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DNA damage and repair in age-related inflammation

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Abstract

Genomic instability is an important driver of ageing. The accumulation of DNA damage is believed to contribute to ageing by inducing cell death, senescence and tissue dysfunction. However, emerging evidence shows that inflammation is another major consequence of DNA damage. Inflammation is a hallmark of ageing and the driver of multiple age-related diseases. Here we review the evidence linking DNA damage, inflammation and ageing, highlighting how premature ageing syndromes are associated with inflammation. We discuss the mechanisms by which DNA damage induces inflammation, such as through activation of the cGAS–STING axis and NF-κB activation by ATM. The triggers for activation of these signalling cascades are the age-related accumulation of DNA damage, activation of transposons, cellular senescence, and the accumulation of persistent R-loops. We also discuss how epigenetic changes triggered by DNA damage can lead to inflammation and ageing via redistribution of heterochromatin factors. Finally, we discuss potential interventions against age-related inflammation.

Introduction

Genomic instability is a hallmark of ageing[1]. DNA in the cell is constantly subjected to damaging agents from the environment as well as damage resulting from intrinsic biological processes. DNA damage, left unrepaired or repaired incorrectly, may cause deleterious mutations. DNA damage was traditionally believed to only contribute to genomic instability. Recently, it was shown that DNA damage is an inducer of inflammation both *in vitro* and *in vivo*[2–5]. In cell culture, DNA damage induces the expression of type I interferons (IFN) and other inflammatory factors[2–4]. Mechanistic studies revealed that the connection between DNA damage and inflammation is through the cytoplasmic DNA sensing pathway[6–8].

Two major events are involved in this process: the presence of DNA in the cytoplasm, and the sensing of cytoplasmic DNA to induce inflammation. DNA damage results in the accumulation of cytoplasmic DNA via two pathways: first, via the formation of micronuclei and second, through the direct leakage of DNA into the cytoplasm[9,10]. Micronuclei emerge

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Conflict of Interest

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from chromosome mis-segregation during mitosis and in response to DNA damage[9,11,12]. When the double-stranded DNA breaks (DSBs) are left unrepaired or mis-repaired, this can lead to formation of acentric chromosomal fragments[9]. These fragments recruit the nuclear envelope to form micronuclei in the subsequent cell division[9]. Micronuclei are nuclei-like structures, consisting of nuclear envelope surrounding chromosome fragments[13]. Roughly 60% of micronuclei undergo a nuclear envelope rupture during interphase[14]. Nuclear envelope rupture is associated with loss of lamina, but the exact mechanism is not clear. Normally, the nuclear envelope is maintained and repaired by the endosomal sorting complexes required for transport III (ESCRT-III) complex[15]. It is possible that this repair is deficient in micronuclei[6]. This results in the leakage of the chromosomal DNA fragments from micronuclei into the cytoplasm. Cyclic GMP-AMP synthase (cGAS) is a DNA sensor that recognizes and binds cytoplasmic DNA in a sequence-independent manner[16]. Upon DNA binding, the cGAS protein changes the conformation of its catalytic centre and mediates the synthesis of cyclic GMP–AMP (cGAMP). cGAMP binds to and activates the adaptor protein stimulator of IFN genes (STING)[16]. Activated STING recruits TANK-binding kinase 1 (TBK1) to phosphorylate IFN regulatory factor 3 (IRF3), which then induces production of type I IFN[17]. STING also activates NF-κB through tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6)[18]. NF-κB cooperates with IRF3 to activate type I IFN and other inflammatory factors[6,19] (Figure 1).

In addition to micronuclei, single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) can be observed in the cytoplasm following DNA damage, possibly occurring due to DNA end resection[10]. The leakage of DNA from the nucleus into the cytoplasm depends on the endonuclease MUS81[20]. MUS81 also promotes type I IFN production in prostate cancer cells[20]. cGAS has little to no affinity for ssDNA but can be activated by the double-stranded secondary structures of ssDNA[21]. This process can also contribute to the inflammation induced by DNA damage. The cGAS-STING pathway recognizes foreign nucleic acids, including DNA from viruses, bacteria, and tumours, as well as self-DNA originating from the nucleus and mitochondria[22]. By detecting foreign DNA and triggering inflammation, cGAS-STING pathway plays a critical role for host defense[22].

Recently, studies have demonstrated direct associations between the DNA damage response and NF-κB-mediated inflammation[23–25]. In response to DNA damage, DSBs and single-stranded DNA breaks recruit ataxia-telangiectasia mutated (ATM) and ATM and RAD3-related (ATR), respectively, to the DNA lesion[26]. The transcription factor GATA4 is normally degraded by autophagy mediated by p62. Upon DNA damage, ATM or ATR inhibit p62 and thus, stabilize GATA4, which activates NF-κB to induce inflammation[23]. ATM was also shown to translocate to the cytosol to activate TRAF6, which eventually activates NF-κB[24]. Remarkably, STING can be activated by ATM independently of cGAS, leading to NF-κB activation[27]. Activated ATM can also activate NF-κB by degrading IκBα and phosphorylating RELA (p65) in the cytoplasm[25]. In summary, DNA damage has emerged as an important inducer of inflammation (Figure 1). In this Review, we will discuss various events associated with DNA damage, including DNA repair deficiencies, activation of transposons, cellular senescence, R-loop formation, and change in chromatin structure, that link inflammation and ageing.

DNA damage and premature ageing

Defective DNA repair, inflammation and premature ageing syndromes.

Mutations in genes involved in DNA repair and genome maintenance are often associated with premature ageing syndromes. Traditionally, it was believed that the pathologies seen in DNA repair disorders arise from cell death and accumulation of mutations. However, emerging evidence points to inflammation as a contributing cause to the pathogenesis of these syndromes. The connection between DNA repair defects and inflammation appears to be mediated, in many cases, by damaged DNA driving activation of the cGAS-STING signalling pathway and interferon response. Below, we review several cases where defects in DNA repair have been linked to both premature ageing and the induction of an inflammatory response.

Nucleotide excision repair syndromes.

Inherited mutations in more than a dozen of nucleotide excision repair (NER) **[G]** genes contribute to several human syndromes, including xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy (TTD), as well as some related syndromes with combined phenotypes[28]. Each syndrome has several complementation groups, with different combinations of mutant NER genes[29]. Xeroderma pigmentosum is characterized by high risk of skin cancer[30,31]. Other NER deficiency syndromes, however, display premature ageing without cancer predisposition. The XPF–ERCC1 heterodimer is a 3'-flap endonuclease that generates the primary DNA nick crucial for NER progression and is also required for DNA inter-strand crosslink (ICL) repair[32–34]. The R153P mutation of *XPF* gene in XFE progeroid syndrome results in severe progeroid symptoms without elevated cancer risk[35].

Several studies showed that mouse models carrying mutations in ERCC1–XPF demonstrate increased inflammation. *Ercc1*$^{-/-}$ mice display persistent DNA damage that triggers a chronic autoinflammatory response in the adipose tissue[36]. The cytoplasmic phosphorylated ATM is believed to induce transcriptional de-repression of inflammatory factors. In cultured human ERCC1-deficient fibroblasts or cultured fibroblasts from the skin of *Ercc1*$^{-/\Delta}$ mice (which carry a null and a hypomorphic Ercc1 alleles), there is elevated cellular senescence and production of senescence-associated secretory phenotype (SASP) **[G]** factors[37]. TNF secreted by the senescent cells induced apoptosis of the neighbouring stem cells, contributing to the premature ageing phenotypes[37]. Fibroblasts and tissues of ERCC1-deficient mice also display ATM-dependent activation of NF-κB, which induces SASP and senescence[38].

