Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: December 31, 2023

Citation: Watcharanurak, P., and Mutirangura, A., 2023. Genome wide hypomethylation and youth-associated DNA gap reduction promoting DNA damage and senescence-associated pathogenesis. Medical Research Archives, [online] 11(12). https://doi.org/10.18103/mra.v11i12.4952

DOI: https://doi.org/10.18103/mra.v11i12.4952

ISSN: 2375-1924

RESEARCH ARTICLE

Genome wide hypomethylation and youth-associated DNA gap reduction promoting DNA damage and senescence-associated pathogenesis

Papitchaya Watcharanurak[1] and Apiwat Mutirangura[2*]

[1]Faculty of Medical Technology, Prince of Songkla University, Hat Yai, Songkhla, Thailand. [2]Center of Excellence in Molecular Genetics of Cancer and Human Disease, Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand.

[*]apiwat.mutirangura@gmail.com

ABSTRACT

Background: Age-associated epigenetic alteration is the underlying cause of DNA damage in aging cells. Two types of youth-associated DNA-protection epigenetic marks, global methylation, and youth-associated genomic stabilization DNA gap (youth-DNA-gap) reduce when cell ages. The epigenomic mark reduction promotes DNA damage and accelerates aging hallmarks. While DNA hypomethylation destabilizes DNA by several mechanisms, the DNA sequence around the youth-DNA-gap is hypermethylated. Therefore, the genomic instability mechanisms underlying DNA hypomethylation and youth-DNA-gap reduction are linked.

Results: DNA gap prevents DNA damage by relieving the torsion forces caused by a twisted wave during DNA strand separation by transcription or replication. When the cells begin to age, hypomethylation and youth-DNA-gap reduction can occur as consequences of the efflux of intranuclear HMGB1. The methylated DNA gaps are formed by several proteins. Box A of HMGB1 possesses a molecular scissor role in producing youth-DNA-gaps. So the lack of a gap-producing role of HMGB1 results in a youth-DNA-gap reduction. The histone deacetylation role of SIRT1, an aging prevention protein, prevents DNA ends of youth-DNA-gaps from being recognized as pathologic DNA breaks. Youth-DNA-gaps are methylated and determined genome distribution by AGO4, an effector protein in RNA-directed DNA methylation. The lack of intranuclear HMGB1 promotes global hypomethylation due to two subsequent mechanisms. First is the loss of AGO4-methylating DNA. The other is the accumulation of DNA damage due to lacking HMGB1-produced DNA gap promoting DNA demethylation while undergoing DNA repair. DNA torsion due to youth-DNA-gap reduction increases DNA damage and, consequently, the DNA damage response (DDR). Persistent DDR promotes cellular senescence. Accumulating senescent cells leads to the deterioration of the structure and function of the human body. Rejuvenating DNA (RED) by adding DNA protection epigenetic marks using genomic stability molecule (GEM) such as box A of HMGB1 increases DNA durability, limits DNA damage, rejuvenates senescence cells, and improves organ structure and function deterioration due to aging.

Conclusion: Reducing youth-associated epigenetic marks is degenerative diseases' primary molecular pathogenesis mechanism. REDGEM is a new therapeutic strategy inhibiting the upstream molecular aging process that will revolutionize the treatment of DNA damage or age-associated diseases and conditions.

Keywords: DNA protection, DNA methylation, youth-associated genomic stabilization DNA gap, youth-DNA-gap, global hypomethylation, DNA damage, genomic instability, senescence, aging, rejuvenation, RIND-EDSB, Box A of HMGB1, Rejuvenating DNA by genomic stability molecule, REDGEM.

Introduction

Age increases the risk of numerous health issues from deterioration and failure of organ functions (1-5). The pathogenesis of these deteriorations may result from the cellular aging process (6, 7). Determining the nidus of the aging process and applying molecular therapy to edit the nidus can completely rejuvenate cells and reverse organ structure and function (8, 9). DNA represents a "book of life" that dictates the phenotype of living organisms. While the aging process changes the structure and function of various molecules in the human body, the DNA alterations, genomic instability and epigenetic changes, found in aging cells is the primary hallmarks of aging (10-12). Damage to DNA can cause a persistent DNA damage response (DDR) that drives cellular senescence (12-15). Therefore, the key mechanism underlying the aging process is likely DNA changes that reduce DNA durability (9, 16, 17). Here, we reviewed two epigenetic marks that drift down with aging. These reductions cause DNA fragility and endogenous DNA damage accumulation. Therefore, the drift of epigenetic marks may be the aging process nidus.

We recently showed that restoring epigenetic marks improves DNA durability, reduces DNA damage and DDRs, and rejuvenates organ structure and function (9, 17). This article reviews the roles of epigenetic marks in DNA protection and rejuvenation and will describe how they are formed, how they protect DNA, how they are drifted down in elderly individuals, and how the drift drives the aging process. In addition, we discuss if editing the epigenetic marks may play a therapeutic role for noncommunicable diseases (NCDs).

Youth-associated DNA-protection epigenetic marks

Two epigenetic marks, genome-wide DNA methylation and naturally occurring DNA gaps, are present in larger numbers in younger individuals relative to older individuals and play a role in DNA protection **(Fig. 1)** (9, 16-18). DNA methylation can be classified depending on the DNA sequences and functions. DNA methylation of unique sequences mainly occurs at regulatory sequences and plays a role in gene regulation (19). Interspersed repetitive sequence (IRS) methylation plays a role in both gene regulation and genomic stabilization (20-27). The DNA hypomethylation of IRSs, particularly Alu elements and human endogenous retrovirus K and long interspersed nucleotide element-1 (LINE-1) to a lesser degree, is a common epigenomic alteration in elderly individuals (28-31). Alu hypomethylation has also been demonstrated in patients with age-associated NCDs, including diabetes, osteoporosis and essential hypertension (32-34). Interestingly, in newborns, Alu methylation is directly associated with the growth rate (35). Therefore, Alu hypomethylation is an epigenotype of aging.

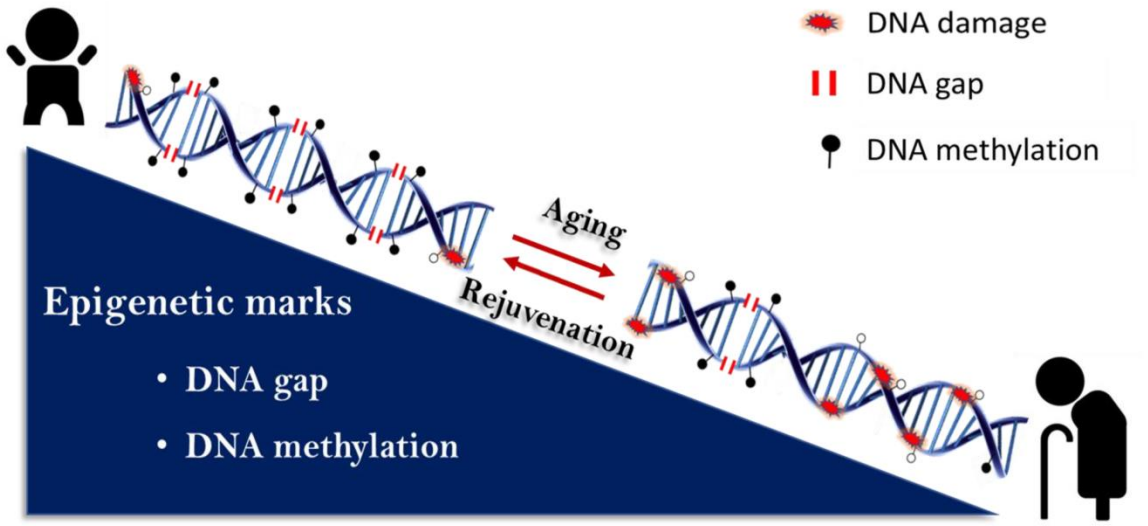

Figure 1. Youth-associated DNA protection epigenetic marks. *In elderly individuals, the number of DNA gaps and the level of DNA methylation decrease, and reductions in both epigenetic marks promote endogenous DNA damage. Epigenetic editing by adding the epigenetic marks can result in rejuvenation.*

Eukaryotic DNA contains naturally occurring DNA gaps, previously named physiological replication-independent endogenous DNA double strand breaks (RIND-EDSBs) (9, 36, 37). The number of DNA gaps is low in chronological aging yeast, old rats, senescent cells, elderly individuals (9, 16). DNA gaps are found to be inversely associated with HbA1c levels in type 2 diabetes mellitus (38). Reducing DNA gaps causes spontaneous DNA shearing (16, 39), while increasing DNA gaps increases DNA durability, decreases endogenous DNA damage and promotes rejuvenation (9). Therefore, we renamed the gaps youth-associated genomic stabilization DNA gaps (youth-DNA-gaps) **(Fig. 1)** (9). HMGB1 acts as molecular scissors to produce DNA gaps **(Fig. 2)** (9). Youth-DNA-gaps are produced by cellular enzymes and prevent DNA damage. Therefore, similar to IRS methylation, youth-DNA-gaps are DNA-protection epigenetic marks. Interestingly, both marks are linked. CpG dinucleotides around youth-DNA-gaps are hypermethylated, and the hypomethylated genome possesses scant youth-DNA-gaps **(Figs. 1, 2 and 3)** (36).

