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Perspective

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Targeting DNA damage in ageing: towards supercharging DNA repair

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Abstract

Ageing is the most important risk factor for many common human diseases, including cancer, diabetes, neurodegeneration and cardiovascular disease. Consequently, combating ageing itself has emerged as a rational strategy for addressing age-related multimorbidity. Over the past three decades, multiple genetic and pharmacologic interventions have led to substantial extension of lifespan and healthspan in model organisms. However, it is unclear whether these interventions target the causal mechanisms of ageing or downstream consequences. Ample evidence suggests that DNA damage to the somatic genome is a major causal mechanism of ageing, which compromises essential cellular functions such as transcription and replication, and leads to cellular senescence, apoptosis and mutations. Recently, new concepts have emerged to target the main consequences of DNA damage and enhance DNA repair capacities, thereby extending maintenance of the genome. Here, we review advances in this field and discuss approaches to pharmacologically mitigate the adverse effects of DNA damage to delay ageing, prevent mutation-driven cancer and mitigate age-related degenerative diseases.

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Introduction

Over the past 150 years, human life expectancy in the developed world has doubled to an average of approximately 80 years, with less developed countries now rapidly catching up, as reported by the United Nations' Global Health Estimates. This longevity surge, coupled with declining fertility rates, has led to a demographic shift that has resulted in nearly 30% of the population in some countries now aged 65 and above[1,2]. This dramatic increase in life expectancy has been largely due to improvements in living conditions, including food security and sanitation. However, longer lifespan is associated with an increased prevalence of chronic age-associated diseases. Although in the past famine, violence and infectious diseases were the most common causes of death, today an increasing fraction of the population suffers from cancer, cardiovascular disease, type 2 diabetes, Alzheimer disease and other debilitating chronic illnesses. This has substantially increased the cost of healthcare, which must be provided by a shrinking fraction of the population being of working age. To effectively tackle this societal challenge, new approaches are required that target the underlying mechanisms of ageing rather than individual age-related diseases. Therefore, understanding the ultimate causes of ageing that are rooted in evolutionary history (outlined in Box 1), dissecting the immediate drivers of the ageing process and, based on this, devising therapeutic strategies targeting the mechanisms of ageing, has become of paramount importance.

DNA damage has been identified as a major, if not the main, cause of ageing[3]. Indeed, there is evidence that somatic mutation rate, the adverse effects of errors in DNA repair, is inversely correlated with species-specific lifespan in mammals[4,5]. As macromolecular structures such as the genome require constant repair, ageing can be considered the default outcome when maintenance and repair falter. Therefore, boosting maintenance and repair of somatic cells is critical for maintaining health during the now greatly extended human lifespan and reducing the risk for age-related diseases.

As DNA is the ultimate template of all biological information, the consequences of DNA damage are profound and far-reaching (Fig. 1). Estimates suggest that up to 100,000 DNA lesions can occur in a single human cell on any given day[6]. The sources of such damage are diverse, varying from endogenous factors, such as spontaneous hydrolysis, oxidation and alkylation[7,8], to exogenous agents, such as ionizing radiation, ultraviolet (UV) light and environmental compounds[9]. They result in a variety of lesions, ranging from single nucleotide modifications, such as those typically caused by deaminations or oxidative modifications, to crosslinks that can occur between nucleotides, such as the UV-induced thymidine dimers, or between opposite strands, such as the cisplatin-induced interstrand crosslinks that result from cancer treatments. Some metabolic components, such as formaldehyde, can induce diverse lesion types, including DNA–protein crosslinks[8], interstrand crosslinks and oxidative lesions[10]. Single-strand breaks occur frequently, whereas double-strand breaks (DSBs) are less common but have far greater consequences, potentially leading to the formation of genome structural variants or mis-segregation of chromosomes[9,11,12].

Depending on the lesion type, DNA damage can impede both DNA replication and transcription. Replication fork stalling is a common occurrence, often resolved through mechanisms such as backtracking and the removal of mis-incorporated dNTPs (deoxyribonucleotide triphosphates) via the exonuclease activity of the replicative DNA polymerases[13,14]. More obstructive base modifications may require the involvement of translesion synthesis by specialized polymerases that pass through the damage, which is typically more error-prone[15]. RNA polymerase (RNAP) complexes can also stall at DNA lesions. Although certain oxidative base modifications can be bypassed, more obstructive lesion types necessitate removal before RNAPII elongation can resume. Transcription-blocking lesions predominantly affect long genes as they are more likely to incur damage. Consistent with an accumulation of such damage, a gene length-dependent transcriptional decline has been observed during ageing in multiple species[16–18], and it is accelerated in progeroid mice carrying nucleotide excision repair

Box 1 | Theories of ageing

When August Weismann demonstrated in the late 19th century that the germline but not somatic cells pass on the genetic information[346], it occurred to him that indefinite maintenance of the soma offered no fitness gain for as long as the germ cells could indefinitely perpetuate their genomes. The ultimate cause of ageing across most multicellular organisms is, therefore, considered by most to reside in the declining force of natural selection with age[3]. Fitness gains of genetic traits acting late in life become negligible when offspring has already been generated and is capable of carrying on the gene pool of the species and thus ensure its survival. Peter Medawar suggested in the 1950s that the contribution of old individuals to fitness would be negligible and thus mutations with detrimental effects in late life could accumulate[347]. Fitness gains are mainly driven by genetic variants with a beneficial effect early in life. According to Williams' antagonistic pleiotropy theory, such early beneficial gene variants could have detrimental effects late in life[348]. Cellular senescence could thus be viewed as an antagonistic pleiotropy process[349]. Cellular senescence is thought to play an important role for tissue remodelling during development, whereas the accumulation of senescent cells with ageing leads to late-life degenerative changes, such as inflammation. Therefore, in humans, somatic maintenance is optimized to span the three to four decades required to raise the subsequent generation. Throughout its ~300,000 years of history, human life expectancy hovered around 30 years, before tremendous gains were made starting in the 19th century, leading to an average lifespan of almost 80 years in many developed countries today[350]. However, these gains were driven by improvements in hygiene, nutrition, vaccines, antibiotics and so on, that is, by changes in living conditions, but not by any genetic changes. Therefore, our genetic composition is still optimized for somatic maintenance lasting a few decades but not the nearly a century of life we are now living. In contrast to the limited somatic repair and maintenance factors, our germ cells continue their indefinite life. A new approach to improve somatic repair is learning from the germline maintenance mechanisms[298]. Failure to repair leads to rapid functional decline, as the incurrence of damage is an inevitable feature of macromolecules[3]. Therefore, transferring germline-like maintenance and repair mechanisms to improve the soma could provide a strategy for extending healthspan far beyond the few decades of somatic maintenance our genomes currently encode for.

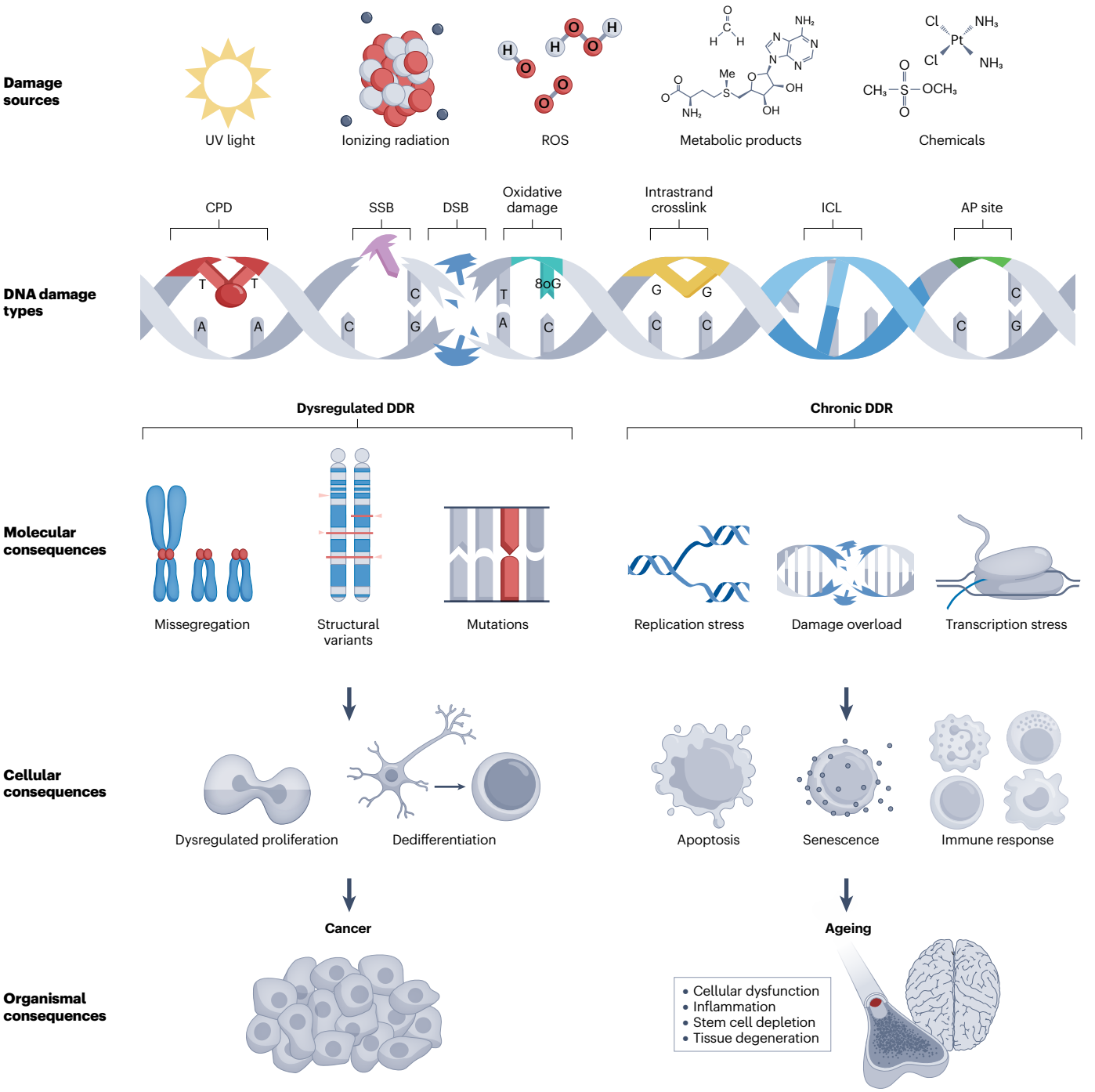

Fig. 1 | Sources and types of DNA damage and the molecular, cellular and organismal consequences. DNA damage is inflicted by exogenous and endogenous sources that lead to distinct lesion types. Errors during repair, recombination or replication can lead to genome sequence alterations, such as structural variants, mutations and missegregation. Mutations in tumour suppressor genes and oncogenes can lead to dysregulation of proliferation and dedifferentiation, fuelling cancer development. Persistent DNA damage can stall replication and transcription, triggering the DNA damage response (DDR) that induces apoptosis, senescence and immune responses. The chronic DDR compromises cellular function, triggers inflammation, deprives stem cell compartments and promotes tissue degeneration. From top to bottom, the reactive oxygen species (ROS) shown are hydrogen peroxide, a hydroxy radical and superoxide. Metabolic product molecules shown are formaldehyde and S-adenosyl methionine. Chemicals are cisplatin and methyl methanesulfonate. AP site, apurinic/apyrimidinic site; CPD, cyclobutane pyrimidine dimers; DSB, double-strand break; 8oG, 8-oxo-guanine; ICL, interstrand crosslink; SSB, single-strand break; UV, ultraviolet.

(NER) defects[19]. The mechanisms underlying gene length-dependent transcriptional decline might depend on the physiological context, as cultured transcription-coupled NER (TC-NER)-defective cells did not lose the expression of long genes[20]. Here, the challenge of transferring mechanistic insights into the physiology of organismal ageing and, vice versa, identifying the mechanisms of homeostatic changes in cell culture becomes apparent as the distinct DNA repair mechanisms and the outcomes of the cellular response to DNA damage can be cell type-specific and dependent on the tissue context and the homeostatic changes occurring in the ageing organism.

