Chapter

A Hypothesis to Explain How the DNA of Elderly People Is Prone to Damage: Genome-Wide Hypomethylation Drives Genomic Instability in the Elderly by Reducing Youth-Associated Gnome-Stabilizing DNA Gaps

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Abstract

Epigenetic changes are how the DNA of elderly people is prone to damage. One role of DNA methylation is to prevent DNA damage. In the elderly and those with aging-associated noncommunicable diseases (NCDs), DNA shows reduced methylation; consequently, the aging genome is unstable and accumulates DNA damage. While the DNA damage response (DDR) of the direct intracellular machinery repairs DNA lesions, too much DDR halts cell proliferation, and promotes senescence. Therefore, genome-wide hypomethylation drives genomic instability, causing aging-associated disease phenotypes. However, the mechanism is unknown. Independent of DNA replication, the eukaryotic genome retains a certain amount of endogenous DNA double-strand breaks (EDSBs), called physiologic replication-independent EDSBs (Phy-RIND-EDSBs), that possess physiological function. Phy-RIND-EDSBs are reduced in aging yeast, and low levels of Phy-RIND-EDSBs decrease cell viability and increase DNA damage. Thus, Phy-RIND-EDSBs have a biological role as youth-associated genomic-stabilizing DNA gaps. In humans, Phy-RIND-EDSBs are located in the hypermethylated genome. Because the genomes of aging people are hypomethylated, the elderly should also have a low level of Phy-RIND-EDSBs. Based on this evidence, I hypothesize that in the human Phy-RIND-EDSBs, reduction is a molecular process that mediates the genome-wide hypomethylation driving genomic instability, which is a nidus pathogenesis mechanism of human body deterioration in aging-associated NCDs.

Keywords: genome-wide hypomethylation, genomic instability, global hypomethylation, DNA damage, youth-associated genomic-stabilizing DNA gaps, youth-DNA-GAPs, physiological replication-independent endogenous DNA double-strand breaks, RIND-EDSBs, Phy-RIND-EDSBs, aging

1. Introduction

As people age, their bodies begin to deteriorate. Understanding how changes in the DNA of aging people affect cellular function will be an important clue for future prevention and treatment of age-associated noncommunicable diseases (NCDs). Genomic instability, a hallmark of cancer and aging, is defined as a high frequency of mutations within the genome [1, 2]. In cancer, the permanent alteration of the nucleotide sequence of DNA, or mutations, occurring in proto-oncogenes and tumor suppressor genes lead to cancer development and progression. In the aging process, however, the accumulation of DNA damage, which is an abnormal chemical structure in DNA and includes base modification, base loss, and DNA breaks (which are precursors of mutations), stimulates the DNA damage repair signal (DDR) to induce cells to repair DNA damage [3, 4]. Nevertheless, DDR arrests the cell cycle, rewires cellular metabolism, promotes senescence, and initiates programmed cell death. As a result, too much DDR drives the cellular aging process [3, 4]. Accumulation of DNA damage is found in the elderly and people with age-associated NCDs (**Figure 1**) [5]. Therefore, DNA damage accumulation is a crucial molecular pathogenic mechanism of the aging process. However, the mechanism by which DNA damage spontaneously accumulates in the aging genome remains to be explored.

Both epigenetic marks and DNA damage or lesions are temporary modifications of DNA. However, both are produced by different mechanisms and play roles in genomic instability in opposite directions. Epigenetic marks are produced by biological processes and possess physiological functions [6, 7]. For example, DNA methylation or methyl CpG is produced by DNA methyltransferase. The molecular function of methyl CpG is to interact with a protein such as methyl-CpG-binding protein. This interaction forms a cascade of molecular biological processes for gene regulation control and genomic stability. DNA lesions, on the other hand, are produced by endogenous or exogenous hazards [8]. For example, pyrimidine dimers, one type of DNA lesion, are formed via photochemical reactions such as exposure to UV light. DNA damage is converted into a mutation during subsequent replication, so accumulation of DNA damage leads to genomic instability. This chapter describes that genomic instability in the elderly should occur by the alteration of epigenetic marks leading to spontaneous accumulation of DNA damage.

Global DNA hypomethylation is an epigenetic change in the elderly and people with NCDs that promotes genomic instability [9–13]. However, the underlying mechanism of how the hypomethylated genome accumulates DNA damage is unknown [14]. In 2008, my group discovered an unprecedented type of endogenous DNA double-strand break (EDSB). These breaks are found in all cells, including nondividing cells, so we named them replication-independent EDSBs (RIND-EDSBs) [15]. RIND-EDSBs are located in hypermethylated DNA. Therefore, cells with global hypomethylation, such as cancer cells, have lower levels of RIND-EDSBs than noncancer cells [15]. After the discovery, we explored several characteristics of RIND-EDSBs and found that the majority of RIND-EDSBs possess physiological functions, namely, physiologic RIND-EDSBs (Phy-RIND-EDSBs), as epigenetic marks in maintaining genomic stability [16–19]. Interestingly, Phy-RIND-EDSBs in yeast decrease when yeast cells age [19]. So here I rename Phy-RIND-EDSBs in accordance with their role as youth-associated genomic-stabilizing DNA gaps (Youth-DNA-GAPs). In this chapter, we propose a hypothesis that the hypomethylated genome of the elderly reduces Phy-RIND-EDSBs and that this reduction causes DNA damage. The accumulation of DNA damage initiates DDR and consequently drives the cellular aging process (**Figure 1**).

In other words, the reduction in Phy-RIND-EDSBs by genome-wide hypomethylation is the underlying molecular pathogenesis mechanism of aging phenotypes.