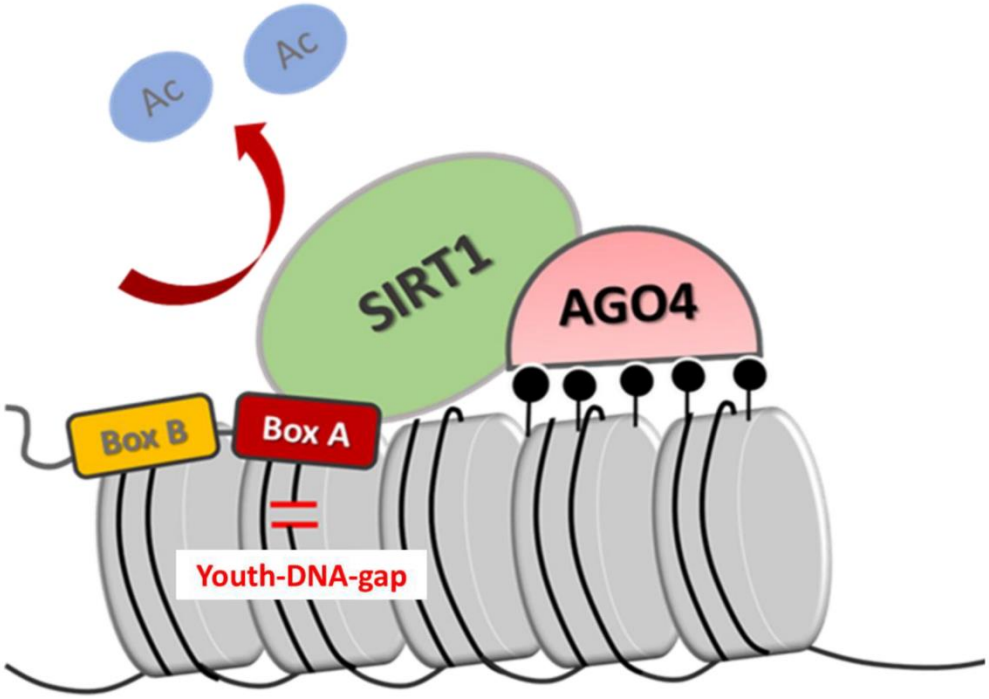

Figure 2. A youth-DNA-gap complex, *Box A of HMGB1 acts as molecular scissors to produce youth-DNA-gaps. AGO4 plays a role in RdDM for methylating DNA around the youth-DNA-gap. SIRT1 deacetylates histones to compact chromatin to hide youth-DNA-gaps from the DDR.*

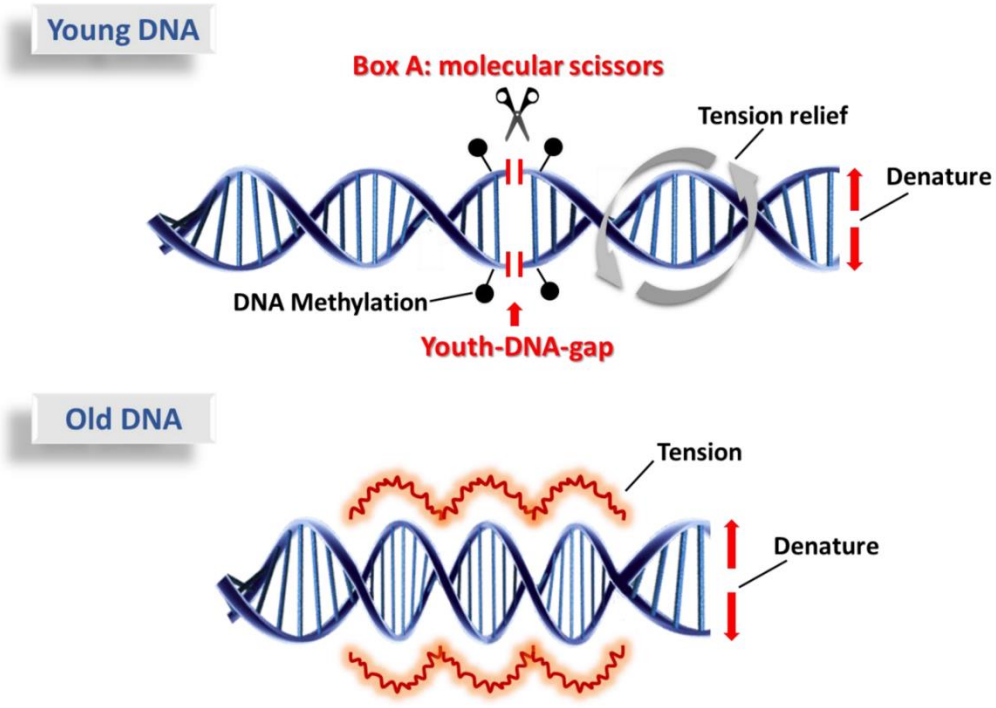

Figure 3. Box A produces youth-DNA-gaps that play a role in DNA protection. *DNA denaturation from DNA replication or transcription promotes torsional stress. The DNA gap helps relieve the torsion force by allowing free spin of DNA. Old DNA has fewer youth-DNA-gaps than young DNA.*

Causes of global hypomethylation

The actual event that causes global hypomethylation in aging has not yet been determined. In early embryogenesis and cell differentiation, ten-eleven translocation (TET) proteins is responsible for the global demethylation process (40, 41). TET also demethylate DNA during the DNA repair process (17, 42-44). TET enzymes generate 5-hydroxymethylcytosine (5hmC) during active DNA demethylation and 5hmC is common at DNA damage foci (45-47). Therefore, when DNA is widely damaged, DNA repair will result in genome-wide hypomethylation **(Fig. 4)**.

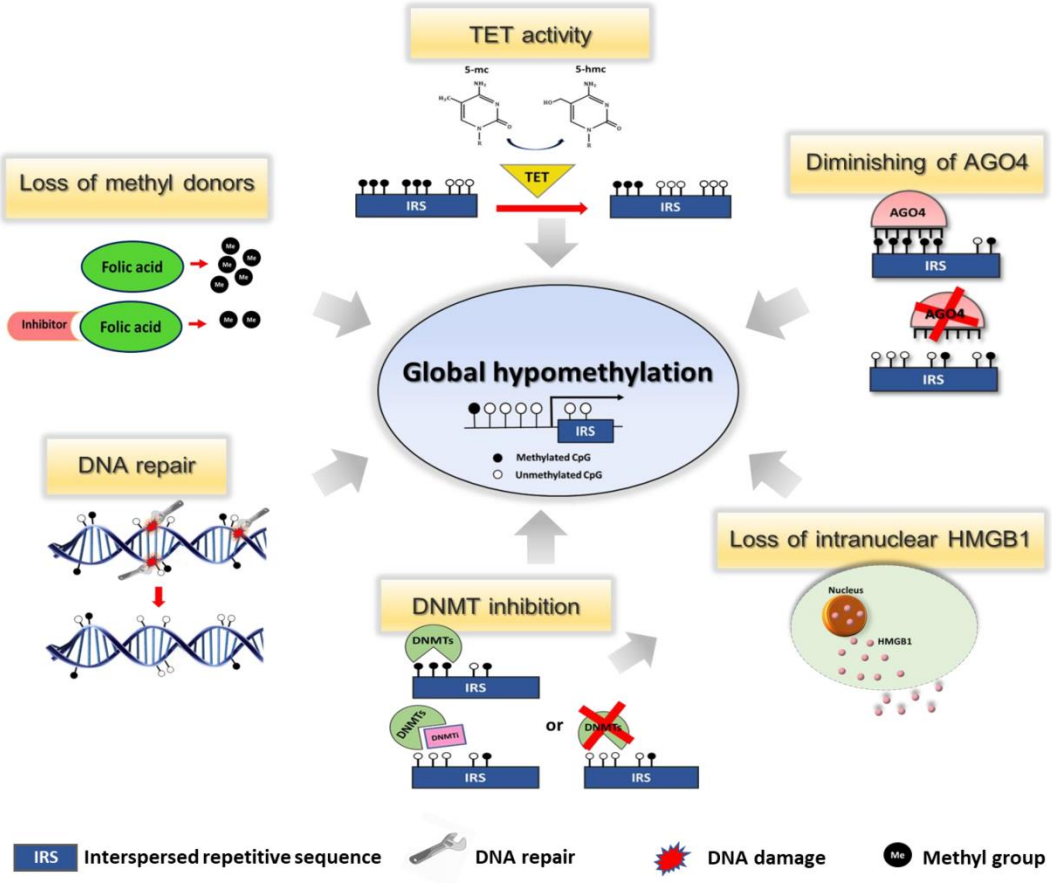

Figure 4. Summary of the causes of global hypomethylation. *The induction of global hypomethylation is caused by several mechanisms, including the demethylation activity of ten-eleven translocation (TET) proteins, loss of methyl donors, the DNA repair process, the inhibition of DNA methyltransferases or AGO4, and loss of intranuclear HMGB1.*