Patients with Cockayne syndrome display accelerated ageing in multiple systems, characterised by hearing loss, compromised vision and cognitive dysfunction, among other symptoms[39,40]. There are two complementation groups for Cockayne syndrome, namely Cockayne syndrome A (caused by mutation of *CSA* (also known as *ERCC8*)) and Cockayne syndrome B (caused by mutation of *CSB* (also known as *ERCC6*)), with Cockayne syndrome B accounting for the majority of Cockayne syndrome cases[41]. In a mouse model of Cockayne syndrome, *Csb*$^{m/m}$ mice carry mutations affecting CSB and develop fibrosis

and inflammation in response to ultraviolet (UV) radiation[42]. In another mouse model with complete loss of NER, namely the *Csa*$^{-/-}$*Xpa*$^{-/-}$ mouse, animals display upregulation of inflammatory markers in the brain, including ICAM1, TNF and phosphorylated NF-κB p65[43]. Although the molecular mechanisms responsible for induction of inflammation in Cockayne syndrome are poorly understood, it is possible that mitochondrial stress is playing a role. CSB interacts with mitochondrial transcription factor A (TFAM) and plays an important role in maintaining mitochondrial integrity[44]. Mitochondrial DNA stress due to loss of TFAM has been shown to trigger cGAS–STING–IFN signalling via the release of mitochondrial DNA into the cytoplasm[45,46]. Going forward, it will be important to test the contribution of mitochondrial DNA stress to the inflammation observed in patients with Cockayne syndrome.

DNA double-strand break repair syndromes.

Defects in DNA double-strand break (DSB) repair **[G]** are implicated in several premature ageing syndromes. Mutations in RecQ family of helicases result in Werner syndrome and Bloom syndrome. Werner syndrome is a segmental progeroid syndrome caused by a null mutation on the gene encoding Werner syndrome ATP-dependent helicase (WRN)[47]. Cells isolated from patients with Werner syndrome display genomic instability, including aberrant recombination, increased spontaneous mutations, and telomere defects[48]. Werner syndrome is associated with strong induction of inflammatory response and was therefore proposed as an example of inflammageing **[G]**[49–52]. Fibroblasts isolated from patients with Werner syndrome express high levels of IFNβ[53]. Likewise, short-term siRNA-mediated knock down of WRN in fibroblasts from healthy young individuals triggered an inflammatory gene-expression profile resembling that seen in older patients, with activated inflammatory pathways including IL-6, NF-κB, and genes associated with the IFN response[48]. The inflammation was believed to be activated through the p38 pathway due to telomere defects and genomic instability-induced intracellular stress[49]. p38 activated inflammation is believed to be largely mediated by increasing the transcriptional activity of NF-κB[54]. However, the induction of an interferon response suggests that the cGAS–STING pathway may also be involved in Werner syndrome, although further studies are required to provide direct evidence. Bloom syndrome is another segmental progeroid syndrome and it results from mutations in BLM helicase, which plays a critical role in genome integrity maintenance[55]. Cells from patients with Bloom syndrome display increased activation of the phosphorylated histone 2A (γH2AX)–ATM– serine/threonine protein kinase CHK2 (CHK2) DNA double-strand break checkpoint response pathway[56]. Importantly, Bloom syndrome cells accumulate micronuclei, which trigger the cytoplasmic DNA sensing cGAS–STING–IFN pathway[57]. While mutation of BLM results in Bloom syndrome, high levels of BLM correlate with poor prognosis in breast cancer[10]. This is possibly because BLM also plays a role in generating ssDNA during DSB repair by mediating end resection[10]. Depletion of BLM decreased cytosolic ssDNA sensing through cGAS-STING pathway[10].

The Ku70–Ku80 heterodimer is required for the non-homologous end joining (NHEJ) pathway of DNA repair. Knockdown of Ku70 or Ku80 in mice results in severe premature ageing. These mice also display airway inflammation and subepithelial fibrosis, which are potentially induced by mitochondrial stress or ER stress[58]. Missense mutations of

DNA-PK catalytic subunit (DNA-PKcs) in mice or patients derepress cGAS-mediated innate immunity[59]. Interestingly, DNA-PK itself has been reported to be a cytoplasmic DNA sensor, which triggers IRF3-dependend innate immunity[60], providing a direct link between DNA repair and inflammation.

ATM is a serine/threonine protein kinase activated by DNA double-strand breaks. ATM initiates DSB repair responses by phosphorylating multiple downstream targets involved in cell cycle arrest, apoptosis and repair[61]. Inherited mutations of ATM cause Ataxia-telangiectasia, a premature ageing disorder characterized by immunodeficiency, progressive neurodegeneration, and increased risk of cancer[62–64]. Studies demonstrate that Ataxia-telangiectasia syndrome is associated with strong pathologic inflammation, which is responsible for many of its symptoms[65]. Serum levels of IL-6 and IL-8 are significantly higher in patients with Ataxia-telangiectasia than in control groups[66,67]. ATM-deficiency also causes elevated and prolonged inflammation in response to lung injury[68]. Type I and type III IFN signatures are elevated in the plasma and peripheral blood cells of patients with Ataxia-telangiectasia resulting from the accumulation of DNA in the cytoplasm[69]. In microglia from patients with Ataxia-telangiectasia, cytoplasmic DNA accumulates and activates the cGAS–STING pathway[70] and the absent in melanoma 2 (AIM2) inflammasome[70]. These studies underlie the contribution of inflammation to the symptoms of Ataxia-telangiectasia syndrome through accumulation of cytoplasmic DNA and activation of the cGAS–STING pathway.

Defects in genome maintenance.

Hutchinson-Gilford progeria syndrome (HGPS) is caused by mutations on the lamin A (*LMNA*) gene[71]. The nuclear lamins are responsible for maintaining nuclear architecture by providing a scaffold for chromatin and all nuclear contents. Lamins play important roles in maintaining nuclear membrane integrity, nuclear envelope assembly, DNA replication, transcription, and maintenance of heterochromatin[72]. The mutant lamin A in patients with HGPS results in a mis-shaped nucleus, genome instability, dysregulated epigenome and gene expression, and premature ageing[73,74]. One of the hallmarks of HGPS is inflammation. Higher basal expression of components of the NLRP3 inflammasome pathway were observed in skin fibroblasts and lymphoblasts from patients with HGPS[75]. In an $LMNA^{G609G/G609G}$ mouse model, inhibition of NLRP3 inflammasome significantly extended lifespan, suggesting that inflammasome activation is a major contributor to the progeria phenotype[75]. In another HGPS model, cells from mice with a $Zmpste24^{-/-}$ mutation (affecting the Zmpste24 protease responsible for processing pre-lamin A), exhibit nuclear blebbing, micronuclei formation, and activation of the cGAS–STING pathway[76]. Both mouse models of HGPS are characterized by elevated expression of inflammatory cytokines and activation of ATM and NF-κB signalling pathways[77].

In summary, deficiency in different genes involved in DNA repair and genome maintenance is associated with a strong induction of inflammation (Table 1). Considering the central role of inflammation in the etiology of age-related disease it is possible the induction of inflammation, rather than accumulation of mutations, plays a key role in pathogenesis of premature ageing syndromes.

Transposons are inducers of inflammation

A major part of the mammalian genome consists of transposable elements (TEs). Mammalian TEs include retrotransposons (RTEs) **[G]**, such as long-interspersed nuclear elements (LINEs) and short-interspersed nuclear elements (SINES), and endogenous retroviruses (ERVs)[78,79]. LINE elements are 6 kb-long, fully autonomous retroelements that encode two proteins necessary for retrotransposition into the genome; ORF1p, which forms homo-trimers that assemble on the LINE RNA as chaperones and ORF2p, which encodes an endonuclease and reverse transcriptase[80–82]. SINE elements are considerably shorter than LINEs and unlike their larger brethren, they do not encode proteins[83]. Rather, SINEs rely upon the protein machinery encoded and expressed by LINE elements in order to copy themselves back into the genome[83,84]. In humans, LINE-1 retrotransposons (L1s) and associated SINEs are the predominantly active elements, with only minor contribution from human ERVs (HERVs)[85]. While the vast majority of LINEs belong to evolutionarily inactive families, there are still hundreds of sequences that remain fully active and capable of autonomous self-replication[86]. The mechanisms by which these elements retro-transpose into the genome has been extensively covered elsewhere and will only be briefly covered here[87]. The protein subunits encoded by L1s preferentially associate *in cis* with L1 mRNA, forming a ribonuclear protein (RNP). The RNP translocates to the nucleus, where it initiates reverse-transcription of the RNA after generating a single-strand break (SSB) in the genomic DNA to prime off of. Integration of the new DNA and the subsequent generation of the second strand complete the process. SINEs retro-transpose in a similar manner, as they also associate with L1-encoded proteins to form their own RNPs. Retrotransposons are potentially mutagenic, generating insertion mutations and DNA breaks. Retrotransposons are considered genomic parasites involved in a perpetual arms race with the host cell.

