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Review article

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Roles of chromatin and genome instability in cellular senescence and their relevance to ageing and related diseases

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Abstract

Ageing is a complex biological process in which a gradual decline in physiological fitness increases susceptibility to diseases such as neurodegenerative disorders and cancer. Cellular senescence, a state of irreversible cell-growth arrest accompanied by functional deterioration, has emerged as a pivotal driver of ageing. In this Review, we discuss how heterochromatin loss, telomere attrition and DNA damage contribute to cellular senescence, ageing and age-related diseases by eliciting genome instability, innate immunity and inflammation. We also discuss how emerging therapeutic strategies could restore heterochromatin stability, maintain telomere integrity and boost the DNA repair capacity, and thus counteract cellular senescence and ageing-associated pathologies. Finally, we outline current research challenges and future directions aimed at better comprehending and delaying ageing.

Sections

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Introduction

Ageing is a slow progressive process encompassing loss of tissue and organ function and ultimately leading to death, which has fascinated human beings since the dawn of civilization. With advancing age, the escalating risk of age-related ailments such as neurodegenerative conditions, metabolic syndromes, cardiovascular disorders and cancer presents significant threats to human health[1–3].

Cellular senescence is a state of irreversible cell-growth arrest, commonly referred to as the Hayflick limit, or replicative senescence[4]. This limit is defined as the number of times a differentiated normal cell will divide until cell division ceases. Cellular senescence is governed by programmes that prevent the proliferation of potentially harmful cells such as pre-cancerous or damaged cells within the body. For example, normal cells respond to oncogene activation by permanently withdrawing from the cell cycle, a process known as oncogene-induced senescence[5]. This senescence, in turn, can activate a potent anticancer mechanism[6,7]. Notably, chemicals that induce cancer-cell senescence have been used as chemotherapeutics for cancer treatment[8,9]. We term this type of senescence chemical-induced or therapy-induced senescence[10,11], which can also be considered a form of stress-induced senescence (a type of senescence triggered by various stressors instead of by the Hayflick limit). Additionally, cell senescence that occurs during the development of the embryo and placenta and in the process of wound healing is identified as developmental or physiological senescence and is crucial for tissue remodelling[10,12–14]. As our understanding of cellular and molecular processes that occur in ageing expands, the definition of cellular senescence is becoming more precise. Particularly, a growing body of evidence suggests that cellular senescence is both an indicator and a driver of ageing: senescent cells release pro-inflammatory cytokines, but also other factors known as the senescence-associated secretory phenotype (SASP), which contribute to chronic inflammation, impaired tissue regeneration, ageing and age-related diseases[7,15]. Understanding the determinants of cellular senescence and their relevance to ageing is crucial to dissect the underlying mechanisms of ageing and age-related diseases and explore potential therapeutic avenues[16,17].

Cellular senescence can result from a range of inter-dependent mechanisms including epigenetic erosion, telomere attrition, DNA damage, metabolic imbalance and loss of proteostasis (Box 1). Notably, the first three have recently garnered substantial interest[18–20] with regard to cell senescence and ageing. Epigenetic erosion, manifested by the deterioration of histone and DNA modifications, notably leads to the disruption of heterochromatin, a densely compacted form of chromatin that is crucial for gene silencing and thus to changes in gene expression patterns, ultimately driving cell dysfunction and senescence[21]. Telomere attrition is the gradual reduction of protective caps at the ends of chromosomes. As telomeres shorten with each cell division, they eventually reach a critical threshold that prompts cell entry into a senescent state[10]. Finally, DNA damage, resulting from endogenous or exogenous stimuli, can induce senescence through activation of DNA damage response (DDR) pathways, such as the canonical tumour suppressor p53 and $p21^{CIP1}$ pathway[22]. These three determinants of cell senescence are interconnected and also engage in crosstalk with other cellular mechanisms dysregulated during senescence, such as metabolism (Box 2), thereby presenting challenges to clear delineation of their separate effects on senescence.

Irrespective of these challenges, recent discoveries in senescence regulation have uncovered new mechanisms and specific therapeutic targets. For instance, heterochromatin loss in senescent cells was found to contribute to derepression of endogenous retroviruses (ERVs), a type of retrotransposon that integrates into the host genome. Once derepressed, ERVs further exacerbate cellular senescence by activating the innate immune system[18]. By contrast, abolishing the silencing of the telomerase reverse transcriptase (*TERT*) gene boosted its expression and alleviated senescence through telomere-maintenance-initiated DNA damage relief, thereby enhancing cell proliferation and reducing the SASP[20]. Through targeting these mechanisms, therapeutic agents could be engineered to specifically rejuvenate or eliminate senescent cells, thereby potentially restoring tissue function and mitigating age-related pathology. Accordingly, a timely and comprehensive review of these three molecular underpinnings of cellular senescence is warranted.

In this Review, we discuss the most recent findings on how heterochromatin loss, telomere attrition and DNA damage cause cell senescence and are correlated with ageing and age-related diseases. Furthermore, we discuss existing therapeutic strategies targeting senescence and ageing-related conditions and highlight their association with these pathways.

Molecular causes of cellular senescence

Cellular senescence is characterized by cell cycle arrest, cell morphology changes, activation of senescence-associated β-galactosidase (SA-β-gal), accumulation of reactive oxygen species (ROS), SASP, mitochondrial dysfunction, metabolic dysregulation, epigenetic alterations (for example, heterochromatin loss), telomere attrition, genome instability (for example, DNA damage) and others[7,21] (Box 1). In this section, we discuss the processes associated with heterochromatin loss, telomere attrition and DNA damage within the framework of cellular senescence. We aim to offer a systematic understanding of the mechanistic roles of these processes in cellular senescence.

Heterochromatin loss

Heterochromatin is a highly conserved and structurally distinctive form of eukaryotic chromatin. Unlike euchromatin, heterochromatin is tightly packed in a way that ensures gene silencing and genome stability[23,24]. Heterochromatin is studded with hypoacetylated histones, DNA methylation and specific histone modifications such as dimethylated and trimethylated histone H3 Lys9 (H3K9me2 and H3K9me3) or Polycomb-mediated H3K27me3 (ref. 25). In mammalian cells, H4K20me3 and H3K56me3 are also present at heterochromatin regions[26]. Histone methylation is catalysed by histone methyltransferases[27] and is recognized by proteins such as heterochromatin protein 1 (HP1), which bind to them and facilitate the formation and stabilization of heterochromatin[28,29]. Based on the disparity in gene silencing-causing modifications and function, heterochromatin is typically classified as either constitutive or facultative[25]. Constitutive heterochromatin is generally enriched in H3K9me3 and H4K20me3 and is typically found in pericentromeric or telomeric regions, which contain satellite DNA sequences and transposable elements[25,30]. During cellular senescence, the loss of constitutive heterochromatin is usually associated with instability of telomeres, centromeres and their adjacent regions, and with derepression of transposons and developmentally restricted genes[18,31–35]. By contrast, facultative heterochromatin can be marked by H3K27me3 and varies between cell types and developmental states, exerting specialized regulatory functions. Upon senescence, aberrantly formed facultative heterochromatin within the nucleus known as senescence-associated heterochromatin foci (SAHF) is implicated in silencing genes that stimulate cell division[36].

Box 1 | Features and inducers of cellular senescence

Listed below are representative features, inducers and biomarkers of cellular senescence.

- Cell cycle arrest is a pivotal feature of cell senescence. Cell cycle arrest is crucially mediated by the p53–$p21^{CIP1}$ and $p16^{INK4a}$–RB (retinoblastoma tumour suppressor) pathways, which can be activated by DNA damage[7]. Cell cycle arrest can be detected by immunostaining as absence of proliferation markers such as Ki67 and PCNA[22].
- Senescent cells often exhibit morphological changes, including a flattened, irregular shape, enlarged nuclei, increased nucleolar size and abnormalities in organelles such as mitochondria and endoplasmic reticulum[10,312,313].
- Senescence-associated β-galactosidase (SA-β-gal) represents the increased activity of β-galactosidase, a lysosomal enzyme, during senescence. SA-β-gal is easily identified at pH 6.0 in senescent cells with X-Gal staining[314,315].
- The senescence-associated secretory phenotype (SASP) involves the release by senescent cells of various pro-inflammatory cytokines, chemokines, growth factors and matrix metalloproteinases. The SASP creates a microenvironment that can promote chronic inflammation and influence neighbouring cells to undergo senescence[7,15].
- In addition to morphological changes, senescent cells exhibit functional impairments in mitochondria, leading to decreased ATP production, altered membrane potential, increased production of reactive oxygen species (ROS) and heightened mitochondrial DNA damage and mutations, which advance the progression of cellular senescence[21,316].
- Paired with mitochondrial dysfunction, metabolic pathways undergo dysregulation during senescence. Changes in glucose metabolism, lipid metabolism and other metabolic processes disrupt energy balance and metabolite abundance within the cell, contributing to the senescent state[21,316].
- Accumulation of ROS within the cell, primarily generated by mitochondria, functions as both a feature and a trigger of cellular senescence. ROS can induce oxidative damage in proteins, lipids and DNA[21,317].
- Senescent cells exhibit loss of proteostasis, leading to the accumulation and aggregation of misfolded or damaged proteins, which can trigger cellular stress responses and disrupt cell function[318].
- Epigenetic changes are considerable in senescent cells, encompassing alterations in modifications of DNA, RNA and histones and dysregulation of non-coding RNAs and chromatin organization. Notably, prominent heterochromatin loss, characterized by erosion of epigenetic marks (for example, histone H3 Lys9 trimethylation (H3K9me3)) and consequently loss of gene silencing, drives the senescence process. Heterochromatin loss typically corresponds to the decreased expression of heterochromatin protein 1 (HP1) and lamin B1, and the activation of certain retrotransposons[32,242]. By contrast, in specific conditions such as oncogene-triggered senescence, irregular heterochromatin clusters known as senescence-associated heterochromatin foci (SAHF) appear in the nuclei of the senescent cells[36].
- Telomere attrition can trigger a DNA damage response, which culminates in cell senescence.
- Genome instability is frequent in senescent cells owing to accumulated DNA damage and is characterized by chromosomal rearrangements and other genomic abnormalities. DNA damage leads to activation of the DNA damage response, which is detected through phosphorylated histone H2AX (γH2AX) or p53-binding protein 1 (53BP1) foci formation and ataxia–telangiectasia mutated (ATM) and p53 activation[319].

Global loss of heterochromatin, particularly constitutive heterochromatin, accompanies senescence in various cell models and diverse species. For example, in *Saccharomyces cerevisiae*, heterochromatin loss is associated with disrupted transcriptional gene silencing and with replicative senescence (when comparing later generations with earlier ones)[37–40]. In addition, heterochromatin erosion as indicated by H3K9me3 loss was reported in enterocytes from aged *Drosophila melanogaster* and in senescent fibroblasts from progeroid mice[41,42]. Moreover, reduced levels of H3K9me3 and HP1 were reported in human mesenchymal stem cell (MSC) models of progeroid disorders such as Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome[31,43], which undergo premature senescence owing to stresses caused by their genetic mutations. Similar observations of heterochromatin loss were documented in primary human MSCs derived from aged individuals[31], bleomycin-induced senescent fibroblasts and human MSCs derived from individuals with HGPS and Cockayne syndrome[44–46], and in senescent human neurons following long-term culture[47]. Senescence-related heterochromatin loss is usually coupled with aberrant expression of histone methyltransferases, such as downregulation of suppressor of variegation 3-9 homologue 1 (SUV39H1) and of SET domain bifurcated histone lysine methyltransferase 1 (SETDB1) and upregulation of histone lysine demethylase 4A (KDM4A) or KDM4B[31,45,48]. Furthermore, manipulation of the expression or activity of these enzymes can reverse or accelerate senescence[31,45,48]. Likewise, HP1 downregulation aggravates heterochromatin disorganization and accelerates senescence, whereas its replenishment restores heterochromatin stability and mitigates premature senescence[31–33,49], further supporting heterochromatin loss as a driving force of cell senescence.