Genome-wide hypomethylation may also be caused by a generalized reduction in the DNA methylation capacity of the cells **(Fig. 4)**. Knocking down DNA methyltransferases (DNMTs) and diminishing methyl donors, such as folic acid, vitamin B12, choline, and DNMT inhibitors, lead to DNA hypomethylation (26, 48-51). A newly described mechanism that methylates IRS is RNA-directed DNA methylation (RdDM) (52-54). Human RdDM is mediated by Argonaut 4 (AGO4), which is primarily bound to IRSs (52). Diminishing AGO4 function also leads to IRS hypomethylation (52). Finally, limited intranuclear HMGB1 causes decreased IRS methylation **(Fig. 4)** (55). The mechanism by which HMGB1 loss leads to genome-wide hypomethylation will be discussed in a later section.

Genome-wide hypomethylation also occurs when cells are exposed to hazardous environments, such as smoke, benzene, burns, and oxidative stress (56-59). Environmental hazards are also associated with DNA damage, which leads to demethylation and repair, and HMGB1 release (60, 61).

DNA hypomethylation causing genomic instability

DNA hypomethylation causing genomic instability has been described for several decades. One of the natural events indicated the link between DNA hypomethylation and genomic instability was reported in ICF syndrome (62, 63). ICF syndrome or immunodeficiency, centromeric region instability, and facial anomalies are caused by DNMT3B mutation (62). ICF patients commonly have DNA hypomethylation and chromosomal rearrangements at the pericentromeric region or satellites. This colocalization between hypomethylation and mutation suggests that hypomethylated DNA is prone to being broken. Similar findings were found in cells treated with a DNA demethylating agent (64). DNA mutations, chromosomal instability, and tumors also developed in cells, and mice were promoted when DNA was hypomethylated (25, 26, 65).

DNA methylation is known to prevent mutations by several mechanisms. First, the mismatch repair (MMR) system requires DNMT1 to form a complex and properly function (66-68). Both MMR and DNMT1 interact with replication machinery, while DNMT1 plays a role in post-replicative maintenance of DNA methylation (69, 70). MMR in repairing replication errors of hypomethylated genome may be limited **(Fig. 5)**. Second, DNA methylation at the promoter of retrotransposable elements, such as LINE-1, prevents the retrotransposable element transcription process and consequently prevents DNA rearrangement from the element genome insertion step (21, 24). LINE-1 may also indirectly promote instability by promoting intracellular inflammation. The intermediate form of LINE-1 retrotransposition also promotes the intracellular inflammation process during late senescence (71). Third, DNA methylation is associated with histone compaction, being essential for the maintenance of genome stability (72). Fourth, the association with heterochromatin also results in different DNA double-strand break (DSB) repair precision, which DSB repair within a heterochromatic region is ATM-mediated and is more precise than general DNA-PKcs mediated nonhomologous end-joining repairs (73-75). Fifth, DNA hypomethylation may alter DNA repair gene regulation, resulting in genomic instability. For example, hypomethylation of intragenic LINE-1 of the PPP2R2B gene downregulates the gene, and the gene's function is to regulate the nuclear translocation of ATM, a DNA repair protein (21, 76). LINE-1 hypomethylation is a generalized process in cancer, meaning that most LINE-1s are synchronously hypomethylated (77). Therefore, global hypomethylation can cause defects in DNA repair by dysregulating the PPP2R2B pathway. Finally, we proposed a new mechanism that weakens the chemical bonds of DNA due to the limited number of methylated youth-DNA-gaps **(Fig. 5)** (9, 36, 55).

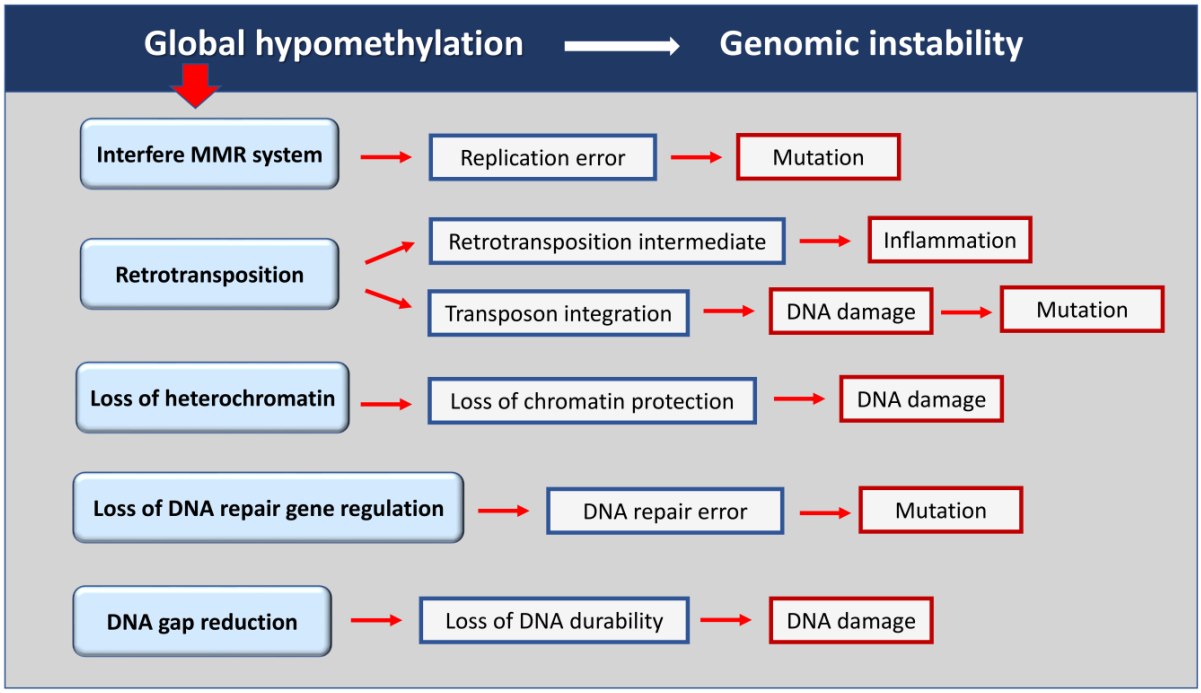

Figure 5. Schematic diagram of the molecular mechanisms by which global hypomethylation drives genomic instability. The mechanisms include MMR system interference, retrotransposition, loss of heterochromatin, loss of DNA repair gene regulation, and DNA gap reduction.

We reported that DNA methylation prevents all kinds of DNA damage, including base change, 8-OHdG, base loss, AP sites, and DNA breaks (17, 55). First, we identified an inverse correlation between Alu element methylation and the endogenous DNA damage level. Then, we used Alu siRNA to increase Alu methylation by AGO4 mediated RdDM (17, 52). The Alu siRNA-transfected cells showed reduced endogenous DNA damage, increased DNA damaging agent resistance, and improved cell proliferation. Under the same mechanism, SINE siRNA was applied to effectively treat burn and diabetic wounds in rats (78, 79). Interestingly, the DNA protection effect of de novo Alu methylation extended far beyond the methylated loci. While Alu siRNA increased Alu methylation over approximately 1/10 of the genome, approximately 100,000 loci, endogenous DNA damage was reduced to 7/10 of the genome. Therefore, each methylation locus extends DNA stabilization by approximately 21 kb indicating that DNA methylation prevents mutations by limiting DNA damage over long distances (17). The expansion size of the genome stabilization effect is far larger than that of the previously described methylation-associated chromatin complex. Therefore, except for DNA gaps, the mechanism of DNA damage prevention by DNA methylation is unlikely to be explained by the other roles of DNA methylation described earlier **(Fig. 5)**. However, similar to DNA methylation, the methylated youth-DNA-gap also protects the DNA long-range and both protein complexes of Alu-siRNA and youth-DNA-gap are composed of AGO4 **(Fig. 2)** (9, 16, 17, 36, 52, 55). Therefore, global hypomethylation accelerating DNA damage may be due to methylated youth-DNA-gap reduction.