When DNA repair mechanisms are insufficient, the DNA damage response (DDR) can drive cells into apoptosis or cellular senescence. The apoptotic DDR has been a major route of cancer treatment for many decades. By contrast, eliminating senescent cells (SnCs) has been more recently explored as a strategy to remove cells that are particularly harmful due to the chronic inflammatory senescence-associated secretory phenotype (SASP). Such chronic inflammatory responses have also been observed as a more general outcome of damaged DNA when it is transferred into the cytosol, termed cytoplasmic chromatin fragments, where it triggers the cGAS-STING pathway (that is, stimulation of protein STING by enzyme cGAS via the synthesis of 2′3′ cyclic GMP–AMP) and other nucleic acid sensors of the immune defence[21].

Some cancer therapies directly target DNA repair enzymes, particularly through PARP inhibition, to harness specific vulnerabilities of cancer cells to abrogate routes of DNA repair. The opposite strategy — boosting DNA repair in order to maintain genome stability — has only recently become experimentally feasible through a better understanding of the regulation of DNA repair genes. In this Perspective, we provide an overview of the roles of genome instability in ageing and discuss how targeting the DDR and DNA repair mechanisms to augment genome stability could provide new routes for anti-ageing therapies. A summary of the approaches and drugs described in this Perspective can be found in Table 1.

Genome instability as a universal cause of ageing

The constant onslaught of DNA damage threatens the most fundamental cellular processes and requires proficient DNA repair mechanisms. Somatic mutations occur due to insufficient somatic DNA repair capacities and explain why ageing is the most important risk factor for cancer. The DDR can alter cell fates, inducing apoptosis or cellular senescence and inflammatory responses that promote ageing. By affecting the most apical component of the biological information hierarchy, DNA damage profoundly impacts cell function, identity and fate. Consequently, DNA damage is a root cause not only of cancer but also of age-associated diseases.

DNA damage and hallmarks of ageing

DNA damage has far-reaching consequences on a wide range of physiological processes (Fig. 1). DNA damage can impact some of the most fundamental cellular processes: when it blocks transcription, gene expression required for cellular function can be impaired; when it halts replication, cell division required for development and homeostatic cell renewal can be hampered. However, it is not only the direct effects of damaged DNA that can contribute to the ageing process but also the cellular response to DNA damage[3]. The DDR is activated upon recognition of lesions, and, depending on damage type and severity, it amplifies the signal and impinges on a variety of cellular processes.

The DDR can trigger the DNA damage checkpoint to halt the cell cycle, thus allowing time for repair before replication and cell division. Amid severe damage, the DDR can trigger apoptosis to eliminate damaged cells or cellular senescence to permanently withdraw compromised cells from proliferation. Although the dysfunction of the DDR is a typical feature of cancer cells, an improved checkpoint response in mice (carrying an extra allele of the central checkpoint regulator TP53) provides improved cancer protection[22]. However, over-activation of the checkpoint (by a hyperactive form of p53) can accelerate ageing, likely by hampering homeostatic cell renewal[23]. Likewise, the apoptotic DDR provides tumour protection, whereas excessive apoptosis promotes degenerative diseases such as Alzheimer disease or immunodeficiencies. Cellular senescence can have more complex outcomes. Although a bona fide tumour suppressor mechanism, SnCs can also promote cancer growth non-cell-autonomously via the secretion of cytokines[24]. The SASP can also have pro-inflammatory consequences and promote age-associated diseases[25,26], while conversely also contributing to tissue remodelling during wound healing[27].

In addition to the SASP, damaged DNA itself can trigger inflammatory responses. DNA damage and R-loops, which are DNA:RNA hybrids that can occur when transcription is impeded, give rise to cytosolic DNA species that activate the cGAS-STING or toll-like receptors, subsequently activating the inflammasome[28,29]. Inflammatory responses to DNA damage have long been observed in UV-irradiated skin[30], and they are now recognized as a major adverse end point of chronic DNA damage, even in neurons, in which they have recently been linked to neurodegeneration[31,32].

DNA damage can also affect the epigenetic code. The ‘access–repair–restore’ model indicates that histone modifications are instrumental in regulating the access of DNA repair machineries[33]. The epigenetic status can be altered during the repair process, most prominently during DSB repair, in which modifications such as γH2AX become predominant around the DSB site[34]. After the lesions have been removed and integrity of the DNA sequence restored, the epigenetic landscape also needs to be reinstated[35]. Post-repair events can involve further epigenetic alterations. For example, in cases of transcription-blocking lesions repaired via TC-NER, the deposition of H3K4me2 along the body of genes involved in protein biosynthesis and homeostasis is necessary to induce their expression. All these steps are required for reinstating cellular protein biosynthesis and homeostasis[36]. The maintenance of the epigenetic code is an essential component of genome stability. It has recently been shown that aberration of epigenetic regulation, such as a transient loss of the polycomb repressor complex, is sufficient to trigger cancer development[37].

In addition to changes in histone marks, alterations in the methylation site patterns of cytosine–guanine dinucleotide (CpG) occur during the ageing process. Age-related changes in some of those CpG methylation marks have allowed the construction of accurate ageing clocks, with linear regression models measuring chronological age and predicting biological age. Interestingly, the polycomb repressor complex sites are over-represented among the CpG sites[38]. DNA repair-deficient mouse models show accelerated ageing as well as accelerated advancement of DNA methylation-based ageing clocks[39]. Consistent with the role of age-dependent damage accumulation, epigenetic clocks were recently shown to be driven by the accumulation of stochastic variation[40]. Moreover, sites of somatic mutations have been associated with areas of age-predictive CpG changes[41]. Epigenetic modifications, in turn, might also affect DNA damage susceptibility, as for instance open chromatin is more vulnerable to damage, whereas closed heterochromatin structures are more refractory to repair, such as homologous recombination repair (HRR)[42].

Table 1 | Selected approaches and example molecules being investigated to decrease DNA damage or ameliorate its negative effects

Approach/drug class	Compound name	Target	Development Stage	Refs.
Senolytic	Dasatinib	Tyrosine kinases	Used for CML and ALL	80–87
	Nintedanib	STAT3	Used for pulmonary fibrosis and NSCL cancer	95–99
	BAY80-6946 (copanlisib)	PI3K	Used for follicular lymphoma	111
	BYL-719 (alpelisib)	PI3K	Used for breast cancer	111,112
	CAL-101 (idelalisib)	PI3K	Used for CLL	111
	IPI-145 (duvelisib)	PI3K	Used for CLL	111
	Umbralisib	PI3K	Used for marginal zone lymphoma	111
	ABT-199	BCL-2	Used in CLL, SLL and AML	118,125
	Tamatinib/fostamatinib	Syk, FAK and p38 MAPK	Used for ITP and clinical trials (phase III)	140–142
	Cardiac glycosides	Na^+/K^+ ATPase pumps	Used in congestive heart failure and arrhythmias	143
	Quercetin	PI3K/AJT/mTOR and STAT3	Clinical trials (phase IV) and available[a]	80–87
	Fisetin	BCL-2, PI3K/AKT, p53	Clinical trials (phase II) and available[a]	80,88–90
	Luteolin	PI3K/AKT	Clinical trials (phase II) and available[a]	91–94
	PCC1	BCL-2	Clinical trial (phase not applicable) and available[a]	127
	Curcumin/EF24	BCL-2	Clinical trials (phase IV) and available[a]	128
	Piperlongumine	OXR1	Mouse models and available[a]	131–134
	PF-04691502	PI3K/AKT and mTOR	Clinical trials (phase II)	102–105
	PX-866	PI3K	Clinical trials (phase II)	106–110
	TAS-116	HSP90	Clinical trials (phase II)	116
	ABT-263	BCL-2 and BCL-xL	Clinical trials (phase II)	119–122
	MIK665	MCL-1	Clinical trials (phase II)	125,126
	UBX0101	MDM2-p53	Clinical trials (phase II)	136,137
	17-DMAG	HSP90	Clinical trials (stopped after phase I)	113–115,117
	CAR T cells	NKG2DL	Mouse and primates[b]	146
	AG490	JAK2/STAT3	Mouse models – preclinical	101
	ABT-737	BCL-2, BCL-xL and BCL-w	Mouse models – preclinical	118,123
	A1331852	BCL-xL	Mouse models – preclinical	124
	A1155463	BCL-xL	Mouse models – preclinical	124
	PROTACs	BCL-2, BCL-xL and BCL-w	Mouse models – preclinical	129,130
	FOXO4-DRI peptide	FOXO4-p53	Mouse models – preclinical	135
	P5091	USP7	Mouse models – preclinical	138
	GMD	β-Galactosidase activated cytotoxin	Mouse models – preclinical	79,139
	CAR T cells	uPAR	Mouse models – preclinical[b]	144,145
	Immune activation	GPNMB	Mouse models – preclinical	148
	Immune activation	CD153	Mouse models – preclinical	149
	Immune activation	B2M	Mouse models – preclinical	150
	Immune activation	ApoD	Mouse models – preclinical	151
Senomorphic	Rapamycin	mTOR, NF-κB	Used in cancer, organ transplant and more	79
	Metformin	Multiple	Used for diabetes	79
cGAS-STING inhibitors	Hydroxychloroquine	cGAS–DNA binding	Used for malaria and autoimmune disorders	174
	Quinacrine	cGAS–DNA binding	Used for malaria and autoimmune disorders	174
	X6	cGAS–DNA binding	Used for malaria	175
	Aspirin	cGAS–DNA binding	Used as anti-inflammatory and more	176
	CXA-10	STING cysteines	Clinical trials (phase II)	182

Table 1 (continued) | Selected approaches and example molecules being investigated to decrease DNA damage or ameliorate its negative effects

Approach/drug class	Compound name	Target	Development Stage	Refs.
cGAS-STING inhibitors (continued)	VENT-03	cGAS catalytic site	Clinical trials (phase I)	173
	RU.521	cGAS catalytic site	Mouse models – preclinical	169
	G140-G150	cGAS catalytic site	Mouse models – preclinical	170
	TDI-6570	cGAS catalytic site	Mouse models – preclinical	170,171
	30d-S	cGAS catalytic site	Mouse models – preclinical	172
	H-151	STING cysteines	Mouse models – preclinical	178
	C-176	STING cysteines	Mouse models – preclinical	179
	BB-Cl	STING cysteines	Mouse models – preclinical	180
	LB244	STING cysteines	Mouse models – preclinical	181
	SN-011	STING-cGAMP binding site	Mouse models – preclinical	182
	T0901317	cGAMP reduction via LXR-SMPDL3A	Mouse models – preclinical	183
	JQ1	BRD4 inhibitor	Mouse models – preclinical	184,185
	GSK8612	TBK1 inhibitor	Mouse models – preclinical	186–189
	AG490	JAK2 inhibitor	Mouse models – preclinical	101
	Viral peptides	Multiple	N/A	180–195
R-loop/ssDNA removal	Nucleases	DNA damage by-products	Mouse models – tool compound	29,197
Repair enzymes	Photolyase	DNA damage	Clinical trials (phase NA) and available[c]	212–214
	T4 endonuclease V	DNA damage	Clinical trials (phase III) and available[c]	217–220
DNA protection	DSUP	DNA	Human cells, plants and flies	224–226
NAD^+ supplementation	NR, NA, NAM, vitamin B3, NAR, NMN	PARP, SIRTs, mitochondria	Clinical trials (phase III and IV) and available[a]	227–253,256–265
SIRT6 activation	UBCS039	SIRT6	Mouse models – preclinical	265–268
Gene therapy	Cas9-derived ABEs	LMNA	Mouse models – preclinical	269
	CRISPR–Cas9	CSB/ERCC6	Cell culture	270
	CRISPR–Cas9	WRN	Cell culture	271
Epigenetic remodellers	DNMTi	DNA methyltransferase	Used for MDS and AML	280,281
	Partial reprogramming	Cell fate	Mouse models – preclinical	274–278
	SRT1720	SIRT1	Mouse models – preclinical	282–288
DREAM complex	EGCG	DYRK1A	Clinical trials (phase II/III) and available[a]	337
	Harmine	DYRK1A	Clinical trials (phase I)	313,318
	Leucettinib-21	DYRK1A	Clinical trial (phase I)	338,339
	INDY	DYRK1A	Cell culture	314
	ID-8	DYRK1A	Cell culture	318

The table has been ordered per drug class by the stage of development of the compound (in use, in clinical trials, in animal models and so on). When in clinical trials, the highest stage of any one clinical trial for the specific compound is indicated (when available). ABEs, adenine base editors; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; cGAMP, 2′3′ cyclic GMP–AMP; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; DREAM complex, dimerization partner, retinoblastoma-like, E2F and multi-vulval class B complex; ITP, immune thrombocytopenia; EGCG, epigallocatechin-3-gallate; MDS, myelodysplastic syndromes; NA, nicotinic acid; NAM, nicotinamide; NAR, nicotinic acid riboside; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside; NSCL cancer, non-small-cell lung cancer; PARP, poly(ADP-ribose) polymerase; PROTACs, proteolysis targeting chimeras; SLL, small lymphocytic lymphoma; ssDNA, single-stranded DNA. [a]Available as a supplement. [b]Being clinically used for other targets. [c]Available in creams.

