a gene may increase transcriptional activity.[27,28]

Conversion of cytosine to 5mC by DNMT occurs almost exclusively in CpG islands, and study showed that around 90% of mammalian CpG islands are marked with methylation.[29] In general, only certain locations in the genome of living organisms have CpGs that are susceptible to methylation. For example, human telomeres do not have CpG sites and are thought to be independent of DNMT activity. However, in mouse embryonic stem cells, telomere length and recombination are regulated by methylation of nearby sub-telomeric regions.[30] The important role of DNA methylation and the enzyme that regulates it, DNMT, has been clearly demonstrated through observations of the phenotype of mice deficient in DNMT activity (Dnmt1, Dnmt3a, or Dnmt3b), where early embryonic death occurs and deletion of Dnmt3a in nerve cells causes mice to have shorter life. In addition, the methylation of DNA is involved in the determination of various biological processes, such as chromosome stability, X chromosome inactivation, genome imprint, differentiation of stem cells, control of gene expression, autoimmune diseases, carcinogenesis, and aging.[31,32]

Aging has a strong correlation with changes in DNA methylation regulation, to the point that some prediction models using a small number of these methylation sites can accurately predict donor chronological age.[33,34] Methylation of DNA and other epigenetic changes have been directly attributed to determine the lifespan of organisms, from low evolutionary levels such as yeast to species with the highest evolutionary level, namely humans. An increasing number of in vitro and in vivo studies show common changes with aging in the form of a global pattern of hypomethylation in non-CpG regions and hypermethylation of CpG islands (Figure 2).[26] Additionally, interventions known to increase life expectancy in humans, such as calorie restriction can directly reduce changes in DNA methylation patterns. [35]Other studies have reported that aging risk factors such as UV-B rays, pollutant, and cigarette smoke can cause genome-wide hypomethylation.[36,37]

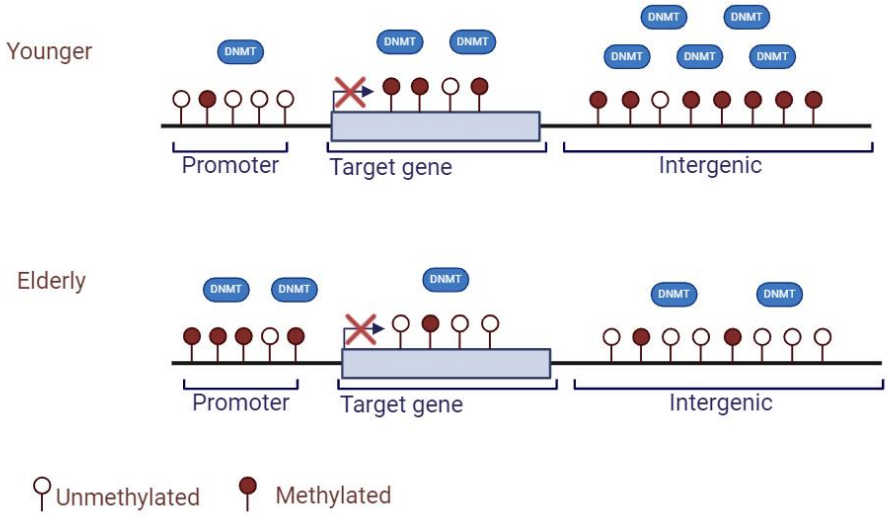

Fig. 2. Overview of the regulatory role of DNA methylation in aging (created with BioRender.com)

The maximum levels of 5mC is recorded during the embryonic stage of life and progressively drops with advancing age in humans.[38] Furthermore, during aging, site-specific hypermethylation is also frequently seen in the genome. For instance, studies show that ribosomal DNA and bivalent chromatin domains are the primary sites of hypermethylation in human aging.[26,39] Based on available data, the reduced global methylation associated with aging could be attributed to either low DNMT expression or inadequate folic acid intake in the elderly.[40] Additionally, studies reveal that hypomethylated regions occur more frequently in regions that have H3K4me1 modifications. [41]However, research on specific hypermethylation mechanisms in certain

regions of the genome is currently still very limited. Some reports indicate that during aging, genomic regions bound by the Polycomb complex are susceptible to hypermethylation.[42]