Role of the DNA gap in DNA protection

The role of DNA gaps is similar to that of the gaps left between successive rails on a railway track, which prevent railway bends from environmental heat. During transcription or replication, the double strands of the helix structure of DNA must be separated. The denaturation inevitably causes a twisted wave. If both ends of DNA are fixed, then the twisted wave will create a torsion force, thereby weakening all chemical bonds of DNA (80-82). The DNA gap helps relieve the torsion force by ending the twisted wave by freely spinning the end of the DNA gap **(Fig. 3)** (9, 16). While youth-DNA-gap helps relieves DNA tension, aged DNA lacks DNA gaps; therefore, twisted waves increase DNA tension. This torsion force weakens DNA chemical bonds, which increases the likelihood of DNA damage. Moreover, DNA torsional force inhibits transcription (83, 84). Consequently, young DNA is more durable and works more smoothly than old DNA **(Fig. 3)** (9).

DNA gaps are DNA modifications that are serendipitously discovered. Because DNA hypomethylation drives genomic instability, including genome deletion, we designed experiments to determine the DNA methylation of DNA sequences near EDSBs to test the hypothesis that EDSBs as DNA damage lesions should have been hypomethylated. We tested DNA from many cell types, and some were nondividing cells. Surprisingly, hypermethylated DNA was observed for all EDSBs (36). So these EDSBs may not be DNA damage but should be DNA modifications that opposite to DNA damage lesions, which are epigenetic marks. The other unexpected finding was that while pathologic EDSBs were predicted to occur exclusively during DNA replication (85), our ligation-mediated PCR from IRS to the EDSB technique, which is called IRS-EDSB PCR or DNA-GAP PCR, detected EDSBs in nondividing cells (36). Therefore, this type of EDSB has a distinct biology from the generally described replication-dependent EDSBs (36, 37, 39, 85, 86). We previously named these DNA gaps RIND-EDSBs. RIND stands for replication independent.

In humans, DNA gaps are generated by the molecular scissoring activity of Box A of HMGB1 **(Fig. 2 and 3)** (9). Transfection of Box A-producing plasmid increases DNA gaps, increases DNA durability, and reduces endogenous DNA damage and DDRs. HMGB1-produced DNA gaps protect DNA over long distances from all kinds of DNA damage, base changes, base losses, and DNA breaks, including single-strand breaks and DSBs. HMGB1-induced DNA gaps also prevent radiation-induced DSBs (9). While HMGB1 can facilitate DNA repair (87), the DNA damage prevention action of HMGB1-produced DNA gap is DNA protection (9).

The DNA gap structure is similar to that of pathological DSBs. Therefore, DNA gaps should have been recognized by the DSB response to gamma-H2AX, which signals immediate DSB repair, and their retention in cells should have been unlikely. DNA gaps are hidden in heterochromatin by histone deacetylases (9, 39), including SIRT1. Heterochromatin action by histone deacetylation hides the DNA gap ends so that cells do not recognize DNA gaps as DSBs (9). Interestingly, SIRT1 has been demonstrated to play essential roles in aging prevention,

including resistance to oxidative stress (88, 89). Therefore, one of the mechanisms by which SIRT1 prevents aging is likely youth-DNA-gap maintenance **(Fig. 2)**.

Additionally, because of the DSB structure of the DNA gap, to avoid chromosome deleterious multiple DNA breakage events, youth-DNA-gaps must be repaired before the DNA replication fork passes through. To prevent DNA repair errors, youth-DNA-gap repair must be more precise. Therefore, error-prone Ku-mediated nonhomologous end-joining repair (NHEJ) may not be acceptable (39). youth-DNA-gaps are also present in G1 and thus may not be repaired by a homologous DSB repair system. We found that retaining youth-DNA-gaps in nonacetylated heterochromatin helps block Ku-mediated NHEJ and allows these breaks to be repaired by a more precise ATM-dependent pathway (39). Therefore, youth-DNA-gaps do not promote the consequences of pathologic DSBs, such as persistent DDRs driving senescence or mutations causing cancer.

In addition to HMGB1 and SIRT1, the DNA gap complex consists of at least another protein, AGO4 **(Fig. 2)**. AGO4 acts as RdDM to methylate DNA around the DNA gap (55). Argonaut proteins play an essential role in the RNA silencing complex, or RISC (90). AGO4, however, is a critical component of RdDM (52). AGO4 binds to SIRT1 in the cytoplasm and forms DNA gap complexes with HMGB1 and SIRT1 in the nucleus (55). AGOs also contain small RNA to determine the binding target. Therefore, youth-DNA-gap locations are determined by the small RNA sequences of AGO4, which primarily binds to IRSs (52). As a result, DNA gap formation can avoid critical locations that would have disrupted essential genome functions, such as mRNA synthesis.

Rejuvenating DNA by genomic stability molecule or REDGEM

Both principle mechanism of action and treatment outcome of both Alu-siRNA and box A of HMGB1 expression plasmid are unprecedented. Both molecules protect DNA (9, 17, 55, 78, 79). For example, the DNA of cells pretreated by box A had higher resistance to radiation than untreated cells (9). We classified these two molecules as **re**juvenating **D**NA by **ge**nomic stability **m**olecules (REDGEM). Rejuvenating DNA or RED means cells treated with REDGEM are added youth-associated epigenetic marks. Genomic stability molecule or GEM means REDGEM protects DNA from being damaged.

Alu or SINE-siRNA is not promising for future clinical use. In addition to AGO4, the siRNA may incorporate into other AGOs and yield different outcomes among different cell types. In addition, SINE loci distribution and sequences among animal species are distinct. So SINE methylation outcome of each species may be different. Therefore, preclinical evaluation of SINE-siRNA may not be fully equivalent to Alu-siRNA.

We showed that Box A rejuvenated cells by producing DNA gaps to increase the durability of old DNA (9). The strengthened DNA eventually reduced endogenous DNA damage and DDR. Consequently, Box A transfected cells' senescent signaling cascade was limited and as a result cells were rejuvenated **(Fig. 6)**. We currently

demonstrated that Box A expression plasmid transfection could rejuvenate senescent cells in two models of aging rats: d-galactose-induced aging and natural-aging rats (9). Box A improved memory and liver function and reduced visceral fat, liver fibrosis, and senescence-associated proteins. The rejuvenation degree was remarkable because Box A reversed all aging markers to that observed in youth groups (9). These experiments indicated that youth-DNA-gap reduction is the nidus of the aging process and the cellular senescence stage is maintained by youth-DNA-gap reduction (9). Improving memory means Box A is a promising therapeutic approach for senile dementia (9). Interestingly, the learning ability of aged rats treated with REDGEM was better than that of the young group. Recently, we proved that Box A of HMGB1 can enhance stem cell properties (91). It is crucial to explore further if REDGEM can promote neurogenesis by enhancing neuronal stem cells in the damaged brain. The reduction of fibrosis expands the treatment potential of Box A to numerous pathological structures in the extracellular space, such as lung fibrosis, amyloid in brain, and fat deposits in arteriosclerosis (9). Interestingly, HMGB1 was shown to prevent and ameliorate heart hypertrophy by inhibiting DDR (92, 93). Therefore, producing DNA gaps may be valuable for treating DNA damage- and age-associated diseases or conditions, including major organ failure (9).

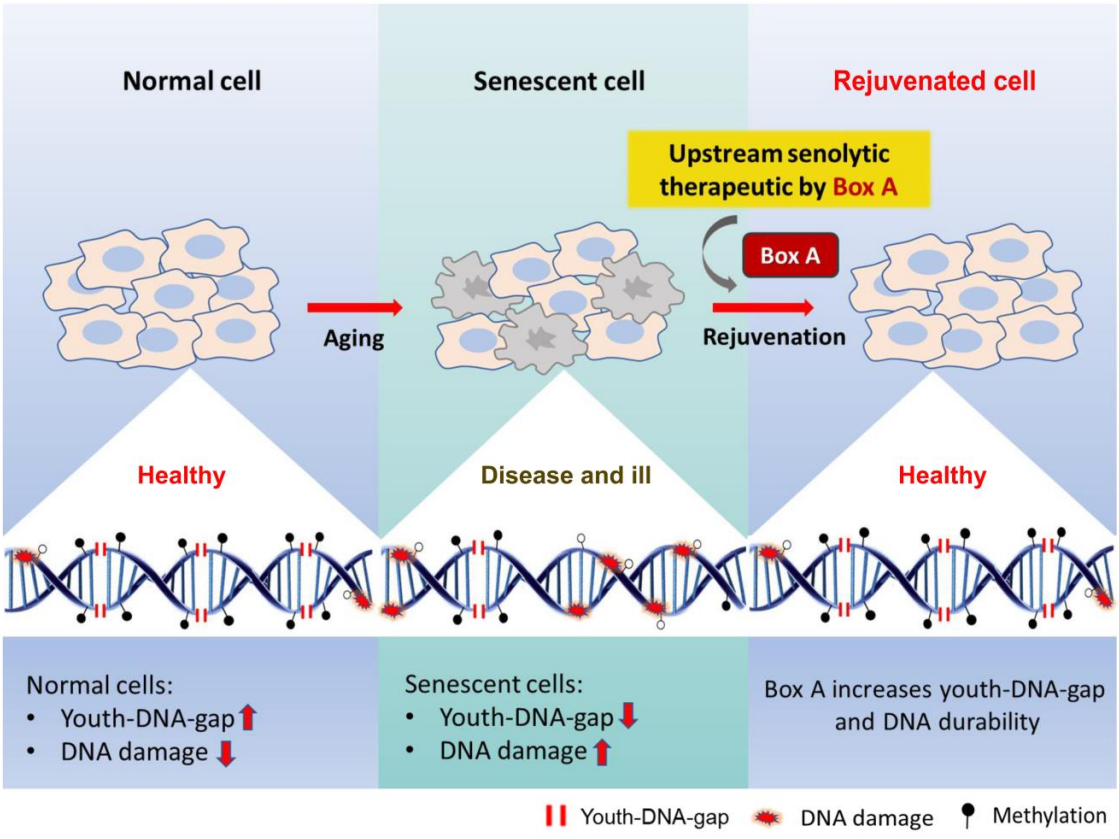

Figure 6. Box A of HMGB1 is an upstream senolytic therapeutic molecule. *Youth-DNA-gap reduction occurs in the elderly, accumulates DNA damage, and drives cells to senescence, leading to body structure and function deterioration and diseases. As REDGEM, box A produces youth-DNA-gaps and reverse aging consequences.*