Erroneous repair of DNA damage, somatic mutations and cancer

Although DNA damage is normally repaired quickly and accurately, errors do occur. Errors, such as misincorporation of bases or annealing the wrong ends of DSBs, can lead to mutations, that is, irreversible changes in DNA information content. The inherent propensity of DNA mutagenesis underlies the basis of genome evolution and, consequently, speciation. However, unlike germline mutations, which are common to all cells originating from a mutated gamete, somatic mutations differ from cell to cell, unless they are clonally amplified, due to genetic drift or a growth advantage. As expected, somatic cells accumulate mutations over their lifetimes as a function of age[43], with

the accumulation rate inversely correlated with the maximum lifespan of the species[5]. This inverse correlation suggests that somatic DNA repair capacity has been subjected to strong evolutionary selection not to exceed species-specific lifespan[5]. Interestingly, in humans, somatic mutations have been found to increase in all tissue types investigated, but at different rates. Mitotically active tissues generally show a higher mutation rate than postmitotic tissues, possibly due to the occurrence of replication errors. However, mutation rates in postmitotic cells can also be high, as in hepatocytes, which have high somatic mutation rates, likely due to their exposure to multiple dietary genotoxins[44]. Surprisingly, neurons also display a relatively high rate of age-related mutation accumulation[43], possibly reflecting their high levels of oxidative metabolism. As expected, stem cells have relatively low somatic mutation burden[45–47].

Typically, mutations have neutral or detrimental effects on the function of the affected gene, although in rare cases they can result in gain-of-function alleles. Somatic mutations may undergo clonal amplification, with the most extreme example being the selective advantage offered by tumour suppressor mutations, leading to clonal expansion of affected cells, a foundational concept of tumour biology[48]. There are also other forms of genetic aberrations, such as structural variants, which can have more severe consequences, as particularly observed in aggressive cancer types[49,50]. Structural variants can give rise to recurrent genetic aberrations through the breakage–fusion–bridge cycle, in which mis-segregation leads to new chromosomal breaks, resulting in new fusion events and promoting tumour genomic complexity[51]. Structural variants are far less numerous than single-nucleotide variants, which make up the majority of somatic mutations. Thus far, the analysis of structural variants is technically more challenging, and it will be highly interesting to further ascertain their role in the ageing process.

The impact of somatic mutations on the ageing process remains less well understood. Humans and mice carrying defects in DNA polymerases or mismatch repair genes linked to higher mutation rates show elevated cancer susceptibility, without overt signs of accelerated ageing[52–54]. However, the susceptibility to cancer in the presence of mismatch repair defects is highly cell type-specific, with colon cancer being more frequent than other tumour types in humans. In addition, neither mice nor human patients have been carefully studied for early age-related symptoms[55].

Genetic defects in DNA repair and premature ageing

The effects of DNA damage on the ageing process can be observed in multiple progeroid ('premature ageing-like') syndromes. Most of these progeroid syndromes are caused by germline mutations that compromise genome maintenance. These syndromes typically exhibit segmental ageing, as specific genetic defects in DNA repair accelerate the ageing process in specific cell types[56]. Investigation of the molecular mechanisms and pathological consequences of those progeroid syndromes has provided important conceptual and mechanistic insight into the role of DNA damage in ageing and age-associated diseases. The Cockayne syndrome, which is caused by genetic defects in TC-NER, leads to premature ageing in multiple different tissue types, underscoring the important role of transcription blockage caused by endogenous damage. Patients with Cockayne syndrome experience severe growth retardation and, in classical cases, succumb before reaching their teenage years, displaying a range of age-related pathologies, including neurodegeneration and arteriosclerosis[57]. Malfunctioning genome maintenance mechanisms also lead to other progeroid syndromes. For instance, in Hutchinson–Gilford progeria syndrome (HGPS), mutations in lamin proteins cause nuclear instability, resulting in elevated DNA damage[58]. Werner syndrome, one of the most recognizable manifestations of accelerated ageing due to its gradual onset typically beginning in the third decade of life, is caused by a defect in a RecQ helicase that leads to recombinational repair defects. Germline mutations in other members of the RecQ helicase family can also lead to Bloom syndrome and Rothmund–Thomson syndrome[59].

Currently, there are no curative therapies for progeroid patients. However, there are interventions that could slow the progression and mitigate pathologies. In NER-deficient prematurely ageing mice that model Cockayne syndrome-like progeroid syndromes, calorie restriction extended the lifespan up to twofold. The mouse studies suggest that calorie restriction reduces the DNA damage load, as assessed by reversing the gene length-dependent transcription decline that is triggered by transcription-blocking lesions[17,19]. In HGPS, a mutation in the *LMNA* gene gives rise to a farnesylated form of lamin A, termed progerin. The farnesyltransferase inhibitor lonafarnib modestly decreased mortality rates in patients with HGPS[60]. The mutations in the LMNA protein alter nuclear morphology and chromatin structure, leading to defects in DNA replication and repair. The similarities between progeroid syndromes and natural ageing, as assessed in mouse models[61], suggest that interventions mitigating premature ageing could also be implemented to decelerate normal ageing and prevent age-related diseases. New gene editing methodologies might eventually prove successful for the treatment of these monogenic progeroid syndromes.

Therapeutic targeting of the DNA damage response

The understanding of the role of genome instability in promoting the ageing process has started to advance the development of therapeutic strategies targeting the cellular response to DNA damage. Indeed, pharmacological targeting of the DDR has been particularly advanced through senolytics that eliminate SnCs and thus curb their antagonistic effect on health. Inflammatory responses have increasingly been linked to DNA damage via the activation of cGAS-STING through cytosolic DNA moieties and R-loops that are triggered by nuclear DNA damage. The DDR is a complex network of response mechanisms that act at the level of cells, tissues and the organism. It will be important to further dissect how pharmacological treatments affect the DDR network in the context of the physiology of the ageing organism.

DNA damage vulnerabilities and adaptation: lessons from cancer therapy

One of the most clinically relevant outcomes of DNA damage and its erroneous repair is mutagenesis as the main cause of cancer. Ironically, inflicting DNA damage also remains the main form of cancer therapy. Both radiation and chemotherapy rely on the induction of DNA damage to trigger a DDR that would lead to the apoptotic killing of cancer cells. However, these therapies also lead to an increase in cellular senescence, secondary cancers and premature ageing in long-term cancer-therapy survivors[62].

Taking advantage of the cancer-specific defects in DNA repair capacity can lead to a more tumour-specific therapy[63]. Defects in certain DNA repair pathways make cancer cells critically dependent on other repair pathways, which can be therapeutically targeted. Given that the DDR is orchestrated by a cascade of post-translational modifications, including phosphorylation, ubiquitylation, sumoylation and PARylation, there are numerous potential strategies to inhibit DNA

repair pathways, achieving synthetic lethality in cancer cells already harbouring specific DNA repair defects. For instance, cancer cells with defects in the HRR factor BRCA1 are particularly susceptible to PARP inhibitors such as olaparib, likely due to exacerbation of the cytotoxic effects of replication fork stalling amid unrepaired DNA damage[64]. Inhibiting the kinase DNA-PK, involved in DSB repair by non-homologous end-joining (NHEJ), has been shown to sensitize cancer cells to radiotherapy and chemotherapy, and shows promise in combination with other DNA repair-inhibiting approaches, such as the above-mentioned PARP inhibition. Together with DNA-PK, ATM and ATR are kinases belonging to the phosphoinositide 3-kinase (PI3K) family with critical roles in the DDR, for which multiple of inhibitors are being developed and used to sensitize cancer cells to DNA damage (reviewed elsewhere[65,66]). Kinase inhibitors that bind to such structurally related kinases can thus be utilized to effectively inhibit the DDR, for example, by simultaneously blocking DNA-PK and ATM. Drugs such as XRD-0394 specifically bind to these kinases and highly sensitize cells to DNA damage[67].

The combined use of DNA-damaging agents and specific repair inhibitors in cancer therapy remains to be fully explored. Cancer cell heterogeneity and high cellular turnover create many scenarios

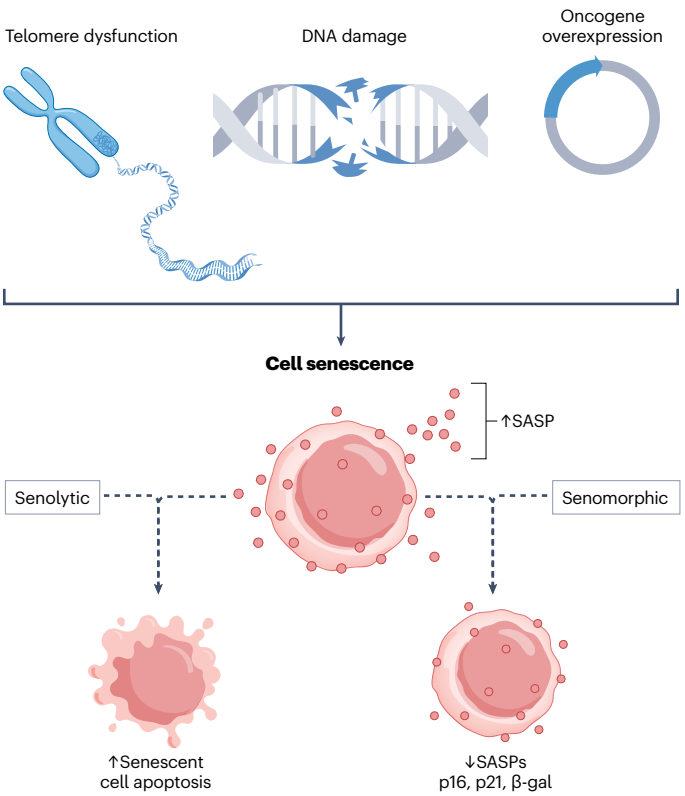

Fig. 2 | Causes of cellular senescence and pharmacological intervention strategies. Cellular senescence can be triggered by telomere dysfunction, both shortening or DNA damage (such as oxidative damage or strand breaks); DNA damage; or oncogene overexpression. Senescent cells are characterized by a permanent cell-cycle arrest and the senescence-associated secretory phenotype (SASP). Two main approaches are being followed to target senescent cells: the use of senolytics, to specifically induce apoptosis in these cells, and the use of senomorphics, to decrease the expression of SASP and senescence markers such as p16, p21 and β-gal.

of cellular adaptations to DNA damage and subsequent cancer recurrence. Some of these cases are directly connected to an altered DDR and include an improved DNA repair capacity. Many cancer stem cells express high levels of DNA repair genes involving all the major repair pathways (reviewed elsewhere[68]), which results in the survival of some of these cells and the later reappearance of the disease. This further connects stemness and repair potential (further discussed below in the section on cellular reprogramming). Interestingly, some cancers that survived DNA-damage-based therapies show similarities with cancer stem cells, including pluripotency markers and a higher expression of DNA repair genes[68]. This is the case in ovarian cancer, in which recurrence correlates with Rad6 expression[69]. Understanding how cancer cells acquire DNA damage resistance will likely lead to the discovery of novel mechanisms to enhance DNA repair in healthy people, thereby promoting resistance to neoplastic transformation of their normal cells.