In addition to well-known regulators, a panel of non-canonical factors identified as heterochromatin stabilizers or destabilizers are reported to significantly affect cellular senescence (Fig. 1). These novel regulators include modulators of the circadian clock such as circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1) (refs. 33,50), sirtuin family members such as SIRT1, SIRT3, SIRT6 and SIRT7 (refs. 32,51–53), and the regulators of the RNA N^6-methyladenosine (m^6A) modification such as METTL3 (m^6A writer)[54,55], FTO (eraser)[56,57] and YTHDC1 (reader)[58,59], the canonical microRNA processing factor DGCR8 (ref. 49), the master transcriptional repressor of autophagy ZKSCAN3 (ref. 60), the long non-coding

Box 2 | Cellular metabolism and senescence regulation

Cellular metabolism involves conversion of nutrients into energy and building blocks that are essential for cellular activities. Senescent cells are both susceptible to and trigger many aspects of metabolic reprogramming. Conversely, metabolic changes can profoundly affect the onset and progression of cellular senescence and contribute to ageing and the development of ageing-related diseases[251,316]. Cellular metabolism is linked to the regulation of senescence through dynamic interplay between key metabolites such as *S*-adenosylmethionine (SAM), NAD^+ and acetyl-coenzyme A (CoA), and signalling pathways such as the insulin–insulin-like growth factor 1 (IGF1), mTOR and sirtuin pathways. Through these molecules and pathways, cellular metabolism influences cellular senescence and ageing either alone or in conjunction with other senescence inducers, including heterochromatin loss, telomere attrition and DNA damage. Below, we provide several examples of metabolic changes that affect cellular senescence by interacting with the three indicated senescence triggers.

SAM is a major methyl donor for DNA and histone methylation, and thus has an important role in the regulation of gene expression and chromatin remodelling. Its production from methionine is catalysed by methionine adenosyltransferase 2A (MAT2A). Methionine deprivation or MAT2A inhibition induces senescence, leading to compromised cell proliferation, increased senescence-associated β-galactosidase (SA-β-gal) staining, upregulated expression of $p16^{INK4a}$ and $p21^{CIP1}$, and accumulation of DNA damage as indicated by the formation of p53-binding protein 1 (53BP1) foci[320]. Depletion of SAM causes heterochromatin loss in senescent muscle stem cells from aged mice. Conversely, restoration of SAM can promote heterochromatin stability by increasing the levels of trimethylated histone H3 Lys9 (H3K9me3) and heterochromatin protein 1 (HP1) and alleviate the senescent phenotypes by decreasing expression of long interspersed element 1 (LINE-1) retrotransposons and accumulation of the DNA damage response factor phosphorylated histone H2AX (γH2AX). Similar results are also obtained in human muscle stem cells upon SAM supplementation[229].

NAD^+ is a coenzyme crucial for glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation. Gene regulation, DNA repair and cellular senescence involve the consumption of NAD^+ by the NADase CD38, by poly(ADP-ribose) polymerases (PARPs) and by sirtuins, which are protein deacylases. Deregulated NAD^+ metabolism is associated with increased CD38 and suppressed PARP and sirtuin activities. NAD^+ level decreases in senescent fibroblasts derived from individuals with dyskeratosis congenita, which is a bone marrow disorder characterized by very short telomeres (Table 1). NAD^+ replenishment or CD38 inhibition significantly alleviates cellular senescence in dyskeratosis congenita fibroblasts, as manifested by reduced telomere damage, attenuated mitochondrial dysfunction and improved proliferative capacity[245]. NAD^+ restoration can also prevent accumulation of DNA damage, cytoplasmic DNA and inflammation, thereby counteracting cellular senescence in a mouse model of Alzheimer disease[321].

Acetyl-CoA serves as a substrate for histone acetylation, thereby connecting cellular metabolism to gene regulation and influencing senescence-associated gene expression patterns and cellular responses[322]. Acetyl-CoA can be generated from acetate by acetyl-coenzyme A synthetase, cytoplasmic (ACSS2). This enzyme is required to increase H4K16 acetylation levels, decrease telomere stability and accelerate cellular senescence in human endothelial cells. ACSS2 depletion alleviates telomere shortening and cellular senescence[116]. Notably, pyruvate metabolism and fatty acid oxidation also contribute to acetyl-CoA synthesis, which involves PARP-mediated DNA damage repair in support of genome stability[323,324].

RNA *KCNQ1OT1* (ref. 61), the E3 ligase substrate adaptor DCAF11 (ref. 62) and apolipoprotein E (APOE)[63]. CLOCK, for example, is essential to stabilize the nuclear envelope and, through it, heterochromatin by interacting with lamin B1, lamin B receptor (LBR) and the transcription repressor KRAB-associated protein 1 (KAP1; also known as TRIM28 or TIF1β). Consequently, deletion of *CLOCK* promotes the loss of constitutive heterochromatin and accelerates senescence in human MSCs[33]. *KCNQ1OT1* binds to HP1 and guides DNA methylation and H3K9me3 in heterochromatin, and repression of *KCNQ1OT1* results in heterochromatin decompaction and detachment from the nuclear envelope and activation of the SASP[61]. By contrast, APOE interacts with LBR, emerin and KAP1 to facilitate their degradation through the autophagy–lysosome pathway and senescence onset in human MSCs, whereas APOE ablation restores constitutive heterochromatin and prevents onset of senescence[63]. Importantly, the mechanism by which APOE induces heterochromatin loss also applies to human fibroblasts undergoing replicative senescence, oncogene-induced senescence and premature senescence triggered by oxidative stress or ultraviolet (UV) irradiation[63], suggesting that they share a common pathway. The identification of these heterochromatin regulators deepens our understanding of how epigenome stability is maintained and suggests potential therapeutic targets.

A consequence of constitutive heterochromatin loss is the derepression of transposable elements, which contributes to activation of the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway and the type I interferon response, and upregulation of SASP factors[64] (Fig. 1). For instance, SIRT7 deficiency is associated with lower H3K9me3 levels at long interspersed element 1 (LINE-1) retrotransposons, leading to increased chromatin accessibility and LINE-1 activation in human MSCs[32]. Subsequently, the increased phosphorylation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3) and activation of nuclear factor κB (NF-κB) signalling, contribute to interferon production, SASP and premature senescence. In a related study, SIRT7 depletion was found to disrupt its interplay with lamin A/C, another nuclear envelope protein, responsible for stabilization of perinuclear heterochromatin, resulting in LINE-1 derepression in mouse fibroblasts and human tumour cells[65]. Supporting these findings, LINE-1 activation and type I interferon response induction are features also of SIRT6-deficient mouse fibroblasts and of oncogene-induced or stress-induced senescent human fibroblasts[66,67]. In mouse stromal cells, deletion of *Yap* and *Taz*, which encode co-activators of Hippo signalling, also stimulates the cGAS–STING pathway and accelerates senescence by downregulating lamin B1 and disrupting the nuclear envelope[68]. ERV expression

is also included upon loss of H3K9me3 and DNA methylation, which increases cGAS–STING activity, inflammation and senescence in human MSCs, fibroblasts and neurons[18,47]. CRISPR-mediated genetic activation of ERVs in proliferating cells induces senescence characteristics such as increased SA-β-gal activity, compromised clonal-expansion capacity, upregulation of cell cycle suppressors such as $p16^{INK4a}$, downregulation of lamin B1, elevated levels of phosphorylated TBK1, IRF3 and NF-κB, and an enhanced SASP[18]. Moreover, activated ERVs can produce retrovirus-like particles, which amplify senescence in neighbouring cells in collaboration with the paracrine effect of SASP factors[18,69,70] (Fig. 1). These senescence phenotypes can be reversed by suppressing ERVs, indicating that the activation of ERVs drives cellular senescence, both replicative senescence and stress-induced premature senescence. Interestingly, there is evidence of feedback regulation between heterochromatin, retrotransposons and cGAS: accumulation of LINE-1 RNA drives heterochromatin loss, as manifested by reduction in H3K9me3 and H3K27me3 levels[71], whereas cGAS inhibits LINE-1 retrotransposition by inducing degradation of its endonuclease ORF2p[72]. These findings suggest that a bidirectional regulatory heterochromatin–transposons–cGAS axis exists, although further experiments would be required to test such a model.

In certain contexts, cellular senescence has also been linked to elevated levels of heterochromatin. For example, SAHF form upon oncogene-induced senescence in human fibroblasts, representing de novo establishment of facultative heterochromatin[73–75] (Fig. 1). During this process, DNA methylation, mediated by DNA methyltransferase 1

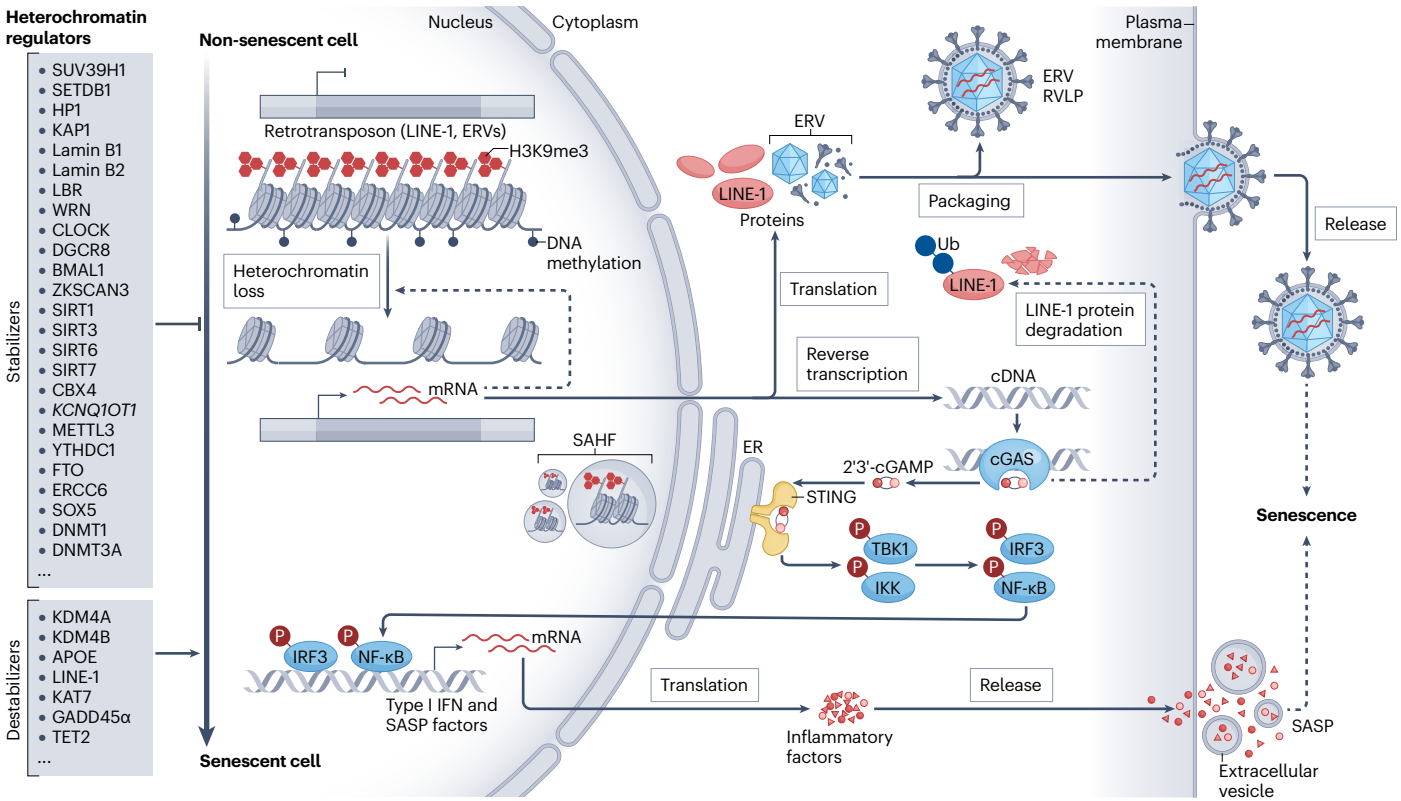

Fig. 1 | Heterochromatin loss and retrotransposon activation in cellular senescence. In non-senescent cells, heterochromatin is well maintained and highly enriched in DNA methylation and gene-repressive histone modifications including trimethylated histone H3 Lys9 (H3K9me3), which mediate the transcriptional repression of retrotransposons such as long interspersed element 1 (LINE-1) and endogenous retroviruses (ERVs). In senescent cells, heterochromatin is severely eroded, leading to the derepression of retrotransposons. Reverse transcription of LINE-1 or ERV mRNAs into cDNA triggers the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway, causing activation of type I interferon (IFN) response and the senescence-associated secretory phenotype (SASP). The activated ERVs can produce retrovirus-like particles (RVLPs), which further amplify the senescence signal. In some contexts (for example, oncogene-triggered senescence), senescence-associated heterochromatin foci (SAHF) comprising newly established facultative heterochromatin domains, might form in the nuclei of senescent cells. The dotted arrows depicted within the cell nucleus and cytoplasm signify feedback regulation.