DNA damage can lead to all aging phenotypes, health deterioration, and senescence-associated diseases (12). Endogenous DNA damage accumulation in aging cells is caused by youth-DNA-gap reduction (9, 16). Therefore, producing DNA gaps rejuvenates cells and improves the health of the elderly by preventing DNA damage **(Fig. 6)**. Senolytic therapeutics is a medical technology that eliminates senescent cells (94, 95). Senescence altering cell and tissue structure and function is common pathogenesis of age-associated NCDs (96). The possible application of the senolytic therapeutic approach is to treat age-associated NCDs. DNA damage leads to several senescence promotion mechanisms, such as DDR, low level of nicotinamide adenine dinucleotide (NAD+), metabolic imbalances such as insulin resistance, telomere attrition by lacking telomerase, the decline in mitochondrial function, *senescence-associated secretory phenotype* or SASP, and repression of autophagy (97-102). So the role of REDGEM in DNA protection prevents senescence by a mechanism that is more upstream than all known targets of current senolytic therapeutic approaches and rejuvenation remedies.

Youth-DNA-gap complex metabolism is associated with a rejuvenation remedy, NAD+. NAD+ is a coenzyme for redox reactions. NAD+ is low in aging cells, and restoration of NAD+ promotes aging reversal (103, 104). SIRT1 uses NAD+ as a substrate to remove acetyl groups from a target protein (105, 106). So, one of the roles of NAD+ in aging prevention may be due to the position of SIRT1 in the youth-DNA-gap complex.

The upstream property possesses several advantages. Inhibiting the downstream process may omit or augment other signaling cascades, so senescence inhibition cannot complete. For example, while the upregulation of p16 or mTORC1 promotes aging, suppression of p16 can induce mTORC1 (107-109). Moreover, facilitating a rejuvenation network may not be effective because of lacking a rejuvenation initiator. For example, although NAD+ supplements activate SIRT1, this action may not be able to rejuvenate cells by creating sufficient new youth-DNA-gap complexes when cells significantly lack intranuclear HMGB1. Because the substrate of the senescence signaling cascade is DNA damage, rejuvenation by inhibiting the DDR may increase the risk of carcinogenesis. Finally, classical senolytic is to kill senescent cells. The killing mechanism may have off targets non-senescent cells or removal of senescence cells could yield harmful effect (110). By targeting late senescent cells or senescent associated molecules, such as SASP, the function of the pre-senescent cell will not be improved. In contrast, maybe due to the upstream role of box A, box A did not harm normal cells and can improve cell proliferation even if the cells have yet to express senescence-associated markers (9). So box A is a very promising medicine in treating age-associated diseases.

DNA gap homeostasis

Youth-DNA-gap reduction in elderly individuals initiates spontaneous DNA damage, resulting in cellular senescence (9). Thus, understanding how the gaps are reduced will provide insights into the mechanisms underlying the aging process.

Three different in vitro experiments demonstrated a reduction in youth-DNA-gaps. The first approach limits histone deacetylation by trichostatin A treatment or SIRT1 deletion; SIRT1 is SIR2 in yeast (39, 111). These approaches inhibited histone deacetylase activity, limited histone deacetylation and limited DNA gap residents. Therefore, cells recognize DNA gaps as DNA breaks and consequently repair the gaps. The second experiment introduced a DSB, which led to global DSB repair (16). Therefore, DSB induction by any causes will result in youth-DNA-gap reduction. The last experiment downregulated HMGB1 (9, 16, 86, 111), which is the DNA gap producers, and limited intranuclear HMGB1 protein will eventually cause a reduction in youth-DNA-gaps **(Fig. 7)**. Notably, intranuclear HMGB1 is reduced in the early senescence process (61). Nuclear HMGB1 of senescent cells relocalizes to the extracellular space, thereby causing intranuclear HMGB1 depletion (61). HMGB1 translocates from the nucleus to the cytoplasm, and its excretion requires posttranslational modifications, including oxidation to the disulfide form and acetylation to the hyperacetylated state (112). The requirement of the hyperacetylate state of HMGB1 release supports the interaction of HMGB1 and SIRT1, a deacetylase protein, in the youth-DNA-gap complex. Finally, HMGB1 release requires the function of p53, activating G1 *checkpoint* as part of the response to *DNA damage* (61, 113). These pieces of evidence support the kinetic role of HMGB1, youth-DNA-gap, and DNA damage in senescence induction.

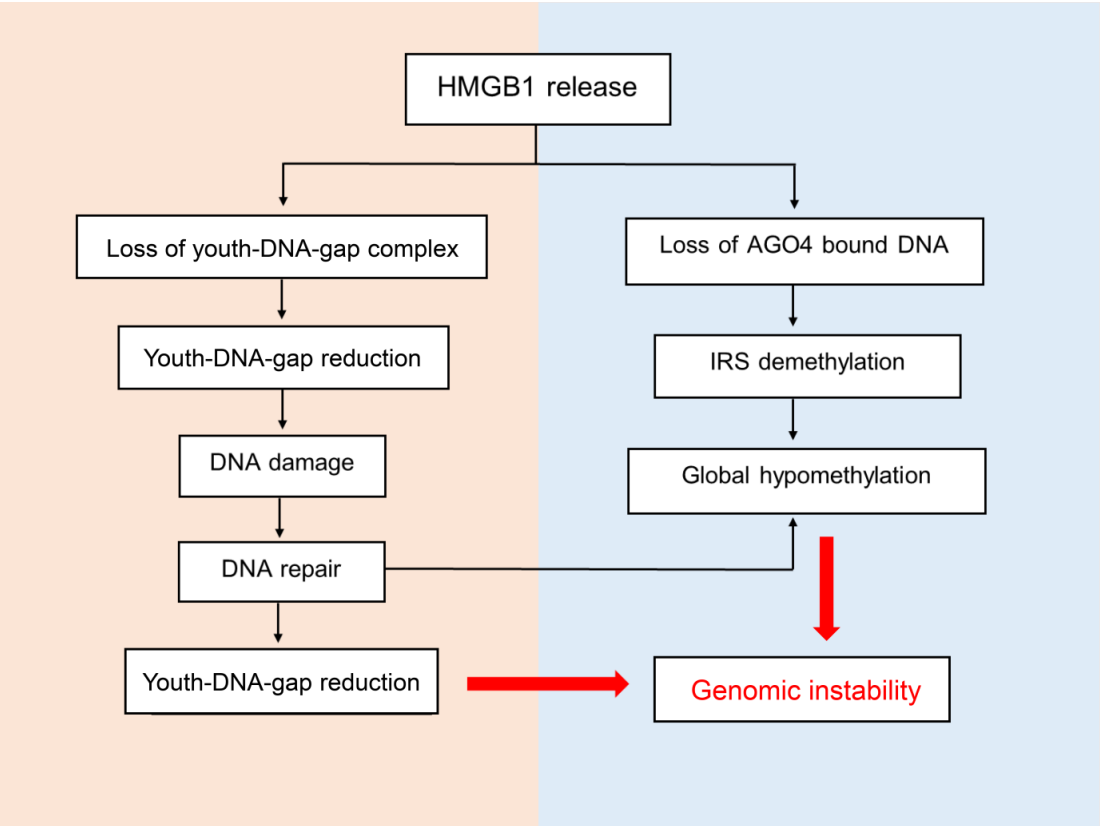

Figure 7. HMGB1 release promotes global hypomethylation and a progressive cycle of genomic instability. *HMGB1 release causes a reduction in AGO4 bound methylated youth-DNA-gaps, which contributes to DNA damage. The DNA repair process demethylates DNA and globally repairing the DNA gaps.*

Although the reduction in youth-DNA-gaps is present in all aging eukaryotes, different species age at different rates, regardless of environmental factors; therefore, the decrease in youth-DNA-gaps may be programmed similarly to other developmental phases. Epigenetic controls gene expression change throughout human life, from embryo to fetus to newborn, from childhood to adolescence to adulthood, and from adult to elderly. Recent studies showed a change in DNA methylation closely associated with age called the epigenetic test clock (114-116). It is interesting to see and differentiate the sets of the epigenetic clocks concerning the cause or consequence of lowering youth-DNA-gaps.