Apoptosis and senescence: pharmacological killing of damaged cells

Given that genomic instability, including DNA damage and the DDR, is a primary hallmark of ageing, interventions that improve DNA repair should extend healthspan and possibly lifespan (Table 1). Among the outcomes of the DDR, cellular senescence has garnered considerable attention. Cellular senescence, first observed by Leonard Hayflick[70], refers to the cessation of cell division after a limited number of replication cycles of human primary fibroblasts. This phenomenon provided evidence that ageing is an inherent property of somatic cells. Cellular senescence is driven by multiple types of stress, especially by the DDR, whether it results from a critically shortened telomere, such as in replicative senescence, or from DSBs, as in oncogene-induced senescence, which is likely triggered by replication stalling amid strong oncogenic signalling[71,72]. In some progeroid mouse models, high levels of cellular senescence have been observed, particularly in the spindle checkpoint-defective BubR1 mouse[73]. Senescence can also be driven by viral infection and potentially other pathogens, including bacteria, through specific pathogen-associated molecular patterns[74–76]. Elimination of SnCs via a suicidal $p16^{Ink4a}$ or $p21^{Cip1}$-driven construct extends healthspan, improves resilience and reduces mortality[26,77].

The targeting of SnCs has been extensively investigated, considering the role of these cells in inflammation and ageing. As DNA damage is a well-known inducer of cellular senescence[78], treating or eliminating these cells is an approach to counter the negative effects of insufficient somatic DNA damage-repair capacities. Two main types of senotherapeutic drugs are being utilized: senolytics – able to selectively eliminate SnCs – and senomorphics – suppressors of specific properties of SnCs, such as the SASP (reviewed elsewhere[79,80]) (Fig. 2). Given that SnCs upregulate anti-apoptotic genes, termed senescent cell anti-apoptotic pathways, many of the identified senotherapeutics are re-purposed cancer drugs. As mentioned below, several of these drugs are currently being tested in clinical trials for many age-related diseases and conditions. These crucial studies also establish the basis of safety and tolerance that will facilitate future trials of these drugs for other age-related diseases. However, it is important to note that, given the heterogeneity in SnCs, leading to a variety of different senotypes, there is currently no single senotherapeutic able to target all types of senescence.

The first senolytic approach identified was the combination of dasatinib and quercetin (D+Q). Dasatinib[81] is a tyrosine kinase inhibitor that includes the anti-apoptotic ephrin receptor family[82] and is being

used to treat myeloid and lymphoblastic leukaemia. Quercetin is a flavonoid that interacts with the PI3K/AKT/mTOR and STAT3 pathways as well as Bcl-2 family members, promoting apoptosis. D+Q has been shown to selectively eliminate SnCs and is being, or has been, evaluated in multiple phase I and II clinical trials, including for age-related diseases such as Alzheimer disease (for example, NCT04685590), chronic kidney disease (NCT02848131) and osteoporosis (NCT04313634)[80,83–87]. Although in a recent phase II trial in postmenopausal women D+Q failed to reduce bone resorption, participants with the highest levels of senescence markers did benefit, suggesting that stratification according to senescence burden could enhance treatment efficacy[86]. Similarly, the anticancer drug navitoclax and related compounds targeting Bcl-2 and Bcl-xL also have senolytic activity, at least on certain types of SnCs (see below). Other senolytic and senomorphic drugs have similar mechanisms of action, also involving PI3K, STAT3 and Bcl-2 pathways. Fisetin is a flavonoid structurally similar to quercetin that triggers apoptosis in SnCs[88] by affecting multiple pathways involved in apoptosis, including BCL-2, PI3K/AKT and p53. It is being tested in I/II phase trials of osteoarthritis, and it is soon starting phase II and II/III trials for sepsis (NCT05758246) and joint injury (NCT05505747)[80,89]. For more information and trials of fisetin, the reader is referred to a published review[90]. Luteolin, reported to inhibit PI3K/AKT[91,92], was recently identified as the most abundant flavonoid in *Salvia haenkei* extract. In mice, luteolin inhibited p16-CDK6 and exerted a senomorphic effect[93]. It is also undergoing several trials[94], some related to its possible effects on behaviour and memory (NCT0504407) or to its neuroprotective effects (dementia, NCT04489017; ischaemic stroke, NCT06777680). Nintedanib is a tyrosine kinase inhibitor that inhibited STAT3 in SnCs, promoting cell death[95]. Success in clinical trials has led to its use in pulmonary fibrosis and non-small-cell lung cancer[96–99]. Novel STAT3 inhibitors also exerted a senomorphic effect[100]. Finally, treatment of a stroke-induced senescence mouse model with a JAK2/STAT3 inhibitor, AG490, also reduced SnCs and inflammation[101].

Multiple inhibitors of PI3K/AKT and mTOR have senolytic or senomorphic effects[102], including drugs previously in clinical trials for different cancers. For instance, PF-04691502 (ref. 103) has been tested in phase I and II trials against advanced tumours[104,105], although some trials were terminated due to poor tolerability (for example, NCT01430585), and PX-866 was tested in phase I and II trials of different cancers[106–110] (such as NCT01331083) and in a phase I trial in healthy individuals (NCT01408316). Several PI3K inhibitors are already being used clinically[111], such as BAY80-6946 (copanlisib) in follicular lymphoma, BYL-719 (alpelisib) in breast cancer, CAL-101 (idelalisib) and IPI-145 (duvelisib) in chronic lymphocytic leukaemia, and umbralisib for relapsed marginal zone lymphoma (for more information on PI3K inhibitors and trials involving new compounds, the reader is referred to another review[111]). Interestingly, albeit not linked to an effect on senescence, alpelisib supplementation extended lifespan in mice[112]. In a screen utilizing fibroblasts derived from prematurely ageing *Ercc1*$^{-/-}$ DNA repair-deficient mice, multiple HSP90 inhibitors, such as 17-DMAG, were effective senolytic agents; they were also active on other SnCs from mouse and human. HSP90 inhibition downregulated the PI3K/AKT pathway, which might explain the senolytic effect of these inhibitors[113]. Multiple HSP90 inhibitors have also undergone clinical trials for inflammatory diseases and cancer[114]. For 17-DMAG, clinical trials stopped after phase I (ref. 115), but other HSP90 inhibitors in trials against some cancers showed better results, such as TAS-116, in phase III against gastrointestinal stromal tumour[116] (JapicCTI-184094). For more information on clinical trials of HSP90 inhibitors, the reader is referred elsewhere[115]. However, little is known about the senolytic or senomorphic effects of many of the newest HSP90 inhibitors. For a review focusing on HSP90 inhibitors, the reader is referred elsewhere[117].

A range of drugs induces apoptosis by inhibiting the Bcl-2 family of anti-apoptotic proteins[118], which show increased expression in SnCs. One of the most studied of these is navitoclax (ABT-263), which can effectively inhibit BCL-2 and BCL-xL, removing SnCs in mice[119], and it recently completed phase II and III clinical trials and is undergoing another phase III trial to treat myelofibrosis (NCT04468984)[120–122]. ABT-737 targets BCL-2, BCL-xL and BCL-w, and exerts effective senolytic activity in cell culture and mice, although it is not orally bioavailable[118,123]. Additional compounds known to inhibit BCL and applied in senescence models include A1331852 and A1155463, selective for BCL-xL[124], and the leukaemia and lymphoma-approved drug ABT-199 (venetoclax), selective for BCL-2 and for which efficacy varies depending on the levels of BCL-2 in the specific SnC[118,125]. Beyond cancer, venetoclax was also recently tested in a phase I trial against pulmonary fibrosis (NCT05976217). Furthermore, the combination of different BCL-2 inhibitors with inhibitors of MCL-1 (such as MIK665) was shown to increase the senolytic effect[125] and has completed phase I and II trials for some cancers[126] (NCT03672695; NCT01989585). The phytochemical procyanidin C1 (PCC1), found in grape seeds, was shown to alter the expression of BCL-2, but its senolytic function is not yet fully understood. At low doses, PCC1 presents senomorphic effects, and it was reported to extend mouse lifespan and healthspan[127]. PCC1 has undergone a clinical trial for skin ageing targeting SnCs, but the results are not yet published (NCT06641869). Curcumin and its analogue EF24 also seem to influence BCL-2 by promoting its degradation, promoting SnC apoptosis[128]. As many of the BCL-XL/BCLl-2 inhibitors exhibit toxicity due to their high expression in platelets, proteolysis-targeting chimeras targeting BCL-2, BCL-xL and even BCL-w utilizing E3 ligases not present in platelets are under development, with promising initial results[129]. For more information on drugs exerting platelet toxicity and strategies to overcome this, the reader is referred to a published review[130].

Another natural compound with interesting senolytic properties is piperlongumine[131]. Applied in combination with ABT-263, this drug synergistically induces apoptosis of SnCs, a finding that led to further research on piperlongumine analogues with increased potency[132,133]. The mechanism of action of these compounds seems to involve oxidative stress and leads to the inhibition of the protein oxidation resistance 1 (OXR1)[134].

Other approaches worth highlighting, although not all clinically tested, include p53-related treatments such as a D-retro inverso FOXO4 peptide to inhibit the FOXO4-p53 interaction[135]; MDM2-p53 inhibitors such as UBX0101 (ref. 136), which has undergone clinical phase I and II trials for osteoarthritis with conflicting results[137] (such as NCT03513016); USP7 inhibitors such as P5091 (ref. 138); and several galactose-modified senolytic drugs, like galactose-modified duocarmycin[139], taking advantage of the high β-galactosidase activity in SnCs (reviewed elsewhere[79]). R406 (tamatinib) is a known Syk inhibitor that in SnCs resulted in inhibition of the focal adhesion kinase (FAK) and p38 MAPK. FAK's function is critical in adherent cells, and its inhibition can induce apoptosis in some cancer cells[140]. Considering that SnCs show a hyper-adhesive phenotype[141], it will be interesting to follow further tests on senolytics involving FAK. The related fostamatinib is being used to treat chronic immune thrombocytopenia and is recruiting for and has undergone multiple clinical trials. This includes a phase III trial for rheumatoid arthritis, which, despite some

positive indications, did not meet efficacy and safety expectations[142] (NCT01197755).

An additional feature of SnCs that can be targeted is their partially depolarized plasma membrane. Cardiac glycosides, used in some heart disorders, inhibit Na^+/K^+ ATPase pumps and are particularly effective at inducing apoptosis in SnCs, probably due to their membrane characteristics[143].

Finally, like most cell types, SnCs have a particular signature of membrane proteins and receptors that distinguishes them from other cells, allowing for approaches that can recognize these proteins and specifically target senescence. For instance, senescence leads to the upregulation of urokinase-type plasminogen activator receptor (uPAR). One of the most studied approaches to target SnCs involves the use of chimeric antigen receptor (CAR) T cells. In this case, when these cells targeted uPAR, SnCs were eliminated both in vitro and in vivo[144]. This study was recently expanded, showing that one single treatment in mice had long-term positive effects in normal ageing and obese mice[145]. The use of CAR T cells was also proven effective when targeting another class of highly expressed protein in SnCs, natural killer group 2 member D ligands (NKG2DLs). This approach eliminated SnCs in vitro, in mice and in nonhuman primates, also ameliorating age-related pathologies in mice[146]. CAR T cells are already being used in many clinical trials for cancer and autoimmune disorders[147] and could also be therapeutic in other DNA damage-related pathologies. The glycoprotein nonmetastatic melanoma protein B (GPNMB) is another transmembrane protein that can be targeted due to its high expression levels in SnCs. Genetic elimination of GPNMB[148] and, more interestingly, also vaccinating the mice against it, improved certain age-related pathologies both in normal and progeroid mice. Another vaccination approach targeting the protein CD153 to eliminate senescent T cells showed interesting results in obese mice[149]. Other antibody–drug conjugates targeting senescence-associated epitopes are directed against B2M, a highly expressed protein in SnCs[150], and against apolipoprotein D (ApoD), which is expressed on senescent dermal fibroblasts, and resulted in reduced dermal SnCs and improved skin phenotypes[151]. However, currently there is not one cell surface protein that is specific for SnCs or expressed on all SnCs.

