Chapter

The Role of DNA Repair in Cellular Aging Process

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Abstract

Aging is defined as the time-dependent decline of functional properties. One common denominator of aging is mitochondrial dysfunction and accumulation of genetic damage throughout life. In fact, the imperfect maintenance of nuclear and mitochondrial DNA likely represents a critical contributor of aging. Each day, the integrity and stability of DNA are challenged by exogenous physical, chemical, or biological agents, as well as by endogenous processes, including DNA replication mistakes, spontaneous hydrolytic reactions, and reactive oxygen species. In this way, DNA repair systems have evolved a complex network that is collectively able of dealing with most of the damages inflicted. However, their efficiency may decrease with age and, therefore, influence the rate of aging. Thus, the purpose of this work is to summarize the recent knowledge in cellular aging process and its link with DNA repair systems, with a particular emphasis on the molecular mechanisms associated.

Keywords: DNA damage, DNA repair, BER, NER, MMR, HR, NHEJ

1. Introduction

Aging is a complex biological process that results in a progressive loss of physiological integrity. Overall, aging is a consequence of accumulation of cellular damage and is characterized by nine hallmarks: genomic instability, telomere attrition, epigenetic alterations, cellular senescence, mitochondrial dysfunction, loss of proteostasis, deregulated nutrient sensing, stem cell exhaustion, and altered intercellular communication (**Figure 1**) [1]. Although aging may involve damage to various cellular constituents, there is evidence suggesting that DNA constitutes the key target in this process [2]; consequently, genomic instability is the main factor of aging [3–5]. Genome instability has been implicated as a cause of aging since unrepaired DNA damage, DNA mutations, and epimutations accumulate in an age-related manner [3]. In the same way, the notion that multi-system premature aging syndromes are mainly caused by defects in genome maintenance or affect genome function highlights the role of genome integrity in aging [6]. Meanwhile, normal aging is accompanied by telomere shortening with cell division due to the "end-replication problem" and telomere end processing. Currently, there is a wide body of evidence associating reduction in the length of telomeres with failure of cell division and senescence of normal cells, and oxidative stress and inflammation can contribute to the rate of attrition of telomere length [7]. Age-related changes involve

alterations in DNA methylation patterns and posttranslational modification of histones such as increased histone H4K16 acetylation [8], H4K20 trimethylation [9], or H3K4 trimethylation [10], as well as decreased H3K9 methylation [11] or H3K27 trimethylation [12]. At the same time, with aging there is also a global heterochromatin loss and redistribution [13], thus affecting the expression of several genes, mainly those involved in DNA repair, cellular proliferation, differentiation, and cell-cycle regulation, and therefore triggering the emergence of other hallmarks of aging [14, 15]. Cellular senescence is a process that has become an important contributor in aging since it imposes a permanent proliferative arrest of cells in response to various stressors such as DNA damage and telomere loss [16]. Furthermore, as cells and organisms age, mitochondria suffer a decline in their integrity and function, tending to diminish the efficacy of the respiratory chain and thus reducing ATP generation, increasing electron leakage and ROS production that can damage DNA, proteins, and lipids, among other important biomolecules [17]. Proteostasis involves mechanisms for correct folding proteins and mechanisms for the degradation of proteins, which act in a coordinated fashion to prevent the accumulation of damaged components and assuring the continuous renewal of intracellular proteins. There is evidence that aging is associated with perturbed proteostasis, thus favoring the development of several diseases [18]. Recent data have shown that anabolic signaling accelerates aging; in agreement with this, caloric-restricted diet decreases nutrient signaling and as a result, a long life span is promoted since DNA repair systems are improved; on the other hand, protein homeostasis decreases ROS production and delays cellular senescence [19]. Decline in the regenerative potential of tissues is one of the most obvious characteristics of aging, where stem cell exhaustion is also important and explained by a decreased cell-cycle activity. Interestingly, this correlates with the accumulation of DNA damage, telomere shortening, and overexpression of cell-cycle inhibitory proteins such as p16INK4a, increasing the relevancy of DNA repair systems [20]. Finally, aging also involves changes at the level of intercellular communication, where neurohormonal signaling tends to be deregulated together with composition of the peri- and extracellular environment