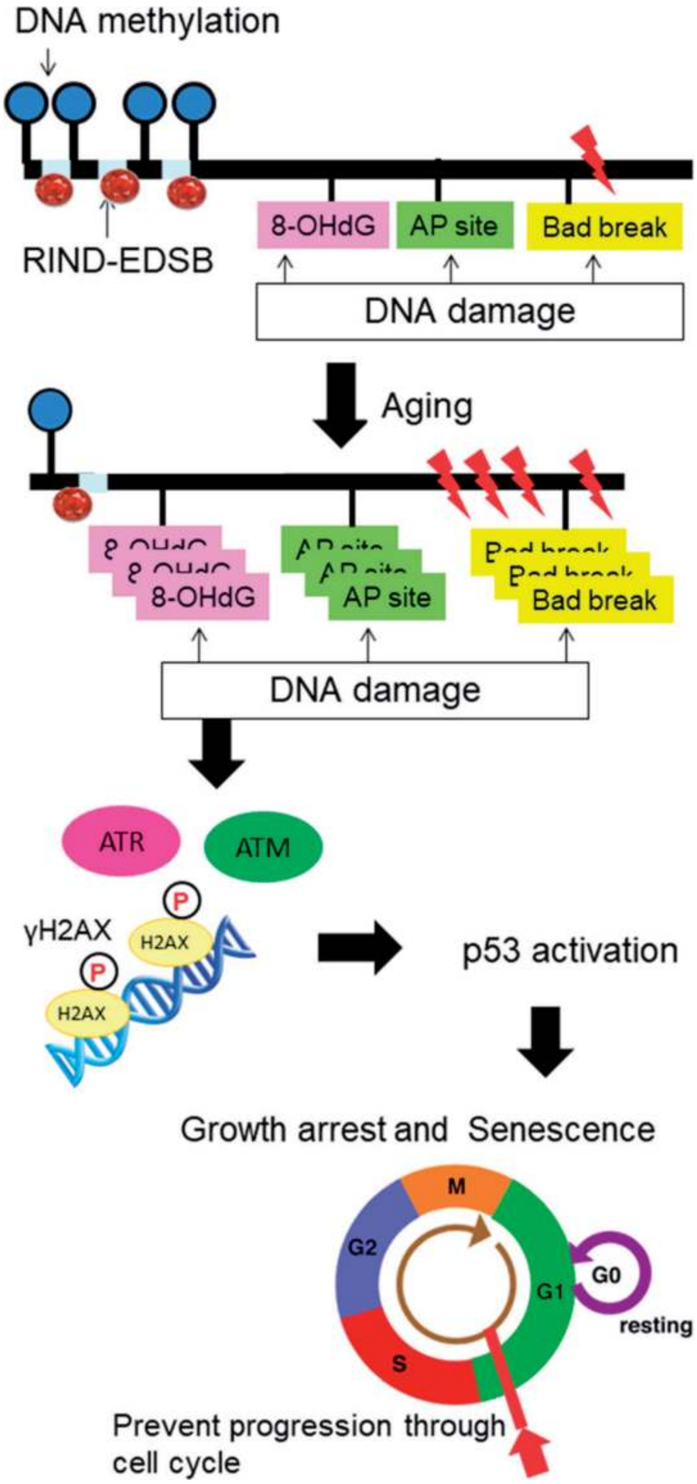

Figure 1.
Genome-wide hypomethylation drives genomic instability in the elderly by reducing youth-associated genome-stabilizing DNA gaps: A hypothesis. DNA methylation in the elderly is generally reduced, genome-wide hypomethylation. A reduction in DNA methylation leads to genomic instability, accumulation of endogenous DNA damage, and sensitivity to DNA-damaging agents. Here, we propose a hypothesis that global hypomethylation causes a reduction in Phy-RIND-EDSBs and that the reduction in Phy-RIND-EDSBs causes DNA damage. The accumulation of endogenous DNA damage will promote DDR, and too much DDR will arrest cells, causing metabolic rewiring and senescence.

2. Genome-wide hypomethylation

Genome-wide hypomethylation reduces the DNA methylation level of the whole genome. DNA methylation possesses two basic roles, gene regulation and the prevention of genomic instability, which we emphasize here [20]. The majority of DNA methylation in the human genome is on interspersed repetitive sequences (IRSs). Genome-wide hypomethylation or global hypomethylation mostly reflects a decrease in the DNA methylation of IRSs [11, 21]. Here, I will describe how IRS methylation occurs, how hypomethylation occurs, and how hypomethylation drives genomic instability in the elderly.

2.1 Interspersed repetitive sequence methylation

To evaluate the global methylation level, most recent studies have used PCR techniques to measure the DNA methylation level of each IRS, including Alu elements (Alu), long interspersed element-1s (LINE-1s), and several types of human endogenous retroviruses (HERVs). A reduction in Alu element methylation represents a genome-wide hypomethylation, driving genomic instability more than that of LINE-1 s and HERVs [11]. Throughout the human genome, there are over 1 million copies of Alu elements [22]. Although there is also a vast number of LINE-1 s, only approximately 3000 copies of LINE-1s contain a 5' UTR where LINE-1 methylation was usually measured [23, 24]. Because there are several classes of HERVs, each PCR measured DNA methylation of one class and as a result, covered a smaller percentage of the genome [25]. Furthermore, methylation of LINE-1 and HERV was reported to possess gene regulation functions [24, 26]. The tissue-specific methylation level of LINE-1 is locus dependent [27, 28]. In contrast, the global hypomethylation occurs as a generalized process [11, 28]. Therefore, methylation of LINE-1 and HERV represents global methylation in a lesser proportion than that of Alu elements.

2.2 Alu hypomethylation in aging and NCDs

Although global hypomethylation has been reported in the elderly, not all IRSs are hypomethylated. We investigated Alu, LINE-1, and HERV-K and found Alu and HERV-K hypomethylation in aging but not LINE-1 [11]. Therefore, methylation of LINE-1 and Alu may possess different roles. Global hypomethylation is also associated with the aging phenotype. First, lower global DNA methylation is associated with higher cardiovascular risk in postmenopausal women [29]. Second, Alu hypomethylation was observed in individuals with lower bone mass, osteopenia, osteoporosis, and a high body mass index [12]. Finally, Alu hypomethylation was reported in diabetes mellitus patients and was directly correlated with high fasting blood sugar, HbA1C, and blood pressure [13]. Interestingly, the Alu methylation level was also high in catch-up growth in a 20-year-old offspring [30]. These studies indicated the positive role of Alu methylation in the human growth process and the role of Alu hypomethylation as an epigenetic cause of the human aging process.