A conceptually complementary approach to target SnCs is the use of senomorphics, which target phenotypes of SnCs rather than driving them into apoptosis, and, most importantly, are known to reduce the SASP. SASP describes the property of SnCs to secrete a range of cytokines, likely in response to persistent DNA damage, such as DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), but it is also triggered by leakage of nuclear or mitochondrial DNA into the cytosol, in which it can evoke inflammatory cGAS-STING signalling. Such senomorphics also include drugs currently studied due to their possible healthspan-extending and lifespan-extending properties, such as rapamycin and metformin (reviewed elsewhere[79]).

A major limitation of senolytic and senomorphic therapies stems from our current incomplete understanding of cellular senescence. Cellular senescence has not only been observed during ageing but also during mammalian development, when it has been suggested to contribute signalling cues for tissue remodelling. Moreover, during wound healing, cellular senescence appears to be essential by secreting PDGF and other factors to support tissue remodelling during the healing process[27]. Additionally, immune cells such as macrophages can obtain properties of SnCs while they exert anti-inflammatory effects on surrounding cells[152]. However, it is unclear if the transient SnCs involved in these beneficial processes express the same senescent cell anti-apoptotic pathways and other pathways that are targeted by known senolytics. At least in older mice, clearance of $p21^{Cip1}$ SnCs improved wound healing[153]. It is thus essential to gain a more complete understanding of the biology of cellular senescence, especially regarding molecular differences between transient and chronic SnCs.

Inflammatory DNA damage responses

Inflammation can drive tissue damage, and there is ample evidence that, during human ageing, chronic inflammation could be a major disease mechanism[154,155]. Inflammation typically arises when an innate immune response, such as that triggered by viral infection, remains unresolved. The anti-viral immune response, however, could also be triggered by cytosolic DNA originating from the nucleus or mitochondria. This has recently been observed to result from various forms of nuclear DNA damage that leads to replication or transcription stress. For instance RNAPII stalling at oxidative lesions, particularly at telomeres, was shown to trigger cytosolic release of telomeric DNA that could even be released via extracellular vesicles, leading to paracrine senescence[156]. DNA leaks in the cytosol can be detected by multiple pathways (see a relevant review[157]), which interestingly includes DNA-sensing proteins from repair pathways, such as Ku70 (ref. 158), DNA-PK[159], MRE11 (ref. 160) and Rad50 (ref. 161). In addition, induction of the retrotransposon LINE-1 transcription occurs in at least some SnCs, leading to an increase in the type 1 interferon response by the cytoplasmic LINE-1 complementary DNA[162,163].

Nucleic acids in the cytosol can be recognized by the cyclic GMP–AMP synthase (cGAS), which synthesizes 2′3′ cyclic GMP–AMP (cGAMP). cGAMP activates STING (stimulator of interferon genes), a well-known inducer of type 1 interferons and other inflammatory cytokines, which results in an immune response[164–166] (Fig. 3). However, the detection of cytosolic DNA can promote multiple human autoimmune and degenerative diseases and, thus, targeting these pathways could ameliorate the consequences of accumulated damaged DNA during ageing and in progeroid syndromes[167]. Interestingly, loss or reduction in cGAS also leads to upregulation of transcription of LINE-1 retrotransposons though loss of H3K9me3 heterochromatin marks[168], suggesting cGAS plays multiple roles in regulating the immune response to exogenous and endogenous pathogens, including regulating heterochromatin marks.

One of the major pharmacological routes to inhibit this pathway are cGAS antagonists that bind to its catalytic site. A high-throughput screening focused on inhibiting the enzymatic activity of cGAS found a class of compounds binding to its catalytic pocket. This study highlighted compound RU.521, which decreased interferon levels in an autoinflammatory disorder mouse model[169]. A similar approach identified other compounds and derivatives with positive results in different mouse models, including G140 and G150 (ref. 170), TDI-6570 (refs. 170,171) and 30d-S[172]. It is worth highlighting that, recently, the cGAS inhibitor VENT-03 (ref. 173) successfully underwent a phase I trial and will initiate phase II during 2025.

Another major pharmacological approach to inhibit the cGAS-STING pathway focuses on targeting the binding between cGAS and DNA. Normally, cGAS dimers bind to two molecules of DNA. Two antimalarial drugs, hydroxychloroquine and quinacrine, were found to bind and disrupt this complex[174]. Further development of this strategy led to the synthesis of a new antimalarial-like drug, X6, with a stronger effect than hydroxychloroquine in inhibiting cGAS and decreasing immune markers in mice with increased cytosolic DNA[175].

As summarized in the reviews mentioned below, other compounds disrupt the binding of cGAS and double-stranded DNA by directly competing with it. Finally, aspirin has been described to acetylate cGAS at critical locations for DNA binding[176]. An increasingly popular approach to inhibit this pathway focuses on targeting STING. The generation of cGAMP and its binding to STING leads to its oligomerization and translocation, and the activation of a downstream signalling cascade, eventually leading to increased expression of interferon and inflammatory cytokine genes[177]. An important discovery in targeting STING came from a series of screenings on molecules that covalently modified the STING cysteine Cys91, blocking the activation-induced palmitoylation of the protein, a modification necessary for its translocation from the endoplasmic reticulum to the Golgi, which is required for STING signalling. This study found several compounds, of which the most potent was H-151, and proved that STING inhibition was achievable pharmacologically, leading to decreased systemic inflammation in an autoinflammatory disease mouse model[178]. Derived from this study, the molecule C-176 was later used in models of neuroinflammation and neurodegeneration in mice[179]. Posterior screenings of molecules to target other STING cysteines include BB-Cl[180] and LB244, which modify Cys148 (ref. 181). Among other compounds, the nitro-fatty acid PPAR agonist CXA-10, which also leads to modifications of cysteines in STING, is worth highlighting, as it has completed phase II trials to treat primary focal segmental glomerulosclerosis (NCT03422510). Other drugs inhibiting STING target the cyclic dinucleotide binding domain where cGAMP binds; among such drugs, SN-011 decreased inflammation and prevented early death in mice with increased cytosolic DNA[182].

The cGAS-STING pathway can also be suppressed by decreasing the cGAMP levels indirectly with the drug T0901317 (ref. 183) by reducing STING transcription through the BRD4 inhibitor JQ1, which was used in retina degeneration models[184,185], or by inhibiting downstream effectors of STING such as TBK1 with drugs such as GSK8612 (refs. 186–189), or JAK2 with its inhibitor AG490 (ref. 101). The fact that many virally encoded peptides inhibit cGAS-STING, including peptides from HIV[190], varicella zoster[191] and the African swine fever virus, suggests drugs based on the mechanism of action of these peptides would be effective inhibitors (reviewed elsewhere[192]). For more details and compounds altering the cGAS-STING pathway, the reader is referred to published reviews[193–195].

A limitation for the repurposing of such drugs as geroprotectors might be a compromised response to infectious viral DNA. Furthermore, cGAS-STING plays a role in repressing early stages of cancer by promoting immune cell-mediated clearance of tumour cells; such role could be compromised[196]. To instead move closer to the instigator of inflammatory signalling, it is possible to reduce the aberrant cytosolic double-stranded DNA and target R-loops, by introducing DNase and RNase enzymes, respectively. Here, the challenges of delivering nucleases could be overcome by extracellular vesicles, exosomes that can be loaded with proteins and deliver their contents even through the blood–brain barrier. Recently, extracellular vesicles loaded with S1 nuclease, RNase H and/or RNase A were used to deliver these proteins to pancreatic cells of progeroid *Ercc1*$^{-/-}$ mice. These cells, with irreparable DNA lesions, accumulate cytosolic single-stranded DNA and promote inflammation. Treatment with the nucleases reduced the cytosolic DNA and the inflammatory signalling produced in the cells[29]. Extracellular vesicle-mediated elimination of cytosolic double-stranded DNA could also prevent neuroinflammation and neuronal cell death, as well as delay neurodegeneration in a mouse model of DNA repair-deficient microglia[197]. These approaches leave the anti-viral immune defence unaffected while taking a more causal targeting approach at the immunogenic cytosolic nucleic acid species. However, whether these strategies also delay the onset of age-related diseases during the normal ageing process still needs to be determined.

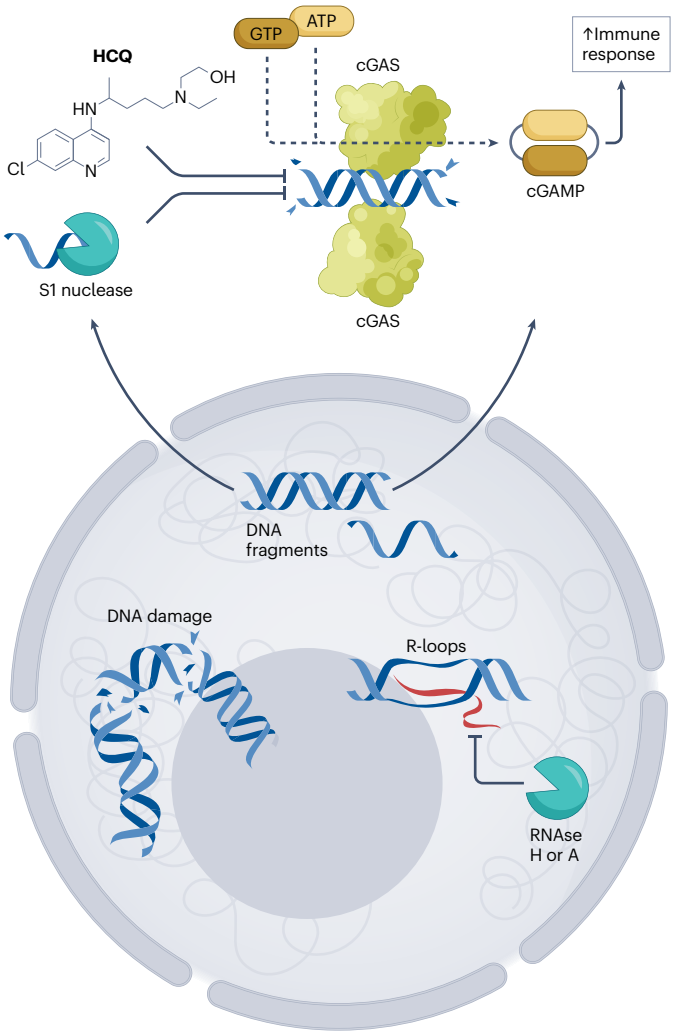

Fig. 3 | Inflammatory responses to DNA damage. DNA damage from different sources, including excessive R-loops, can lead to cytosolic DNA or DNA:RNA hybrids. Extraneous DNA-sensing proteins, such as cGAS, can detect this DNA and induce an immune response by generating 2′3′ cyclic GMP–AMP (cGAMP), which interacts with STING, activating the cGAS-STING pathway. Reducing the amounts of R-loops and cytosolic DNA with nucleases and RNAses or the inhibition of cGAS-STING (with molecules such as hydroxychloroquine (HCQ)) are possible approaches to reduce immune disorders associated with DNA damage. Blue ribbons represent DNA strands and the red ribbon represents an RNA strand.