REGULATION OF CHROMATIN REMODELING IN AGING

DNA and histones make up the dynamic structure known as chromatin, which is found in the nucleus of eukaryotic cells and can be either in euchromatin or heterochromatin states. The portion of the chromosome that lacks DNA sequences encoding proteins is known as heterochromatin, whereas the portion that does has protein-coding DNA sequences is known as euchromatin. The nucleosome core particle, the fundamental building block of chromatin, is surrounded by a histone octamer made up of two histone dimers, H2A-H2B, on either side of a tetramer of histones H3 and H4.[43,44] One of the most important methods that cells use to control access to proteins involved in all DNA-related functions, such as gene transcription, DNA replication, and DNA repair, is chromatin architecture. Histone modifications, chromatin rearrangement, and histone chaperons are just a few of the many dynamic regulators of chromatin that occur to allow physical access to DNA.[43]

Chromatin remodeling is a biological process in which chromatin undergoes confirmation changes from a condensed to relaxed (physically accessible) state, or vice versa, that allow regulation of the physical access of transcription-regulating factors to DNA to control the activity of transcription of the genes. Chromatin rearrangement is a change in genome architecture at chromosomal levels or specific domains (e.g. centromeres). Epigenetic modifications of histone proteins can alter chromatin structure resulting in transcriptional activation or repression of genes.[45] Chromatin remodeling that is repressive on global gene expression has been implicated in the natural aging process.[46] The most convincing evidence that failure in chromatin remodeling can trigger the aging process was the discovery that Sirtuins, which are histone deacetylases (HDACs), directly regulate the aging process in experimental animals and humans.[47] In addition, the role of chromatin remodeling in aging is further proven by the existence of Hutchinson-Gilford progeria syndrome, a congenital disease that causes children to experience premature aging.[48]

Significant remodeling of chromatin structure has been identified as a factor influencing cellular aging, ranging from modifications of histone components to changes in chromatin compartments and topological association domains (TADs).[49] Globally reduced regulation of chromatin, especially histones, across the genome is considered to be a key feature of the cellular aging process.[50] This is supported by

studies showing that increasing histone H3/H4 can extend the life expectancy of animal models, indicating that increasing free histones can facilitate nucleosome exchange and post-transcriptional chromatin repackaging.[51] The loss of approximately 50% of nucleosomes throughout the genome and the redistribution of the remaining nucleosomes during aging can lead to increased transcription of various genes and result in genome instability.[52] One study reported that histone transcription decreases in muscle stem cells during chronological aging.[53]

Progeria or premature senescence is a phenotype displayed by individuals with deletions in one of the isoforms of histone H1. Additionally, fibroblast cells exhibit increased nucleolar instability, less condensed chromosomes, and increased nucleoid relaxation.[54] Aging is accompanied by a general loss of heterochromatin and a separation of lamina-associated domain (LAD) structures from the nuclear lamina. Age-related changes in chromatin structure are correlated with the redistribution of different histone modifications, such as acetylation of histones 3 and 4, which includes H3K9ac, H3K56ac, H4K16ac, and H3K18ac, and methylation of histone 3, including H3K4me3, H3K27me3, H3K36me3, and H3K9me3. Because of the aberrant chromatin accessibility, this results in dysregulated gene expression (Figure 3).[14]