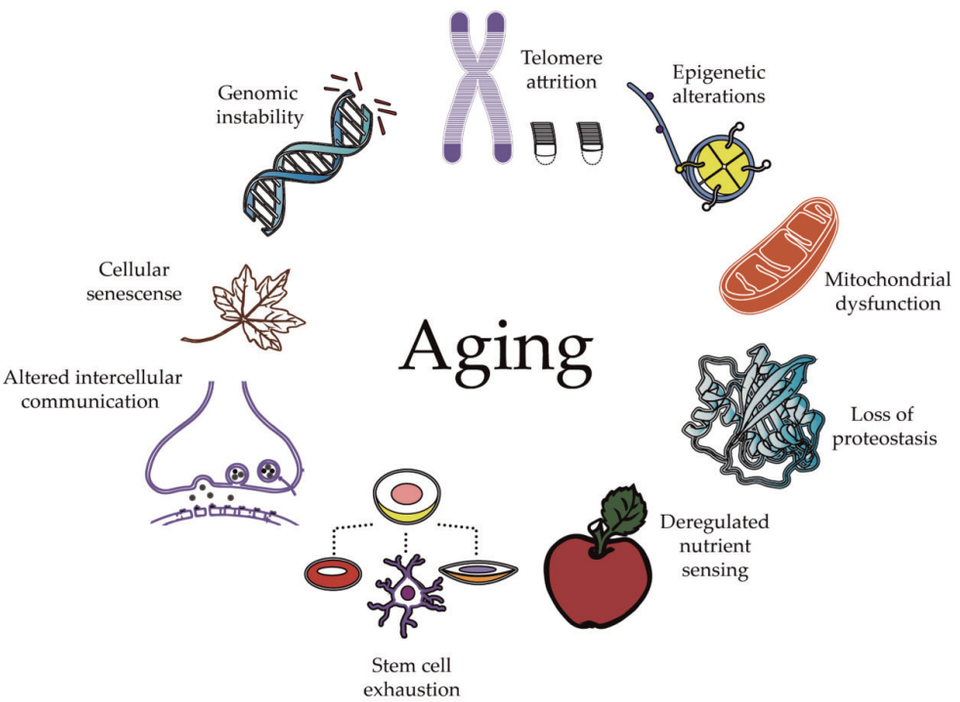

Figure 1.
The hallmarks of aging. The figure illustrates nine hallmarks previously described [1] and where age-related changes in DNA repair systems have important roles to promote the development of this phenotype.

and immune system, specially increasing inflammatory reactions and declining immunosurveillance against pathogens and premalignant cells [21]. In this way, our work focuses on describing the molecular bases that associate DNA damage and the cell aging process, with a special emphasis in DNA repair systems.

2. Age-related changes in DNA repair

Each day, the integrity and stability of DNA are challenged by exogenous physical, chemical, or biological agents, as well as by endogenous processes, including DNA replication mistakes, spontaneous hydrolytic reactions, and reactive oxygen species (ROS). Thus, depending on the source of damage, DNA can be affected in different ways, including nucleotide alterations, bulky adducts, single-strand breaks (SSB), and double-strand breaks (DSB). To combat threats posed by DNA damage, cells have evolved complex and finely regulated mechanisms collectively referred to as DNA damage response (DDR) which detects DNA lesions, signals their presence, and promotes their repair [22–24]. However, according with the genome maintenance hypothesis of aging, DNA repair can itself be subject to age-related changes and deterioration, allowing accumulation of damages (**Figure 2**). The wide diversity of DNA-lesion types requires multiple, largely distinct DNA repair mechanisms that differ in their components, whereas some lesions are subject to direct protein-mediated reversal, most are repaired by a sequence of catalytic events mediated by multiple proteins [22]. Thus, cells with defects in key proteins involved in DDR have been shown an accelerated aging phenotype caused by the accumulation of mutations and epimutations that eventually cause malfunction of the cells, senescence, or apoptosis [25].