APOE, apolipoprotein E; BMAL1, brain and muscle ARNT-like protein 1; CBX4, chromobox protein homologue 4; 2′3′-cGAMP, 2′,3′-cyclic GMP–AMP; CLOCK, circadian locomotor output cycles kaput; DGCR8, DiGeorge syndrome critical region gene 8; DNMT1, DNA methyltransferase 1; ER, endoplasmic reticulum; ERCC6, excision repair cross-complementation group 6; FTO, fat mass and obesity-associated protein; GADD45α, growth arrest and DNA damage-inducible 45α; HP1, heterochromatin protein 1; IKK, IκB kinase; IRF3, interferon regulatory factor 3; KAP1, KRAB-associated protein 1; KAT7, lysine acetyltransferase 7; KCNQ1OT1, KCNQ1 opposite strand/antisense transcript 1; KDM4A, lysine demethylase 4A; LBR, lamin B receptor; METTL3, methyltransferase like 3; NF-κB, nuclear factor κB; P, phosphorylation; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; SIRT1, sirtuin 1; SOX5, SRY-box transcription factor 5; SUV39H1, suppressor of variegation 3-9 homologue 1; TBK1, TANK-binding kinase 1; TET2, ten-eleven translocation 2; Ub, ubiquitylation; WRN, Werner syndrome RecQ helicase; YTHDC1, YTH domain-containing 1; ZKSCAN3, zinc-finger protein with KRAB and SCAN domains 3.

(DNMT1), is also redistributed, which in turn leads to the derepression of HMGA2, an essential factor for SAHF formation, making oncogene-induced senescence and replicative senescence distinguishable in terms of chromatin remodelling dynamics[74]. The formation of SAHF coincides with the recruitment of the retinoblastoma (RB) tumour suppressor to transcriptionally repress E2F target genes, which promote the cell cycle, thereby impeding proliferation and leading to senescence[36]. Additionally, in senescent mouse MSCs deficient for KDM4B, H3K9me3 levels increase concomitant with the formation of SAHF and the repression of stem cell signature genes[76]. Heterochromatin variability in senescent cells suggests the existence of context-specific mechanisms that orchestrate spatial rearrangements of heterochromatin domains or relocalization of heterochromatin modifications, as opposed to a general decrease or increase in heterochromatin levels. For instance, TET2-mediated DNA demethylation contributes to the spatial redistribution of H3K9me3-marked heterochromatin and activation of ERVs and to an interferon response in senescent mouse haematopoietic stem and progenitor cells; disruption of DNMT1 or DNMT3A function had similar outcomes[77]. A multi-omics study also linked stem cell senescence with increased chromatin entropy and epigenome instability, characterized by the loss of boundaries and diminished insulation or discrimination between heterochromatin and euchromatin domains, as well as between constitutive heterochromatin and facultative heterochromatin[34,78]. This domain blurring results in suppression of genes that were initially active, such as genes related to cell cycle progression and DNA repair, and in activation of suppressed genes, including retrotransposons and placenta-specific genes. Interestingly, the high expression of the latter genes owing to disrupted heterochromatin silencing is not only present in the context of replicative senescence and stress-induced premature senescence, but is also correlated with the physiological senescence-like phenotype in syncytiotrophoblast during pregnancy[34], potentially contributing to the remodelling of maternal tissues, including the placenta, and to the complex biological processes that facilitate parturition. These discoveries highlight the intricate spatial alterations that the genome undergoes during, and the mechanisms involved in preserving epigenome homeostasis through, senescence.

Telomere attrition

Telomeres, the cap structures at chromosome ends, are composed of repetitive DNA sequences (for example, TTAGGG in mammals) and the protective shelterin complex[79]. Shelterin comprises six proteins — telomeric repeat binding factor 1 (TRF1), TRF2, TRF1-interacting nuclear protein 2 (TIN2), protection of telomeres 1 (POT1), repressor/activator protein 1 (RAP1) and POT1 and TIN2-interacting protein (TPP1; also known as ACD) — and safeguards telomeres from damage during cell division. However, owing to incomplete DNA-end replication, telomeres shorten with each division, ultimately becoming critically short and triggering cell-growth arrest (replicative senescence). To counteract shortening, telomerase, which consists of TERT and telomerase RNA component (TERC), adds DNA at the ends of chromosomes. Whereas the expression of the non-coding RNA TERC is common in normal human cells, TERT is primarily expressed at a high level in cancer cells, germ cells and stem and progenitor cells, displaying extremely low or undetectable levels in differentiated cells such as fibroblasts and keratinocytes[80–83]. To prevent excessive telomere extension, the CTC1–STN1–TEN1 (CST) complex terminates telomerase activity[84]. Additionally, the long non-coding telomeric repeat-containing RNA (TERRA) contributes to telomere maintenance through both telomerase-dependent and telomerase-independent mechanisms[85,86]. Overall, telomere stability is regulated by mechanisms that involve both RNA and protein molecules.

Before replicative senescence, progressive telomere attrition functions as a timer that tracks the cumulative number of cell divisions (Fig. 2a). When telomeres reach a critically short length, they are recognized as damaged DNA, which subsequently activates a DDR that leads to cellular senescence[87]. Remarkably, from yeast to human cells, the presence of the shortest telomere, or of just a few critically short telomeres, is enough to trigger senescence[88–91]. Telomere attrition occurs concurrently with downregulation of certain telomere regulators, such as TRF1, TRF2 and TPP1, and with senescence in various cell types, including human fibroblasts and endothelial cells[35,92,93], demonstrating inadequate shelterin loading at critically short telomeres. Defects in TRF1, TRF2 or TPP1 can induce premature senescence in various cells such as human fibroblasts and vascular smooth muscle cells, and mouse bone marrow cells[94–96], whereas overexpression of these factors is sufficient to extend telomere length and/or delay senescence in certain contexts, as observed in human endothelial cells (TRF1), stem cell-derived cardiomyocytes (TRF2) and fibroblasts (TPP1)[92,97,98]. Similarly, downregulation of TERT accelerates onset of senescence in both human melanoma cells and mouse fibroblasts[99,100]. Interestingly, a recent study has demonstrated that in normal human fibroblasts, TERT expression can be detected using nested PCR, albeit at a very low level, and repression of TERT induces senescence characteristics in these cells[99]. These findings indicate that, similar to mouse fibroblasts, human fibroblasts require TERT function for the regulation of senescence. By contrast, upregulation of TERT promotes telomere elongation and alleviates senescence phenotypes in normal human cells, including fibroblasts[20,101]. However, several studies have reported findings inconsistent with these relationships. For example, overexpression of TRF1 and TRF2 in tumour cells is associated with shorter telomeres, which is seemingly opposite to their antagonistic effects on cellular senescence[102–104]. Furthermore, although evidence suggests that TRF1 overexpression can delay senescence in human endothelial cells, in other cells, elevated levels of TRF1 might actually accelerate senescence by inducing recombination-mediated DNA damage at subtelomeric regions, particularly in human WI-38 fibroblasts and U2OS cancer cells[92,104]. These phenomena could be explained by excessive TRF1 or TRF2 restricting cellular capacity to extend or stabilize telomeres, potentially through the inhibition of telomerase by TRF1 or the activation of telomere degradation by TRF2 (refs. 102,105), or through other mechanisms yet to be fully understood. In addition, the role of RAP1 in telomere maintenance and senescence is inconsistent across studies: RAP1 deficiency extends telomeres and delays senescence in human stem cells[106], but does not affect telomere length and accelerates senescence in fibroblasts[93]. These discrepancies may originate from biological differences, experimental conditions and research methods.

Apart from replicative senescence and senescence caused by mutations in canonical telomere regulators, telomere attrition occurs in other cellular senescence contexts. For example, in human fibroblasts, oxidative stress causes accelerated telomere shortening, leading to compromised proliferation and premature senescence; by contrast, reducing ROS levels reverses telomere shortening and enhances their proliferation[107,108]. Beyond impairing proliferation through DDR activation, critically short telomeres can also derepress retrotransposons and activate innate immunity responses including

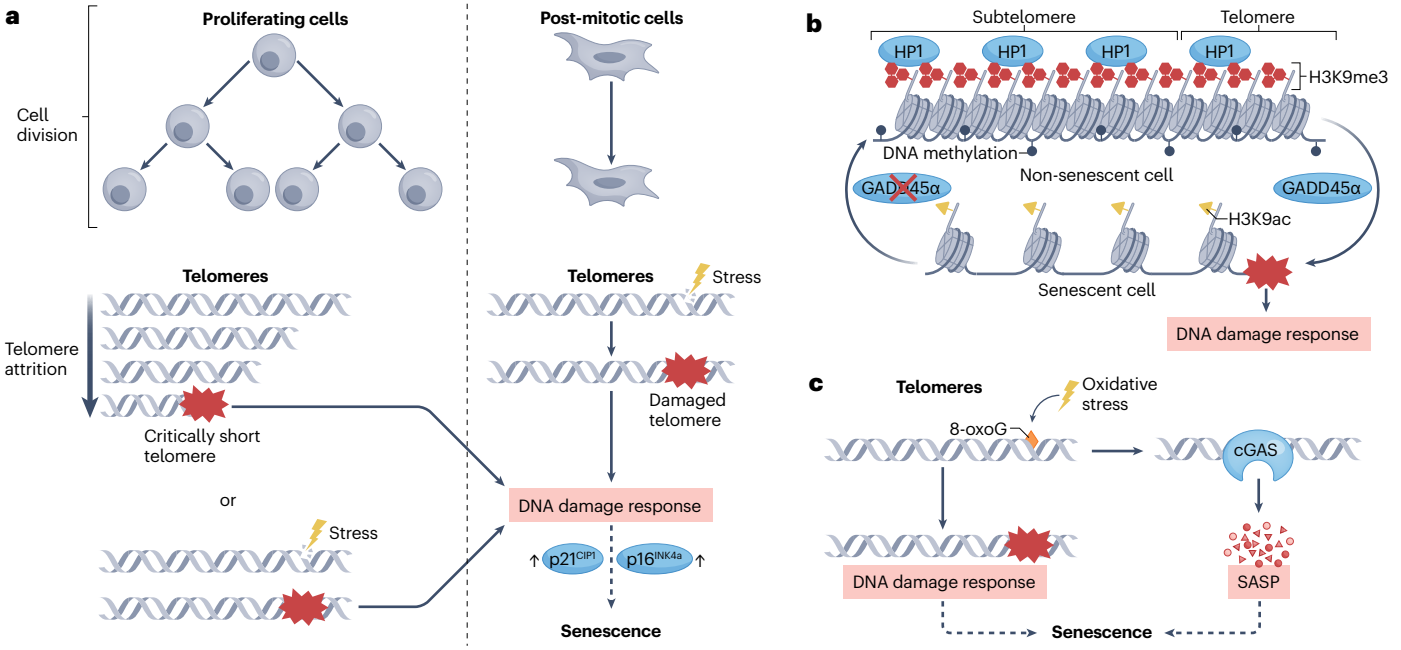

Fig. 2 | Telomere dysfunction in cellular senescence. a, Telomere dysfunction induces senescence in proliferating and post-mitotic cells through distinct mechanisms. In proliferating cells, telomeres gradually shorten with each cell division, but can also suffer damage from various internal or external stresses. In post-mitotic cells, telomere dysfunction arises in response to stress-induced damage, regardless of alterations in telomere length. Consequently, critically shortened or damaged telomeres prompt activation of the DNA damage response (DDR), resulting in the upregulation of $p21^{CIP1}$ and $p16^{INK4a}$, ultimately leading to cellular senescence. **b**, Growth arrest and DNA damage-inducible protein 45α (GADD45α)-induced heterochromatin loss at subtelomeric and telomeric regions contributes to senescence by driving telomere attrition and DNA damage. Heterochromatin loss is indicated by enhanced DNA demethylation, reduced levels of trimethylated histone H3 Lys9 (H3K9me3) and heterochromatin protein 1 (HP1), and increased deposition of acetylated H3K9 (H3K9ac). These changes lead to telomere shortening and persistent DNA damage, thereby promoting entry into a senescent state. GADD45α depletion restores telomeric/subtelomeric heterochromatin and delays the onset of cellular senescence. '×' represents the shutdown of gene expression. **c**, Length-independent telomere dysregulation can accelerate cellular senescence. Under oxidative stress, telomeric DNA can accumulate the oxidized base 8-oxo-guanine (8-oxoG), which triggers a persistent DDR and increases the levels of cytoplasmic DNA. Cytoplasmic DNA is recognized by cyclic GMP–AMP synthase (cGAS), further activating type I interferon genes and the senescence-associated secretory phenotype (SASP), and ultimately triggering cellular senescence.

cGAS–STING signalling[109,110], probably further exacerbating senescence in an inflammation-dependent manner. Moreover, in prematurely senescent human MSCs lacking WRN, a RecQ-like helicase mutated in Werner syndrome, telomeres are shortened but can be rescued by gallic acid, a natural antioxidant proved to alleviate cellular senescence[31,111]. The mechanism by which gallic acid rescues the telomeres is not yet fully elucidated, but it is thought to involve its ability to reduce oxidative stress, DNA damage and inflammation, and to increase heterochromatin stability[111]. Similar telomere attrition occurs in stem cells deficient in FTO or ZKSCAN3 (refs. 57,60), suggesting that telomere attrition is a common feature of cellular senescence and that therefore targeting telomere shortening could potentially be a strategy to prevent senescence, although this approach poses risks of malignant transformation, particularly through activation of telomerase[112]. Excitingly, a recent study has demonstrated that T lymphocytes can acquire elongated telomeres through fusion with telomeres carried in vesicles derived from antigen-presenting cells, thereby postponing senescence without the need for telomerase activity[113] and unveiling a novel mechanism for delaying senescence.