2.3 Mechanism causing global hypomethylation

The direct correlation between IRS methylation levels suggests that the mechanisms causing global hypomethylation in both aging cells and cancer are a generalizing process [11, 28]. The actual mechanism causing global hypomethylation in aging remains to be explored. Nevertheless, exposure to oxidative stress, benzene, air pollution, UV light, radiation, smoke, and folate deficiency facilitates genome-wide hypomethylation processes [31–37]. Therefore, the accumulation of

DNA damage, oxidative stress, or a lack of DNA methylation precursors can lead to genome-wide hypomethylation.

Evidence suggests that DNA damage drives the demethylation process. DNA repair, which is how cells remove DNA lesions, is also a demethylation mechanism that directly removes 5-methylcytosine. Methylcytosine is a DNA base that is prone to be deaminated and must be fixed by base excision repair (BER) machinery to prevent cytosine-to-thymine substitution. However, BER replaces the DNA lesion with an unmethylated form of cytosine. As a result, the methylcytosine is demethylated. The other mechanism is to remove the entire DNA patch and refill with unmethylated nucleotides by nucleotide excision repair (NER) or mismatch repair (MMR) [38].

For oxidative stress, oxidation of 5-methylcytosine forms 5-hydroxymethylcytosine. There are several mechanisms for removing 5-hydroxymethylcytosines and replacing them with unmethylated forms, AID/APOBEC enzymes and TET enzymes followed by BER [39–44]. Alternatively, oxidative stress may interfere with the DNA methylation protein machinery. For example, oxidative stress depletes the synthesis of glutathione and decreases the availability of S-adenosylmethionine for DNA methylation [45]. This proposed mechanism is similar to DNA demethylation in depletion of the methyl pool in folate-deficient models [46, 47].

2.4 Hypomethylation accumulates multiple kinds of DNA lesions

The hypomethylated genome is prone to accumulating multiple kinds of DNA damage, which is an abnormal chemical structure in DNA and includes oxidative damage, depurination, depyrimidination, and pathologic EDSBs [10, 14]. Alu methylation levels in white blood cells were found to inversely correlate with 8-hydroxy-2′-deoxyguanosine (8-OHdG) oxidative damage and apurinic/apyrimidinic sites (AP sites) [37]. Transfection of cells with Alu small interfering RNA (Alu siRNA) increased Alu methylation and reduced endogenous 8-OHdG and AP sites [37]. Interestingly, Alu siRNA also increased cell division and resistance to DNA damage-causing agents [37]. This evidence indirectly suggests that Alu methylation stabilizes the human genome. DNA methylation also prevents pathologic EDSBs. The chromosomal rearrangements and deletions of DNA commonly found in cancer cells treated with DNA demethylating agents and DNA methyltransferase (DNMT) knockout mice and naturally occurring mutations in the cytosine DNA methyltransferase DNMT3B suggest that pathologic EDSBs are the intermediate products of hypomethylation that drive genomic instability [10, 48–50].

2.5 DNA lesions as a molecular pathogenesis mechanism of the aging process and NCDs

A number of studies support the idea that accumulation of DNA damage drives the aging process. First, congenital defects in DNA repair accelerate aging. For example, progeroid syndrome patients with ERCC4 mutations have premature aging of many organs. ERCC4 is a protein designated as the DNA repair endonuclease XPF that is critical for many DNA repair pathways, including NER [51]. Second, genotoxic agents accelerate the aging process in cancer survivor patients. For example, 50-year-old survivors of childhood cancer have an increased incidence of age-related diseases compared to their siblings [52]. Third, there is evidence of DNA damage accumulation when cells age. Pathologic EDSBs are accumulated in chronological aging yeast [17]. Many kinds of DNA damage from base modifications to γH2AX foci, representing pathologic EDSBs, have been reported in several organs of animals and humans [53–56]. Finally, a reduction in DNA repair efficiency was reported in aging cells of many organisms [57–59]. In NCDs, the accumulation of oxidative DNA damage has been reported in patients with cardiovascular disease, diabetes and metabolic syndrome, chronic obstructive pulmonary disease, osteoporosis, and neurological degeneration, including Alzheimer's disease and Parkinson's disease [5]. DNA damage triggers DDR. To facilitate DNA repair and prevent mutation accumulation, DDR arrests cell cycle progression until repair is complete. While DDR can prevent cancer development, DDR leads to many unwanted effects, including inflammation, metabolic rewiring, senescence, apoptosis, and aging [60–62]. The DDR signaling pathway consists of signal sensors, transducers, and effectors. The sensors of this pathway are proteins that recognize DNA damage. The main transducers are ATM and ATR and their downstream kinases. The effectors of this pathway are substrates of ATM and ATR and their downstream kinases. These effectors of DDR involve many proteins, including P53, BRCA1, and CDC25s [60–63].

2.6 DNA methylation possesses a long-range effect in stabilizing the human genome in cis

A direct association between loss of DNA methylation and rearrangements in the pericentromeric heterochromatin was demonstrated in ICF syndrome (immunodeficiency, chromosomal instability, and facial anomalies) and loss-of-function mutations in DNMT3B [50, 64]. Therefore, hypomethylation could lead to spontaneous mutations in cis, which are epigenetic and genetic events occurring in the same chromosome. Notably, Alu siRNA increased Alu methylation levels in HEK293 cells from 60 to 70% [14]. Because there are approximately 1 million copies of Alu, by rough estimation, Alu siRNA methylates 10% of Alu elements or approximately 100,000 Alu elements in 3000 Mb of the human genome. In other words, Alu siRNA transfection methylated one locus of every 30 kb of human genome on average. Furthermore, Alu siRNA reduced 75% of endogenous 8-OHdG [14]. Therefore, even if Alu siRNA increases methylation in a limited location, the transfection stabilized the genome far beyond the methylated Alu elements (**Figure 2**).