2.1 Response to DNA single-strand breaks (SSBs)

2.1.1 Base excision repair (BER)

BER pathway corrects DNA damage from oxidation, deamination, alkylation, and other small DNA alterations that do not distort the overall structure of double helix. In general, BER is initiated by a DNA glycosylase that recognizes and

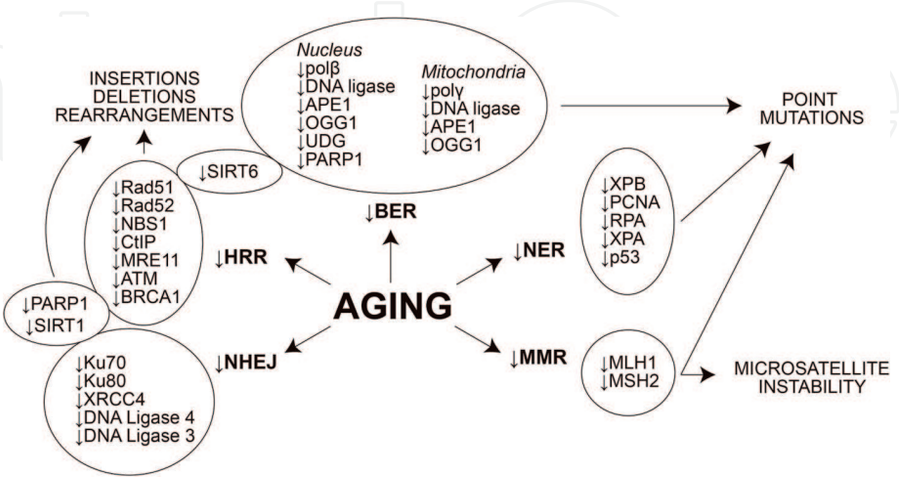

Figure 2.
Age-related changes in DNA repair and their consequences. Aging involves deterioration of DNA repair systems allowing the damages to accumulate and eventually cause a malfunction of the cells. In general, all age-related changes in DNA repair pathways promote genomic instability in different ways. Decline in efficiency and fidelity of BER and NER leads to point mutations, whereas inefficient MMR leads to microsatellite instability and point mutations. Meanwhile, deficiencies in NHEJ and HRR result in deletions and genomic rearrangements.

removes the damaged base, leaving an abasic (apurinic/apyrimidinic; AP) site that is subsequently processed by an AP endonuclease (APE), an exonuclease, a DNA polymerase, a ligase, and many other ancillary factors in a short-patch repair or long-patch repair [26]. Notably, several pieces of evidence indicate that the efficacy of BER may negatively change with age, and it has a significant impact in longevity together with homologous recombination repair (HRR) [27]. Age-related changes in the BER mechanism have been studied mainly in neuronal extracts where it constitutes the main repair pathway. In this way, an overall deficiency in several factors has been observed [28], where DNA polymerase β (pol β) together with DNA ligase [29] and APE1 activities [30, 31] seem to be the most limiting factors. Interestingly, an age-dependent attenuation in the transcriptional activation of pol β and APE1 was observed in response to DNA damage [32] together with APE1 accumulation in the nucleus and mitochondria [33]. Aging has also been shown to have a significant effect on cleavage efficacy of tetrahydrofuran:A, U:G mispair, U:A base pair, thymine glycol:A, and 8-oxo-7,8-dihydroguanine:C [34]. Thus, senescent human fibroblasts as well as leukocytes from old donors showed higher basal level of AP sites than young donors. However, after a challenge with the oxidizing agent H_2O_2 or the alkylating agent methyl methanesulfonate (MMS), the number of AP sites increased quickly in young cells, whereas in senescent and older cells, they were observed to grow slowly with a concomitant loss of viability, suggesting a decrease in DNA glycosylase activity, mainly in OGG1 8-oxoguanine and 3-methyladenine DNA glycosylases [35], although other reports have also mentioned a decrease in the UDG uracil-DNA glycosylase [28]. Because polyADP-ribosylation (PARylation) levels are linked to downstream mechanisms in DNA repair together with other cellular deficiencies as cell-cycle arrest, cell survival, cell death, and/or cell transformation, a decline in PARP1 activity is important since it has been linked with the age in humans and rats [36]. Further, a decrease in the interaction between the endonuclease VIII-like NEIL1 and PARP1 was observed in old mice when compared to young mice [37], which also could be associated with the decrease in PARP1 activity. Meanwhile, a significant decrease in the expression of SIRT6 has been reported to have a relevant role in BER because it regulates repair activity through a PARP1-dependent pathway [38]. Since sirtuins can function as metabolic sensors, they could also be related with a significative increase in pol β [39] and APE activities [30] under caloric restricted diets. Consequently, BER pathway showed to be deficient when repairing age-downregulated genes in comparison with genes that are not affected by age [40].