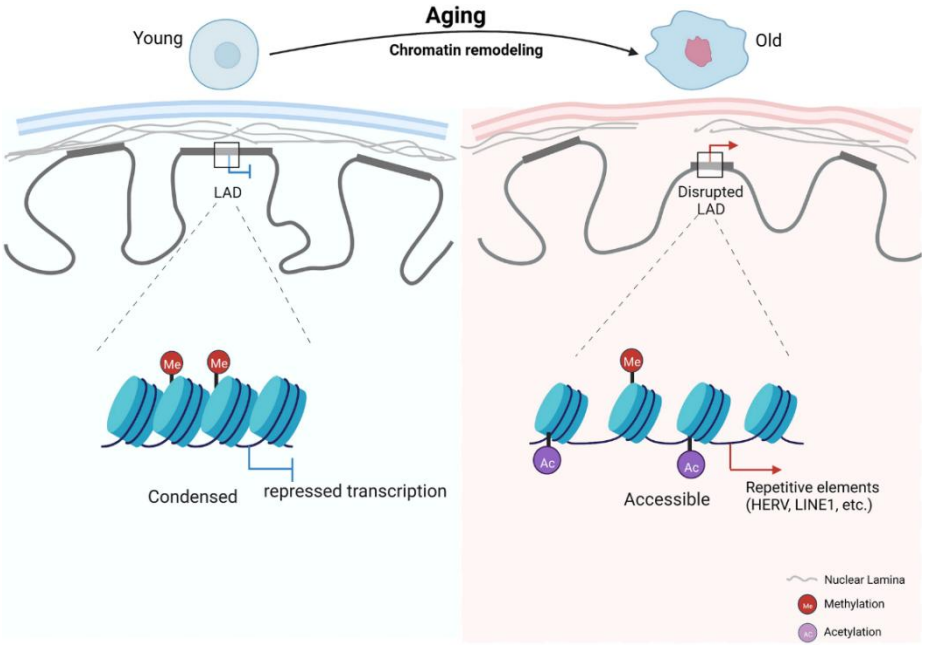

Fig. 3. Impact of aging on chromatin structure.[14]

THE INVOLVEMENTS OF MicroRNA IN AGING PROCESS

RNA that does not code for proteins is called non-coding RNA (ncRNA) and is usually less than 200 nucleotide bases in size, which was previously considered "junk DNA". Since their discovery, ncRNAs have become recognized as crucial regulators of a wide range of biological processes in diverse cell types and tissues, and many diseases has been implicated as a result from ncRNA dysregulation. While other ncRNAs like long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) have also emerged as significant contributors to human disease, many studies have concentrated on the relationship between human cancer and miRNAs.[55,56] Compared to protein-coding RNAs, all ncRNAs are extremely specific for particular tissue types, underscoring their significance in regulating tissue identity and function, particularly as aging occurs.[57]

Among the ncRNAs that have recently been studied the most are miRNAs. MicroRNA is a non-coding single stranded RNA with a length of around 17-25 (generally 22) nucleotide base pairs that controls gene expression in humans, animals, plants and eukaryotic unicellular organisms. In humans, miRNAs play various role in physiological and pathological conditions, including embryogenesis, tissue homeostasis, toxicity and viral infection. In controlling gene expression, microRNA binds to its target mRNA. One miRNA can target hundreds of mRNAs, and acts on its target mRNA transcript causing inhibition or degradation in the translation process according to the level of binding and compatibility between the miRNA and its target.[58–60]

MicroRNA is transcribed as a precursor molecule which is cut by the endonuclease enzyme Drosha and Dicer. Mature miRNA is bound by the Argonaute (AGO) protein family to form a silencing complex, namely the miRNA induced silencing complex (miRISC), which is called RISC loading, the process of entering/loading pri-miRNA into the RISC. The development of knowledge in the analysis of Drosha and Dicer structures and the RISC loading process as key factors in the stages of miRNA biogenesis has revealed this mechanism in more detail.[61]

Certain miRNAs have the ability to bind complementary to the sequences of their target mRNA after they have matured, which can be used to inhibit translation or cause mRNA degradation. Three distinct routes are used to package and deliver additional miRNAs to the extracellular environment: Exosomes, shedding vesicles, apoptotic bodies, and other closed membrane vesicles; (2) lipoproteins like high-density lipoproteins (HDL); or (3) complex bonds to RNA binding proteins like nucleophosmin 1 (NPM1) and Argonaute2 (AGO2).[62]

In general, miRNAs bound to 3′ untranslated region (UTR) of their target mRNAs to trigger degradation, de-capping of the mRNA thereby thwarting translation or deanylation to inhibit translation. However, recent report has found that miRNAs can interact with regions other than 3' UTR, including the 5' UTR, gene body (coding sequence), and gene promoter. Binding to the 5'UTR and coding region causes silencing or no gene expression, while interaction in the promoter region induces transcription.[58–60,63]

It has been shown that miRNAs measure the levels of miRNA expression during tissue aging and also target various factors involved in the molecular pathways of cell aging. Senescent cells accumulate over time and are involved in the regulation of an organism's life span as well as tissue-specific aging. Nowadays, aging is widely thought to be a predestined state of existence that includes the process of growth cessation, which is thought to act as a cancer-prevention mechanism. MiRNAs target molecules involved in the stress response to control the transition from replicating cells to senescent cells, as well as signaling pathways that have been shown to control aging and cancer.[58,64]