2.7 Hypotheses: DNA methylation prevents genomic instability mechanisms

There are at least three possible mechanisms by which Alu methylation reduces endogenous DNA damage and increases resistance to DNA damage-causing agents. The extension of genomic stability from methylated Alu loci supports my first hypothesis that DNA methylation stabilizes the genome by homing Youth-DNA-GAPs, Phy-RIND-EDSBs, and that the gaps extended the stabilizing effect to the entire genome [14]. Another reason that supports the Phy-RIND-EDSBs mediating the DNA methylation role in stabilizing the genome is that Phy-RIND-EDSBs are localized in hypermethylated DNA [15]. Moreover, Phy-RIND-EDSBs possess a redundant topoisomerase which relieve tension of double-helix spin and torsion from any DNA activity [17]. The second hypothesis would be the spreading of DNA methylation and consequently heterochromatin [65]. However, this mechanism is unlikely because the spreading would need to extend to cover the whole genome and would interfere with cellular function. A reduction in cell viability by Alu siRNA was not observed. The last and unlikely hypothesis was that DNA methylation somehow enhanced DNA repair activity [66], although this mechanism is also unlikely because most DNA repair machinery starts with specific sensors to recognize DNA lesions.

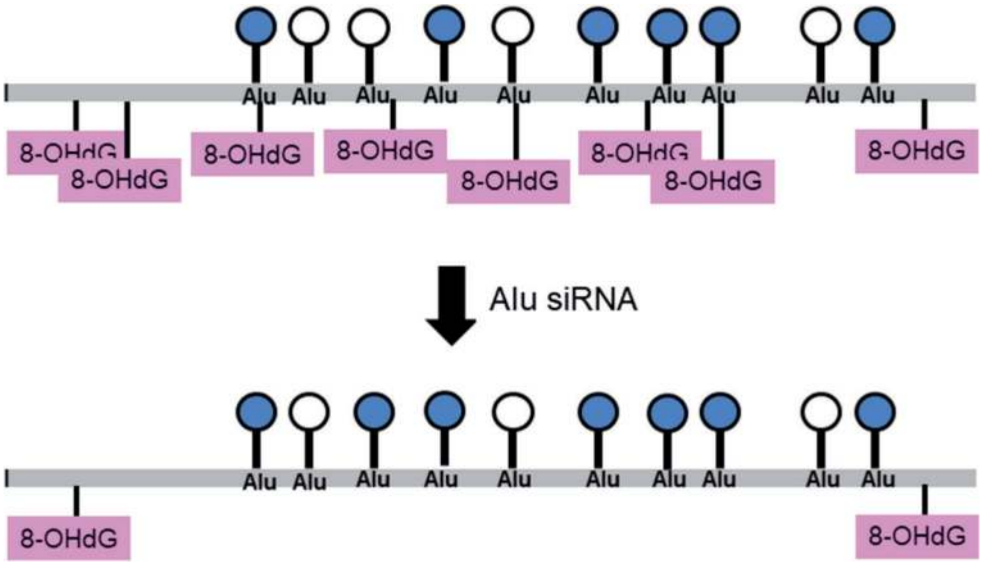

Figure 2.
DNA methylation possesses a long-range effect in stabilizing the human genome in cis. This diagram represents a fraction of the human genome before and after Alu siRNA transfection. While Alu siRNA methylated only 10% of Alu loci, Alu siRNA reduced 75% of the 8-OHdG in the entire genome [14]. Therefore, DNA methylation possesses a long-range effect in stabilizing the human genome. Blue circles are DNA methylation and white circles are unmethylated DNA.

3. Phy-RIND-EDSBs represent epigenetic marks as youth-DNA-GAPs

Phy-RIND-EDSBs are found in all eukaryotic cells, produced by certain proteins, and reduced in chronological aging yeast [15, 17, 19]. A reduction in Phy-RIND-EDSBs decreased cell viability and augmented pathologic EDSB production [19]. Phy-RIND-EDSBs are devoid of DDR and are repaired by the error-free repair pathway [16]. Therefore, Phy-RIND-EDSBs are Youth-DNA-GAPs epigenetic marks that prevent genomic instability in eukaryotic genomes.

3.1 IRS-EDSB ligation-mediated PCR (IRS-EDSB-LMPCR) to measure EDSBs

Ligation-mediated PCR (LMPCR) is the method that we used for EDSB detection [15]. Previously, this PCR technique was used to characterize the signal end and coding end of EDSBs occurring during the V(D)J recombination process [67]. For V(D)J recombination, the signal end and coding end of EDSBs occur at the T-cell receptor or antibody genes in lymphoblasts. To detect the signal end and coding end, DNA from lymphoblasts was ligated to a linker, and PCR was performed using linker primer and oligonucleotide sequences of T-cell receptor or antibody genes. Generalized EDSBs can occur anywhere in the genome. Therefore, we replaced IRS as a primer instead of T-cell receptor or antibody genes [67, 68]. As a result, IRS-EDSB-LMPCR yields two types of amplicons, IRS-EDSB and IRS-IRS sequences, and we detected linker sequences that represent EDSB amplicons. In brief, IRS-EDSB-LMPCR was performed as follows. First, the oligonucleotide linker, EDSB linker, was ligated to high-molecular-weight DNA (HMWDNA) or nucleus. Second, real-time quantitative PCR was performed using two PCR primers. The first was homologous to IRS, and the other had the same sequence as the 5′ end of the ligation linker. The number of EDSBs could be measured by Taqman probe homology to the 3′ end of the ligation linker sequence. The HMWDNA or nucleus served as a source of EDSBs, and the EDSB linker detected and ligated EDSBs. The first PCR cycle polymerized DNA from genome-wide distributed IRSs. The polymerization through EDSBs generated an EDSB-LMPCR linker template. The IRS-EDSB-linker sequences were generated, detected, and quantitated by the Taqman probe during PCR cycle (**Figure 3**) [15].

Common criticism of IRS-EDSB-LMPCR is the possibility of DNA shearing from HMWDNA preparation. However, the characteristics of the DSBs generated by DNA preparation are different from RIND-EDSBs. In humans, the sequence around RIND-EDSBs is always hypermethylated, whereas methylation levels of DSBs from mechanical shearing possess less methylation than RIND-EDSBs [15]. To prove that the RIND-EDSBs are real, we compared EDSBs from linker ligated to HMWDNA and nucleus and found that RIND-EDSBs analyzed directly from in situ ligation displayed the same pattern as IRS-EDSB-LMPCR from HMWDNA [17]. Therefore, DSBs detected by IRS-EDSB-LMPCR were endogenous in origin.