On the other hand, the mitochondrial free radical theory of aging states that free radicals generated in mitochondria are strongly related with the intrinsic aging process, mainly due to the accumulation of oxidative damage and derived mutations in mitochondrial DNA (mtDNA) mainly in D-loop region. mtDNA is more susceptible to oxidative damage than the nuclear genome, presumably because of the physical proximity of the source of ROS and lack of histones [41]. BER is the predominant and best understood DNA repair pathway in mitochondria involving at least four components, a DNA glycosylase, an AP endonuclease (or other mechanism for processing abasic sites), DNA polymerase γ (pol γ), and DNA ligase [42]. Recently, pol β was also detected in mitochondrial protein extracts, where it is required to provide enhanced mtDNA BER activity [43]. In a similar way to nuclear BER, in rat brain mitochondria, there is a marked age-dependent decline in mitochondrial BER activity, as indicated by a pol β, pol γ, ligase, APE1 endonuclease, and OGG1 glycosylase activities [44]. Interestingly, activity of mitochondrial OGG1 AE8-oxoguanine DNA glycosylase increases in mouse liver mitochondria according with the age [45]. However, a significant fraction of the OGG1 remains in the outer membrane and intermembrane space in an immature form, presumably because

its import into the mitochondrial matrix is impaired as a consequence of aging. In addition, a nearly identical phenomenon was observed with the mitochondrial uracil-DNA glycosylase [46].

2.1.2 Nucleotide excision repair (NER)

NER is the primary pathway for repairing a wide range bulky DNA lesions, including UV-induced photoproducts (cyclopyrimidine dimers [CPDs], 6–4 photoproducts [6-4PPs]), adducts formed by mutagens in the environment such as benzo[a]pyrene or some aromatic amines, some oxidative endogenous lesions such as cyclopurines, and adducts formed by cancer chemotherapeutic drugs such as cisplatin. NER can be initiated by two subpathways: global genome NER (GG-NER) where the participation of XPC-RAD23B is involved and the transcription-coupled NER (TC-NER) where RNA polymerase interacts with CSA, CSB, and XAB2. Both converge to complete the excision process requiring the core NER factors RPA, XPA, TFIIH, XPD, XPB, XPG, and ERCC1–XPF, among other auxiliary proteins [47]. NER activity decreases with aging possibly because there is a transcriptional downregulation of NER genes together with an altered protein function or processing and a decrease in energy production [48]. In this manner, it was previously observed that aged human skin [49] and fibroblasts [50] showed decreased levels of XPB, PCNA, RPA, XPA, and p53, and more importantly the UVB-induced pyrimidine dimers were removed in a slower manner than in younger counterparts [50]. Interestingly, the effect of age on the repair of UV-induced DNA damage varies for transcribed and nontranscribed DNA, decreasing considerably in unexpressed DNA [51, 52] but improving in both cases under calorie restricted diets [52]. Furthermore, UV-induced damage and repair in telomeres showed to be slower and less frequent than in other regions of the genome such as active genes [53]. Additionally, ERCC1 and XPF, which are considered as the rate-limiting members in NER, also showed an age-dependent decline in their relative expression levels [54]. Because XPC, XPB, and XPF appear to be dependent on the activation status of the IGF-1R, decreased levels of IGF-1R observed with aging also contributed with the decline of NER pathway [55]. Meanwhile, in an assay based in plasmid reactivation after UV damage, cells from older donors introduced an increased number of mutations in the transfected plasmid, which suggests that not only the repair is less efficient with age but also more mistakes are made [51].