MiRNAs have been linked to various cellular functions and the general aging process in humans. It is well known that aging characteristics are related to one another. For instance, telomere shortening, a response to DNA damage, is a hallmark of cellular aging, as are alterations in the cytokine profile released by aging-related cells. Similarly, several miRNAs linked to aging are regulators that coincide with the characteristics of aging. For instance, the miR-29 family controls DNA methylation genes and is linked to stem cell weakness; miR-34a has been linked to mitochondrial dysfunction and telomere shortening. These show that many miRNAs work together to play an integrated role in the aging process, though it's possible that the effects of individual miRNAs vary somewhat depending on the type of tissue.[64] MiRNAs appear to have a dual function in the context of tissue aging, meaning they can have both positive and negative effects on the aging of different human organs and tissues (Figure 4).[65]

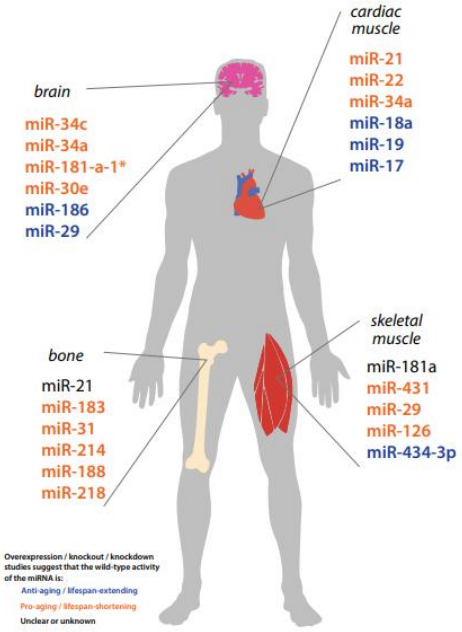

Fig. 4. Some examples of miRNAs involved in organ aging.[65]

It has been demonstrated that miRNAs target different genes involved in the molecular pathways of cell aging in addition to measuring the levels of miRNA expression during tissue aging. Senescent cells gradually build up and play a role in both tissue-specific aging and the overall control of an organism's life span. These days, aging is commonly understood to be a predetermined state of living things that includes the process of growth cessation—a mechanism believed to serve as a defense against cancer. MiRNAs target molecules that suppress cancer, signaling pathways that have been demonstrated to control aging, and components involved in the stress response to regulate the transition from replicating cells to senescent cells.[66–68]

Various miRNAs can regulate the expression of genes involved in aging pathways at the cellular level. Stress-induced cellular senescence, for example, can be regulated by MAPK and interleukin pathways. Several miRNAs, such as miR-15b, miR-24, miR-25 and miR-141 can target MAP2K4 for degradation. Some miRNAs also regulate the p53 and p21 pathways which are apoptotic pathways. In this context, p53 activates the synthesis of miR-34a which can target SIRT1. p21 is negatively correlated with the activity of miR-108b, miR-130b, miR-20a, and so on. Some miRNAs regulate factors associated with mitochondrial aging, for example miR-34a and miR-335 target important factors in free radical metabolism in the mitochondrial respiratory chain. MiR-29 and miR-30 can directly target retinoblastoma protein (pRB).[69,70]In general, at the molecular level, miRNAs can directly or indirectly influence cellular mechanisms involved in cell aging programs, namely the p53-p21 and p16-pRB pathways (Figure 5).