Telomere dysfunction

One of the best-understood drivers of cellular senescence are critically shortened telomeres. This shortening occurs due to telomeric DNA damage, particularly induced by reactive oxygen species, and the end-replication problem, caused by the inability to completely replicate the lagging strand at chromosome ends and end resection of the leading strand. Consequently, chromosomes shorten with

each round of replication. Telomerase, primarily active in germ cells and to some extent in somatic stem cells, can compensate for this by efficiently extending telomeric sequences. Somatic cells, however, switch off telomerase, likely as a tumour suppressor mechanism to limit somatic-cell proliferation. The vast majority of cancer cells reactivate telomerase to support their indefinite growth[198]. Normal telomeres are protected by the shelterin complex from eliciting the DDR. When, however, a telomere becomes critically shortened, shelterin no longer provides protection, leading to DDR activation, the formation of telomere-associated foci or telomere-induced foci, and induction of cellular senescence. An unprotected and thus dysfunctional telomere thereby becomes an important DNA damage type. Interestingly, telomeres present low repair capacity and might even serve as a 'sponge' of DNA damage that can also induce a persistent DDR[199]. Indeed, the majority, if not all, SnCs have telomere-associated foci.

Telomere shortening has consistently been observed during human ageing of mitotically active cells or tissues, and the relative shortening correlates with mortality risk, suggesting a causal relationship between the speed of shortening and ageing[200]. The concept of extending lifespan by elongating telomeres was proposed decades ago, and the reports of lifespan extension in mice overexpressing telomerase indicate that increasing telomerase activity could be therapeutic[201,202]. However, caution is warranted due to the recognition that telomerase activity is a critical factor supporting tumorous growth[203]. Furthermore, as telomeres are particularly relevant for replicative capacity, the direct benefits of such therapies are likely to be rather specific to dividing cells. In humans, dyskeratosis congenita is a disorder with decreased telomere maintenance that results in defects in muco-cutaneous and bone marrow systems, and an increased risk of some cancers, with rare cases presenting other phenotypes[204].

Mechanisms to boost DNA repair

The age acceleration effect of unrepaired DNA damage is particularly apparent in progeroid syndromes that are caused by defective DNA repair. The critical question remains as to whether age deceleration can be achieved through improved DNA repair mechanisms. Overexpressing DNA repair genes has been attempted, with mixed results, with some studies demonstrating beneficial effects[205–207] and others showing no significant or even antagonistic outcomes[206,208–210]. This variability in outcomes can be understood when considering the complexity of DNA repair mechanisms. For instance, the core NER pathway involves more than 30 different proteins[211] that operate in concert, requiring a balanced stoichiometry, which could easily be disturbed by overabundance of a single NER protein, especially if the NER protein is an enzyme with DNA endonuclease activity.

Notable advances have recently been made in therapeutically targeting the DNA repair mechanisms, from improving the repair function of nicotinamide adenine dinucleotide (NAD^+)- utilizing enzymes to now augmenting the overall armament of DNA repair systems via dimerization partner, retinoblastoma-like, E2F and multi-vulval class B (DREAM) inhibition. Distinct cell types show distinct DNA repair requirements and restrictions, and it will thus be important to establish how cell types ranging from highly proliferative haematopoietic cells to terminally differentiated cell types could benefit from boosting DNA repair. Furthermore, given the complexity of DNA repair mechanisms, it will be important to study those approaches in a physiological context and determine beneficial and potentially adverse effects.

Usage of highly effective DNA repair enzymes

One approach to enhance DNA repair, which is already commercially available, revolves around the use of non-human proteins that use a particularly efficient DNA repair mechanism (Fig. 4). Photolyase proteins were among the first repair enzymes discovered, and they have been characterized for their highly efficient removal of UV-induced lesions[212]. The different kinds of photolyases bind with high selectivity to the UV-induced cyclobutane pyrimidine dimers (CPDs) or pyrimidine-(6-4)-pyrimidone photoproducts and use visible light to confer the electron transfer to split those dimers into the pre-existing undamaged bases[212–214]. Photolyases are used by species that are exposed to high levels of UV light. CPD lesions are the culprit of UV-induced carcinogenesis, and expressing photolyases in keratinocytes is sufficient to effectively protect mice from UV-induced carcinogenesis[215,216]. In addition to photolyases, the T4 endonuclease V, which was originally isolated in *Escherichia coli* that had been infected with the T4 bacteriophage, splits CPDs, but without the requirement of energy from blue light[217,218]. The delivery of such enzymes has been achieved in human skin by encapsulating them in liposomes that, upon application on the skin, can pass through the stratum corneum and reach epidermal cells[219]. These treatments are limited to the repair of UV-induced damage in the skin, and even though there has been some debate over their effectivity in preventing skin ageing compared with regular sunscreen, improved lesion removal has been reported in healthy individuals and in those with NER-defective xeroderma pigmentosum (reviewed elsewhere[220]).

An intriguing strategy for improving genome stability involves harnessing repair enzymes from species known for their remarkable resistance to DNA damage. *Deinococcus radiodurans* is a bacterium that has adapted to living in some of the harshest ecological niches and has evolved very high radiation resistance. The DNA-damage resilience of *D. radiodurans* is supported by a combination of effective antioxidant defence and repair enzymes[221–223]. Examples of radioresistant animals are tardigrades, invertebrates that have been linked to the DSUP protein that protects the genome structure of these water bears[224]. Although initial reports suggested that DSUP expression in human fibroblasts could confer radiation resistance, later findings that DSUP triggers death of cultured primary murine neurons hampered the prospects of transferring this species-specific damage resistance mechanism to humans[225]. A recent multi-omics study of the tardigrade DDR has suggested a combination of radical scavenging, DNA repair and NAD^+-generating processes that contribute to their DNA-damage resistance[226], and it will be highly interesting to further explore how such response mechanisms could be transferred to human cells.

NAD^+ supplementation to enhance PARP-dependent DNA repair

The NAD^+ molecule has indeed been proposed as an approach to improving DNA repair (Fig. 5). The oxidized NAD^+, reduced NADH and phosphorylated forms, $NAD(P)^+$/NAD(P)H, have important roles in energy production, redox reactions, and as cofactors and substrates of many enzymes[227]. In relation to DNA repair, a crucial role of NAD^+ is as a substrate for PARPs. These enzymes cleave NAD^+ producing nicotinamide and ADP-ribose, followed by the binding of the ADP-ribose to PARP itself or other acceptor proteins (alone or as part of a polymer of ADP-riboses), in a process called PARylation. PARP proteins can recognize DNA strand breaks, leading to PARylation and recruitment of DNA repair proteins involved in single-strand break repair by base excision repair and DSB by homologous recombination and NHEJ, and by promoting chromatin remodelling in NER (reviewed elsewhere[228,229]).

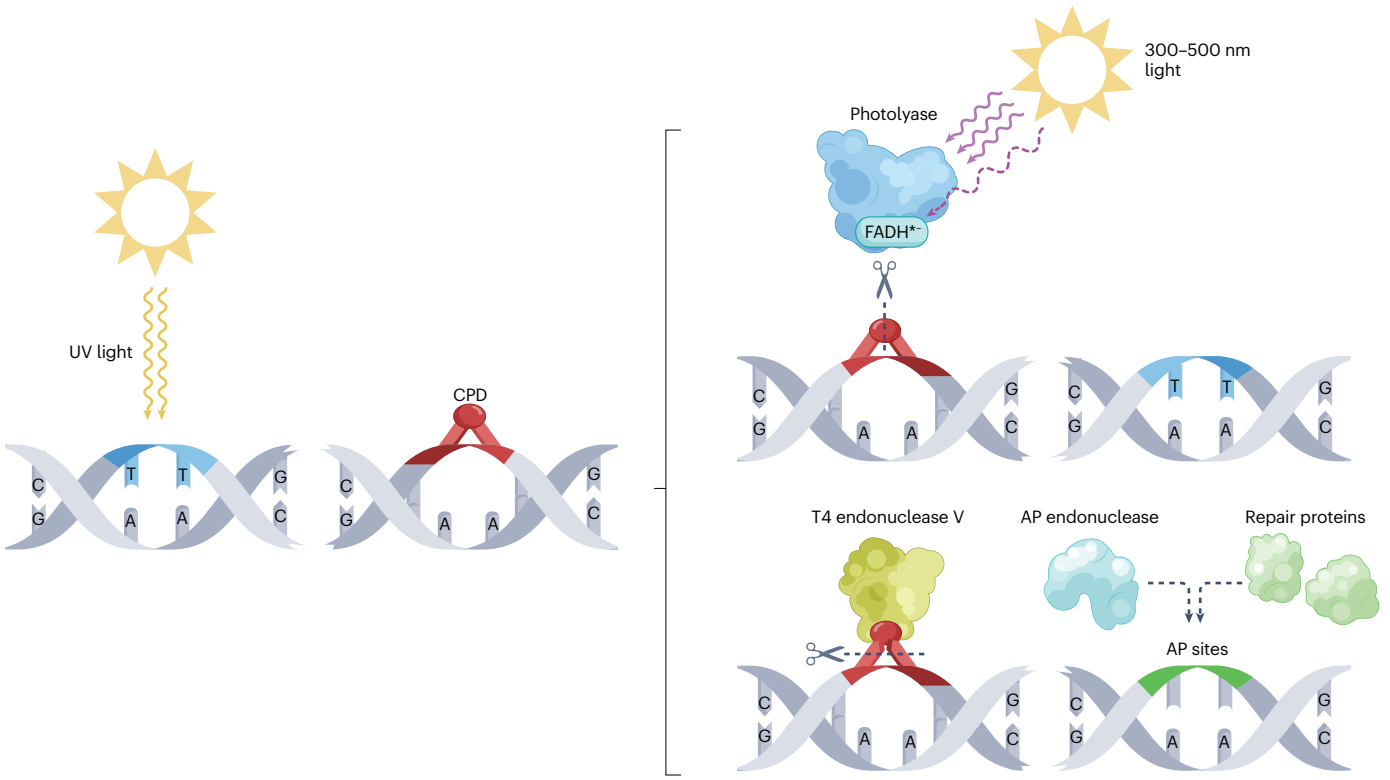

Fig. 4 | Single-enzyme DNA repair reactions. Bulky DNA lesions, such as cyclobutane pyrimidine dimers (CPDs), can be repaired by externally applying enzymes such as photolyase or T4 endonuclease V. Photolyase flips the CPD lesion accommodating it to its catalytic site. This enzyme absorbs photons at wavelengths around the 300–500 nm range and transfers the energy to a flavin ($FADH^-$), which transfers the electron to the CPD, splitting it back to two pyrimidines[343,344]. The T4 endonuclease V flips the CPD, and, by DNA glycosylase activity, it removes the damage, leaving apurinic/apyrimidinic sites (AP sites), which will be repaired by the base-excision repair pathway[345]. UV, ultraviolet.

Furthermore, NAD^+ is also a required cofactor of the protein deacetylase family of sirtuins, involved in multiple metabolic processes, stress responses and ageing[230]. In DNA repair, the most studied sirtuins are SIRT1 and SIRT6, although other sirtuins may also play a role by regulating cell cycle, reactive oxygen species production or mitochondrial DNA repair (reviewed elsewhere[231–233]). SIRT1 is recruited to areas with damaged DNA, promoting gene silencing and DNA repair[234–237], such as homologous recombination, NHEJ and NER, by deacetylating WRN[238], Ku70 (ref. 239), and XPA[240] and XPC[241], respectively. SIRT6 is involved in DSB repair, and its deficiency leads to accelerated ageing in mice[242]. SIRT6 activity correlates with lifespan of different species[243], and its overexpression can extend lifespan in mice[244]. SIRT6 was reported to impact several repair mechanisms, base excision repair, homologous recombination and NHEJ, by influencing PARP1 activity[243,245], global genome NER via deacetylating DDB2 (ref. 246), and NHEJ by stabilizing DNA-PK[247]. SIRT6 might also promote DNA repair by indirectly increasing H3K36me2 levels in damaged areas[248], and, by interacting with CHD4, it opens chromatin, allowing DNA repair recruitment[249]. Recently, a rare genetic variant of SIRT6 has been identified in the human centenarian genome that increases the mono-ribosylation of SIRT6 and is suggested to enhance genome stability, increasing HRR and NHEJ activity in cell culture assays[250]. Recently, fucoidan, a sugar found in brown seaweed, was shown to stimulate the mono-ribosylation and DNA repair activities of SIRT6 and increase healthspan in naturally aged mice[251].