Retrotransposons and disease.

Cells have evolved multiple overlapping mechanisms to suppress TE activity[88,89]. Failure of these mechanisms can lead to inappropriate transposon activation, which has been documented in several diseases. Historically, the contributions of RTEs to disease were primarily linked to their mutagenic activity[90]. Elevated activity of RTEs, especially L1s, is a hallmark of many cancers[91–93]. In fact, over 40% of neoplasms demonstrate elevated L1 activity[94,95]. However large-scale sequencing efforts showed that the majority of RTE insertions found in tumours are benign passenger events[96]. While several cases of insertions into tumour suppressor genes had been documented, these events are rare[96]. Thus, elevated RTE activity in tumours is likely a consequence rather than a cause of tumorigenesis.

In recent years, the deleterious impact of RTE activity outside of mutagenesis has become center-stage. Primarily, it appears that disruption of mechanisms of RTE suppression and the subsequent unregulated retrotransposition result in sterile inflammation **[G]**. Dysregulation of L1s has been demonstrated to drive inflammation in Trex1-dependent autoimmune disorders and initiate pro-inflammatory interferon expression in senescent cells[97–99]. Loss of tumour suppressors and disruption of genes associated with DNA repair, such as p53 and SIRT6, also elicit increased L1 activity and inflammation[100,101]. This pro-inflammatory activity driven by L1 is also observed in cancers with elevated L1 expression[102,103]. In

addition to L1-induced autoimmune and pro-inflammatory diseases, Alu SINE elements in humans induce inflammation resulting in macular degeneration and Aicardi-Goutières syndrome[97,104–109].

Mechanistically, RTE-induced inflammation results from RTE cDNA triggering anti-viral surveillance mechanisms in the cytoplasm, though there is also evidence that RTE-induced genomic instability may also contribute to pro-inflammatory activity through the formation of micronuclei[110,111] (Figure 2). The cGAS–STING axis plays a pivotal role in the sensing of RTE cDNAs and triggering a sterile inflammation response[22,111]. This pathway can detect a host of extrinsic (viral, retroviral, bacterial, tumoural) and intrinsic (nuclear, mitochondrial and cytoplasmic) DNA sources. In any event, the activation of an immune response mediated by NF-κB and IRF3 produces pro-inflammatory cytokines such as IFN-α, IFN-β, IL-6, and TNF[112]. How RTE-derived cDNA arrives in the cytoplasm is still not fully understood. While it has been suggested that failed integration events accumulate in the nucleus and 'leak' out, there is recent evidence that demonstrates cytoplasmic synthesis can occur in the case of Alu elements associated with macular degeneration[109].

Retrotransposons promote ageing via induction of inflammation.

While the immediate impact of genetic and stress-induced RTE activation and inflammation are clearly demonstrated in the pathologies they induce, there lies a more insidious threat within the RTE-induced inflammation pathway. Indeed, loss of heterochromatin, sterile inflammation and the mobilization of RTEs are also hallmarks of ageing. Aside from the RTE-induced inflammatory signature observed in senescent cells (which accumulate with age), progeroid SIRT6-knockout mice and aged wild-type mice also demonstrate elevated interferon production triggered by cGAS-sensing of LINE cDNAs in the cytoplasm[98,100,113]. In cell-free DNA detected in blood, L1 methylation changes and circulating Alu DNAs are also observed to correlate with advanced age[114–116]. SINE elements also contribute to ageing pathologies, as is the case with age-related macular degeneration[104,109]. Additionally, SINEs carry over one third of total CpG methylations within the genome, which become hypomethylated with age[117–119]. The ubiquitous nature of age-related sterile inflammation gave rise to the concept of inflammageing, where sterile inflammation serves as a driving force of multiple age-related pathologies and the ageing process itself[120]. The sources of sterile inflammation are not fully understood and are believed to result from cell debris, accumulation of senescent cells among other factors. RTE-induced inflammation points to a novel source of age-related inflammation where ageing cells gradually lose control over endogenous genomic parasites triggering a response similar to that of a persistent viral infection (Figure 2).

Tumour suppression by retrotransposons: the bright side.

Besides the deleterious effects triggered by RTEs in the context of ageing, emerging evidence indicate beneficial effects of transposons as tumour suppressors. This role of RTEs may be the reason these parasites have not been eliminated from the mammalian genomes. In human cancer cells, DNA-demethylating agents activate ERVs, which form double-stranded RNA (dsRNA) and trigger IFN response to kill the cancer cells[121,122]. Likewise, ablation of lysine-specific histone demethylase 1A (LSD1) derepresses ERVs and

triggers cancer cell death through cytoplasmic dsRNA sensing pathway, where the dsRNA sensors TLR3 and MDA5 trigger IFN response[123]. Forced reactivation of Alu-derived dsRNA inhibited human cancer cell growth, which was further synergized by loss of ADAR1, a dsRNA-editing enzyme[124,125]. These studies suggest that transposons may have a potential tumour suppressive function through viral mimicry. Recently, it was demonstrated that L1s trigger genomic instability in myeloid leukemia, showing a tumour-suppressive effect[126].

A recent study using a naturally long-lived and cancer-resistant rodent, the blind mole rat **[G]** (*Spalax*), revealed that RTEs can serve as a tumour-suppressor through a natural process[127]. Blind mole rats are extremely resistant to both spontaneous and induced tumorigenesis. Their fibroblasts display a unique phenotype termed concerted cell death upon over-proliferation[128]. It was demonstrated that, due to their naturally weak DNA methyltransferase 1 (DNMT1) activity, rapidly proliferating cells in blind mole rats lose global DNA methylation and derepress RTEs. Activated RTEs form cytoplasmic RNA–DNA hybrids and activate the cGAS–STING–IFN pathway to kill the over-proliferating cells and prevent cancer[127]. Remarkably, pro-inflammatory genes are repressed in the normal tissue of middle-aged blind mole rats, indicating that concerted cell death is activated specifically in over-proliferating cells to avoid widespread inflammation. In summary, these results suggest that ‘domesticated’ RTEs were naturally co-opted to serve as a tumour suppressor. Hence, RTEs can be seen as a double-edged sword, acting as a tumour-suppressor in young age, but later contributing to inflammageing.

Cellular senescence and inflammation

Cellular senescence is a status of permanent cell-cycle arrest in response to stress or telomere shortening. Senescence has evolved as a tumor-suppressor mechanism to limit cell proliferation[129]. However, over time accumulation of senescent cells compromises tissue function and is one of the hallmarks of ageing[1]. First observed in replicative exhaustion of human primary fibroblasts (replicative senescence)[130], cellular senescence was later identified to also result from activation of oncogenes (oncogene-induced senescence, OIS) and sustained DNA damage (stress-induced premature senescence, SIPS)[26].

Senescent cells exhibit inflammatory secretory phenotypes.

Senescent cells were traditionally viewed as zombie cells, contributing to ageing by altering tissue function and integrity[131]. Later studies, however, revealed more complicated effects beyond the cell-autonomous level. Senescent cells were shown to secrete a spectrum of inflammatory factors, which were termed the senescence-associated secretory phenotype (SASP)[132]. SASP has deleterious effects by promoting inflammation and, potentially, tumour progression in neighbouring cells[133]. Both the overexpression of the oncogene RAS and the loss of the tumour suppressor p53 can enhance the development of SASP, suggesting that SASP may be a consequence of a persistent DNA-damage response[134].