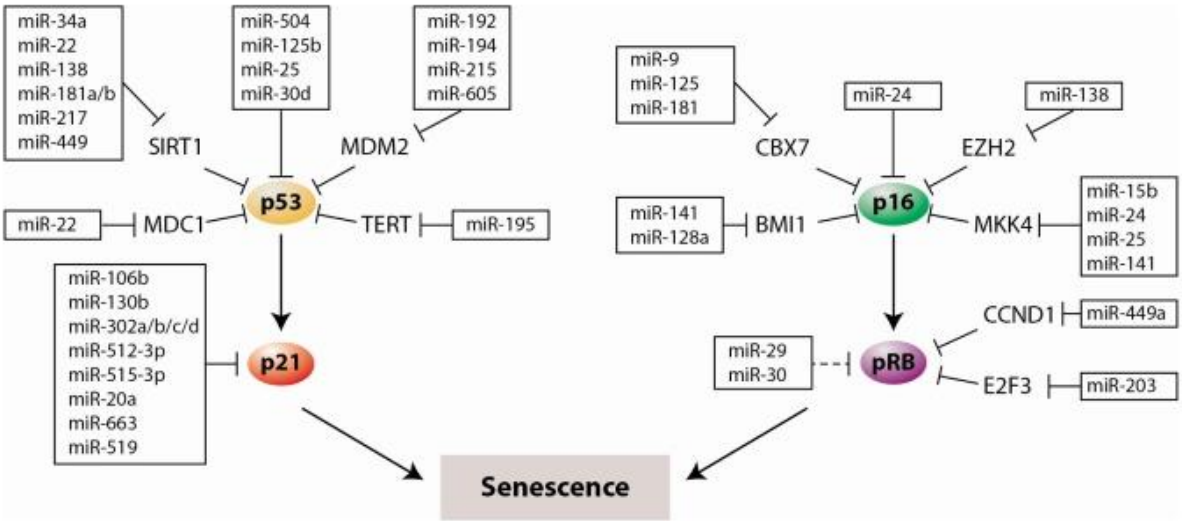

Fig. 5. List of known miRNAs involved in the molecular pathways of cell aging.[70]

Numerous studies have documented the secretion of miRNAs from cells into peripheral body fluids through the use of microvesicles and exosomes, whereby the microRNAs bind to lipoproteins or Argonaute 2.[71] Blood, saliva, cerebrospinal fluid, urine, and at least 12 other human bodily fluids have all been found to contain these systemic circulating miRNAs. Because miRNAs are present in the circulatory system at very high levels, are stable over time, and have been shown to alter with advancing age, scientists and medical professionals are keen to learn more about them and apply them as noninvasive aging biomarkers.[4] Recent research has examined the differences in miRNA expression in a variety of peripheral fluids, such as serum, plasma, and saliva, between young and elderly people. Attempts to link miRNA profiles in the circulatory system directly to lifespan in humans have also been reported.[65]

In addition to their potential as aging biomarkers, our understanding of the role of miRNAs in aging is expanding, and with it, so is the therapeutic potential of modulating specific miRNAs to change the course of aging. The anti-aging role of advantageous miRNAs can be replaced by synthetic RNA molecules that are engineered to resemble endogenous miRNAs. In contrast, compounds known as anti-miRNAs (antimiRs) and/or synthetic miRNA targets that decrease active miRNAs may be employed to lessen the effect of miRNAs on aging.[72,73]

CONCLUSION

Numerous *in vitro*, *in vivo*, and clinical investigations have demonstrated epigenetic alterations in DNA, RNA, and histone modifications, along with modifications in the state of chromatin structure, that are associated with aging. Consequently, it can be referred that these epigenetic modifications could serve as biomarkers or targets for anti-aging therapies. Numerous epigenetic alterations associated with aging have been extensively researched, such as a genome-wide reduction in the methylation of genomic DNA, a genome-wide loss of histones, chromatin landscape rearrangement, and ncRNA regulation of genes linked to aging. More recent and sophisticated biomolecular technologies, like single-cell omics sequencing, offer a greater resolution for deciphering the epigenetic traits associated with aging. Furthermore, additional information regarding the interplay between epigenomic factors and other aging-related factors can be obtained from spatiotemporal genomic, transcriptomic, and proteomic atlases in diverse mammalian tissues. Many lifestyle modification techniques can be used to treat and prevent age-related diseases based on epigenetic changes in cells during aging. For instance, aging can be effectively postponed with diet and exercise that modify epigenetic landscape. Promising clinical treatment strategies to intervene in aging and treat diseases associated with aging

also include drugs that target important molecular and cellular changes linked to aging.

It is still unknown how epigenetic modifications interact with other factors, including their relationship to genetic factors and even the microbiome, despite significant scientific advancements at the molecular level of aging. It is also unknown exactly how different lifestyle choices, physiological, and psychological factors affect aging at the level of epigenetic modifications. In order to gain a better understanding of whether and how epigenetic regulation affects each stage of aging, long-term observational cohort studies are required because aging is a continuous process that takes place in humans over many years.

ACKNOWLEDGMENTS

This manuscript was supported by Grant-in-Aid from Atma Jaya Catholic University of Indonesia (Hibah Dosen Pemula tahun 2024), grant no. 162.24/III/LPPM-PM.10.01/02/2024, to FMS.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

REFERENCES

1. Heryanah, H. Ageing Population Bonus Demografi Kedua di Indonesia. *Populasi* **23**, 1 (2015).

2. Van Hoof, J., Kazak, J. K., Perek-Białas, J. M. & Peek, S. T. M. The Challenges of Urban Ageing: Making Cities Age-Friendly in Europe. *Int. J. Environ. Res. Public Health* **15**, 2473 (2018).