It is important to note that heterochromatinization is indispensable for telomere stability, involving DNA methylation, the histone modifications H3K9me3 and H4K20me3, and low acetylation levels of histones H3 and H4 at telomeric and subtelomeric regions[114]. Conversely, disruption of this silencing mechanism contributes to senescence-associated telomere dysregulation. For example, in telomerase-null cells with short telomeres, growth arrest and DNA damage-inducible protein 45α (GADD45α) drive subtelomeric DNA demethylation and loss of gene silencing, which are accompanied by reduced levels of H3K9me3 and HP1 and increased levels of H3K9ac (Fig. 2b). GADD45α depletion restores telomeric/subtelomeric heterochromatin marks in these cells and delays onset of cellular senescence in normal human fibroblasts[115]. Acetate also disrupts telomere silencing by boosting H4K16ac levels, causing shorter telomeres and faster senescence in yeast cells and in human vein endothelial cells[116]. Remarkably, heterochromatin not only prevents telomere shortening but also impedes lengthening by involving H3K9me3 and the H3K9me3 facilitator KAP1: overexpression of DCAF11 in mouse stem cells results in KAP1 degradation and H3K9me3 loss, thereby initiating telomere extension, enhancing proliferation and countering senescence[62]. Similarly, a recent study has presented a compound called TERT activator compound (TAC), which activates *TERT* transcription by replacing the gene-repressive H3K9me3 with the gene-activating H3K27ac[20]. The resulting TERT-driven telomere elongation contributes to the rejuvenation of prematurely senescent Werner syndrome fibroblasts,

as manifested by a reduction in telomere-associated DNA damage foci and an increase in proliferative capacity.

Despite the conventional link between telomere attrition and cellular senescence, telomere dysfunction can occur independently of its length and contribute to senescence. For example, oncogene activation can induce extensive replication fork stalling, thereby inducing a robust DDR, which in turn results in growth arrest and senescence in normal human fibroblasts, without involving telomere attrition[117–119]. Similar outcomes are observed in cell senescence triggered by genotoxic stress. In this process, acute stress induced by X-ray radiation or genotoxic agents causes the formation of persistent DNA damage foci associated with telomeres and thus triggers premature senescence in human fibroblasts[120]. This process suggests that telomeric DNA damage, occurring independently of or before reaching the division limit, is sufficient to initiate cellular senescence (Fig. 2a). Relative to the rest of the genome, telomeric regions accumulate damage over time, potentially owing to their encapsulation by shelterin, which prevents DNA repair[121,122]. For instance, accumulation of the oxidized base 8-oxo-guanine (8-oxoG) at telomeres triggers persistent DDR and elevates cytoplasmic DNA levels, resulting in cGAS–STING pathway activation, upregulation of SASP factors and rapid onset of senescence in human fibroblasts and epithelial cells with no telomere shortening[123] (Fig. 2c). Furthermore, in senescent post-mitotic cells such as neurons and cardiomyocytes, persistent telomeric DNA damage, irrespective of length, is frequently observed[122,124] (Fig. 2a). These findings support the notion that telomere-associated pathways that regulate cell senescence are not solely affected by telomere attrition.

DNA damage

Over time, accumulating DNA damage such as base modifications, DNA single-strand breaks (SSBs) or DNA double-strand breaks (DSBs) erode genome stability. To detect and counteract such damage, cells use a cascade of sensing and repair mechanisms that involve a range of well-known DDR proteins. Among these, the kinases ataxia–telangiectasia mutated (ATM) and ataxia–telangiectasia and RAD3-related (ATR) are master DDR coordinators activated upon detection of DSBs or SSBs. Upon their activation, ATM and ATR phosphorylate multiple substrates, including the tumour suppressor protein p53, which is a transcription factor that orchestrates the expression of genes involved in cell cycle arrest, DNA repair or apoptosis, chief among them *CDKN1A*, which encodes $p21^{CIP1}$ (ref. 125). Major DNA repair pathways include nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR), non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ)[126] (Fig. 3a). A spectrum of critical players is implicated in these pathways. For example, the recombinase RAD51 is a central component of HR, which is crucial for DSB repair during the S and G2 cell cycle phases[127]. DNA-dependent protein kinase (DNA-PK) is a key factor in the NHEJ pathway, which is active throughout the cell cycle and is required for DSB repair in post-mitotic cells[128,129]. Beyond these established processes, novel DNA repair mechanisms have been recently identified, such as NPAS4–NuA4-coupled neuronal DSB repair[130].

Whether caused by endogenous or exogenous factors, unrepaired DNA damage causes genome instability and initiates cell senescence (Fig. 3a). For instance, excessive ROS levels due to inflammation or unbalanced metabolism trigger the formation of 8-oxoG and accelerate senescence in human fibroblasts[123]. In adipose-derived stem cells from aged individuals, the decline of X-ray repair cross-complementing protein 1 (XRCC1) levels impairs BER, resulting in DNA damage accumulation and senescence-related phenotypes[131]. Senescence-induced TRF2 loss at pericentromeres triggers DNA breaks and ATM activation, which in turn dissolves the pericentromeric heterochromatin by releasing KAP1 and lamin B1. As a result, pericentromeric satellite DNA is released into the cytoplasm and activates the cGAS–STING pathway, which functions as a pro-inflammatory trigger of senescence[35] (Fig. 3b). Intriguingly, in addition to sensing cytoplasmic DNA, cGAS enters the nucleus and suppresses poly(ADP-ribose) polymerase 1-assisted HR, thereby aggravating genome instability[132]. Furthermore, specific nucleic acid structures in the nucleus, such as the G-quadruplex — a G-rich four-stranded DNA structure — and the R-loop — comprising a DNA–RNA hybrid and single-stranded DNA — are vulnerable to damage and synergistically increase the risk of genome instability and the onset of senescence[133,134].

Senescence-associated DNA damage typically occurs in DNA repair mutant cells. For example, *Rad51* deletion in mouse hepatocytes triggers a persistent DDR, as evidenced by the increased levels and distribution into foci of phosphorylated histone H2AX (γH2AX; an H2A variant) and p53-binding protein 1 (53BP1), two typical DSB markers[135]. This DDR aligns with a compromised proliferation capacity, $p21^{CIP1}$ upregulation, SASP activation and a premature senescence phenotype. Defects in the *FANCA* gene, which also functions in DNA repair, are associated with rapid senescence in human MSCs. This senescence phenotype can be reversed through targeted gene correction[136]. Human MSCs containing mutations in *ERCC6* (also known as *CSB*) also display aberrant accumulation of γH2AX foci, indicating DDR activation. Concurrently, these cells display premature onset of senescence at a relatively early passage, as manifested by impaired proliferation, enhanced SA-β-gal activity, reduced lamin B1 levels and upregulation of $p16^{INK4a}$ and the SASP factor IL-6 (ref. 137). ERCC6 also acts as a heterochromatin stabilizer in combating senescence, as its deficiency results in H3K9me3 loss and susceptibility to stress-induced premature senescence in human fibroblasts[45]. Hence, ERCC6 might regulate cellular senescence by orchestrating both heterochromatin stabilization and DNA repair. Similarly, the heterochromatin stabilizer SIRT6 also performs diverse functions in DNA repair through sensing DNA damage, deacetylating histones and coordinating the activity of other repair proteins[138–141]. Defects in *SIRT6* induce human vascular smooth muscle cell senescence through telomeric H3 hyperacetylation and 53BP1 binding[142], indicating loss of gene silencing and DDR activation. This process is also accompanied by increased SASP. Conversely, SIRT6 overexpression or activation restores genome integrity and mitigates cellular senescence[142,143], representing a promising therapeutic target in senescence-associated conditions. These findings underscore the intersection between heterochromatin loss, DNA damage and genome instability.

Senescence-associated DNA damage can also arise from exogenous factors such as chemicals, irradiation and viral infections. For example, treatment with hydroxyurea, doxorubicin or etoposide drives DNA damage-induced senescence in human cancer cells and mouse fibroblasts, manifested by increased γH2AX, SA-β-gal and $p16^{INK4a}$ levels[144]. These senescent cells also exhibit accumulation of cGAS in cytoplasmic DNA foci and elevated SASP, signalling the activation of innate immunity and inflammation (Fig. 3a). Deletion of *CGAS*, *STING1* or *TBK1* lessens senescence following DNA damage[144]. In DDR triggered by etoposide, p53-mediated transcriptional activation of $p21^{CIP1}$ is traditionally viewed as a protective mechanism for promotion of cell senescence over apoptosis. Upregulation of $p21^{CIP1}$ can also be sparked post-transcriptionally by RNA-binding motif protein 42 (RBM42), independently of p53 (ref. 145), providing novel insights into the mechanism that underlies senescence-associated DNA damage. UV irradiation