2.1.3 Mismatch repair (MMR)

The mismatched nucleotides in the DNA can result from polymerase misincorporation errors, recombination between imperfectly matched sequences, chemical or physical damage to nucleotides, and deamination of 5-methylcytosine (5mC) mostly during replication. MMR pathway consists of four major heterodimeric complexes, MutL homolog (MutL)α, MutLβ, MutS homolog (MutS)α, and MutSβ. MutLα involves MLH1 and PMS2, whereas MutLβ consist of MLH1 and PMS1. Meanwhile, MutSα consists of MSH2 and MSH6, and MutSβ is constituted by MSH2 and MSH3. Thus, MutSα complex recognizes single mispaired bases, whereas MutSβ detects mispaired runs of 3–6 bases. MutSα or MutSβ recruits MutLα or MutLβ and forms a tetrameric complex that serves as a base for the recruitment of excision and repair machinery [56]. MMR removes mispaired bases preventing mutations [57], and defects in this pathway are strongly associated with a substantial destabilization of microsatellites, which are tandemly repeated sequences (from 1 to 6 bp), highly polymorphic, interspersed in the genome, and susceptible to slippage during replication [58]. Previously, a decline in MMR function and efficiency correlation with age

was observed [59, 60], especially in microsatellite sequences [61] where age-related methylation of the MLH1 [62, 63] and MSH2 [64] promoters could be associated to microsatellite instability (MSI). Interestingly, MLH1 shores showed a decrease in methylation with increasing age [65]. Shores are regions of the genome around CpG islands with lower GC content and with the ability to control gene expression.

2.2 Response to DNA double-strand breaks (DSBs)

2.2.1 Homologous recombination repair (HRR)

With aging there is an increase in DNA double-strand breaks [66]. However, it is unknown whether this increase is a consequence of accumulation of unrepaired DSBs or progressively delayed repair events, possibly as a reflection of an inherently limited capacity to process DSBs [67]. To repair this kind of DNA damage, HRR, considered a highly reliable pathway, allows the cell to access and copy information from the intact DNA sequence into the sister chromatid. Notably, HRR is restricted to late S to G2 phases when chromosomes are aligned [68]. RAD51 and other members of the RAD52 epistasis group as RAD50, MRE11, and XRS2 are needed for HRR. The efficiency of HRR is enhanced by mediator proteins that promote the loading of RAD51 onto ssDNA, RAD52 among them [69]. HR-mediated repair efficiency declines precipitously during cellular aging together with a decline of RAD51, RAD51C, RAD52, NBS1, CTIP, and MRE11 levels [66, 70]. Furthermore, in human and mice oocytes, a decrease in expression of BRCA1 and ATM [71] and an impaired recruitment of RAD51 to DNA damage sites during aging [72] were observed, which could force cells to utilize the error-prone NHEJ pathway. At the same time, in older mice a lower activity of the ATM kinase that results in less p53 phosphorylation was reported, thus affecting apoptosis, cell-cycle arrest, and senescence [73]. In addition to the above, the decrease in the levels of PARP1 [36] and SIRT6 [38] not only affects BER pathway but also has a relevant role in HRR since supplementation of recombinant SIRT6 was able to partly restore HR activity [70]. This could be related to a higher binding of DBC1 to PARP1 inhibiting its enzymatic activity as well as the change in NAD+ levels [74]. Decreased NAD+ levels observed with age also reduce activity of other sirtuins as SIRT1 and SIRT7 together with PARP1, reducing NHEJ and HRR pathways [75]. Although HRR is essential, its activity must be carefully controlled in order to maintain genomic integrity [76]. Previously, it has been demonstrated that frequency of recombinant cells is highly variable among tissues, from very low levels in the brain and stomach to very frequent in the pancreas and spleen. Additionally, de novo recombination events indeed accumulate in mice colonic somatic stem cells with age [77].