3. Guo, J. *et al.* Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduct. Target. Ther.* **7**, 391 (2022).

4. Kumar, S., Vijayan, M., Bhatti, J. S. & Reddy, P. H. *MicroRNAs as Peripheral Biomarkers in Aging and Age-Related Diseases*. *Progress in Molecular Biology and Translational Science* vol. 146 (Elsevier Inc., 2017).

5. Rando, T. A. & Chang, H. Y. Aging, Rejuvenation, and Epigenetic Reprogramming: Resetting the Aging Clock. *Cell* **148**, 46–57 (2012).

6. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The Hallmarks of Aging. *Cell* **153**, 1194–1217 (2013).

7. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: An expanding universe. *Cell* **186**, 243–278 (2023).

8. Tartiere, A. G., Freije, J. M. P. & López-Otín, C. The hallmarks of aging as a conceptual framework for health and longevity research. *Front. Aging* **5**,

(2024).

9. Gonzalo, S. Epigenetic alterations in aging. *J. Appl. Physiol.* **109**, 586–597 (2010).

10. Kanherkar, R. R., Bhatia-Dey, N. & Csoka, A. B. Epigenetics across the human lifespan. *Front. Cell Dev. Biol.* **2**, (2014).

11. la Torre, A., Lo Vecchio, F. & Greco, A. Epigenetic Mechanisms of Aging and Aging-Associated Diseases. *Cells* **12**, 1163 (2023).

12. Pal, S. & Tyler, J. K. Epigenetics and aging. *Sci. Adv.* **2**, (2016).

13. Jung, A. M. *et al.* Associations Between Epigenetic Age Acceleration and microRNA Expression Among U.S. Firefighters. *Epigenetics Insights* **16**, (2023).

14. Wang, K. *et al.* Epigenetic regulation of aging: implications for interventions of aging and diseases. *Signal Transduct. Target. Ther.* **7**, 374 (2022).

15. Deichmann, U. Epigenetics: The origins and evolution of a fashionable topic. *Dev. Biol.* **416**, 249–254 (2016).

16. Mc Auley, M. T. An evolutionary perspective of lifespan and epigenetic inheritance. *Exp. Gerontol.* **179**, 112256 (2023).

17. Peixoto, P., Cartron, P.-F., Serandour, A. A. & Hervouet, E. From 1957 to Nowadays: A Brief History of Epigenetics. *Int. J. Mol. Sci.* **21**, 7571 (2020).

18. Agarwal, G. *et al.* Epigenetics and epigenomics: underlying mechanisms, relevance, and implications in crop improvement. *Funct. Integr. Genomics* **20**, 739–761 (2020).

19. Teschendorff, A. E. & Relton, C. L. Statistical and integrative system-level analysis of DNA methylation data. *Nat. Rev. Genet.* **19**, 129–147 (2018).

20. Schübeler, D. Function and information content of DNA methylation. *Nature* **517**, 321–326 (2015).

21. Nazor, K. L. *et al.* Recurrent Variations in DNA Methylation in Human Pluripotent Stem Cells and Their Differentiated Derivatives. *Cell Stem Cell* **10**, 620–634 (2012).

22. Atsem, S. *et al.* Paternal age effects on sperm FOXK1 and KCNA7 methylation and transmission into the next generation. *Hum. Mol. Genet.* ddw328 (2016) doi:10.1093/hmg/ddw328.

23. Potabattula, R. *et al.* Allele-specific Methylation of Imprinted Genes in Fetal Cord Blood is Influenced By Cis-Acting Genetic Variants and Parental Factors.

Epigenomics **10**, 1315–1326 (2018).

24. Mattei, A. L., Bailly, N. & Meissner, A. DNA methylation: a historical perspective. *Trends Genet.* **38**, 676–707 (2022).

25. Baghel, V. S. *et al.* Inhibitors targeting epigenetic modifications in cancer. in *Transcription and Translation in Health and Disease* 287–324 (Elsevier, 2023). doi:10.1016/B978-0-323-99521-4.00007-6.

26. Xiao, F.-H., Wang, H.-T. & Kong, Q.-P. Dynamic DNA Methylation During Aging: A "Prophet" of Age-Related Outcomes. *Front. Genet.* **10**, (2019).