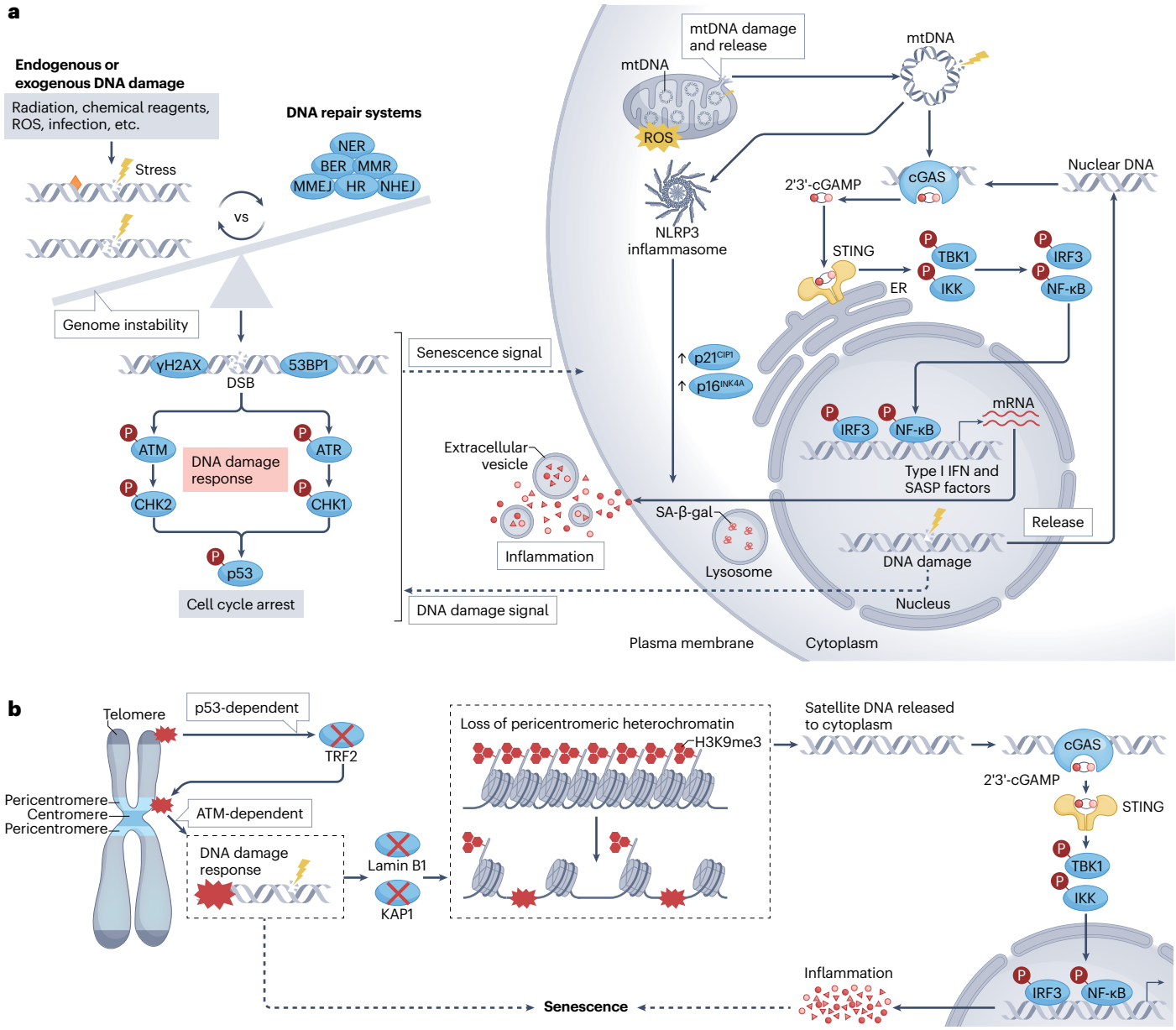

Fig. 3 | DNA damage signalling in cellular senescence. a, Elevated endogenous or exogenous stress-induced damage and/or inadequate DNA repair contribute to persistent genome instability and activation of the DNA damage response (DDR). During DDR activation, DNA double-strand breaks (DSBs) trigger a cascade of events that include the formation of phosphorylated histone H2AX (γH2AX) foci and the recruitment of p53-binding protein 1 (53BP1). Recruitment of γH2AX and 53BP1 to DSB sites leads to activation of the kinases ataxia–telangiectasia mutated (ATM) and ataxia–telangiectasia and RAD3-related (ATR); ATM and ATR phosphorylate and activate checkpoint kinase 2 (CHK2) and CHK1, respectively. These kinases activate effectors that include the tumour suppressor protein p53. The activation of p53 initiates a series of responses aimed at maintaining genome integrity, such as cell cycle arrest and the induction of cellular senescence features, which include upregulation of the cyclin-dependent kinase inhibitors $p21^{CIP1}$ and $p16^{INK4a}$ and activation of senescence-associated β-galactosidase (SA-β-gal). Concurrently, genome instability promotes release to the cytoplasm of nuclear DNA and mitochondrial DNA (mtDNA), which in turn activates the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway and initiates the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome-mediated response, resulting in increased levels of inflammation. **b**, Telomere shortening or genotoxic stress downregulate the expression of telomeric repeat binding factor 2 (TRF2) in a p53-dependent manner. The resulting loss of TRF2 in pericentromeres triggers an ATM-mediated DDR, which in turn leads to heterochromatin instability by reducing lamin B1 and KRAB-associated protein 1 (KAP1) levels and to the release of satellite DNA into the cytoplasm. Consequently, the cGAS–STING pathway is activated, followed by inflammation and cellular senescence. BER, base excision repair; 2′3′-cGAMP, 2′,3′-cyclic GMP–AMP; ER, endoplasmic reticulum; H3K9me3, trimethylated histone H3 Lys9; HR, homologous recombination; IKK, IκB kinase; IRF3, interferon regulatory factor 3; MMEJ, microhomology-mediated end joining; MMR, mismatch repair; NER, nucleotide excision repair; NF-κB, nuclear factor κB; NHEJ, non-homologous end joining; P, phosphorylation; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; TBK1, TANK-binding kinase 1.

also links DNA damage to senescence, by inducing the formation of G-quadruplexes and increasing DNA damage. G-quadruplex formation is suppressed by ZRF1 (also known as DNAJC2), and loss of ZRF1 leads to upregulation of the UV-damage repair factor DNA damage-binding protein 2 (DDB2), which drives cell senescence[134]. Additionally, SARS-CoV-2 infection elicits DNA damage by degrading the ATR effector checkpoint kinase 1 (CHK1), causing a shortage of dNTPs, hindering the cell cycle, activating inflammation and accelerating senescence[146].

In comparison with nuclear DNA, mitochondrial DNA (mtDNA) is more prone to damage and remains damaged for longer periods[147]. This vulnerability is likely caused by proximity to the primary source of ROS and inherent limitations of mtDNA repair mechanisms. Hence, mitochondrial ROS damage mtDNA, resulting in oxidized DNA base lesions and increased levels of cytosolic DNA fragments, which in turn activate the NLRP3 inflammasome and cGAS–STING and initiate senescence-related inflammatory responses[148,149] (Fig. 3a). Likewise, replication stress caused by depletion of mitochondrial transcription factor A (TFAM) increases mtDNA damage and activates cGAS–STING and a pro-inflammatory response[150]. Endolysosomes potentially aid in the disposal of cytoplasmic mtDNA, as well as promote mtDNA sensing by cGAS–STING[150]. Nuclease-induced mtDNA breaks can also trigger structural and functional defects in mitochondria, leading to metabolic dysregulation and inflammation[151–154]. Additionally, apoptotic stress enables damaged mtDNA to escape from the mitochondria, thus driving innate immunity and the SASP[155]. Nonetheless, understanding mtDNA damage regulation and its impact on cellular senescence requires further exploration.

Relevance of cellular senescence to ageing and ageing-related diseases

As mentioned above, the SASP amplifies the effects of cellular senescence to neighbouring cells and thus promotes dysfunctional tissue regeneration, chronic age-related disorders and ageing. In this section, we discuss the relevance of cellular senescence mediated by heterochromatin loss, telomere attrition and DNA damage to ageing and ageing-related diseases (Table 1, Fig. 4a and Supplementary Table 1).

Heterochromatin loss

The loss of heterochromatin, particularly constitutive heterochromatin, and the resulting retrotransposon derepression have been widely implicated in ageing-related processes. For example, in *D. melanogaster*, levels of constitutive heterochromatin decrease with age (from 3 days to 35 days) and this decline is associated with SA-β-gal upregulation[156]. In the liver of ageing cynomolgus monkeys (18–21 years old versus 4–6 years old), heterochromatin loss is correlated with increased levels of various senescence markers, including SA-β-gal, $p21^{CIP1}$ and SASP factors, which can amplify senescence signals and ultimately lead to tissue ageing[157]. Similar observations were also made in aged skeletal muscles and hearts of cynomolgus monkeys[158,159]. Age-related heterochromatin decondensation is often accompanied by the activation of LINE-1 or ERVs, which can prompt inflammation and senescence, thereby contributing to the ageing process in various tissues such as the brain, skin, synovium and articular cartilage of mice, non-human primates and/or humans[18,47,53,66,67,160–163]. Additionally, disrupted heterochromatin with insufficient H3K9me2 or H3K9me3 decoration, along with senescence-related traits in oocytes and haematopoietic stem cells, is characterized in reproductive and haematopoietic ageing[164,165]. By contrast, stable heterochromatin is typically linked to longevity. For example, the naked mole rat, which is a rodent celebrated for its exceptional longevity and resistance to cancer, displays higher levels of H3K27me3 and decreased chromatin accessibility[166]. These characteristics indicate a more stable heterochromatin, which may partially explain the species' increased resistance to cell senescence and ageing[166,167]. In humans, stabilization of heterochromatin is associated with healthy ageing in long-lived individuals[168]. This improved healthspan involves the regulation of DNA methylation and the repression of abnormal gene expression, potentially offsetting gene expression patterns related to senescence and the ageing process[168,169].

Heterochromatin disorganization is also linked to age-related diseases such as progeria, neurodegenerative disorders and cancer (Table 1 and Supplementary Table 1). For instance, in cells modelling progeroid disorders such as HGPS, Werner syndrome and Cockayne syndrome as mentioned earlier, a collection of senescence phenotypes has been documented, alongside the abnormal nuclear architecture and decreased constitutive heterochromatin marks[31,43,45], providing support for understanding the pathogenesis of these premature ageing diseases. Reduced H3K9me3 and HP1 is associated with LINE-1 activation and β-amyloid deposition in physiologically aged brains of non-human primates[161], and loss of heterochromatin is widespread in human tau transgenic *Drosophila* and mouse models, as well as in brains from individuals with Alzheimer disease[170]. In these contexts, heterochromatin disruption is responsible for neuronal senescence, which may further initiate or escalate the development of brain ageing and neurodegenerative disorders. In cancer, destabilized heterochromatin contributes to replication stress, genome instability and gene expression leakage, thus exerting a pro-cancer effect[171,172].

Telomere attrition

Telomere attrition considerably influences the ageing process across tissues and organisms (Fig. 4). In rats, telomere length in the lung, liver, pancreas and kidney decreases with age (from 21 days to 15 months)[173]. Similarly, telomere shortening occurs during human ageing in the liver, kidney and leukocytes[174], and leukocyte telomere length is even used as a clock to predict human biological age. Telomere length varies between different human tissues, with telomeres in most tissue shortening between the ages of 20 and 70 years[175,176]. It is reasonable to speculate that telomere attrition triggers cellular senescence through activation of the DDR, which then drives tissue and even organismal ageing, yet direct evidence under physiological conditions for this hypothesis is still lacking. In mice, telomere shortening is less pronounced owing to their longer telomeres and widespread telomerase expression, despite their shorter lifespans compared with humans. Similarly, in various species, the length of telomeres alone does not necessarily reflect the lifespan of an organism[91]. Instead, the rate at which telomeres shorten over time may serve as a more accurate parameter to predict lifespan, as it accounts for the dynamic nature of telomere length and the balance between telomerase activity and DNA-end replication repair mechanisms[177–179]. Noteworthy is that telomerase inactivation through deletion of *TERT* or *TERC* results in shortened telomeres and premature ageing in mice and zebrafish[80,180,181]. Furthermore, there is evidence that, in these animals, telomere shortening can prompt ageing, at least partially by activating the p53 and $p21^{CIP1}$ cell-senescence pathways[181–184]. Additionally, senescence-associated telomere damage without shortening escalates with age in multiple tissues and species, from mice to non-human primates and humans[120,185–187]. For example, senescent melanocytes positive for $p16^{INK4a}$ staining accumulate in human skin with age; the melanocytes increase telomere-associated DNA damage foci and induce senescence in surrounding cells in a paracrine manner, thereby driving skin ageing[186].