SASP is triggered by cytoplasmic DNA sensing.

Recent studies have shown that SASP is dependent on cytoplasmic nucleic acid-sensing pathways in senescent cells[111,135]. One characteristic of senescent cells is the micronuclei-like structures observed in the cytoplasm, termed cytoplasmic chromatin fragments (CCFs)[111,136]. The CCFs contain fragments of chromatin blebbing from the nucleus to cytoplasm. The mechanism of CCF formation in senescent cells is incompletely understood and is associated with the loss of nuclear membrane integrity[136]. This is associated with the loss of lamin B1, a biomarker of senescence[136,137]. It has been shown that in RAS-induced senescence, the degradation of lamin B1 is mediated by the autophagy-related protein LC3/ATG8, which directly interacts with lamin B1 and the lamin-associated domains on the chromatin[138] targeting them to the lysosome for degradation.

The CCFs in senescent cells are positive for the DNA damage response marker γH2AX and negative for DNA repair protein 53BP1[136], suggesting that the CCF formation is associated with DNA damage. 53BP1 is a DNA repair protein that inhibits resections of DSBs[139]. Interestingly, 53BP1 is also shown to suppress the formation of CCFs[140,141]. It was shown that irradiation-induced senescence was associated with mitochondrial dysfunction and production of reactive oxygen species (ROS), which activated JUN N-terminal kinase (JNK). JNK interacts with 53BP1 to regulate the formation of CCFs[140]. However, the exact mechanism of JNK–53BP1 interaction and its regulation of CCF formation remain unclear.

Cytoplasmic DNA, the DNA part of CCFs, is sensed by the cytoplasmic DNA sensor cGAS, which triggers STING and innate immune response[16] (Figure 3). This model is supported by the observation that DNA damage-induced cytoplasmic DNA fragments contain both γH2AX and cGAS[142]. Importantly, while cGAS senses cytoplasmic DNA presented in senescence, cGAS is also essential for maintenance of senescence[142]. Downstream of STING are two signalling pathways, namely the IFN pathway mediated by IRF3 and the SASP pathway induced by NF-κB[143]. The *IFN* promoter contains elements responsive to both IRF3 and NF-κB; therefore, mechanistically, both pathways can activate IFN responses, and the combination of IRF3 and NF-κB results in a synergetic effect[19,144]. The induction of senescence in mouse embryonic fibroblasts (MEFs) by different methods activated both IFN and SASP responses, which promoted senescence in a paracrine manner[145]. However, in a similar study using primary human lung fibroblasts from the IMR90 cell line, senescence induction using HRASV12 **[G]** transduction or treatment with the chemotherapeutic drug etoposide only activated the SASP, but not an IFN response[135]. The conditioned media from senescent cells, which contains SASP factors and especially IL-1α, suppressed IFN induction by double-stranded DNA transfection[135]. It was speculated that the failure to induce a type I IFN response resulted from the inhibition of STING by p38 MAP kinase (MAPK) in senescent cells[54,135]. Indeed, it was shown that activated p38 phosphorylates USP21, stimulating deubiquitination of STING leading to in STING inhibition[146]. Interestingly, exogenous IL-1β was recently reported to activate an IFN response through the release of mtDNA, which was recognized by cGAS[147]. These different observations may have arisen from the use of different cell types and/or methods to induce senescence. Recently it was reported that topoisomerase 1 (TOP1)–DNA cleavage complex (TOP1cc) is necessary and sufficient for cGAS to recognize cytoplasmic

chromatin to trigger SASP during senescence[148]. This process is stabilized by the chromatin architecture protein HMGB2, suggesting chromatin-associated proteins are involved in cGAS activation in senescence[148]. Studying the mechanisms responsible for the choice of the downstream pathways will provide a better understanding for the role of inflammation in senescence. Interestingly, recent evidence showed that cGAS-STING pathway also activates autophagy through LC3 lipidation independent of the TBK1 and interferon induction[149]. More strikingly, the ancient origin of STING homologue (*Nv*STING) identified from the sea anemone *Nematostella vectensis* lacks the C-terminal domain required for TBK1 activation[150]. *Nv*STING still possesses the ability to induce autophagy, suggesting that the cGAS-STING pathway has originally evolved as part of autophagic response but was later co-opted to serve as a DNA sensor for immune response[149,150]. Thus, providing a link between autophagy, another established hallmark of aging, and inflammation.

Negative regulation of cytoplasmic DNA.

To prevent unintended activation of inflammation, deoxyribonucleases (DNases) in the cytoplasm digest excessive DNA, serving as a negative regulator of cytoplasmic DNA. There are two major DNases in the cytoplasm: the DNase 2α (encoded by *DNaseII*) and Trex1 (originally designated DNaseIII). DNase 2α is located in the lysosome and plays an important role in engulfment-mediated DNA degradation[151]. Deficiency of DNase 2α results in type I IFN-mediated autoinflammation and promotes an ageing phenotype in mice[152,153]. The ageing phenotype can be rescued in Dnase2α$^{-/-}$ STING$^{-/-}$ mice, suggesting its dependence on cGAS–STING pathway[153]. Similarly, deficiency of Trex1, a DNase located in the cytosol, also induces accumulation of cytoplasmic DNA that triggers innate immune responses[154,155]. Intriguingly, both DNases are down-regulated in senescent cells, contributing to aberrant cytoplasmic DNA sensing and inflammation[156,157]. The down-regulation of DNase 2α and Trex1 in senescent cells is believed to occur on the mRNA level, through the repression of the transcriptional factor E2F, which is repressed by the p16-pRb pathway during senescence[156]. It is tempting to propose that the down-regulation of the cytoplasmic DNases synergizes with the loss of nuclear membrane integrity, to contribute to inflammation in senescence through cytoplasmic DNA sensing (Figure 3).

R-loops in age-related inflammation

R-loops as indicators of genomic integrity.

Both DNA synthesis and the transcription of RNA involve accessing chromatin-bound DNA and unwinding of the double helix to form DNA–RNA hybrids. These three-stranded structures, known as R-loops, are frequently found at sites of high transcriptional activity and repetitive sequences[158]. Additionally, R-loops are formed during immunoglobulin class-switching providing access to ssDNA necessary for activation-induced cytidine deaminase to initiate this process[159]. R-loops also play a prominent role at telomeres, where they are formed by association of the TERRA long noncoding RNA (lncRNA) with telomeric DNA[160]. Thus, R-loops play critical roles in normal physiology and constitute ~5% of the human genome at any given time[161].

R-loops are thought to be transitory and are removed once their function is completed. RNaseHs are employed by the cell to remove the RNA from these structures[162,163]. Several DNA-RNA unwinding factors, such as SENATAXIN, BLM and WRN also contribute to the breakdown and resolution of R-loops[164–166]. This tight regulation of R-loop number, location and persistence is necessary to maintain genomic integrity. Indeed, perturbation of several chromatin maintenance pathways, including BRCA1 and BRCA2, the NER pathway via XPG and XPF, and Fanconi anemia pathway increase R-loop abundance[167–171].

Inappropriate R-loop formation and accumulation portends imminent disaster for a cell. Several recent studies have demonstrated R-loops to be drivers of genomic instability and abundant sources for DNA damage. A large portion of the genomic instability attributed to aberrant R-loop activity has been ascribed to stalled replication fork progression, though more recent reports indicate that specific subsets of R-loops and those associated with DNA damage sites may be the main drivers[172–174]. The exposure of ssDNA in R-loops allows endonucleases, such as XPG, XPF, and FEN1, to cleave the exposed DNA, producing single-strand and double-strand breaks[166,174–176]. Loss of tumour suppressors BRCA1 and BRCA2 coincide with genomic instability and over-abundance of R-loops, which are believed to mediate the loss of stability and an increase in DSBs[169].

R-loops drive sterile inflammation and intersect several ageing pathways.