27. Yang, X. *et al.* Gene Body Methylation Can Alter Gene Expression and Is a Therapeutic Target in Cancer. *Cancer Cell* **26**, 577–590 (2014).

28. Neri, F. *et al.* Intragenic DNA methylation prevents spurious transcription initiation. *Nature* **543**, 72–77 (2017).

29. Kondo, M. *et al.* A newly developed age estimation method based on CpG methylation of teeth-derived DNA using real-time methylation-specific PCR. *J. Oral Sci.* **63**, 54–58 (2021).

30. Johnson, A. A. *et al.* The Role of DNA Methylation in Aging, Rejuvenation, and Age-Related Disease. *Rejuvenation Res.* **15**, 483–494 (2012).

31. Reale, A., Tagliatesta, S., Zardo, G. & Zampieri, M. Counteracting aged DNA methylation states to combat ageing and age-related diseases. *Mech. Ageing Dev.* **206**, 111695 (2022).

32. Lu, A. T. *et al.* Universal DNA methylation age across mammalian tissues. *Nat. Aging* **3**, 1144–1166 (2023).

33. Jones, M. J., Goodman, S. J. & Kobor, M. S. <scp>DNA</scp> methylation and healthy human aging. *Aging Cell* **14**, 924–932 (2015).

34. Horvath, S. & Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* **19**, 371–384 (2018).

35. Maegawa, S. *et al.* Caloric restriction delays age-related methylation drift. *Nat. Commun.* **8**, 539 (2017).

36. De Prins, S. *et al.* Influence of ambient air pollution on global DNA methylation in healthy adults: A seasonal follow-up. *Environ. Int.* **59**, 418–424 (2013).

37. Puttipanyalears, C., Subbalekha, K., Mutirangura, A. & Kitkumthorn, N. Alu Hypomethylation in Smoke-Exposed Epithelia and Oral Squamous Carcinoma. *Asian Pacific J. Cancer Prev.* **14**, 5495–

5501 (2013).

38. Goel, N., Karir, P. & Garg, V. K. Role of DNA methylation in human age prediction. *Mech. Ageing Dev.* **166**, 33–41 (2017).

39. Kumar, D., Cinghu, S., Oldfield, A. J., Yang, P. & Jothi, R. Decoding the function of bivalent chromatin in development and cancer. *Genome Res.* **31**, 2170–2184 (2021).

40. Ciccarone, F. *et al.* Age-dependent expression of DNMT1 and DNMT3B in PBMCs from a large European population enrolled in the MARK-AGE study. *Aging Cell* **15**, 755–765 (2016).

41. Fernández, A. F. *et al.* H3K4me1 marks DNA regions hypomethylated during aging in human stem and differentiated cells. *Genome Res.* **25**, 27–40 (2015).

42. Jung, M. & Pfeifer, G. P. Aging and DNA methylation. *BMC Biol.* **13**, 7 (2015).

43. Chen, P., Li, W. & Li, G. Structures and Functions of Chromatin Fibers. *Annu. Rev. Biophys.* **50**, 95–116 (2021).

44. Nothof, S., Magdinier, F. & Van-Gils, J. Chromatin Structure and Dynamics: Focus on Neuronal Differentiation and Pathological Implication. *Genes (Basel).* **13**, 639 (2022).

45. Tabassum, H. & Parvez, S. Translational epigenetics in neurodegenerative diseases. in *Epigenetics and Metabolomics* 297–313 (Elsevier, 2021). doi:10.1016/B978-0-323-85652-2.00020-8.

46. Purohit, J. S. & Chaturvedi, M. M. Chromatin and Aging. in *Topics in Biomedical Gerontology* 205–241 (Springer Singapore, Singapore, 2017). doi:10.1007/978-981-10-2155-8_11.

47. Zhao, L. *et al.* Sirtuins and their Biological Relevance in Aging and Age-Related Diseases. *Aging Dis.* **11**, 927 (2020).

48. Batista, N. J. *et al.* The Molecular and Cellular Basis of Hutchinson–Gilford Progeria Syndrome and Potential Treatments. *Genes (Basel).* **14**, 602 (2023).

49. Liu, Z. *et al.* Large-scale chromatin reorganization reactivates placenta-specific genes that drive cellular aging. *Dev. Cell* **57**, 1347-1368.e12 (2022).