Table 1 | Examples of diseases associated with heterochromatin disorganization, telomere dysfunction and DNA repair defects

Molecular process	Disease	Defective gene	Clinical features	Refs.
Heterochromatin disorganization	Werner syndrome	*WRN*	Premature ageing, premature greying and loss of hair, cataracts, diabetes mellitus, skin changes, increased risk of cancer	31
	HGPS	*LMNA*	Rapid ageing in children, short stature, alopecia, joint abnormalities, cardiovascular problems, atherosclerosis	43
	Cockayne syndrome	*ERCC6*, *ERCC8*	Premature ageing, growth failure, neurological abnormalities, hearing loss, photosensitivity	45
	Alzheimer disease	*APP*, *APOE*, others	Progressive cognitive decline, memory loss, disorientation, behavioural changes	170
	Huntington disease	*HTT*	Involuntary movements, cognitive impairment, psychiatric symptoms, progressive neurodegeneration	292
	Rett syndrome	*MECP2*	Developmental regression, loss of purposeful hand skills, repetitive hand movements, loss of communication abilities, breathing problems, seizures, intellectual disability	293
	Facioscapulohumeral muscular dystrophy	Genes related to D4Z4 repeats, such as *DUX4*, *FRG1* and *FRG2*	Progressive muscle weakness beginning in the face and shoulders, difficulty swallowing, foot drop, asymmetrical muscle wasting	294
	Friedreich ataxia	*FXN*	Progressive ataxia (loss of balance and coordination), muscle weakness, heart problems, scoliosis, diabetes	295,296
	Prader–Willi syndrome	Deletion or inactivation of genes on chromosome 15 (paternally inherited)	Hyperphagia leading to obesity, hypogonadism, cognitive impairment, behavioural issues, short stature	295,297
	Angelman syndrome	*UBE3A*	Intellectual disability, developmental delay, speech impairments, ataxia, frequent laughter/smiling, seizures	295,298
	Fragile X syndrome	*FMR1*	Intellectual disability, developmental delays, social difficulties, hyperactivity, distinctive physical features	299
	Breast cancer	*BRCA1*, *BRCA2*, *TP53*, others	Tumours or lumps in breast tissues	171
Telomere dysfunction	Dyskeratosis congenita	*DKC1*, *TERC*, *TERT*, others	Skin pigmentation abnormalities, oral leukoplakia, nail dystrophy, bone marrow failure, pulmonary fibrosis, increased cancer risk	188
	Hoyeraal–Hreidarsson syndrome	*DKC1*, *TERC*, *TERT*, others	Growth retardation, microcephaly, intellectual disability, bone marrow failure, immunodeficiency, skin pigmentation abnormalities, nail dystrophy	300
	Werner syndrome	*WRN*	See above	31
	HGPS	*LMNA*	See above	301
	Alzheimer disease	*APP*, *APOE*, others	See above	189
	Parkinson disease	*LRRK2*, *PARK2*, *DJ-1*, others	Tremors, bradykinesia, rigidity, postural instability, cognitive impairment	302
	Pulmonary fibrosis	*TERT*, *TERC*, others (for idiopathic pulmonary fibrosis)	Progressive scarring of lung tissue, shortness of breath, chronic cough, fatigue	190
	COVID-19	NA	Fever, cough, shortness of breath, loss of taste or smell	197
	Duchenne muscular dystrophy	*DMD*	Progressive muscle weakness, gait abnormality, cardiomyopathy, respiratory insufficiency	97
	Osteoarthritis	NA	Joint pain, stiffness, swelling, reduced range of motion	303
	Type 2 diabetes	NA	Increased thirst and urination, fatigue, unexplained weight loss or gain, hyperglycaemia	304
	Squamous cancers	*TP53*, *NOTCH1*, others	Squamous cell carcinoma in various organs such as skin, head, neck, lungs and cervix	192

Table 1 (continued) | Examples of diseases associated with heterochromatin disorganization, telomere dysfunction and DNA repair defects

Molecular process	Disease	Defective gene	Clinical features	Refs.
DNA repair defects	Werner syndrome	*WRN*	See above	31
	HGPS	*LMNA*	See above	43
	Cockayne syndrome	*ERCC6*, *ERCC8*	See above	137
	Ataxia telangiectasia	*ATM*	Progressive cerebellar ataxia, telangiectasia (dilated blood vessels), immunodeficiency, increased cancer risk	305
	Xeroderma pigmentosum	*XPA*, *XPB*, *XPC*, others	Extreme sensitivity to sunlight, freckling and pigmentation changes in skin, increased risk of skin cancers	306
	Bloom syndrome	*BLM*	Growth deficiency, photosensitivity, increased cancer risk, immunodeficiency	307
	Alzheimer disease	*APP*, *APOE*, others	See above	211,308
	Parkinson disease	*LRRK2*, *PARK2*, *DJ-1*, others	See above	309
	Cardiac atrophy	NA	Reduction in mass and function of heart muscle, symptoms similar to heart failure	213
	Liver fibrosis	NA	Scarring and hardening of liver tissue, impaired liver function	144,310
	Infertility	NA	Symptoms vary based on the specific condition and the individual's biological sex	214
	Various cancers	Multiple genes associated with DNA repair and tumour suppression	Symptoms depend on the type and stage of cancer	311

APOE, apolipoprotein E; APP, amyloid precursor protein; ATM, ataxia–telangiectasia mutated; BLM, Bloom syndrome helicase; BRCA1, breast cancer susceptibility gene 1; DJ-1, protein deglycase DJ-1 (also known as Parkinson disease protein 7); DKC1, dyskerin pseudouridine synthase 1; DMD, dystrophin; DUX4, double homeobox 4; ERCC6, excision repair cross-complementation group 6; FXN, frataxin; HGPS, Hutchinson–Gilford progeria syndrome; HTT, huntingtin; LMNA, lamin A/C; LRRK2, leucine-rich repeat kinase 2; MECP2, methyl-CpG-binding protein 2; NOTCH1, Notch receptor 1; PARK2, Parkinson disease protein 2 (also known as parkin); TERC, telomerase RNA component; TERT, telomerase reverse transcriptase; TP53, tumour suppressor protein p53; UBE3A, ubiquitin protein ligase E3A; WRN, Werner syndrome RecQ helicase; XPA, xeroderma pigmentosum group A.

In addition to causing diverse telomere biology disorders such as dyskeratosis congenita[188], telomere dysregulation is also a common feature in various age-related diseases such as Alzheimer disease[189], pulmonary fibrosis[190], Duchenne muscular dystrophy[97], metabolic diseases[191], osteoarthritis[91] and cancers[192] (Table 1 and Supplementary Table 1). For example, short telomere length elevates the risk of Alzheimer disease[189], whereas reactivation of *TERT* expression can mitigate neurodegeneration associated with Alzheimer disease in both human neurons and mouse models[193]. Telomere attrition may contribute to neurodegeneration in part by inducing senescence in Alzheimer disease neurons through the activation of cell cycle inhibitors and inflammation genes, which can be reversed by TERT reactivation[193]. In pulmonary fibrosis, the E3 ligase FBW7 mediates pulmonary epithelial stem cell senescence and fibrosis in mice by causing telomere uncapping and shortening through TPP1 degradation[190]. This finding provides potential targets for the treatment of age-associated lung diseases. Interestingly, SARS-CoV-2 infection is associated with cellular senescence and accelerated ageing[194–196], and in individuals with coronavirus disease 2019 (COVID-19), shorter telomeres are associated with increased severity of disease[197,198].

DNA damage

DNA damage also has a pivotal role in the ageing process, affecting the health of tissues, organs and organisms (Fig. 4). For instance, in aged mice (42 months old), the levels of DNA damage γH2AX foci in various tissues including lung, liver, spleen, dermis and gut epithelium, are higher compared with young mice (12 months old)[199]. A correlation between γH2AX-positive cells and SA-β-gal-positive cells is observed in tissues of aged mice (36 months old), suggesting that DDR-driven senescence might be accountable for ageing[199]. Interestingly, persistent DNA damage and cellular senescence are also detected in the placenta of mice and humans, suggesting that these processes have a role in tissue remodelling related to embryonic development[34,200]. Inducing DNA breakage through controlled expression of restriction nucleases accelerates cellular senescence and tissue ageing in mice[19,201,202]. These non-mutagenic breaks instigate cellular senescence and ageing by elevating the DNA methylation age, reducing lamin B1 levels and boosting the expression of LINE-1 and SASP factors. However, these phenotypes were reversed by resetting the epigenetic state to a younger configuration, suggesting that senescence or ageing is caused by the loss of epigenetic information rather than by DNA damage or mutation in and of themselves[19]. This model also supports the hypothesis that the relocalization of chromatin modifiers is involved in DNA damage and repair[52,203]. The relocalization hypothesis, which is an early form of the 'information theory of ageing', posits that, in response to DNA damage, chromatin-associated factors move away from their genomic positions towards the sites of DNA lesions. This translocation may sometimes be unsuccessful in reversing the DNA damage, leading to an ongoing deterioration of the youthful epigenetic landscape and a gradual erosion of the youthful expression profiles of genes, particularly those at hot-spot regions such as developmental genes and transposons, which ultimately contribute to the onset of senescence and ageing[203]. Furthermore, impaired DNA repair resulting from loss of the endonuclease ERCC1 or of the transcription factor

NPAS4 accelerates ageing in multiple tissues and reduces lifespan in mice[130,204]. Mice with ERCC1 deficiency aged 8–10 months exhibit an increased DDR with a premature senescence phenotype in immune cells, thereby resembling 24-month-old wild-type mice[204]. Persistence of *Npas4*-depletion-induced DDR may trigger premature ageing in mice by accelerating neuronal senescence[130]. Although tissue-specific DNA mutations increase with age in both humans and mice, indicative of inadequate DNA repair[205,206], mechanisms that ensure efficient DNA repair contribute to longevity in humans and rodents[138,207]. For example, an elevated burden of mtDNA mutations correlates with the elevated expression of the senescence marker p21^{CIP1} and a decrease in telomere length during intestinal ageing in mice[208]. In rodent species with diverse lifespans, SIRT6 contributes to efficient DSB repair in long-lived species, such as the beaver[138], potentially through combating cellular senescence, making SIRT6 a promising target for prolonging healthspan of short-lived mice[209]. The naked mole rat also exhibits a higher DNA repair efficiency than mice, a trait that, along with its stable epigenome, could underpin its remarkable resistance to stress-induced senescence and ageing[167,210].

Age-related diseases are also associated with accumulation of DNA damage and/or DNA repair defects (Table 1 and Supplementary Table 1). For example, mouse models of Alzheimer disease display elevated oxidative DNA damage and age-related brain function impairments[211], similar to observations in individuals with Alzheimer disease[212].