3.2 Phy-RIND-EDSBs are evolutionarily conserved epigenetic marks

Nature has conserved all epigenetic marks by conserving the genes that produce epigenetic marks [7]. Epigenetic marks have a specific biological role, whether it is gene expression, genomic stability, or interacting with DNA. Therefore, the genome distribution of epigenetic markers will not be random. Finally, epigenetic marks are usually crucial for cell survival and therefore should be ubiquitously present in all cells. To search for genes that produce or maintain Phy-RIND-EDSBs, we evaluated RIND-EDSB levels in yeast strains that lack functional mutation genes encoding various DNA repair regulators, chromatin formation, endonucleases, topoisomerase, and chromatin-condensing proteins [17]. We found low levels of RIND-EDSBs in cells lacking high-mobility group box (HMGB) proteins and Sir2. Thus, HMGB proteins and Sir2 play roles in producing and maintaining Phy-RIND-EDSBs [17]. Phy-RIND-EDSBs are distributed in the genome nonrandomly [18]. In humans, Phy-RIND-EDSBs are localized within hypermethylated DNA [15]. In yeast, DNA sequences 5′ end to RIND-EDSBs were not random; certain four-nucleotide sequences were more likely to be present immediately prior to the breaks. Moreover, RIND-EDSBs were prevented from occurring or were never observed following certain four-base combinations [18]. RIND-EDSBs were found in yeast and in the human genome, and therefore, Phy-RIND-EDSBs are conserved in eukaryotic organisms [15, 17]. In humans, RIND-EDSBs were detectable in all cell types and found within the hypermethylated genome in all phases of the cell cycle [15]. In yeast, we found a very strong direct correlation between cell viability and Phy-RIND-EDSB levels ($r = 0.94$, $p < 0.0001$) [19]. In other words, the more Phy-RIND-EDSBs a cell possesses, the better the cell survives [19]. When Phy-RIND-EDSB levels were reduced by homothallic switching (HO) endonuclease induction or NHP6A gene deletion, cell viability decreased [19]. In conclusion, Phy-RIND-EDSBs are epigenetic markers that are important in all eukaryotic cells [19].

Most of the RIND-EDSBs under normal physiologic conditions are not DNA damage, signals of the DDR, or precursors of mutations [16]. While sequences around human RIND-EDSBs are hypermethylated, γH2AX-binding DNA is hypomethylated. Therefore, most RIND-EDSBs are devoid of γH2AX [16]. γH2AX is a H2AX molecule that is phosphorylated at serine 139 by the signaling cascade of DDR of pathologic DSBs [69]. Most RIND-EDSBs are repaired by a more precise ATM-dependent pathway, and therefore, most RIND-EDSBs under normal physiologic conditions are Phy-RIND-EDSBs [16].

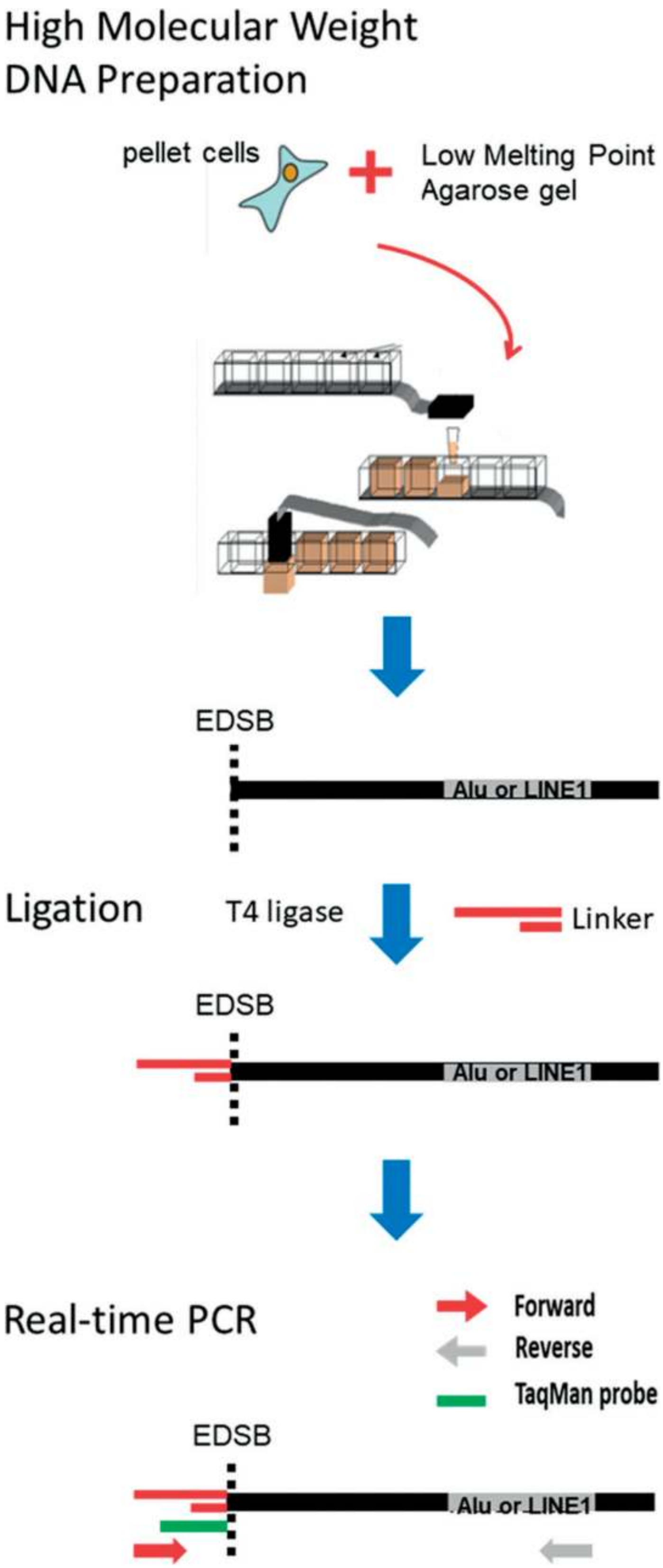

Figure 3.
IRS-EDSB-LMPCR diagram demonstrating IRS-EDSB-LMPCR. LMPCR linker ligates to EDSB. The 5′ end of the LMPCR linker is the same sequence as the PCR primer. The 3′ end of the LMPCR linker is homologous to the Taqman probe. The Taqman probe is used for quantitation of EDSBs by real-time PCR. The IRS primer is a PCR primer with IRS sequences to polymerize numerous locations of the genome [15].

