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STATE-OF-THE-ART REVIEW

Dysregulated RNA processing and metabolism: a new hallmark of ageing and provocation for cellular senescence

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The human genome is capable of producing hundreds of thousands of different proteins and non-coding RNAs from <20 000 genes, in a co-ordinated and regulated fashion. This is achieved by a collection of phenomena known as mRNA processing and metabolism, and encompasses events in the life cycle of an RNA from synthesis to degradation. These factors are critical determinants of cellular adaptability and plasticity, which allows the cell to adjust its transcriptomic output in response to its internal and external environment. Evidence is building that dysfunctional RNA processing and metabolism may be a key contributor to the development of cellular senescence. Senescent cells by definition have exited cell cycle, but have gained functional features such as the secretion of the senescence-associated secretory phenotype (SASP), a known driver of chronic disease and perhaps even ageing itself. In this review, I will outline the impact of dysregulated mRNA processing and metabolism on senescence and ageing at the level of genes, cells and systems, and describe the mechanisms by which progressive deterioration in these processes may impact senescence and organismal ageing. Finally, I will present the evidence implicating this important process as a new hallmark of ageing, which could be harnessed in the future to develop new senotherapeutic interventions for chronic disease.

The hallmarks of ageing

The hallmarks of ageing are a series of basic health maintenance mechanisms that together describe the molecular, cellular and systemic effects that drive or result from the ageing process in multiple species. At the time of writing, there have been nine interconnected and multifaceted hallmarks described which are as follows: genomic instability, epigenetic alterations, mitochondrial dysfunction, altered intercellular communication, deregulated proteostasis, deregulated nutrient sensing, telomere attrition, stem cell exhaustion and cellular senescence [1]. The interconnections between hallmarks means that impacting one hallmark can have significant effects on other hallmarks. The criteria for a phenomenon to be defined as a

Abbreviations
ARE, A-rich element; DR, dietary restriction; HNRNP, heterogeneous nuclear ribonucleoprotein particle; ISE/ESE, intron/exon splicing enhancer; ISS/ESS, intron/exon splicing silencer; NMD, nonsense-mediated decay; ROS, reactive oxygen species; SAFH, senescence-associated heterochromatic foci; SASP, senescence-associated secretory phenotype; SA-β-Gal, senescence–associated beta galactosidase; SRSF, serine Arginine rich splicing factor.

hallmark are (a) it should occur during normal ageing, (b) its experimental induction should result in accelerated ageing and (c) its experimental abrogation should bring about improvement to aspects of the ageing phenotype. The hallmarks of ageing may represent useful points of future therapeutic intervention for the diseases of ageing.

Cellular senescence; a driver of organismal ageing

Cellular senescence is one of the most intensively studied hallmarks of ageing and is described as a permanent cell cycle arrest, which occurs in response to cellular damage or cell stress [2]. Senescent cells have important functions in young systems, including roles in protection from tumorigenesis, and tissue remodelling and repair during development and wound healing [3]. However, during the ageing process, senescent cells accumulate in response to cumulative cellular insult and impaired immune clearance. There are multiple types of senescent cells and senescence can be provoked by multiple stimuli [4]. Senescent cells have many features that differentiate them from their non-senescent counterparts [5], which are depicted in Fig. 1 and described in Table 1. Importantly, senescence has since been linked with multiple common, chronic diseases of ageing in animal models and in humans including lung fibrosis [6], osteoarthritis [7], age-related macular degeneration [8], neurodegeneration [9], vascular dysfunction [10], cardiovascular disease [11], chronic kidney disease [12], diabetes [13] and non-alcoholic fatty liver disease (NAFLD) [14]. Selective removal of senescent cells has been demonstrated to result in improved lifespan and healthspan in animal models [15]. Subsequent work in these systems has