50. Dubey, S. K., Dubey, R. & Kleinman, M. E. Unraveling Histone Loss in Aging and Senescence. *Cells* **13**, 320 (2024).

51. Feser, J. *et al.* Elevated Histone Expression Promotes Life Span Extension. *Mol. Cell* **39**, 724–735 (2010).

52. Hu, Z. *et al.* Nucleosome loss leads to

global transcriptional up-regulation and genomic instability during yeast aging. *Genes Dev.* **28**, 396–408 (2014).

53. Liu, L. *et al.* Chromatin Modifications as Determinants of Muscle Stem Cell Quiescence and Chronological Aging. *Cell Rep.* **4**, 189–204 (2013).

54. Flex, E. *et al.* Aberrant Function of the C-Terminal Tail of HIST1H1E Accelerates Cellular Senescence and Causes Premature Aging. *Am. J. Hum. Genet.* **105**, 493–508 (2019).

55. Nemeth, K., Bayraktar, R., Ferracin, M. & Calin, G. A. Non-coding RNAs in disease: from mechanisms to therapeutics. *Nat. Rev. Genet.* **25**, 211–232 (2024).

56. Arif, K. M. T., Elliott, E. K., Haupt, L. M. & Griffiths, L. R. Regulatory Mechanisms of Epigenetic miRNA Relationships in Human Cancer and Potential as Therapeutic Targets. *Cancers (Basel).* **12**, 2922 (2020).

57. de Goede, O. M. *et al.* Population-scale tissue transcriptomics maps long non-coding RNAs to complex disease. *Cell* **184**, 2633-2648.e19 (2021).

58. Melo, C. A. & Melo, S. A. Biogenesis and Physiology of MicroRNAs. in *Non-coding RNAs and Cancer* (ed. Fabbri, M.) 5–25 (Springer Science+Business Media, 2014). doi:10.1007/978-1-4614-8444-8.

59. Zaravinos, A. The Regulatory Role of MicroRNAs in EMT and Cancer. *J. Oncol.* **2015**, 3–4 (2015).

60. Ni, W. & Leng, X. Dynamic miRNA – mRNA paradigms : New faces of miRNAs. *Biochem. Biophys. Reports* **4**, 337–341 (2015).

61. Treiber, T., Treiber, N. & Meister, G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat. Rev. Mol. Cell Biol.* **20**, 5–20 (2019).

62. Liang, H., Gong, F., Zhang, S., Zhang, C. & Zen, K. The origin , function , and diagnostic potential of extracellular microRNAs in human body fluids. **5**, (2014).

63. O'Brien, J., Hayder, H., Zayed, Y. & Peng, C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front. Endocrinol. (Lausanne).* **9**, 1–12 (2018).

64. Harries, L. W. MicroRNAs as Mediators of the Ageing Process. 656–670 (2014) doi:10.3390/genes5030656.

65. Kinser, H. E. & Pincus, Z. MicroRNAs as modulators of longevity and the aging process. *Hum. Genet.* **139**, 291–308 (2020).

66. Wang, Z., Gao, J. & Xu, C. Tackling cellular

senescence by targeting miRNAs. *Biogerontology* **23**, 387–400 (2022).

67. Potter, M. L., Hill, W. D., Isales, C. M., Hamrick, M. W. & Fulzele, S. MicroRNAs are critical regulators of senescence and aging in mesenchymal stem cells. *Bone* **142**, 115679 (2021).

68. Nikolajevic, J. *et al.* The Role of MicroRNAs in Endothelial Cell Senescence. *Cells* **11**, 1185 (2022).

69. Smith-Vikos, T. & Slack, F. J. MicroRNAs and their roles in aging. *J. Cell Sci.* **125**, 7–17 (2012).

70. Suh, N. MicroRNA controls of cellular senescence. *BMB Rep.* **51**, 493–499 (2018).

71. Jung, H. J. & Suh, Y. Circulating miRNAs in Ageing and Ageing-Related Diseases. *J. Genet. Genomics* **41**, 465–472 (2014).

72. Koch, L. microRNAs as systemic regulators of ageing. *Nat. Rev. Genet.* **24**, 415–415 (2023).

73. Eshkoor, S. A., Ghodsian, N. & Akhtari-Zavare, M. MicroRNAs influence and longevity. *Egypt. J. Med. Hum. Genet.* **23**, 105 (2022).