3.3 Phy-RIND-EDSB or youth-DNA-GAP complex

Human Phy-RIND-EDSBs are localized in hypermethylated DNA regions and deacetylated histones [15, 16]. Phy-RIND-EDSBs are reduced in cells lacking HMGB proteins and Sir2 and NAD-dependent deacetylase [17, 70]. The human Sir2 homolog, sirtuin 1 (SIRT1), binds to the HMGB1 protein and deacetylates DNMT1 [71, 72]. Furthermore, HMGB1 possesses deoxyribophosphate lyase activity [73]. Therefore, we propose a hypothesis here that HMGB1 cuts DNA to produce Phy-RIND-EDSBs. SIRT1-bound HMGB1 deacetylates histones, keeping Phy-RIND-EDSB ends within the heterochromatin to shield them from the DDR signal. Finally, the interaction between SIRT1 and DNMT1 or deacetylated histone and DNA methylation may be the reason why sequences around human Phy-RIND-EDSBs are hypermethylated (**Figure 4**).

Interestingly, both HMGB1 and Sir2 have other functions that can be related to Phy-RIND-EDSBs. HMGB1 is a protein in another physiologic EDSB complex, the signal end and coding end of V(D)J recombination [74]. Moreover, yeast lacking the NHP6A protein, a type of yeast HMGB, shows increased endogenous DNA damage and sensitivity to UV light [75]. Finally, HMGB1 has the ability to bend DNA [76]. To prevent DNA torsion, it is reasonable to create Phy-RIND-EDSBs while bending DNA. Sir2 can deacetylate the histone, while Phy-RIND-EDSBs are localized in the deacetylated histone. Interestingly, Sir2 and SIRT1 are known to prevent the aging process [77, 78].

3.4 Spontaneous pathologic RIND-EDSBs and modified ends with insertion at the breaks

Independent of DNA replication, EDSB-LMPCR could detect pathologic RIND-EDSBs (Path-RIND-EDSBs) as excess EDSBs when DSB repair was inhibited by chemical inhibition or DSB repair gene mutation [19]. When we treated G0 yeast cells with caffeine, a DSB repair inhibitor, we observed a spontaneous increase in RIND-EDSBs [19]. These excess RIND-EDSBs did not possess the same 5′ end-sequence-four-base combinations as Phy-RIND-EDSBs odds ratio (OR) > 1 breaks. Notably, we called four-base combinations that are unlikely to be found in Phy-RIND-EDSBs as OR ≤ 1 breaks [18]. Moreover, we also observed that the 5′ end sequence downstream of the break did not match with the genomic sequence from the first base as reads with modified ends with insertion at the breaks (MIBs) [57]. We found that caffeine treatment increased the proportion

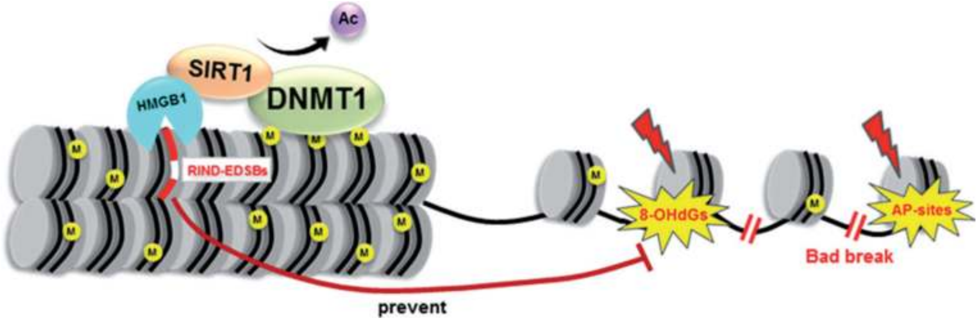

Figure 4.
Phy-RIND-EDSB or youth-DNA-GAP complex: I hypothesize that the HMGB group initiates Phy-RIND-EDSB and that HMGB interacts with SIR2 or SIRT1. SIRT1 deacetylates histones and DNMT1, and DNMT1 methylates DNA. The role of Phy-RIND-EDSB or youth-DNA-GAP is to prevent DNA damage anywhere along the same chromosome.

of MIBs [57]. Therefore, MIBs might be a mechanism that compensates for repair defects, such as alternate repair of the DSB pathway, or prevents EDSB ends from stimulating DDR. Seven repair defect yeast strains, *mec1*Δ, *mre11*Δ, *nej1*Δ, *rad51*Δ, *tel1*Δ, *yku70*Δ, and *yku80*Δ, were studied. Except for *nej1*Δ, the percentages of OR ≤ 1 breaks and MIBs were significantly increased in all samples when compared to the wild type. We also examined whether there was an association between MIBs and types of breaks (OR > 1 breaks and OR ≤ 1 breaks) and found that in the wild type, MIBs occurred at OR ≤ 1 breaks. In contrast, in *mec1*Δ, *mre11*Δ, *rad51*Δ, *tel1*Δ, *yku70*Δ, MIBs occurred at both OR > 1 breaks and OR ≤ 1 breaks [57]. Therefore, both Phy-RIND-EDSBs and Path-RIND-EDSBs are produced in the genome independent of DNA replication. However, most Path-RIND-EDSBs are immediately repaired, while Phy-RIND-EDSBs are retained [57].