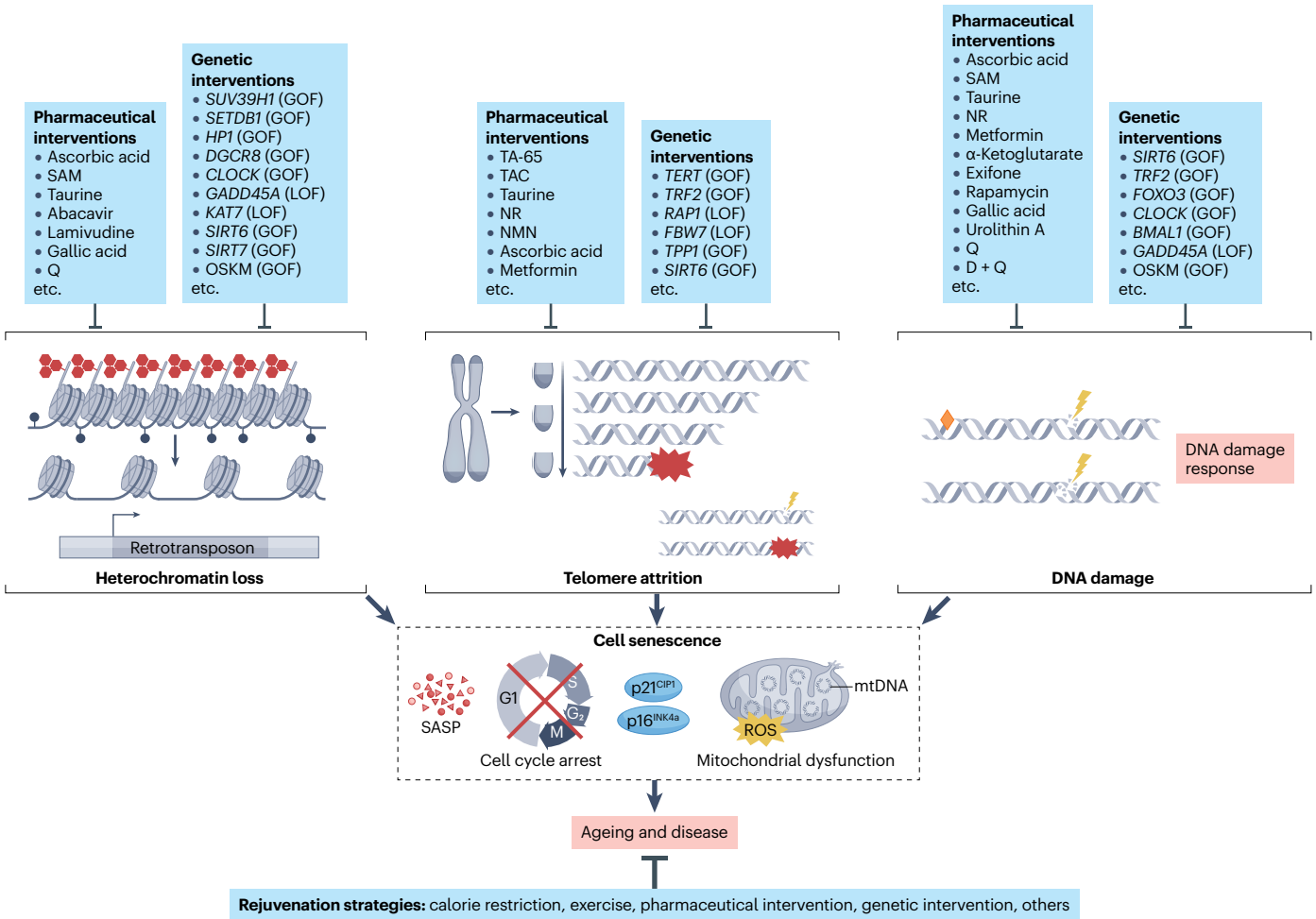

Fig. 4 | The relevance of cellular senescence pathways in ageing, related diseases and associated therapeutic interventions. Molecular pathways linked to heterochromatin loss, telomere attrition and DNA damage responses, have a significant role in the progression of cellular senescence, in tissue and organismal ageing, and in the development of various age-related diseases. As our understanding of cellular senescence has deepened, various therapeutic strategies associated with cell and tissue rejuvenation have been identified. These therapeutics include calorie restriction, exercise, pharmacological treatments, genetic interventions and other therapeutic avenues. We depict representative small molecules and target genes for specific pharmacological or genetic interventions. BMAL1, brain and muscle ARNT-like protein 1; CLOCK, circadian locomotor output cycles kaput; DGCR8, DiGeorge syndrome critical region gene 8; D, dasatinib; FBW7, F-box/WD repeat-containing protein 7; FOXO3, forkhead box protein O3; GADD45A, growth arrest and DNA damage-inducible 45 alpha; GOF, gain of function; HP1, heterochromatin protein 1; KAT7, lysine acetyltransferase 7; LOF, loss of function; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; OSKM, the Yamanaka factors including OCT4, SOX2, KLF4 and MYC; Q, quercetin; RAP1, repressor/activator protein 1; SAM, *S*-adenosylmethionine; SASP, senescence-associated secretory phenotype; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; SIRT6, sirtuin 6; SUV39H1, suppressor of variegation 3-9 homologue 1; TA-65, telomerase activator 65; TAC, TERT activator compound; TERT, telomerase reverse transcriptase; TPP1, POT1 and TIN2-interacting protein; TRF2, telomeric repeat binding factor 2.

Hence, the pathogenesis of Alzheimer disease could be linked to sustained DDR-induced neuronal senescence. In progeroid mice, dysfunctional NHEJ and the consequent increase in DSBs contribute to cardiac atrophy, a process that may involve cardiomyocyte senescence owing to persistent DDR activation[213]. DNA damage also causes activation of cGAS–STING signalling, senescence and liver fibrosis in mice[144]. In humans, ageing-related phenotypes such as increased levels of γH2AX foci and heightened inflammation, have been observed in the somatic testicular niche of men with infertility, indicating senescence of the testicular somatic cells[214]. Similarly, elevated DNA damage and diminished DNA repair, along with activation of cellular senescence pathways in various ovarian cell types, can be detected in the ovaries of older women who underwent oophorectomy[215]. DNA damage can upregulate the expression of the SARS-CoV-2 receptor ACE2 in senescent cells and during ageing[216], potentially increasing COVID-19 severity in the elderly[217,218]. Additionally, mtDNA damage is associated with ageing and related disorders[219–221], possibly depending on cGAS–STING inflammation-triggered senescence, highlighting that maintaining both nuclear and mitochondrial genome stability is important for healthy ageing.

Therapeutic interventions relevant to cellular senescence

Various therapeutic approaches or interventions have been considered to slow down ageing, including healthy lifestyles, pharmaceuticals, genetic interventions and more (Fig. 4 and Supplementary Table 2). Some of these potential therapies have completed or are presently being investigated in clinical trials. In this section, we provide a brief overview of therapeutic interventions designed to mitigate cellular senescence and ageing-related conditions, focusing on how they can influence heterochromatin, telomeres and DNA repair.

Therapeutics related to restoring heterochromatin stability

Lifestyle medicine is a rapidly growing field that aims to counter chronic diseases and achieve healthy ageing by changing behaviours or life habits. Adopting healthy lifestyles, such as specific dietary regimes and exercise, is a convenient and effective approach to countering ageing-related conditions[222–224] that involves preservation of heterochromatin stability (Fig. 4 and Supplementary Table 2). For example, calorie restriction, which is a dietary regimen that involves reduction of food intake without malnutrition, alleviates age-related heterochromatin loss in flies, and exercise increases H3K9me3 heterochromatin in the rat hippocampus, which otherwise declines with ageing[225,226]. A panel of pharmacological compounds also exhibit geroprotective effects, partly by restoring heterochromatin structure (Fig. 4 and Supplementary Table 2), thereby providing potential therapeutic insights into the prevention of ageing and related diseases. Ascorbic acid (vitamin C), for example, can elevate H3K9me3 levels and restore the nuclear lamina, thus attenuating senescence in human MSCs and motor neurons and rejuvenating the aged primate spinal cord[227,228] (Fig. 5a). Similarly, in aged mice, administration of *S*-adenosylmethionine (SAM) restored heterochromatin to a youthful level in muscle stem cells and improved muscle regeneration[229]. Recently, the amino acid taurine was reported to have rejuvenation effects by alleviating senescence in multiple species including rodents and primates, and it also facilitated heterochromatin restoration and physiological function in muscles of aged mice[230]. Uridine, a pyrimidine nucleoside, was shown to restore heterochromatin and enhance DNA repair in senescent human MSCs, and thereby to promote their rejuvenation; in mouse models, uridine has been conceptually demonstrated to have a role in improving multi-tissue regeneration and treating age-related disorders such as osteoarthritis and liver fibrosis[231]. As consequences of heterochromatin loss, retrotransposon derepression and inflammation can be targeted for rejuvenation at both cellular and tissue levels using pharmacological compounds, such as abacavir (for ERV inhibition) and lamivudine (for LINE-1 inhibition), two well-known nucleoside reverse transcriptase inhibitors[18,32,66,67]. Major strides have been made recently in targeting transposable elements to combat age-related diseases, including neurodegenerative disorders and cancer[232].

Modulation of the function of chromatin factors could delay cellular senescence and alleviate ageing-related conditions (Fig. 4 and Supplementary Table 2). For example, lentiviral delivery of *DGCR8* cDNA stabilized constitutive heterochromatin through DGCR8 interaction with KAP1, lamin B1 and HP1, thereby counteracting senescence in human MSCs and ameliorating osteoarthritis in aged mice[49] (Fig. 5b). Similar effects were reported following genetic interventions with the master circadian regulation gene *CLOCK* and the Polycomb repressive complex 1 component *CBX4* (refs. 33,233), representing possible drug targets and therapeutics against ageing and related diseases. Moreover, partial cell reprogramming induced by controlled expression of the 'Yamanaka factors' (the pluripotency transcription factors OCT4 (also known as POU5F1), SOX2, KLF4 and MYC) contributes to increased H3K9me3 levels and attenuated senescence phenotypes when applied to relieve age-associated impairments in mice[19,42]. Conversely, genetic inactivation of the histone acetyltransferase *KAT7* in senescent human MSCs mitigates heterochromatin loss by upregulating HP1, lamin B1 and lamina-associated polypeptide 2 (LAP2). In ageing mouse models, targeted gene therapy through *Kat7* loss of function alleviates hepatocyte senescence and liver ageing and extends lifespan in both prematurely and physiologically aged mice[234]. As mentioned above, depletion or deletion of *GADD45A* restores telomeric heterochromatin and ameliorates senescence in both human and mouse fibroblasts[115]. Furthermore, *Gadd45a* depletion enhances the function of intestinal stem cells and prolongs lifespan in telomerase-deficient mice, offering a new avenue for development of therapeutics for telomere-related disorders.

Therapeutics related to maintaining telomere integrity

Calorie restriction also counteracts ageing by supporting telomere maintenance (Fig. 4 and Supplementary Table 2). For example, it attenuates ageing-associated telomere attrition and synergizes with increased TERT expression in extending healthspan and lifespan in mice[235]. In humans, exercise bolsters telomere integrity and defends against ageing, potentially through telomerase or TERRA-dependent mechanisms[236–239]. These outcomes may be achieved by reducing short-telomere-induced DDR activation and cellular senescence. Likewise, hyperbaric oxygen therapy can elongate telomeres and reduce senescence in immune cells in older people[240]. The activation of telomerase through genetic or pharmaceutical avenues can reverse ageing-related ailments as well. For example, in Tert-deficient zebrafish, gut-specific Tert expression mitigates cellular senescence, rescues gut microbiota dysbiosis, restores systemic tissue integrity and prolongs lifespan[181]. In mice, in vivo *Tert* gene delivery diminishes the levels of $p16^{INK4a}$-expressing senescent cells in various tissues and improves glucose tolerance, physical performance and median lifespan[180,241]. Clinical trials using gene therapy to activate telomerase, such as delivering human *TERT* by adeno-associated viruses, are underway to combat ageing and Alzheimer disease[242], and TA-65, a small-molecule telomerase activator, has been applied to reduce immunosenescence and