2.2.2 Nonhomologous end joined (NHEJ)

In human cells, NHEJ is the major pathway for the repair of DSBs, where two ends of DNA with little or no sequence homology are brought together and repaired. NHEJ can act throughout most of the cell cycle but predominantly in G1 phase [68]. NHEJ is divided into two subpathways: the classical NHEJ pathway (c-NHEJ), in which DNA-PKcs, Ku70/Ku80 heterodimers, Artemis, XRCC4, XLF, and DNA Ligase 4 are involved, and the alternative NHEJ pathway (alt-NHEJ), comprised of the repair factors PARP1 and DNA ligase 3 [78]. Both NHEJ pathways are associated with changes in DNA sequence, where c-NHEJ causes deletions and insertions, whereas alt-NHEJ propitiates the loss of genetic information between microhomologies on chromosomes [79]. NHEJ becomes inefficient and more error-prone during cellular senescence, thus favoring genomic instability and higher

incidence of cancer in the elderly [80, 81]. Furthermore, NHEJ-mediated VDJ recombination in B lymphocytes is impaired, reducing class switch recombination efficiency and contributing to reduced humoral repertoire and impaired immunity with aging [82]. Frequency of microhomology-mediated end joining (MMEJ) increases as a compensatory mechanism; however, at the same time, it favors that more mistakes are generated [81]. Ku 70 and 80 proteins decreased their expression at least twofold in two lines of senescent human fibroblast; at the same time, their localization was changed concentrating them in the nucleus when compared with young cells where they are present in both the nucleus and cytoplasm [83]. Cytoplasmic Ku proteins could serve as a reserve (pool) that is recruited to the nucleus upon DNA damage; therefore in senescent cells these proteins are unavailable to repair new lesions [25]. Additionally, binding activity of the Ku 70/80 heterodimers to broken DNA ends also declines with aging [66]. Notably, mice and cells deleted for either Ku70 or Ku80 exhibited not solely NHEJ disruption but also altered BER [84]. On the other hand, decreased expression of XRCC4, DNA ligase 4, and DNA ligase 3 has been observed, and this implicates that during the aging process, NHEJ becomes more inefficient and inaccurate, leaving more damage sites repaired with a loss of additional genetic information [72]. Interestingly, aging increases DNA-PK activity phosphorylating HSP90α and decreasing its chaperone function in AMPK, which is critical for mitochondrial biogenesis and energy metabolism [85]. Consistently, DNA ligase 4 and Ku80 gene promoters were frequently observed as hypermethylated in elderly people, which could be associated with the silencing expression of both genes [86]. However, as mentioned for other DNA repair mechanisms, caloric restriction diet improves NHEJ activity possibly through SIRT1 and FOXO activity [87].

3. Conclusions

Aging is a consequence of damage accumulation in different cellular constituents and where DNA damage is one of the most important. Every day there are thousands of insults that affect DNA, either due to endogenous factors (such as metabolism) or exogenous factor like contact with radiation sources or exposure to toxic substances; but only a minimal amount (less than 0.02%) accumulates as permanent damage, while the rest is totally repaired. However, if only one gene is not repaired and its function is important as that of a proto-oncogene, a tumor suppressor, or any DNA repair genes, this could lead to accumulation of mutations, and then DNA damage checkpoints can halt the cell cycle and induce cellular senescence or apoptosis, or well erroneous repair or replicative bypass of lesions can result in mutations and chromosomal aberrations leading the cells to transform into cancer cells.

Notably, DNA repair systems are able of dealing with most of the damages inflicted to DNA; however, their efficiency decrease with age, permitting that point mutations, insertions, deletions, and rearrangements, among others, occur more frequently and accumulate over time. This is due in part to the fact that critical proteins involved in DNA repair significantly decrease their expression in an age-related manner. In **Figure 2**, the main age-related changes reported over the different mechanisms of DNA repair together with their consequences that globally cause genomic instability and favor cellular senescence and cancer are summarized.

Overall, this area needs to be more exploited in order to improve our quality of life and prevent or delay the harmful effects of aging. Thus, the more knowledge we acquire about the natural cell aging process and its interrelation with the mechanisms of DNA repair, the closer we will be to develop drugs, therapies, or even vaccines that could help us to prolong our life.

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Conflict of interest

The authors declare no conflict of interest.

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