3.5 Variation in RIND-EDSB level and reduction in Phy-RIND-EDSBs in aging and hypomethylated cells

Path-RIND-EDSBs are spontaneously produced and immediately repaired, while Phy-RIND-EDSBs are produced and retained by the Phy-RIND-EDSB complex formation process. We observed an increase in RIND-EDSB levels in yeast lacking a DSB repair gene, topoisomerase and endonuclease. Analysis of EDSB sequences suggested that DSB repair inhibition causes retention of both Phy-RIND-EDSBs and Path-RIND-EDSBs. For topoisomerase and endonuclease mutants, we postulated that Phy-RIND-EDSBs may have redundant roles with topoisomerase and endonuclease in stabilizing the genome. Nevertheless, sequence analysis is needed to prove this hypothesis. As mentioned earlier, one yeast strain lacking the *HMGB* gene or *SIR2* possessed a low level of RIND-EDSBs. Therefore, we hypothesized that HMGB and Sir2 play roles in PHY-RIND-EDSB complex formation and retention. We observed that three chemicals can alter RIND-EDSB levels. Whereas caffeine and vanillin, DSB repair inhibitors, increased RIND-EDSB levels, trichostatin A, a histone deacetylase inhibitor, decreased the EDSBs. The reduction in RIND-EDSBs by trichostatin A suggested that Phy-RIND-EDSBs are retained within facultative heterochromatin. This result is similar to the low level of RIND-EDSBs in yeast lacking *SIR2*.

DDR signals to repair Path-RIND-EDSBs can repair and consequently reduce Phy-RIND-EDSBs [19]. The retention of Phy-RIND-EDSBs and the immediate repair of Path-RIND-EDSBs led to the finding that the majority of RIND-EDSBs under normal physiologic conditions are Phy-RIND-EDSBs and that a reduction in RIND-EDSBs in any condition is a reduction in Phy-RIND-EDSBs. In addition to gene mutation and histone acetylation, we could reduce RIND-EDSB levels in yeast by inducing a Path-RIND-EDSB by HO endonuclease induction [19]. HO endonuclease is a site-specific endonuclease that cleaves a site in the MAT locus on chromosome III [79]. After induction in nondividing yeast, we observed a sustained reduction in RIND-EDSBs for up to 4 days. However, when we induced HO in yeast lacking *MEC1*, a DSB repair protein, the reduction was not observed [19]. These experiments suggested that Path-RIND-EDSB production can ignite the global DSB repair process, and consequently, the retained Phy-RIND-EDSBs are repaired [19]. This mechanism is one possible explanation for the reduction in RIND-EDSBs in chronologically aging yeast.

Phy-RIND-EDSB levels in the elderly should be low. We found low levels of RIND-EDSBs in chronologically aging yeast and in the human cancer cells, HeLa and SW480, which are cervical cancer and colon cancer cell lines, respectively [15, 19]. Phy-RIND-EDSBs are localized in hypermethylated genomic regions [15].

Therefore, cancer genome hypomethylation may explain why RIND-EDSB levels in cancer cells were low [15, 27]. We have not reported RIND-EDSB levels in the elderly. However, our unpublished data demonstrated results similar to those in chronologically aging yeast and in cancer cells.

3.6 Reduction in Phy-RIND-EDSBs augments pathologic EDSB production

To define the molecular mechanism by which the reduction in Phy-RIND-EDSBs in chronological aging in yeast reduced cell viability and to evaluate the consequences of Phy-RIND-EDSB reduction, we analyzed yeast cells with low levels of Phy-RIND-EDSBs, including HO endonuclease and *nhp6a*Δ, a high-mobility group box protein mutant [19]. Very high levels of Path-RIND-EDSBs were observed in both strains possessing low levels of Phy-RIND-EDSBs after treatment with caffeine, a DSB repair inhibitor. The new Path-RIND-EDSBs were not in the same location as Phy-RIND-EDSBs. Therefore, similar to DNA methylation, Phy-RIND-EDSB stabilizes the genome far beyond the Phy-RIND-EDSB complex (**Figure 4**). These experiments led to my conclusion that the role of Phy-RIND-EDSBs is similar to that of EDSBs induced by topoisomerase, which is DNA torsion prevention and DNA tension reduction from DNA spinning due to any DNA activity, including transcription, replication, and repair. The role of Phy-RIND-EDSBs can be imagined as gaps in a railroad track that prevent track torsion from track expansion by heat. Phy-RIND-EDSB levels decreased in chronologically aging yeast, and the reduction was directly correlated with reduced cell viability. Therefore, Phy-RIND-EDSBs play a Youth-DNA-GAPs role in preventing Path-RIND-EDSBs and DNA damage lesions [19]. Moreover, the *nhp6a* gene is known to prevent other types of DNA lesions, such as pyrimidine dimers [75]. Therefore, it is reasonable to hypothesize that the scatter distribution of the Phy-RIND-EDSB complex prevents all kinds of DNA damage along the length of the whole genome (**Figure 4**).

3.7 DNA repair activity may be compromised in aging cells by a reduction in Phy-RIND-EDSBs

A reduction in Phy-RIND-EDSBs during chronological aging may be a cause of DNA repair defects in the elderly. DNA repair machinery is known to be compromised and error-prone with age [59, 75]. Numerous studies have found a significant decline in all commonly known repair pathway activities with aging, including double-strand break repair activities [53–56]. We demonstrated that the reduction in the Phy-RIND-EDSB complex will increase the production of DNA damage [19]. Therefore, aging cells have to repair DNA damage more often than younger cells. As a result, more DNA repair machinery is required for older cells. Consequently, DNA repair substrates are consumed more quickly than they are produced, resulting in DNA repair defects in the elderly.