Given the mounting body of evidence that links DSBs with genomic instability and the onset of ageing pathologies, it appears highly probable that aberrant R-loop formation and persistence may contribute to ageing[177,178]. Indeed, R-loops are already associated with cancer and neurodegenerative diseases, as well as autoimmunity[169,170,179–183]. Defects in genes involved in R-loop biology, such as *WRN*, *ERCC1*, *EPF* and *XPG* are also associated with progeria in human and/or model organisms[35,166,184,185]. In *C. elegans*, loss of H3K9 histone methylation results in shortened lifespan, infertility and R-loop accumulation[186,187]. Loss of histone methylation is also correlated with aging and the erroneous expression of transposons and satellite repeat elements in C. elegans[186–188]. In fact, it appears that increased R-loop formation and the expression of TE and satellite DNA are closely linked, both in their occurrence and their correlation with aging, and future work elucidating the contributions from each to inflammation will be of high interest. Additionally, a recent study in zebra fish has indicated that R-loops are capable of triggering inflammatory cascades via the cGAS–STING pathway[110,189]. In *Ercc1*$^{-/-}$ knockout mice, R-loop accumulation results in the release of ssDNA to the cytoplasm, where it is recognized by the canonical cGAS–STING pathway, triggering the innate immune response[190]. Curiously, cGAS has recently been shown to function as a suppressor of genomic instability by slowing replication forks, which suggests that cGAS may be directly inhibiting R-loop formation[191]. Given their ability to be recognized by cGAS and activate an innate immune response, R-loops have entered the stage as potential drivers of inflammageing (Figure 4) though much work has yet to be done to determine their contribution.

Age-related epigenetic changes

Organismal ageing and cellular senescence are associated with changes in chromatin structure. Recently, it was shown that DNA damage-induced epigenetic drift and loss of cellular identity play causal roles in promoting ageing[192,193]. Mice in which mild levels of DNA breaks were induced by the I-PpoI homing endonuclease displayed an altered epigenetic landscape and premature ageing phenotype[192,193]. It has to be noted, however, that in a similar mouse model, the I-PpoI induced DSB may result in significant DNA fusions[194] which may also contribute to the aging phenotype. Many of the changes occurring in the epigenome are stochastic in nature; however, there is a well-documented trend for the loss of heterochromatin on RTEs and other repetitive sequences. One of the theoretical mechanisms proposed to underlie ageing-associated loss of heterochromatin is referred to as ‘redistribution of heterochromatin modifiers’. This theory suggests a link between DNA damage and ageing involving the Sirtuin family members SIRT1 and SIRT6[195,196]. SIRT1 and SIRT6 are epigenetic enzymes responsible for the formation and maintenance of heterochromatin[197,198]. Both SIRT1[199] and SIRT6[200] have the second job, where they are recruited to DSBs to promote DNA repair in response to DNA damage. Aged and senescent cells accumulate higher levels of DNA damage which cause re-localization of SIRT1 and SIRT6 to DSBs, limiting Sirtuins’ availability for binding to heterochromatin, resulting in loss of heterochromatin[199]. SIRT6 is specifically implicated in repressing RTEs[100,113]. Upon DNA damage, SIRT6 relocalizes to DSBs leaving RTEs unattended, which leads to RTE activation[113] and induction of IFN responses[98,100].

In summary, DNA damage promotes epigenetic changes that lead to the redistribution of epigenetic modifies such as SIRT1 and SIRT6, loss of heterochromatin and RTE silencing. RTE activation results in formation of cytoplasmic dsRNA or DNA, which are recognized by dsRNA- and DNA-sensing mechanisms leading to activation of IFN responses and fueling age-related pathologies (Figure 5).

Translation to the clinic

What can be done to suppress age-related inflammation? Multiple anti-inflammatory drugs are available and new interventions are being developed. Remarkably, several well-known drugs such as aspirin and quinacrine attenuate the cGAS–STING signallingaxis, and new inhibitors are being actively developed[201–204]. Recent insights into the role of cytoplasmic nucleic acid-sensing have also provided new targets for attenuating inflammation. Members of the PYHIN family of nucleic acid sensors — which includes AIM2 and IFI16 — play a pivotal role in clearing exposed cytosolic DNA and maturing pro-inflammatory cytokines such as IL-1β and IL-18[205]. This pathway has gained attention in recent years and due to its role in DNA sensing, its components are attractive candidates for inhibiting inflammatory responses causes by cytoplasmic DNA species. Additionally, inhibitors of the NLRP3 inflammasome, which lies downstream of nucleic acid sensing pathways, are currently in development and have demonstrated promising results in suppressing pro-inflammatory disorders such as autoimmune encephalomyelitis[206]. More recently, it was demonstrated that extracellular vesicles (EVs) can be utilized to deliver nucleases to cells with cytoplasmic DNA-induced inflammation, resulting in suppression of chronic inflammation and NF-κB

nuclear localization[190]. What's more, the use of EV-delivered nucleases was demonstrated to work in live animals lacking Ercc1, where they successfully suppressed pro-inflammatory factors such as IFNα and matrix metalloproteinase 9 (MMP9)[190].

The repurposing of anti-HIV drugs, such as nucleotide reverse transcriptase inhibitors (NRTIs), has shown promise in treating RTE-related inflammation[97,98,100,207]. NRTI treatment has been shown to reduce Alu-induced macular degeneration and SASP factors in aged animals[98,100,106]. However, the chronic use of NRTIs can elicit several side effects, including hepatotoxicity and may even contribute to neuronal inflammation and chronic pain[208]. It is worth noting that current NRTI use in patients targets high retroviral load pathologies associated with HIV. As such, lower doses may be sufficient for suppressing endogenous RTEs and carry a lower risk of side effects. In additional, other anti-retroviral drugs, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs) have fewer side effects compared to NRTIs and have also been demonstrated to suppress RTE activity[209]. NNRTIs specific to the L1 reverse transcriptase could be developed to provide effective protection from RTE-induced inflammation with minimal off-target effects.

Outside of targeting the initiators of inflammation, strategies that support regulators of the genome and epigenome have shown promise. The most safe and accessible strategies involve lifestyle modification, such as exercise and caloric restriction. Indeed, it has been shown that patients that introduce exercise into their routines show reduced inflammation signals, and caloric restriction studies also show a reduction in TNF and C-reactive protein (CRP) levels[210–212]. Alternatively, caloric restriction mimetics, such as supplemental NAD+, metformin, and rapamycin, have become attractive candidates for eliciting the beneficial results of caloric restriction, including reduced inflammation[213]. As many of these substances are already in use in the clinic, developing safe regimens of these drugs to treat age-related inflammation could be the most immediate approach.

Future perspectives

Genomic instability and inflammation are two major hallmarks of ageing. Only recently, however, has the link between DNA damage and inflammation been revealed. This link helps explain the age-related changes in these pathways and provides yet another axis to the underlying causes of ageing. While anti-inflammatory drugs are an obvious solution to suppress age-related inflammation, they overwhelmingly target the downstream effectors of inflammation. The most common class, NSAIDs, are known to illicit many negative side effects, including kidney, gastrointestinal, and cardiovascular issues[214]. The more desirable approach would be to target the root causes of inflammageing.

Developing strategies to improve DNA repair and ameliorate age-related genomic instability may be the most attractive line of research. While all animal species experience DNA damage, not all species are equal in their DNA repair capacity, indicating that there exists room for improvement[177]. Remarkably, the ability to repair DNA double strand breaks correlates positively with species longevity[177] with SIRT6 being the key factor responsible for more efficient DNA repair in longer-lived species.

The levels of many DNA repair proteins decline with age along with the DNA repair capacity, but rescue of individual factors does not necessarily result in improved repair[215]. Remarkably, overexpression of regulators of DNA repair and chromatin, such as SIRT1 and SIRT6, rescues DNA repair in pre-senescent cells[215–217]. SIRT1 and SIRT6 also play important roles in epigenome maintenance. DNA damage-induced redistribution of these factors results in RTE activation leading to inflammation. Another outstanding question remains in how regulators of RTEs change with age and the relationship between different suppression mechanisms. While there are several seemingly independent pathways involved in RTE suppression, including TREX1, APOBEC3 family members, RNAaseL, ADAR1, MOV10, SIRT1 and SIRT6 little is known about how these factors change with age in terms of their abundance and expression.