HMGB1-produced youth-DNA-gap reduction promoting global hypomethylation

HMGB1 reduction can cause DNA hypomethylation by two mechanisms **(Fig. 7)** (55). First, a reduction in DNA gaps will reduce AGO4-bound DNA and consequently reduce DNA methylation. Second, a reduction in DNA gaps increases DNA damage, and the subsequent DNA repair leads to DNA demethylation **(Fig. 7)** (55). This demethylation process may initiate DNA hypomethylation that drives genomic instability pathways, as mentioned in the previous section **(Fig. 5)**.

In addition to promoting the accumulation of DNA damage and global hypomethylation, reducing DNA gaps may alter gene regulation. Recently, a study reported that the RNA synthesis rate of aged DNA, in general, is faster than that of young DNA (117). Since the role of youth-DNA-gaps is to relieve torsion force, DNA double helix possessing more DNA gaps has stronger synchronizing bonds. This may be the mechanism causing the RNA polymerase of the youth to move slower and smoother than that of older people.

In addition to DNA gap formation, HMGB1 plays several other roles in aging. Intracytoplasmic HMGB1 promotes autophagy (118). Extracellular HMGB1 acts as a senescence-associated protein and sends a signal to immune cells about the senescence process (119, 120). Moreover, HMGB1 induces inflammation by binding to TLR4 receptors (121). Therefore, HMGB1 release initiates and maintains cellular senescence by promoting genomic instability, autophagy and inflammation.

Conclusion

DNA from young individuals is composed of methylated DNA gaps, and these youth-DNA-gaps protect DNA from damage. The youth-DNA-gap complexes formed by HMGB1 produced DNA gaps, AGO4 methylated DNA, and SIRT1 deacetylated histones. Aging stimulation, such as oxidative stress, modifies HMGB1 and causes HMGB1 release. Depleting intranuclear HMGB1 causes youth-DNA-gap reductions, DNA damage, DNA repair, and DNA hypomethylation. DNA repair also results in DNA demethylation and DNA gap reduction. As a result, DNA from the elderly has a limited number of methylated DNA gaps and thus spontaneously accumulates DNA damage. Introducing exogenous Box A of HMGB1 can edit aging DNA to a younger state by producing DNA gaps. The DNA gaps increase DNA durability, resulting in rejuvenation. Box A is a medicine

that rejuvenates DNA by acting as a genomic stabilizing molecule called REDGEM. REDGEM is a promising epigenetic editing technology for treating DNA damage or age-associated diseases and conditions.

Competing interests:

"The authors declare that they have no conflict of interest."

Acknowledgements Statement:

None

Funding Statement:

"Not applicable"

Ethical Approval

"Not applicable"

Authors' contributions

P.W. draw all figures. A.M. wrote the article. Both authors reviewed and approved the article content.

Availability of data and materials

"Not applicable"

List of Abbreviations

5hmC 5-hydroxymethylcytosine
8-OHdG 8-hydroxy-2′-deoxyguanosine
AGO4 argonaute 4
AP apurinic/apyrimidinic
ATM ataxia-telangiectasia mutated
DDR DNA damage response
DNA-PKcs DNA-dependent protein kinase catalytic subunit
DNMT DNA methyltransferase
DSB DNA double-strand break
HMGB1 high-mobility group box 1
IRS interspersed repetitive sequence
LINE long interspersed nucleotide element
MMR mismatch repair
NAD+ nicotinamide adenine dinucleotide
NCDs noncommunicable diseases
NHEJ nonhomologous end-joining repair
RdDM RNA-directed DNA methylation
RIND-EDSB replication-independent endogenous DNA double strand break
RISC RNA silencing complex
SASP senescence-associated secretory phenotype
SINE short interspersed nucleotide element
SIRT1 sirtuin 1
TET ten-eleven translocation

References:

1. Li Z, Zhang Z, Ren Y, Wang Y, Fang J, Yue H, et al. Aging and age-related diseases: from mechanisms to therapeutic strategies. Biogerontology. 2021;22(2):165-87.

2. Jaul E, Barron J. Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. Front Public Health. 2017;5:335.

3. Tseng WI, Hsu YC, Kao TW. Brain Age Difference at Baseline Predicts Clinical Dementia Rating Change in Approximately Two Years. J Alzheimers Dis. 2022;86(2):613-27.

4. Chae CU, Pfeffer MA, Glynn RJ, Mitchell GF, Taylor JO, Hennekens CH. Increased pulse pressure and risk of heart failure in the elderly. JAMA. 1999;281(7):634-9.

5. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol. 2020;146(1):110-8.

6. Crews DE. Senescence, aging, and disease. J Physiol Anthropol. 2007;26(3):365-72.

7. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med. 2015;21(12):1424-35.

8. Mutirangura A. Is global hypomethylation a nidus for molecular pathogenesis of age-related noncommunicable diseases? Epigenomics. 2019;11(6):577-9.

9. Yasom S, Watcharanurak P, Bhummaphan N, Thongsroy J, Puttipanyalears C, Settayanon S, et al. The roles of HMGB1-produced DNA gaps in DNA protection and aging biomarker reversal. FASEB BioAdvances. 2022;4(6):408-34.

10. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153(6):1194-217.

11. Gonzalo S. Epigenetic alterations in aging. J Appl Physiol (1985). 2010;109(2):586-97.

12. Niedernhofer LJ, Gurkar AU, Wang Y, Vijg J, Hoeijmakers JHJ, Robbins PD. Nuclear Genomic Instability and Aging. Annu Rev Biochem. 2018;87:295-322.

13. Ou HL, Schumacher B. DNA damage responses and p53 in the aging process. Blood. 2018;131(5):488-95.

14. Fumagalli M, Rossiello F, Mondello C, d'Adda di Fagagna F. Stable cellular senescence is associated with persistent DDR activation. PLoS One. 2014;9(10):e110969.

15. Galbiati A, Beausejour C, d'Adda di Fagagna F. A novel single-cell method provides direct evidence of persistent DNA damage in senescent cells and aged mammalian tissues. Aging Cell. 2017;16(2): 422-7.

16. Thongsroy J, Patchsung M, Pongpanich M, Settayanon S, Mutirangura A. Reduction in replication-independent endogenous DNA double-strand breaks promotes genomic instability during chronological aging in yeast. The FASEB Journal. 2018;32(11):6252-60.

17. Patchsung M, Settayanon S, Pongpanich M, Mutirangura D, Jintarith P, Mutirangura A. Alu siRNA to increase Alu element methylation and prevent DNA damage. Epigenomics. 2018;10(2):175-85.

18. Wilson VL, Smith RA, Ma S, Cutler RG. Genomic 5-methyldeoxycytidine decreases with age. J Biol Chem. 1987;262(21):9948-51.

19. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science. 2001;293(5532):1068-70.

20. Zheng Y, Joyce BT, Liu L, Zhang Z, Kibbe WA, Zhang W, Hou L. Prediction of genome-wide DNA methylation in repetitive elements. Nucleic Acids Res. 2017;45(15):8697-711.

21. Aporntewan C, Phokaew C, Piriyapongsa J, Ngamphiw C, Ittiwut C, Tongsima S, Mutirangura A. Hypomethylation of intragenic LINE-1 represses transcription in cancer cells through AGO2. PLoS One. 2011;6(3):e17934.

22. Tirado-Magallanes R, Rebbani K, Lim R, Pradhan S, Benoukraf T. Whole genome DNA methylation: beyond genes silencing. Oncotarget. 2017;8(3):5629-37.

23. Sheaffer KL, Elliott EN, Kaestner KH. DNA Hypomethylation Contributes to Genomic Instability and Intestinal Cancer Initiation. Cancer Prev Res (Phila). 2016;9(7):534-46.

24. Kitkumthorn N, Mutirangura A. Long interspersed nuclear element-1 hypomethylation in cancer: biology and clinical applications. Clin Epigenetics. 2011;2(2):315-30.

25. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science. 2003;300(5618):455.

26. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature. 1998;395(6697):89-93.

27. Tongyoo P, Avihingsanon Y, Prom-On S, Mutirangura A, Mhuantong W, Hirankarn N. EnHERV: Enrichment analysis of specific human endogenous retrovirus patterns and their neighboring genes. PLoS One. 2017;12 (5):e0177119.

28. Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, et al. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech Ageing Dev. 2009;130 (4):234-9.