4. Conclusion

All evidences described in this chapter suggest that genomic instability in the elderly is a vicious cycle of interactive networks among DNA damage, DNA repair, DNA demethylation, and reduction in Youth-DNA-GAPs (**Figure 5**). DNA damage occurs spontaneously. Then, the DNA repair process, in addition to repairing DNA damage, has consequences of reducing epigenetic marks. While NER demethylates DNA, the DSB repair pathway will repair Phy-RIND-EDSBs. DNA demethylation

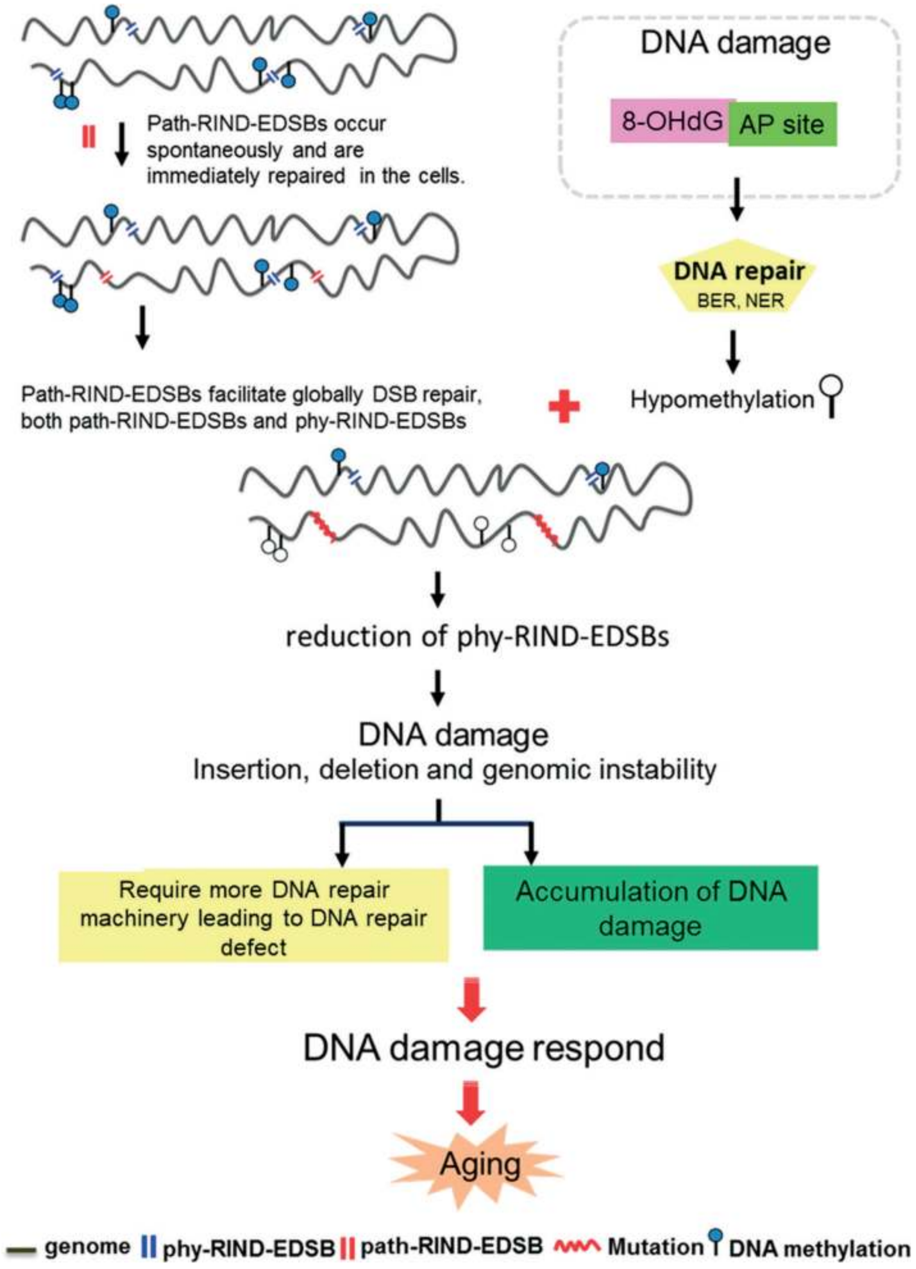

Figure 5.
Destructive network of aging DNA. DNA damage can occur spontaneously. The base modification repair consequence is DNA demethylation, and DSB repair for pathologic DSB will also globally repair Phy-RIND-EDSBs. Continuous DNA demethylation results in genome-wide hypomethylation, which, together with global Phy-RIND-EDSB repair, reduces the Phy-RIND-EDSB complex. A reduction in the Phy-RIND-EDSB complex augments DNA damage, and a large amount of DNA damage requires extensive DDR. Cells extensively use DDR until the DNA repair machinery is exhausted and defective, at which point, DNA damage accumulates and DDR arrests and ages cells.

results in global hypomethylation and consequently reduces the homing of Phy-RIND-EDSBs. The depletion of the Phy-RIND-EDSB complex will then augment DNA damage production. Cells need to use many DNA repair substrates to eliminate DNA damage faster than these substrates are produced and eventually lose the capability of DNA repair. As a result, aging cells continue to accumulate DNA damage and send DDR signals, halting the cell cycle, causing metabolic rewiring, and eventually driving cells to enter senescence.

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Conflict of interest

The author declares no conflict of interest.

Abbreviation list

NCDs	noncommunicable disease
DDR	DNA damage repair signal
EDSB	endogenous DNA double-strand break
RIND-EDSB	replication-independent endogenous DNA double-strand break
Phy-RIND-EDSBs	physiologic RIND-EDSBs
Youth-DNA-GAPs	youth-associated genomic-stabilizing DNA gaps
IRSs	interspersed repetitive sequences
Alu	Alu elements
LINE-1s	long interspersed element-1s
HERVs	human endogenous retroviruses
BER	base excision repair
NER	nucleotide excision repair
MMR	mismatch repair
8-OHdG	8-hydroxy-2′-deoxyguanosine
AP sites	apurinic/apyrimidinic sites
DNMT	DNA methyltransferase
Alu siRNA	Alu small interfering RNA
LMPCR	ligation-mediated PCR
HMW DNA	high-molecular-weight DNA
IRS-EDSB-LMPCR	IRS-EDSB ligation-mediated PCR
HMGB	high-mobility group box
SIRT1	sirtuin 1
Path-RIND-EDSBs	pathologic RIND-EDSBs
HO	homothallic switching
OR	odds ratio
MIBs	modified ends with insertion at the breaks

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