More research needs to be done to understand how epigenome maintenance can be improved and ageing chromatin rejuvenated to restore the youthful order. Exploration into differences in epigenetic regulation between animal species with diverse lifespans is likely to reveal critical components that impact the ageing process. Epigenome could be rejuvenated and RTEs silenced by strategies such as partial reprogramming[218,219] or activation of SIRT6. Recently, several chemical activators of SIRT6 have been reported[220–223], including natural compounds such as the seaweed polysaccharide fucoidan[224,225]. These new and promising interventions, combined with regiments of older drugs repurposed for ageing systems may provide a new approach to attenuating age-related inflammation.

Glossary terms:

Nucleotide excision repair (NER)
a DNA repair pathway that recognizes and removes bulky DNA lesions such as those formed by UV light. NER involves damage recognition, nicks flanking the lesion and removal of the damaged DNA strand, filling in the gap by DNS polymerase and ligation of the nicks to restore intact DNA molecule. Deficiency in NER is associated with cancer predisposition and premature aging syndromes. For review see [226]

senescence-associated secretory phenotype (SASP)
a phenotype associated with cellular senescence, which express and secrete a wide variety of inflammatory factors including cytokines, chemokines, and growth factors.

Double-stranded DNA break (DSB) repair
DNA repair pathway that repairs lesions where both DNA strands are broken. There are two major pathways of DSB repair, homologous recombination (HR) that uses sister chromatid as a template for repair and nonhomologous DNA end joining (NHEJ) that ligates the broken ends without regard for homology. Defects in DNA repair are associated with several premature aging and cancer predisposition syndromes. For review see [227]

Inflammageing
chronic, low-grade sterile inflammation frequently observed during ageing

HRASV12

an oncoprotein of small GTPase HRAS carrying a constitutively activated mutation on codon Val-12.

Sterile Inflammation
inflammation arising in the absence of a pathogen or external inflammatory stimuli.

Retrotransposons (RTEs)
genetic elements abundant in mammalian genomes that move throughout the genome by copy-paste mechanism involving reverse transcription and may accumulate in the cytoplasm triggering inflammation

Blind mole rat
a subterranean rodent of the Muroidea superfamily, characterized by long maximum lifespan (>21 years) and resistance to cancer.

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Box:

Histone modifications, DNA damage and age-related inflammation:

DNA damage induces rapid changes to histone marks surrounding the damage site.[228,229] Most of these changes are reversed after repair is complete, however, some may persist and accumulate during lifetime. Histone marks undergo changes during aging that includes loss of histone H3K9me3 as well as epigenetic drifts in H3K4me3, H3K27me3, and H3K36me3 marks.[230–232] Repetitive regions and TEs tend to lose repressive marks, while some gene promoters gain repressive marks[230,231]. It has also been observed that aging-related chromatin remodeling correlates with transcription of endogenous retroviruses and pro-inflammation pathways[233]. Some of these changes may be attributed to clonal selection such as repressive marks on promoters of genes that negatively regulate cell cycle, while others may represent the scars left after repair of DNA damage. While both DNA repair and aging lead to changes in histone marks, and the loss of repressive marks on TEs is likely to promote inflammation through TE activation and R-loop formation, the exact histone code connecting these events remains to be determined.

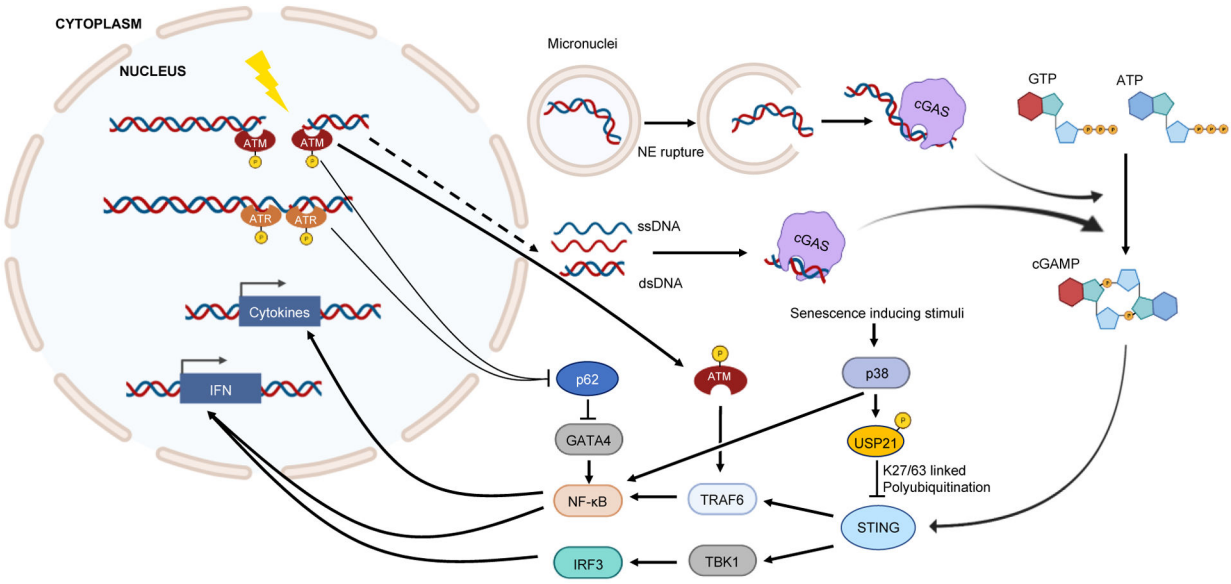

Figure 1: DNA damage as an inducer of inflammation.

Multiple mechanisms connect DNA damage to inflammation. DNA damage may result in chromosome fragments that do not segregate properly during subsequent cell division. These chromosome fragments become surrounded by nuclear envelope forming. When the micronuclei undergo nuclear envelope rupture, the chromosome DNA is exposed to the DNA sensor cGAS. Alternatively, during DNA damage or subsequent resection and repair, DNA fragments may directly leak into the cytoplasm through a less understood mechanism (dashed line). ssDNA fragments may form double-stranded secondary structures, which can also be recognized by cGAS. DNA-bound cGAS converts GTP and ATP into cGAMP, which activates STING. Activated STING through TBK1-IRF3 and TRAF6-NF-κB, induces transcription of IFN genes and other cytokines. Additionally, proteins involved in DNA damage response can directly trigger inflammation. Depending on the type of DNA damage, ATM or ATR are activated and recruited to the DNA break sites. Activated ATM or ATR can activate NF-κB by stabilizing GATA4 protein. ATM can also stimulate NF-κB by activating TRAF6. Senescence inducing stimuli, including sustained DNA damage and telomere defects, may activate p38 pathway. p38 induces inflammation through NF-κB; it also inhibits STING-dependent IFN response through USP21-mediated K27/63 linked polyubiquitination. NE: nuclear envelope. Image was generated using biorender.com.