29. Jintaridth P, Mutirangura A. Distinctive patterns of age-dependent hypomethylation in interspersed repetitive sequences. Physiol Genomics. 2010;41(2):194-200.

30. Cardelli M. The epigenetic alterations of endogenous retroelements in aging. Mech Ageing Dev. 2018;174:30-46.

31. Lu S, Niu Z, Chen Y, Tu Q, Zhang Y, Chen W, et al. Repetitive Element DNA Methylation is Associated with Menopausal Age. Aging Dis. 2018;9(3):435-43.

32. Thongsroy J, Patchsung M, Mutirangura A. The association between Alu hypomethylation and severity of type 2 diabetes mellitus. Clin Epigenetics. 2017;9:93.

33. Jintaridth P, Tungtrongchitr R, Preutthipan S, Mutirangura A. Hypomethylation of Alu elements in post-menopausal women with osteoporosis. PLoS One. 2013;8(8):e70386.

34. Thongsroy J, Mutirangura A. The association between Alu hypomethylation and the severity of hypertension. PLoS One. 2022;17(7):e0270004.

35. Rerkasem K, Rattanatanyong P, Rerkasem A, Wongthanee A, Rungruengthanakit K, Mangklabruks A, Mutirangura A. Higher Alu methylation levels in catch-up growth in twenty-year-old offsprings. PLoS One. 2015; 10(3):e0120032.

36. Pornthanakasem W, Kongruttanachok N, Phuangphairoj C, Suyarnsestakorn C, Sanghangthum T, Oonsiri S, et al. LINE-1 methylation status of endogenous DNA double-strand breaks. Nucleic Acids Res. 2008;36(11):3667-75.

37. Pongpanich M, Patchsung M, Thongsroy J, Mutirangura A. Characteristics of replication-independent endogenous double -strand breaks in Saccharomyces cerevisiae. BMC Genomics. 2014;15:750.

38. Thongsroy J, Mutirangura A. The inverse association between DNA gaps and HbA1c levels in type 2 diabetes mellitus. Sci Rep. 2023;13(1):18987.

39. Kongruttanachok N, Phuangphairoj C, Thongnak A, Ponyeam W, Rattanatanyong P, Pornthanakasem W, Mutirangura A. Replication independent DNA double-strand break retention may prevent genomic instability. Mol Cancer. 2010;9:70.

40. Arand J, Chiang HR, Martin D, Snyder MP, Sage J, Reijo Pera RA, Wossidlo M. Tet enzymes are essential for early embryogenesis and completion of embryonic genome activation. EMBO Rep. 2022;23(2):e53968.

41. Guo JU, Su Y, Zhong C, Ming GL, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell. 2011;145(3):423-34.

42. Schuermann D, Weber AR, Schar P. Active DNA demethylation by DNA repair: Facts and uncertainties. DNA Repair (Amst). 2016;44:92-102.

43. Grin I, Ishchenko AA. An interplay of the base excision repair and mismatch repair pathways in active DNA demethylation. Nucleic Acids Res. 2016;44(8):3713-27.

44. Gehring M, Reik W, Henikoff S. DNA demethylation by DNA repair. Trends Genet. 2009;25(2):82-90.

45. Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet. 2017;18(9):517-34.

46. Melamed P, Yosefzon Y, David C, Tsukerman A, Pnueli L. Tet Enzymes, Variants, and Differential Effects on Function. Front Cell Dev Biol. 2018;6:22.

47. Kafer GR, Li X, Horii T, Suetake I, Tajima S, Hatada I, Carlton PM. 5-Hydroxymethylcytosine Marks Sites of DNA Damage and Promotes Genome Stability. Cell Rep. 2016;14(6):1283-92.

48. Mahmoud AM, Ali MM. Methyl Donor Micronutrients that Modify DNA Methylation and Cancer Outcome. Nutrients. 2019;11(3).

49. Zeisel S. Choline, Other Methyl-Donors and Epigenetics. Nutrients. 2017;9(5).

50. Miller JW, Nadeau MR, Smith J, Smith D, Selhub J. Folate-deficiency-induced homocysteinaemia in rats: disruption of S-adenosylmethionine's co-ordinate regulation of homocysteine metabolism. Biochem J. 1994;298 (Pt 2):415-9.

51. Yang AS, Doshi KD, Choi SW, Mason JB, Mannari RK, Gharybian V, et al. DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia. Cancer Res. 2006;66(10):5495-503.

52. Chalertpet K, Pin-On P, Aporntewan C, Patchsung M, Ingrungruanglert P, Israsena N, Mutirangura A. Argonaute 4 as an Effector Protein in RNA-Directed DNA Methylation in Human Cells. Front Genet. 2019;10:645.

53. Chen L, Dahlstrom JE, Lee SH, Rangasamy D. Naturally occurring endo-siRNA silences LINE-1 retrotransposons in human cells through DNA methylation. Epigenetics. 2012;7(7):758-71.

54. Castanotto D, Tommasi S, Li M, Li H, Yanow S, Pfeifer GP, Rossi JJ. Short hairpin RNA-directed cytosine (CpG) methylation of

the RASSF1A gene promoter in HeLa cells. Mol Ther. 2005;12(1):179-83.

55. Watcharanurak P, Mutirangura A. Human RNA-directed DNA methylation methylates high-mobility group box 1 protein-produced DNA gaps. Epigenomics.0(0):null.

56. Caliri AW, Caceres A, Tommasi S, Besaratinia A. Hypomethylation of LINE-1 repeat elements and global loss of DNA hydroxymethylation in vapers and smokers. Epigenetics. 2020;15(8):816-29.

57. Fustinoni S, Rossella F, Polledri E, Bollati V, Campo L, Byun HM, et al. Global DNA methylation and low-level exposure to benzene. Med Lav. 2012;103(2):84-95.

58. Meevassana J, Serirodom S, Prabsattru P, Boonsongserm P, Kamolratanakul S, Siritientong T, et al. Alu repetitive sequence CpG methylation changes in burn scars. Burns. 2021.

59. Wongpaiboonwattana W, Tosukhowong P, Dissayabutra T, Mutirangura A, Boonla C. Oxidative stress induces hypomethylation of LINE-1 and hypermethylation of the RUNX3 promoter in a bladder cancer cell line. Asian Pac J Cancer Prev. 2013;14(6):3773-8.

60. da Silva J. DNA damage induced by occupational and environmental exposure to miscellaneous chemicals. Mutat Res Rev Mutat Res. 2016;770(Pt A):170-82.

61. Davalos AR, Kawahara M, Malhotra GK, Schaum N, Huang J, Ved U, et al. p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. J Cell Biol. 2013;201(4):613-29.

62. Hu H, Chen C, Shi S, Li B, Duan S. The gene mutations and subtelomeric DNA methylation in immunodeficiency, centromeric instability and facial anomalies syndrome. Autoimmunity. 2019;52(5-6):192-8.

63. Ehrlich M, Jackson K, Weemaes C. Immunodeficiency, centromeric region instability, facial anomalies syndrome (ICF). Orphanet J Rare Dis. 2006;1:2.

64. Tuck-Muller CM, Narayan A, Tsien F, Smeets DF, Sawyer J, Fiala ES, et al. DNA hypomethylation and unusual chromosome instability in cell lines from ICF syndrome patients. Cytogenet Cell Genet. 2000;89(1-2):121-8.

65. Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene. 2002;21(35):5400-13.

66. Kim M, Trinh BN, Long TI, Oghamian S, Laird PW. Dnmt1 deficiency leads to enhanced microsatellite instability in mouse embryonic stem cells. Nucleic Acids Res. 2004;32(19):5742-9.

67. Wang KY, James Shen CK. DNA methyltransferase Dnmt1 and mismatch repair. Oncogene. 2004;23(47):7898-902.

68. Loughery JE, Dunne PD, O'Neill KM, Meehan RR, McDaid JR, Walsh CP. DNMT1 deficiency triggers mismatch repair defects in human cells through depletion of repair protein levels in a process involving the DNA damage response. Hum Mol Genet. 2011;20(16):3241-55.

69. Schermelleh L, Haemmer A, Spada F, Rosing N, Meilinger D, Rothbauer U, et al. Dynamics of Dnmt1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation. Nucleic Acids Res. 2007;35(13):4301-12.

70. Kunkel TA, Erie DA. Eukaryotic Mismatch Repair in Relation to DNA Replication. Annu Rev Genet. 2015;49:291-313.

71. De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NJ, Criscione SW, et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature. 2019;566 (7742):73-8.

72. Janssen A, Colmenares SU, Karpen GH. Heterochromatin: Guardian of the Genome. Annu Rev Cell Dev Biol. 2018;34:265-88.

73. Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Lobrich M, Jeggo PA. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol Cell. 2008;31(2):167-77.

74. Goodarzi AA, Jeggo PA. The heterochromatic barrier to DNA double strand break repair: how to get the entry visa. Int J Mol Sci. 2012;13(9):11844-60.