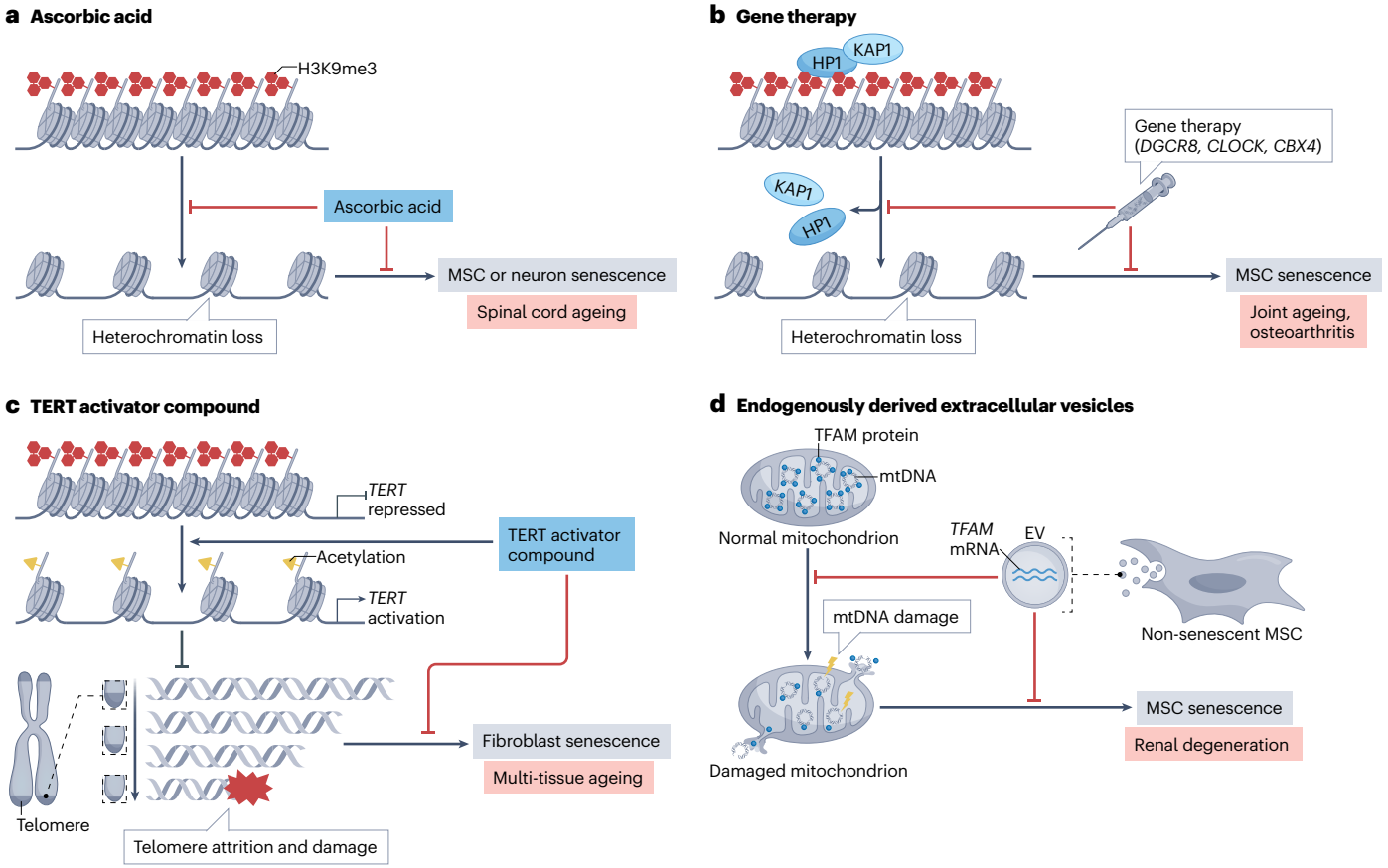

Fig. 5 | Representative cell senescence and/or ageing therapeutic interventions targeting heterochromatin loss, telomere attrition or DNA damage. a, Ascorbic acid restores heterochromatin stability and alleviates senescence in human mesenchymal stem cells (MSCs) and neurons or ageing in the spinal cord of non-human primates. **b**, Genetic interventions based on lentiviral delivery of *DGCR8*, *CLOCK* or *CBX4* cDNA rejuvenate senescent human MSCs and aged mouse joints (or alleviate mouse osteoarthritis). **c**, Administration of a molecule called telomerase reverse transcriptase (TERT) activator compound (TAC) activates *TERT* transcription by reducing the levels of the gene-repressive trimethylated histone H3 Lys9 (H3K9me3) and increasing the levels of the gene-activating acetylated H3K27 (H3K27ac), which contributes to telomere elongation and DNA damage relief, thereby attenuating cellular senescence in human fibroblasts and ameliorating ageing features in multiple tissues of mice. **d**, Extracellular vesicles derived from non-senescent human or mouse MSCs contain mitochondrial *TFAM* mRNA; the translated TFAM protein preserves mitochondrial DNA (mtDNA) stability. Treatment with these vesicles alleviates mtDNA damage, restores mitochondrial function and mitigates MSC senescence and renal degeneration or dysfunction in mice. CBX4, chromobox protein homologue 4; CLOCK, circadian locomotor output cycles kaput; DGCR8, DiGeorge syndrome critical region gene 8; EV, extracellular vesicle; HP1, heterochromatin protein 1; KAP1, KRAB-associated protein 1; TFAM, transcription factor A, mitochondrial.

inflammation in elderly individuals with myocardial infarction[243]. In mice, inhibition of *Fbw7* or overexpression of *Tpp1* has been reported to increase telomere stability and length, and to improve lung function and resilience to cell senescence and fibrosis[190], representing potential targets for the treatment of age-associated pulmonary diseases.

Interestingly, pharmacological interventions that can stimulate rejuvenation similarly to telomerase activation have been reported (Fig. 4 and Supplementary Table 2). For instance, taurine treatment suppresses cellular senescence and improves the survival of Tert-deficient zebrafish[230]. NAD^+, a crucial coenzyme in redox reactions, can prevent the onset of senescence by promoting mitochondrial homeostasis and DNA repair[244]. In fibroblasts from individuals with dyskeratosis congenita, dietary supplementation of nicotinamide riboside, a precursor of NAD^+, enhances telomere integrity, improves mitochondrial function and attenuates cellular senescence[245]. Short-term administration of nicotinamide mononucleotide (NMN), another NAD^+ precursor, also considerably increases telomere length in pre-ageing mice and human volunteers[246]. Similarly, TAC treatment induces TERT expression, extends telomeres and reduces telomere-associated DNA damage foci, thereby alleviating fibroblast senescence and blunting ageing hallmarks in multiple tissues of mice[20] (Fig. 5c). The consumption of ascorbic acid or metformin (an antidiabetes medication) prevents telomere shortening in humans as well[247,248].

Therapeutics related to alleviating DNA damage

Consistent with its role in heterochromatin stabilization and telomere maintenance, calorie restriction also enhances DNA repair in ageing models (Fig. 4 and Supplementary Table 2). For example, in progeroid mice lacking ERCC1, calorie restriction reduced the number of γH2AX DNA damage foci and extended healthspan and lifespan[249]. This process

Glossary

Biological age
An indicator of the physiological state of an organism relative to its chronological age, reflecting the overall health and ageing of the body's systems.

Chromatin entropy
The degree of disorder within the chromatin structure, which may increase with cellular senescence and affect gene expression.

Cockayne syndrome
A rare genetic disorder typically caused by mutations in genes such as *ERCC6* and *ERCC8*, characterized by premature ageing and various developmental abnormalities.

DNA methylation age
An estimate of an individual's biological age based on the methylation patterns of their DNA.

Dysbiosis
An imbalance in the microbiome of an organism, which can negatively affect health and is associated with various diseases.

Gene expression leakage
Unintended expression of genes that are supposed to be repressed or inactive, contributing to background noise in cellular processes.

Heterochronic parabiosis
An experimental system that involves surgically connecting two animals of different ages (typically young and old) to establish a shared circulatory system. This set-up enables the transfer of youthful substances, including blood factors, into the older animal, which can rejuvenate senescent cells and revitalize ageing tissues.

Hyperbaric oxygen therapy
A medical treatment that involves breathing pure oxygen in a pressurized room or chamber, used to treat various conditions by increasing the oxygen levels in the blood and tissues.

Immunosenescence
The gradual deterioration of the immune system associated with ageing, leading to increased susceptibility to infections and diseases.

Information theory of ageing
A theory proposing that the ageing process is propelled by the progressive loss of youthful epigenetic information, which can be restored by rejuvenation through epigenetic reprogramming.

NLRP3 inflammasome
A multiprotein complex that triggers inflammation in response to pathogens or other danger signals, such as cytoplasmic DNA fragments released from damaged mitochondria.

Progeroid
Describes disorders that resemble premature ageing, typically owing to mutations that accelerate the ageing process. The term encompasses premature ageing diseases such as Hutchinson–Gilford progeria syndrome and Werner syndrome.

Reactive oxygen species
(ROS). Chemically reactive molecules containing oxygen, such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-), which have important roles in cell signalling and homeostasis.

Retrovirus-like particles
Structures assembled from endogenous retroviral components that resemble infectious retroviruses but are unable to replicate and are thus non-infectious.

Senescence-associated secretory phenotype
(SASP). A mixture of secreted factors produced by senescent cells, such as cytokines, growth factors and metalloproteinases, that contribute to inflammation and influence the tissue microenvironment.

Senolytics
Agents that selectively target and eliminate senescent cells by inducing apoptosis.

Senomorphics
Agents that target the mechanisms that underlie senescence to promote rejuvenation instead of directly eliminating senescent cells.

involves the repression of p53, $p21^{CIP1}$, $p16^{INK4a}$ and the SASP factor IL-6, indicating mitigation of cellular senescence. Although exercise is widely acknowledged for its positive effects on health, and moderate and regular exercise reduces DNA damage, intense exercise can exacerbate DNA damage[250]. Thus, further research is warranted to better understand the benefits of physical activity in alleviating senescence and countering ageing.

A range of pharmacological and genetic interventions targeting senescence and ageing promote genome stability by decreasing DNA damage (Fig. 4 and Supplementary Table 2). For instance, metformin promotes health and lifespan in model organisms[251], alleviates cellular senescence and DNA damage in MSCs derived from individuals with chronic kidney disease[252] and is under investigation in clinical trials for its potential to mitigate aspects of ageing[242]. In Alzheimer disease mice, the histone deacetylase 1 (HDAC1) activator exifone can reduce oxidative DNA damage in the ageing brain and improve spatial memory, perhaps by reducing DDR-related neuronal senescence[211]. In addition, α-ketoglutarate, an endogenous metabolite, reverses cellular senescence by decreasing γH2AX and SASP levels, thereby ameliorating age-related osteoporosis and extending lifespan in mice[253,254]. Other geroprotective compounds, such as SAM[229], nicotinamide riboside[255], NMN[208], ascorbic acid[228], rapamycin[204], taurine[230] and urolithin A[220], have also shown efficacy in mitigating genome instability and stimulating cell and tissue rejuvenation. Moreover, treatments with extracellular vesicles from non-senescent MSCs or an antibiotics cocktail are capable of reducing DNA damage and inflammation and ameliorating senescence and ageing phenotypes in mice[256,257]. Notably, extracellular vesicles from proliferating MSCs contain high levels of *TFAM* mRNA, which can restore TFAM protein levels and alleviate mtDNA damage, thereby exerting a rejuvenating effect on senescent cells and degenerating or damaged tissues[257] (Fig. 5d). Interestingly, the combination of dasatinib and quercetin, the first senolytics identified to induce apoptosis of senescent cells, seems to be effective in improving DNA repair capacity and alleviating age-dependent intervertebral disc degeneration in mice and ageing of human brain organoids[258,259]. Genetic interventions that promote the function of SIRT6, TRF2, CLOCK, the longevity factor forkhead box protein O3 (FOXO3), the transcription factor SOX5 and Yamanaka factors, can also attenuate DNA damage in senescence or ageing contexts and achieve rejuvenating outcomes in human cells and mouse tissues[19,35,42,142,260–265]. These findings lay the theoretical basis for the clinical development of gene therapies targeting age-related disorders.

Taken together, the studies discussed here reveal a rich array of options for mitigation of cell senescence and ageing by targeting heterochromatin, telomere and DNA repair factors. Additional therapeutic interventions that can remove or rejuvenate senescent cells and alleviate ageing-related pathologies include heterochronic parabiosis or administration of youthful blood factors[266,267], faecal microbiota transplantation[268,269], immunotherapies such as senolytic vaccines, antibodies and chimeric antigen receptor (CAR)-T cell therapies, which can

eliminate senescent cells by targeting specific antigens on them[270–272], and senomorphics, which disrupt the production of SASP factors and their secretion from senescent cells without eliminating the cells themselves[22]. Although these strategies have been extensively proved effective in animal models, primarily in mice, further investigations are required to determine their direct effects on the three aforementioned senescence mechanisms. Regardless, the advancements in preclinical settings provide promising druggable targets and therapeutic strategies for the prevention and treatment of diseases associated with ageing.

Conclusions and future perspective

In recent years, significant progress has been made towards advancing our understanding of determinants of cellular senescence and their relevance to ageing. We discuss cellular senescence mechanisms spanning heterochromatin loss, telomere attrition and DNA damage, and their roles in ageing and related conditions. Particularly noteworthy is their shared role in promoting inflammation, which contributes to ageing and disease, potentially through amplifying senescence signals across cells and tissues through paracrine signalling. Moreover, epigenetics alteration emerges as a shared theme underlying these molecular processes, highlighting how interplay between genetic and epigenetic instability triggers the initiation of cellular senescence and ageing. At least in part, the role of epigenetics aligns with the information theory of ageing[19,203], offering potential explanations of the root cause of senescence or ageing. By discussing known interventions and their effects on specific molecular and cellular pathways, we offer insights into the measurability and intervenability of cellular senescence and ageing. However, assessing the safety and effectiveness of the vast majority of these therapeutic strategies in clinical settings remains essential.

Looking ahead, cellular senescence and ageing research faces several challenges. For instance, the heterogeneity in the manifestation of cellular senescence across cells, tissues, individuals and populations or ethnicities, heightens the complexity and challenges associated with understanding, quantifying and intervening in senescence and ageing. Additionally, molecular changes during senescence and ageing do not happen in isolation, rendering reductionism inadequate to conclusively delineate all aspects of cell senescence. Therefore, comprehending the integration of regulatory mechanisms at multiple hierarchical scales is necessary to understand the dynamics of cellular senescence and organismal ageing[273]. Furthermore, the lack of universal, precise and validated biomarkers and biochronometric indicators to measure cellular senescence and organ ageing in diverse contexts poses substantial challenges in tracking ageing progression and disease, and in assessing therapeutic efficacy[274–277]. The development of multimodal ageing clocks based on DNA methylation and other molecular processes may offer a new avenue for these assessments[278,279]. To address these challenges, future research must integrate high-throughput functional genomics screening[234,280,281], single-cell and multi-omics methods[282,283], gender- and ethnicity-inclusive cohort studies[278,279], searchable big data repositories[284–286] and artificial intelligence-assisted methodologies to advance ageing research[287,288]. We also view fostering interdisciplinary collaborations as paramount for advancing research on cell senescence and ageing-related diseases, with a specific focus on non-human primate studies to enable possibly easier clinical translation of diagnostics and therapies[228,289–291]. With ongoing efforts in this area, we will delve into the essence of ageing with increased precision, aspiring to establish a global community centred on human healthspan and well-being in the foreseeable future.

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All authors researched data for the article and contributed substantially to discussion of the content. Z.W. wrote the article. All authors reviewed and/or edited the manuscript before submission.

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