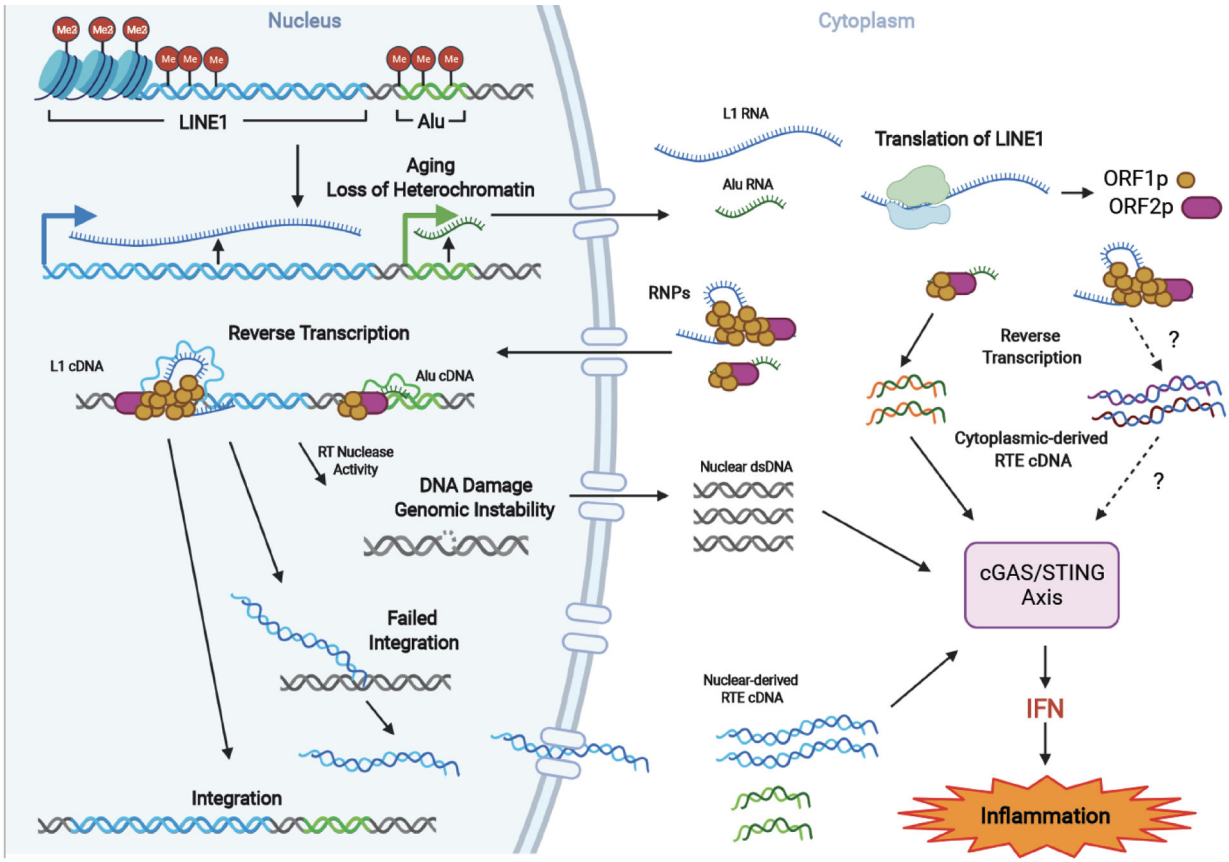

Figure 2: RTE mobilization triggers inflammation via cytoplasmic DNA.

Ageing-related loss of heterochromatin marks on RTEs results in their transcriptional activation and retrotransposition. In the cytoplasm, Alu elements produce ssDNA via self-priming utilizing LINE1 RT machinery. Simar process may take place for L1s. In the nucleus, RNPs initiate RTE integration by generating DNA nicks and reverse transcription, which induces DNA damage. Additionally, unsuccessful integration produces DNA flaps, which are processed to RNA-DNA hybrid fragments. The RTE RNA-DNA hybrids resulting from reverse transcription either in the nucleus or cytoplasm and DNA fragments resulting from genomic damage are recognized by cytoplasmic cGAS, triggering the cGAS-STING signaling, leading to IFN production and sterile inflammation. Dashed lines indicate steps that require further experimental evidence.

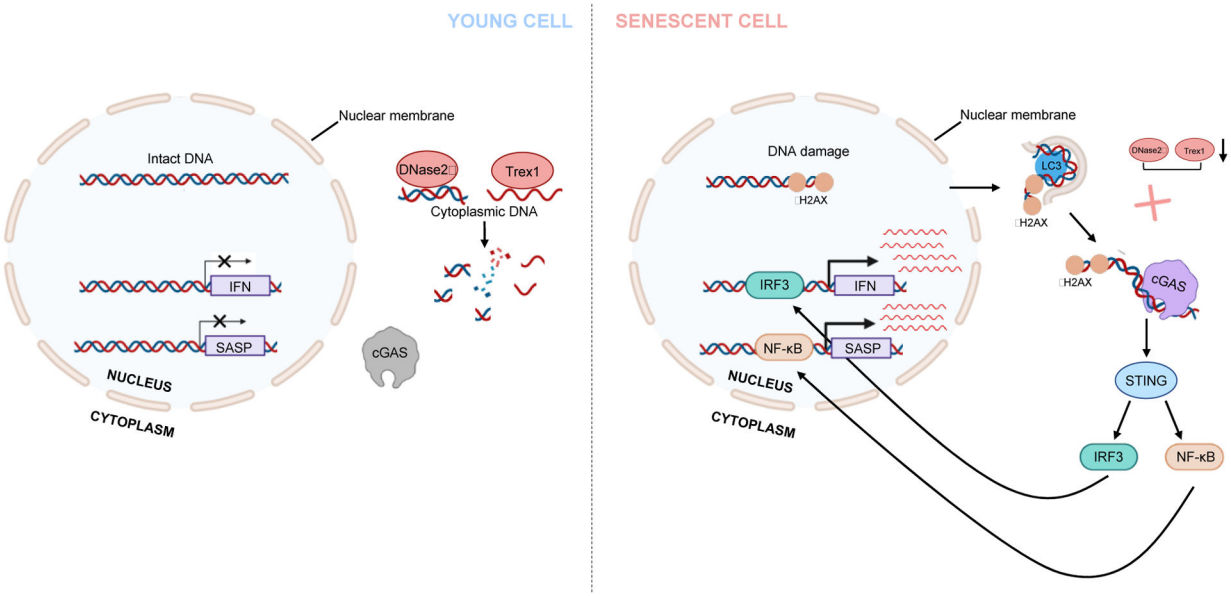

Figure 3: Mechanisms of senescence induced inflammation.

Cellular senescence induces inflammation via cytoplasmic nucleic acid sensing pathway. Three major events participate in the generation of cytoplasmic DNA. One of the hallmarks of senescence is the loss of lamin B1. The autophagy-related protein LC3 interacts with lamina and lamina-associated domains (LADs) of the chromatin and directs lamin B1 to autophagy. This process results in the loss of nuclear membrane integrity, contributing to the leaking of chromatin to the cytoplasm. Sustained DNA damage response (DDR) is another hallmark of senescence. The cytoplasmic DNA is positive for γH2AX and negative of 53BP1, suggesting that the formation of cytoplasmic DNA is associated with DDR. Two cytoplasmic DNases, DNase 2α and Trex1, are responsible for degrading excessive cytoplasmic DNA. Both enzymes are down-regulated in senescence through a negative transcriptional regulation. Therefore, in senescence, loss of nuclear membrane integrity promotes the accumulation of cytoplasmic DNA, which is further stabilized by the down-regulation of DNases. Cytoplasmic DNA is sensed by the DNA the sensor cGAS, which triggers STING and its downstream pathways, including IRF3-IFN and NF-κB-SASP pathways.