75. Wang HC, Chou WC, Shieh SY, Shen CY. Ataxia telangiectasia mutated and checkpoint kinase 2 regulate BRCA1 to promote the fidelity of DNA end-joining. Cancer Res. 2006;66(3):1391-400.

76. Suyarnsestakorn C, Thanasupawat T, Leelahavanichkul K, Gutkind JS, Mutirangura A. Ataxia telangiectasia mutated nuclear localization in head and neck cancer cells is PPP2R2B-dependent. Asian Biomedicine. 2010;4(3):373-83.

77. Phokaew C, Kowudtitham S, Subbalekha K, Shuangshoti S, Mutirangura A. LINE-1 methylation patterns of different loci in normal and cancerous cells. Nucleic Acids Res. 2008;36(17):5704-12.

78. Yasom S, Khumsri W, Boonsongserm P, Kitkumthorn N, Ruangvejvorachai P, Sooksamran A, et al. B1 siRNA Increases de novo DNA Methylation of B1 Elements and Promotes Wound Healing in Diabetic Rats. Front Cell Dev Biol. 2021;9:802024.

79. Meevassana J, Nacharoenkul P, Wititsuwannakul J, Kitkumthorn N, Hamill KJ, Angspatt A, Mutirangura A. B1 repetitive sequence methylation enhances wound healing of second-degree burns in rats. Biomed Rep. 2022;16(3):20.

80. Skoruppa E, Nomidis SK, Marko JF, Carlon E. Bend-Induced Twist Waves and the Structure of Nucleosomal DNA. Phys Rev Lett. 2018;121(8):088101.

81. Ramstein J, Lavery R. Energetic coupling between DNA bending and base pair opening. Proc Natl Acad Sci U S A. 1988;85(19):7231-5.

82. Teves SS, Henikoff S. DNA torsion as a feedback mediator of transcription and chromatin dynamics. Nucleus. 2014;5(3):211-8.

83. Roca J. Transcriptional inhibition by DNA torsional stress. Transcription. 2011;2(2):82-5.

84. Joshi RS, Pina B, Roca J. Positional dependence of transcriptional inhibition by DNA torsional stress in yeast chromosomes. EMBO J. 2010;29(4):740-8.

85. Vilenchik MM, Knudson AG. Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. Proc Natl Acad Sci U S A. 2003;100(22):12871-6.

86. Pongpanich M, Patchsung M, Mutirangura A. Pathologic Replication-Independent Endogenous DNA Double-Strand Breaks Repair Defect in Chronological Aging Yeast. Front Genet. 2018;9:501.

87. Lange SS, Mitchell DL, Vasquez KM. High mobility group protein B1 enhances DNA

repair and chromatin modification after DNA damage. Proc Natl Acad Sci U S A. 2008;105(30):10320-5.

88. Chen C, Zhou M, Ge Y, Wang X. SIRT1 and aging related signaling pathways. Mech Ageing Dev. 2020;187:111215.

89. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, et al. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res. 2007;100(10):1512-21.

90. Moazed D. Small RNAs in transcriptional gene silencing and genome defence. Nature. 2009;457(7228):413-20.

91. Ei ZZ, Mutirangura A, Arunmanee W, Chanvorachote P. The Role of Box A of HMGB1 in Enhancing Stem Cell Properties of Human Mesenchymal Cells: A Novel Approach for the Pursuit of Anti-aging Therapy. In Vivo. 2023;37(5):2006-17.

92. Takahashi T, Shishido T, Kinoshita D, Watanabe K, Toshima T, Sugai T, et al. Cardiac Nuclear High-Mobility Group Box 1 Ameliorates Pathological Cardiac Hypertrophy by Inhibiting DNA Damage Response. JACC Basic Transl Sci. 2019;4(2):234-47.

93. Funayama A, Shishido T, Netsu S, Narumi T, Kadowaki S, Takahashi H, et al. Cardiac nuclear high mobility group box 1 prevents the development of cardiac hypertrophy and heart failure. Cardiovasc Res. 2013;99(4):657-64.

94. Serrano M, Barzilai N. Targeting senescence. Nat Med. 2018;24(8):1092-4.

95. Di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol. 2021;22(2):75-95.

96. Amaya-Montoya M, Perez-Londono A, Guatibonza-Garcia V, Vargas-Villanueva A, Mendivil CO. Cellular Senescence as a Therapeutic Target for Age-Related Diseases: A Review. Adv Ther. 2020;37(4):1407-24.

97. Ying W, Alano CC, Garnier P, Swanson RA. NAD+ as a metabolic link between DNA damage and cell death. J Neurosci Res. 2005;79(1-2):216-23.

98. Shimizu I, Yoshida Y, Suda M, Minamino T. DNA damage response and metabolic disease. Cell Metab. 2014;20(6):967-77.

99. Chen RJ, Wu PH, Ho CT, Way TD, Pan MH, Chen HM, et al. P53-dependent downregulation of hTERT protein expression and telomerase activity induces senescence in lung cancer cells as a result of pterostilbene treatment. Cell Death Dis. 2017;8(8):e2985.

100.Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll UM. p53 has a direct apoptogenic role at the mitochondria. Mol Cell. 2003;11(3):577-90.

101.White E. Autophagy and p53. Cold Spring Harb Perspect Med. 2016;6(4): a026120.

102.Mirzayans R, Andrais B, Hansen G, Murray D. Role of p16(INK4A) in Replicative Senescence and DNA Damage-Induced Premature Senescence in p53-Deficient Human Cells. Biochem Res Int. 2012;2012:951574.

103.Belenky P, Bogan KL, Brenner C. NAD+ metabolism in health and disease. Trends Biochem Sci. 2007;32(1):12-9.

104.Mendelsohn AR, Larrick JW. Interacting NAD(+) and Cell Senescence Pathways Complicate Antiaging Therapies. Rejuvenation Res. 2019;22(3):261-6.

105.Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and

longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature. 2000;403(6771): 795-800.

106.Canto C, Auwerx J. Targeting sirtuin 1 to improve metabolism: all you need is NAD(+)? Pharmacol Rev. 2012;64(1):166-87.

107.Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. Int J Cancer. 2012;130(8):1715-25.

108.Papadopoli D, Boulay K, Kazak L, Pollak M, Mallette F, Topisirovic I, Hulea L. mTOR as a central regulator of lifespan and aging. F1000Res. 2019;8.

109.Buj R, Chen CW, Dahl ES, Leon KE, Kuskovsky R, Maglakelidze N, et al. Suppression of p16 Induces mTORC1-Mediated Nucleotide Metabolic Reprogramming. Cell Rep. 2019;28(8):1971-80 e8.

110.Grosse L, Wagner N, Emelyanov A, Molina C, Lacas-Gervais S, Wagner KD, Bulavin DV. Defined p16(High) Senescent Cell Types Are Indispensable for Mouse Healthspan. Cell Metab. 2020;32(1):87-99 e6.

111.Thongsroy J, Matangkasombut O, Thongnak A, Rattanatanyong P, Jirawatnotai S, Mutirangura A. Replication-independent endogenous DNA double-strand breaks in Saccharomyces cerevisiae model. PLoS One. 2013;8(8):e72706.

112.Yu Y, Tang D, Kang R. Oxidative stress-mediated HMGB1 biology. Front Physiol. 2015;6:93.

113.Vaziri C, Saxena S, Jeon Y, Lee C, Murata K, Machida Y, et al. A p53-dependent checkpoint pathway prevents rereplication. Mol Cell. 2003;11(4):997-1008.

114.Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet. 2018;19(6):371-84.

115.Rakyan VK, Down TA, Maslau S, Andrew T, Yang TP, Beyan H, et al. Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res. 2010;20(4):434-9.

116.Horvath S, Zhang Y, Langfelder P, Kahn RS, Boks MP, van Eijk K, et al. Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol. 2012;13 (10):R97.

117.Debes C, Papadakis A, Gronke S, Karalay O, Tain LS, Mizi A, et al. Ageing-associated changes in transcriptional elongation influence longevity. Nature. 2023;616(7958): 814-21.

118.Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, et al. Endogenous HMGB1 regulates autophagy. J Cell Biol. 2010;190(5):881-92.

119.Lu H, Zhang Z, Barnie PA, Su Z. Dual faced HMGB1 plays multiple roles in cardiomyocyte senescence and cardiac inflammatory injury. Cytokine Growth Factor Rev. 2019;47:74-82.

120.Andersson U, Yang H, Harris H. Extracellular HMGB1 as a therapeutic target in inflammatory diseases. Expert Opin Ther Targets. 2018;22(3):263-77.

121.Paudel YN, Angelopoulou E, Piperi C, Othman I, Aamir K, Shaikh MF. Impact of HMGB1, RAGE, and TLR4 in Alzheimer's Disease (AD): From Risk Factors to Therapeutic Targeting. Cells. 2020;9(2).