Decreased levels of NAD^+ have been associated with multiple diseases and ageing, and research to increase NAD^+ levels has gained momentum with the use of the NAD^+ precursors niacin, nicotinamide riboside, nicotinic acid, nicotinamide (vitamin B3), nicotinic acid riboside and nicotinamide mononucleotide, among other compounds (reviewed elsewhere[228]). The use of these supplements has shown some results in cell culture, with reduction of DNA damage and increased repair in lymphocytes[252] and peripheral blood mononuclear cells[253]. However, most of the research involving DNA-damaged cells with NAD^+ supplementation indicate that the positive effects might be due to its roles in energy metabolism and mitochondrial homeostasis. This is the case for NAD^+ supplementation in XPA-deficient animals due to the effects of DNA damage in PARP overactivation and the consequent negative effects in mitophagy[254,255]. Similarly, ATM-deficient cells and mice supplemented with NAD^+ showed improvements in DNA repair and mitophagy[256]. NAD^+ supplementation also rescues the mitochondrial dysfunction and transcriptomic profile in Cockayne syndrome models that present PARP overactivation[257], preventing hearing loss of Cockayne syndrome mice[258] and improving the cognitive function of an Alzheimer disease model by decreasing damage, inflammation and apoptosis of hippocampal neurons[259].

The NAD^+ supplement nicotinamide has undergone a phase III clinical trial in patients with nonmelanoma skin cancer, showing a

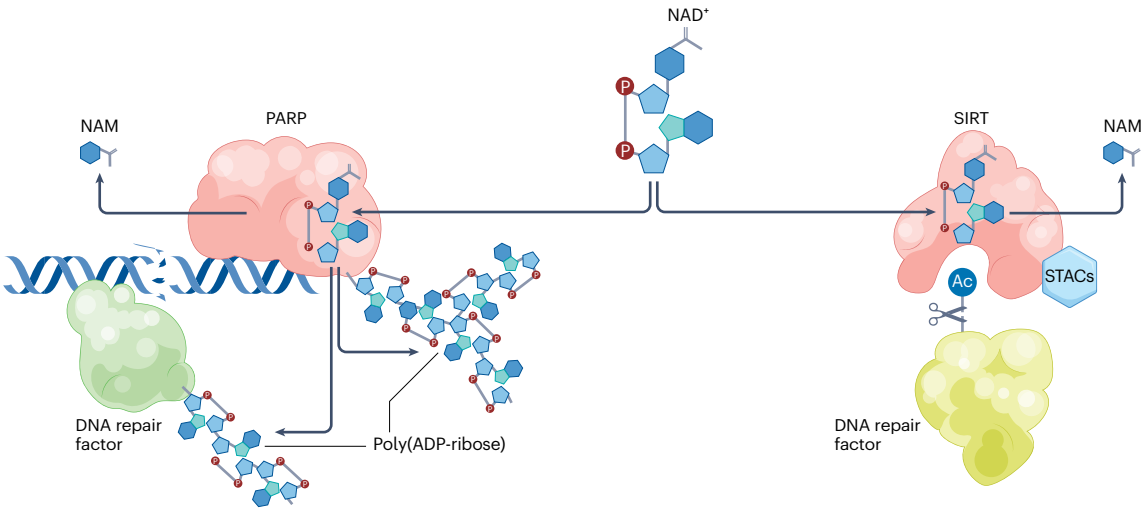

Fig. 5 | Promotion of DNA repair regulator activity by nicotinamide adenine dinucleotide (NAD^+). NAD^+ is used as a substrate by poly(ADP-ribose) polymerases (PARPs), which form chains of ADP-riboses and produce nicotinamide (NAM). The ADP-ribosylation of proteins around DNA damaged sites serves as signalling to recruit DNA repair and chromatin-remodelling machinery. NAD^+ is also consumed by sirtuins (SIRT), which catalyse a deacetylation reaction that activates proteins involved in DNA repair. NAD^+ supplementation or the use of sirtuin-activating compounds (STACs) is being explored. Ac, acetyl group.

decrease in the rate of cancer reappearance (20–30% decrease) and actinic keratoses (11–20% decrease)[260]. Patients with Alzheimer disease receiving nicotinic acid or vitamin B3 in a phase II trial did not show improved cognitive performance[261]. However, patients with ataxia–telangiectasia receiving nicotinamide riboside for 2 years in a phase II trial showed improvements in coordination[262]. A similar study with patients with ataxia–telangiectasia receiving vitamin B3 is undergoing phase II trial (NCT03962114), and nicotinamide riboside supplements in patients with Parkinson disease have been tested in a phase I trial, with a phase III trial initiating soon (NCT03568968)[263]. Recently, a phase II trial using nicotinamide riboside treatments in patients with chronic obstructive pulmonary disease, a highly prevalent disease associated with DNA damage, resulted in a reduction of inflammatory markers (NCT04990869)[264]. Other age-related diseases are being pursued through increasing NAD^+ levels, such as a phase II/III trial against chronic kidney disease (NCT06866236) using the NAD oxidase modulation agent QRX-3, and nicotinamide riboside supplement in a phase IV trial to improve brain health in elderly people (NCT05483465). For more examples of clinical trials, many of which are in early stage or have yielded mixed results, the reader is referred elsewhere[265].

Therefore, NAD^+ supplementation is a promising approach, but it is currently difficult to discern how much of the positive effects of NAD^+ supplementation in DNA damaged cells involves DNA repair or the downstream consequences of the damage in energy production and mitochondrial homeostasis. Future phase II and III clinical trials will further evaluate the efficacy of this approach.

The pharmacological activation of SIRT6 has been mostly studied around the compound UBCS039 (ref. 266). Activated SIRT6 has shown some positive effects, such as reduced senescence induction in cultured myocardial cells[267] and ameliorated liver damage in a liver failure model[268], although more research is needed to elucidate the effect of SIRT6 in DNA repair and its possible uses in human disease.

DNA repair by gene therapy

Increasing DNA repair capacity is of particular relevance in the case of genetic disorders characterized by increased genome instability, such as HGPS, Werner syndrome and Cockayne syndrome. Recent advances in gene therapy approaches for such genetic diseases have been spurred by the revolutionary progress in gene editing techniques witnessed over the past decade. HGPS mouse models carrying the human *LMNA* gene with the c.1824 C>T mutation have been treated with adeno-associated virus encoding Cas9-derived adenine base editors targeting this mutation. Treated mice were healthier, and their lifespans were more than doubled[269]. Similarly, induced pluripotent stem cell-derived mesenchymal stem cells and neural stem cells from a patient with Cockayne syndrome, with the genetic defect corrected by CRISPR–Cas9, presented less senescent markers, less γH2AX, better differentiation potential and decreased attrition, when the mesenchymal stem cells were implanted in mice[270]. In induced pluripotent stem cell-derived mesenchymal stem cells from a patient with Werner syndrome, the correction of the mutation with CRISPR–Cas9 led to improved differentiation, angiogenesis and wound healing[271]. Challenges remain, particularly in terms of gene therapy delivery, given that many progeroid syndromes just like the normal ageing process affect a multitude of cell types.

The rationale for using gene editing in patients with currently incurable genetic diseases caused by a mutation in a single DNA repair gene in a relevant percent of cells is, although practically challenging, conceptually straightforward. However, improving overall genome stability beyond the normally limited somatic DNA repair represents a more complex undertaking. Altering a single gene within a repair pathway in a healthy individual is unlikely to cause substantial improvements, considering the complexity of repair pathways and the likelihood of stoichiometrically limiting elements.

Epigenetics and cellular reprogramming

Cellular reprogramming of differentiated somatic cells into induced pluripotent stem cells is currently pursued as a therapeutic strategy for

age-related diseases[272] and was suggested to have potential for rejuvenating cells in the ageing organism[273]. Indeed, cellular reprogramming by the Yamanaka factors has been observed to reverse the epigenetic clock and the age-associated stochastic variation, suggesting that the epigenetic code could be reset[40,274–276]. During reprogramming, the p53-mediated DDR elicits a strong selection process that only allows a very limited number of cells to assume stem-cell properties[277]. Genomically compromised cells might thus be weeded out. Not only did partial reprogramming of progeroid Ercc1 mutant mice promote DNA repair, but it also restored the epigenetic clock of these animals[278]. As epigenetic clocks are based on changes in the CpG modifications[279], it will be interesting to explore how far resetting of the CpG methylation landscape, for instance through DNA methyltransferase inhibitors, could be effective. DNA methyltransferase inhibitors are being used in therapies for myelodysplastic syndrome and acute myeloid leukaemia, and they have been suggested to hold therapeutic potential for applications in various diseases, including other cancers, and cardiovascular or neurological diseases[280]. More research is required to assess whether DNA methyltransferase inhibitors could have positive effects in healthy individuals, but there are important advancements in developing inhibitors with fewer side effects and decreased toxicity that could be used to study the impact of methylation levels in ageing[281]. Likewise, loss of heterochromatin is also a hallmark of ageing cells and contributes to their functional decline, as for instance described for ageing mammalian oocytes. Pharmacological compounds, for example SIRT1 activators such a SRT1720, can restore heterochromatin structure and improve oocyte maturation[282]. On the organismal level, SRT1720 has also been reported to extend lifespan and healthspan in mice[283]. There has been controversy on the mechanism of SRT1720 and related molecules regarding their direct activation of SIRT1 (refs. 284,285), although later research indicated that these types of molecules could interact with SIRT1 (refs. 286–288).

It will be interesting to explore further how far DNA repair mechanisms are triggered during cellular reprogramming or the more recently pursued partial reprogramming in vivo, and how effectively DDR activation could remove the cells that have accumulated most DNA damage.

Hyperactivating all major repair pathways by inhibiting the DREAM complex

Different cell types have distinct capacities to repair DNA, and we propose that augmenting the overall repair capacity in those cells whose repair repertoire is limited could be a viable strategy to achieve improved genome maintenance to benefit the organism. For instance, some stem cell types, such as the haematopoietic stem cell, mostly reside in quiescence, during which they have limited DNA repair capacities, in contrast to progenitor cells that actively cycle. Haematopoietic stem cells tend to utilize error-prone NHEJ, leading to the age-dependent increase in structural variants[289]. Postmitotic neurons particularly rely on TC-NER, as they predominantly require repair of transcribed genes, but do not necessarily need to survey their entire genome as replicating cells do.

The most distinguished maintenance of genome stability can be found in the dichotomy of germ and somatic cells. As germ cells perpetuate the genome indefinitely throughout the existence of a species, they require high genome maintenance mechanisms. Indeed, germline mutation rates are at least an order of magnitude lower than somatic mutation rates, arguing for more effective DNA repair[290]. In addition, the germline also controls the stability of gametes through a sensitive DDR that triggers the apoptotic demise of genomically compromised cells. Germline genomes in modern *Homo sapiens* have been perpetuated for hundreds of thousands of years, and in some species for hundreds of millions of years, without striking alterations, suggesting a highly efficient combination of DNA repair, in addition to the selection for gametes with the most stable genomes. The distinct DNA repair capacities between germline and somatic cells suggest the presence of a common regulatory mechanism that may restrict somatic repair.