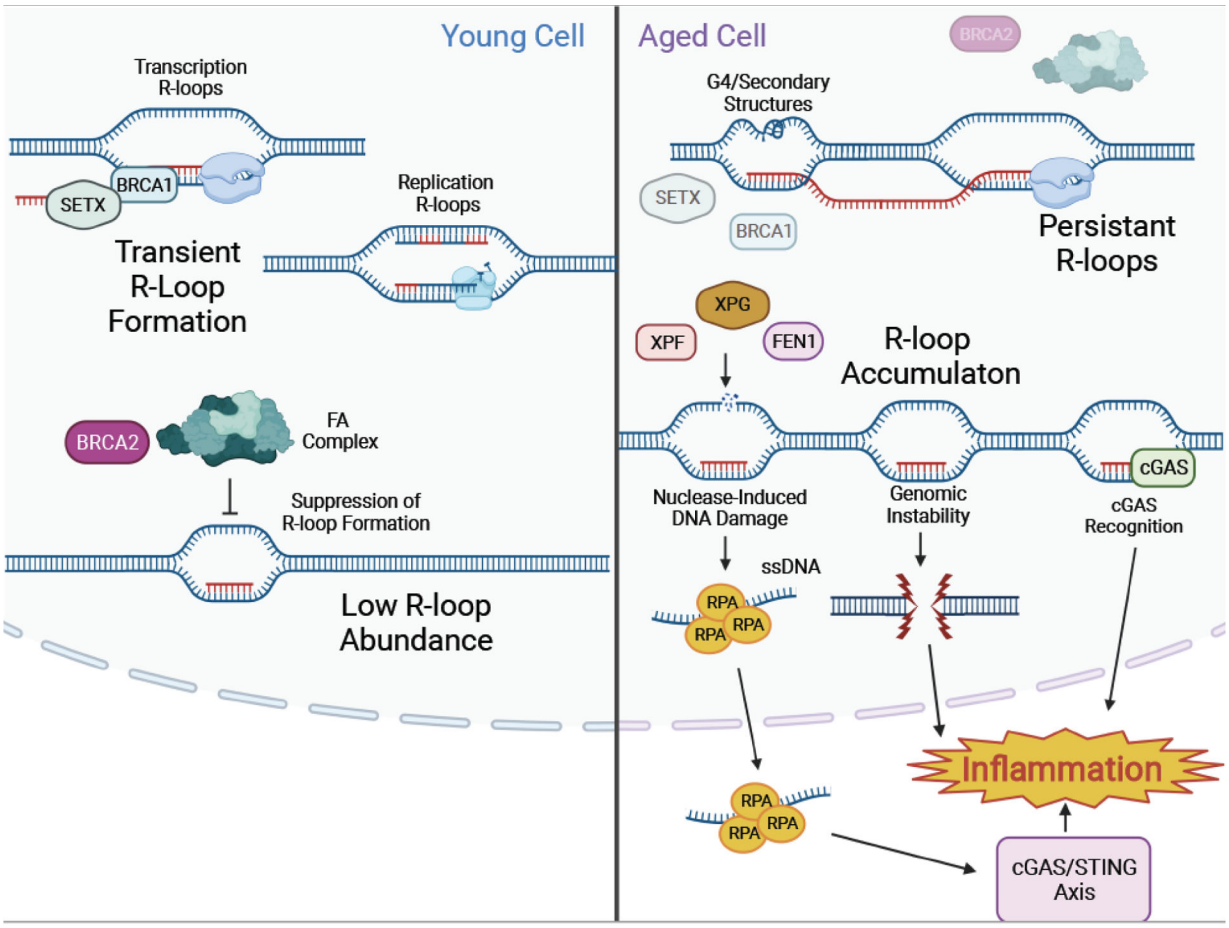

Figure 4: R-loops accumulate with age and drive inflammation.

R-loops form in healthy young cells during regular cellular processes, including transcription and DNA replication. Proteins such as BRCA1 and SETX ensure RNA displacement and R-loops resolution. FA complex proteins and BRCA2 also ensure that R-loops remain transitory. In older cells, R-loops accumulate and persist within the genome, due to failure of processes responsible for their resolution. Secondary DNA structures, such as G-quadraplexes, contribute to unresolved R-loop accumulation. Persistent R-loops leave displaced ssDNA strands exposed to endonucleases, such as XPF, XPG and FEN1, leading to genomic instability and inflammation. Persistent R-loops can also be recognized by cGAS-STING and trigger sterile inflammation.

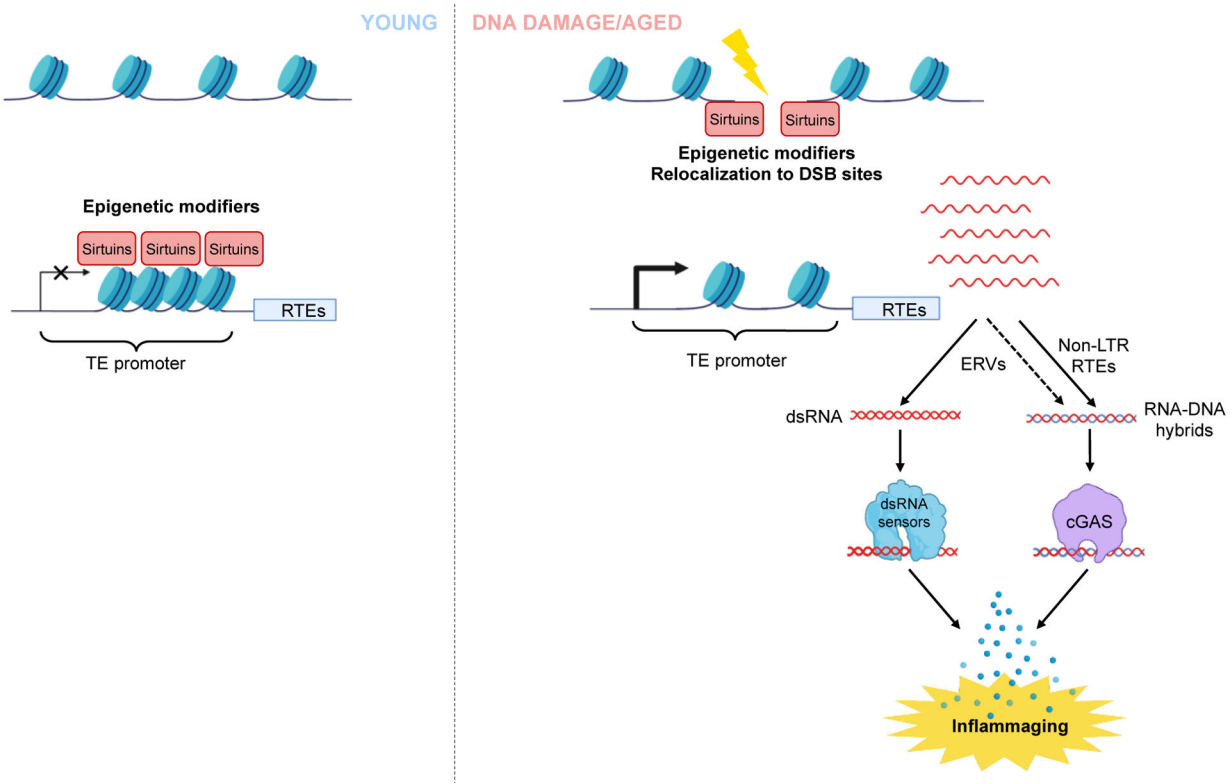

Figure 5: Epigenetic changes triggered by life-long DNA damage lead to induction of RTEs and inflammaging.

DNA damage can induce redistribution of heterochromatin modifiers such as proteins of the Sirtuin family, SIRT1 and SIRT6. In young cells, these Sirtuins silence RTEs and other repetitive elements by maintaining heterochromatin. Upon DNA damage, Sirtuins re-localize to DSB sites, leading to the activation of RTEs. Activated RTEs result in the formation of cytoplasmic RNA or DNA, which trigger the RNA or DNA sensing pathway to induce inflammation. This mechanism provides a link between DNA damage and inflammation through chromatin change.

Table 1.

Syndromes of DNA repair deficiency and their association with inflammation

DNA repair pathway	Genes mutated	Syndrome	Cancer risk	Premature ageing	Inflammation	Mechanisms of inflammation
NER	various NER genes	Xeroderma pigmentosum (XP)	Yes	Yes	No	NA
NER	*XPF* (severe mutation)	XPF-ERCC1 progeroid (XFE)	No	Yes	Yes	Cytoplasmic p-ATM; SASP
NER	*csa*, *csb*	Cockayne syndrome (CS)	No	Yes	Yes	Mitochondrial stress
DSB	*WRN*	Werner syndrome (WS)	Yes	Yes (segmental)	Yes	telomere defect; possibly cGAS-STING
DSB	*BLM*	Bloom syndrome (BS)	Yes	Yes (segmental)	Yes	Micronuclei
Indirect	*LMNA*	Hutchinson-Gilford Progeria Syndrome (HGPS)	No	Yes	Yes	DNA leakage; micronuclei
Indirect	*ATM*	Ataxia-telangiectasia (AT)	Yes	Yes	Yes	Cytoplasmic DNA