To identify such regulatory mechanisms, the simpler genome structure of more ancestral species could provide interesting new insights. Indeed, the nematode *Caenorhabditis elegans* shows some of the most distinct properties of germ and somatic cells. Most cell divisions are completed during embryonic development and in adult worms, all somatic cell types are postmitotic, whereas the germline retains its dynamics with ongoing germ stem cell proliferation followed by meiotic differentiation during gametogenesis. Here, DNA repair mechanisms such as HRR and global genome NER are exclusively expressed in germ cells but absent in the post-embryonic somatic cell types[291,292]. Whether the limited expression of DNA repair genes in somatic cells compared with the germline might be regulated by a common transcription factor was recently investigated. A promoter analysis indeed identified the cycle-dependent element (CDE) and cell-cycle gene homology region (CHR) elements in the majority of DNA repair genes in *C. elegans*. The CDE-CHR motif is recognized by the DREAM repressor complex[293–297]. Consistent with DREAM-mediated repression, loss-of-function mutants of the DREAM complex show an upregulation of multiple genes involved in all main pathways of DNA repair, leading to elevated resistance to all DNA damage types tested[298] (Fig. 6).

The DREAM complex is a highly conserved gene repressor first described in *Drosophila melanogaster*, and subsequently in *C. elegans* and humans[299–301]. In *C. elegans*, DREAM gene repression has been described to involve H3K9me2 in the promoter mediated by MET-2, the major H3K9me2 histone methyltransferase[302,303] and H2A.Z gene body enrichment[303,304]. The most well-described function of the DREAM complex is the repression of cell-cycle genes involved in G1-S transition and the promotion of cellular quiescence (reviewed elsewhere[305]). Interestingly, in *C. elegans*, mutations in the DREAM complex components lead to resistance to UV with increased repair capacity, and to DSBs repaired by HRR and NHEJ, cisplatin-mediated crosslinks, and MMS-induced alkylation damage[298]. Transcriptomic and proteomic analysis combined with promoter analysis and ChIP-seq data from multiple studies revealed that the DREAM complex targets and represses multiple genes involved in all major repair pathways[297,298,304,306,307]. Interestingly, this also rescued the sensitivity to DNA damage of animals lacking TC-NER, global genome NER or HRR, highlighting how certain damage types can be redundantly repaired by multiple mechanisms that, when upregulated, can partially compensate for the lack of another[298]. The transcriptional upregulation of DNA repair genes thus far has been reported as limited, responsive to damage and specific to it, and with often limited biological relevance[308,309]. By contrast, mutations in DREAM result in the upregulation of the basal expression levels of multiple DNA repair genes without previous damage. The resulting increased DNA repair efficiency of multiple pathways makes targeting the DREAM complex a promising new approach to improve overall genome maintenance. Interestingly, the DREAM-targeted DNA repair genes are normally predominantly expressed in the germline, suggesting that, indeed, DREAM mutations lead to germline-like DNA repair capacities in the soma.

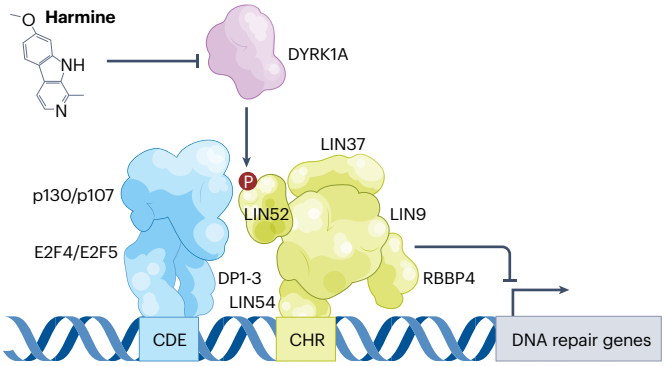

Fig. 6 | Regulation of DNA repair capacities by the DREAM repressor. The dimerization partner (DP), retinoblastoma-like, E2F and multi-vulval class B (DREAM) repressor complex can repress DNA repair genes by binding to cycle-dependent element (CDE) and cell-cycle gene homology region (CHR) motifs in the promoter of genes. The dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) regulates DREAM assembly by phosphorylating the protein LIN52, which allows the binding with the pocket protein p130 or p107. The use of inhibitors of DYRK1A such as harmine can inhibit DREAM formation, leading to the upregulation of DNA repair genes. RBBP4, retinoblastoma binding protein 4.

The assembly and function of this complex is regulated mostly at the binding between the protein LIN52, which is part of the CHR motif-binding multi-vulval class B (MuvB) subcomplex, and the pocket protein p130/p107, which, in complex with E2F and the dimerization partner, binds CDE sites. First, upon phosphorylation of the pocket protein p130 by cyclin-dependent kinases (CDKs), there is a reduction in DREAM formation[310,311], suggesting an expected cell-cycle control of DREAM assembly. Second, the phosphorylation of the LIN52 protein at serine 28 is crucial for the specific interaction of LIN52 with p130 and p107. In mammals, this phosphorylation is mediated by the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A)[310,312]. The inhibition of this kinase using two compounds, harmine[313] and INDY[314], in quiescent human cells also lead to a DREAM de-repression of DNA repair genes and a consequent resistance to UV and alkylation damage[298]. Furthermore, the treatment of NER-deficient mice carrying an *Ercc1*$^{-/-}$ mutation with harmine decreased DNA damage in the retina and photoreceptor loss, which is a hallmark pathology in patients with progeroid Cockayne syndrome and also normally occurs particularly in the dry form of age-related macular degeneration[298].

DYRK1A plays an important role in Down syndrome that could further highlight the relevance of DREAM in the regulation of DNA repair and its consequences. Down syndrome is caused by a full or partial trisomy 21, which results in intellectual disability, shortened lifespan and increased likelihood of developing several conditions, such as Alzheimer disease[315], among others. Down syndrome has been considered by many as the most frequently occurring segmental progeroid syndrome[316–319] with accelerated ageing clocks[318,320], increased cellular senescence[321] and, interestingly, increased DNA damage and/or decreased damage repair[318,322–333]. DYRK1A, located in chromosome 21, contributes to multiple Down syndrome phenotypes[334–336], and a patient with a duplication of chromosome 21, comprising only 31 genes that included DYRK1A, presented increased senescence and DNA damage. Treating cells derived from this patient with the DYRK1A inhibitors harmine and ID-8 reduced the damaged DNA[318]. These results support the role of DYRK1A in repressing DNA repair, likely by promoting DREAM-complex assembly and the repression of its target genes[298]. It is worth highlighting that harmine has undergone phase I clinical trials and, more importantly, that phase II trials using the DYRK1A inhibitor epigallocatechin-3-gallate have been conducted in patients with Down syndrome (NCT01394796); in patients with Down syndrome aiming to delay Alzheimer disease progression, leading to improvements in some cognitive tests[337] (NCT01699711); and in phase II/III trials for early-stage Alzheimer disease (NCT00951834). However, harmine and, especially, epigallocatechin-3-gallate have multiple targets beyond DYRK1A, which complicates the utility of these compounds. Motivated by the relevance of DYRK1A in several phenotypes of Down syndrome and other diseases, multiple inhibitors with increased efficiency and fewer side effects have been developed. This is the case for leucettinib-21, entering phase I clinical trials[338], and many others with interesting properties yet to be clinically tested (reviewed elsewhere[339]).

DYRK1A itself might have a range of targets affecting multiple pathways and many of its inhibitors have off-targets[339]. It will thus be important to further improve the specificity of DYRK1A inhibitors and ascertain how improved DNA repair via DREAM inhibition could be achieved. To this end, more specific strategies to target the DREAM complex and also to mitigate potential non-DNA-repair effects of targeting DREAM are needed. Here, opportunities to use antisense oligonucleotides, small interfering RNA knockdown or proteolysis-targeting chimeras targeting components of the DREAM complex could improve specificity. LIN52, as a bridging component between the MuvB and p130/p107, might provide a particularly interesting drug target, also due to its DYRK1A-mediated S28 phosphorylation that determines the dynamics of DREAM assembly[312]. Improved molecular specificity of targeting DREAM function might be achieved by gene editing methods, including CRISPR–Cas9 edits of such regulatory sites, for example, on LIN52.

The function of the DREAM complex as a master regulator that curbs overall DNA repair capacities opens a new avenue for augmenting genome stability, which could potentially both reduce somatic mutation accumulation and thus lower cancer risk, and preserve an intact genome, thus reducing the risk of age-related diseases and extending healthy lifespan. However, due to the role of the DREAM complex in regulating the expression of cell-cycle genes, its inhibition could lead to other challenges in cells that are not fully differentiated or in cells prone to division. Further studies at the organismal level are needed to discern which cells and tissues could benefit most and tolerate better the inhibition of this complex, without compromising their function or progression through cell cycle. Given that DREAM abrogation itself is usually insufficient to drive cells into S-phase, however, suggests that DREAM inhibition could offer a route for harnessing the benefits of improved DNA repair.

Outlook

The recognition of the widespread consequences of DNA damage to the functional integrity of cells has provided a more complete picture of the underlying mechanisms of ageing and risk factors for age-related diseases[3]. DNA repair is one of the most conserved complex molecular processes in a cell. The plethora of distinct lesion types and the irreplaceability of the nuclear genome underscore the unique role of genome maintenance among longevity assurance systems. As human life expectancy continues to increase, the need for effective interventions to maintain genome integrity becomes ever more critical for sustaining health and longevity in ageing populations and mitigating

the age-dependent cancer risk. Today, strategies that target some of the phenotypic consequences of DNA damage, collectively called the DNA damage response or DDR, including cancer, inflammation and cellular senescence, are in clinical trials or close to clinical translation. Moving forward, there is growing interest in developing strategies to enhance somatic DNA repair capacities in normal somatic cells, akin to those seen in germ cells, to better preserve genomic integrity throughout an individual's lifespan. Some of these strategies, such as the use of NAD^+ supplements or SIRT6 activators, are already being pursued. However, the ultimate goal would be to increase genome maintenance capacity globally, across all somatic cells, but this has been essentially constrained by the lack of a master regulator. The DREAM complex provides the first major target for such systematic strategies aimed at conferring germline-like repair capacities to the soma, with the potential to drastically increase human lifespan.

How could such geroprotective strategies be implemented into preventive medicine? One important hurdle to overcome is the implementation of ageing biomarkers instead of specific diseases as endpoints[340]. However, there are clinical indications that are closely linked with the slowing of age-related degenerative pathologies. For instance, trials on senolytics have targeted specific age-associated diseases, such as osteoarthritis or age-related macular degeneration. Here, clinical endpoints of a reduced disease progression, such as a reduced expansion of the geographic atrophy area in the retina, could provide important proof-of-concept trial models. A reduction of disease progression might turn out to be sufficient to delay age-related functional decline and frailty. In this regard, chronic kidney disease could serve as a clinical paradigm in which the maintenance even of a reduced number of functional glomeruli might be sufficient to maintain kidney function. Moreover, targeting such chronic diseases could also reduce co-morbidities. For instance, chronic kidney disease has been linked to an increased risk of numerous other co-morbidities, suggesting that maintaining kidney function could mitigate overall risk of multimorbidity[341]. In addition, some metabolic interventions have shown protective effects from age-related multimorbidity, such as the recently reported geroprotective properties of GLP-1 agonists[342].

Medicine may gradually migrate from the current exclusive treatment of specific diseases to the era of geroprotective medicine, in which at least some aspects of multimorbidity can be targeted simultaneously to promote health instead of treating disease. Conventional disease treatment will overburden a society in which a third of the population is elderly, of whom more than half suffer from multimorbidity. It is thus pertinent to implement ageing biomarkers, such as biological ageing clocks, as clinical study endpoints. Clearly, the ageing clocks need clinical validation and must be able to detect individual ageing trajectories and personal disease risks. Such ageing biomarkers could then be applied to ascertain geroprotective effectiveness and control side effects of such long-term treatments. The emerging concept of treating ageing at its root cause, by improving overall DNA repair, could enable a healthy ageing society of the future.

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Author contributions

All authors contributed to writing the manuscript.

Competing interests

J.V. is co-founder of Singulomics Inc. and Mutagentech Inc, and P.D.R. is co-founder of Genascence and Itasca Therapeutics. The other authors declare no competing interests.

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