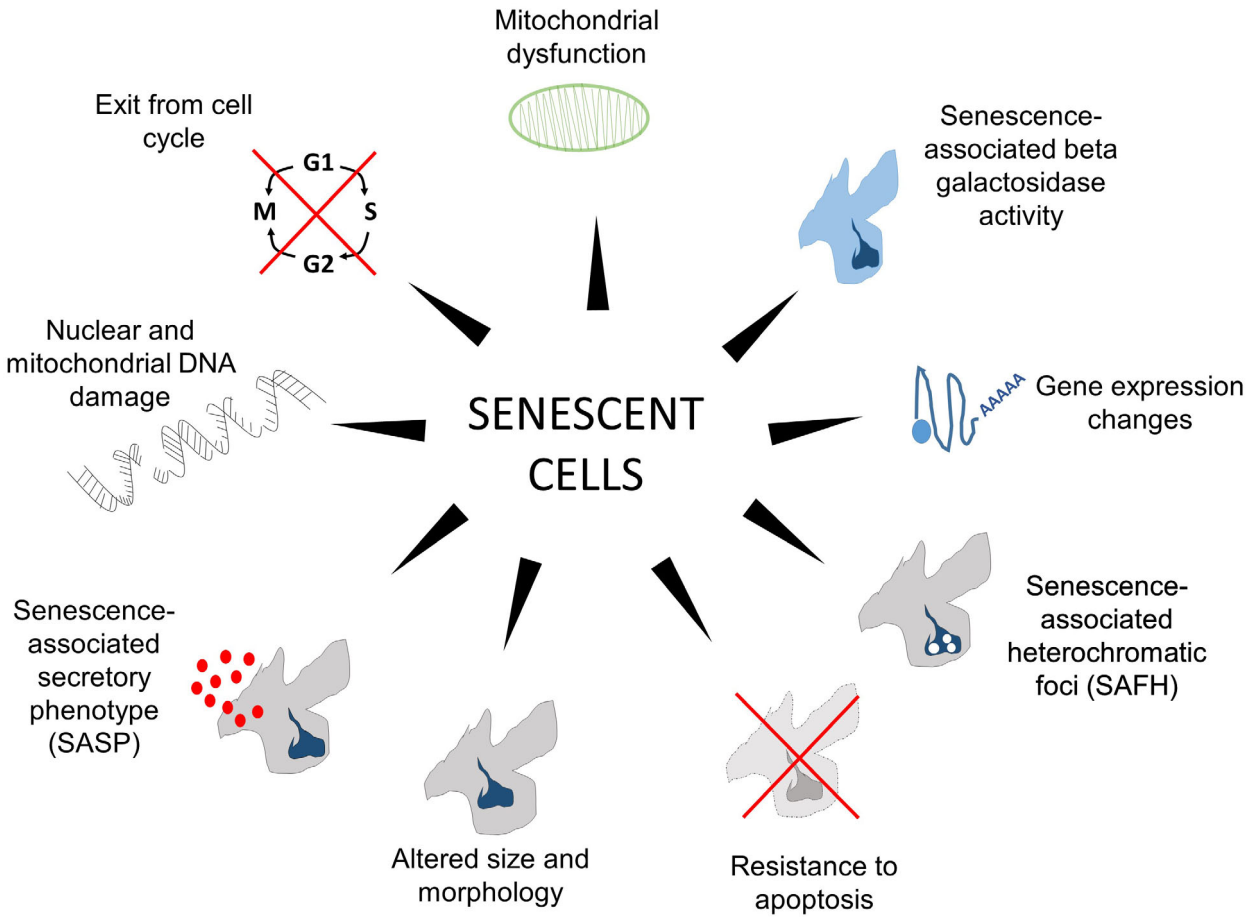

Fig. 1. The characteristics of senescent cells. This figure illustrates the characteristics of senescent cells. These include mitochondrial dysfunction, secretion of the senescence-associated secretory phenotype, characteristic changes to gene expression, the presence of senescence-associated heterochromatic foci (SAFH), increased resistance to apoptosis, altered size and morphology, the high lysosomal content indicated by high levels of senescence-associated beta galactosidase, genetic damage and exit from cell cycle.

Table 1. Characteristics of senescent cells. The characteristics of senescent cells are given below. BrdU, bromodeoxyuridine; EdU, 5-ethynyl-2′-deoxyuridine; MMP, Matrix metalloproteinases; ROS, reactive oxygen species; SAHF, senescence-associated heterochromatic foci; SA-β-Gal, senescence-associated beta galactosidase.

Characteristic	Marker	References
Exit from cell cycle	Ki67/EdU/BrdU negative	[107]
Dysfunctional mitochondria	Elevated ROS, aberrant mitochondrial structure	[108]
Resistance to apoptosis	TUNEL assay negative	[109]
High lysosomal content	SA-β-Gal positivity	[110]
Morphological change	Light/confocal Microscopy	[111]
Chromatin reorganisation	Presence of SAHF	[112]
Altered gene expression	Elevated p16, p21, p15 expression	[113]
Secretion of SASP	Altered IL6, IL8, IL-1β, IL-1α, MMP expression plus others, often cell type specific	[114]

demonstrated beneficial effects on brain ageing and neurodegenerative disease [16] and musculoskeletal function [17]. More recently, selective removal of senescent cells by induced apoptosis (senolysis) has been demonstrated to result in clinical improvement in humans in the context of idiopathic lung fibrosis [18] and diabetic kidney disease [19].

Dysregulated mRNA processing and metabolism: a link between splicing and stress

A unifying feature of most of these provocations for senescence is an aberrant response to different types of cellular stress. Eukaryotic cells have several mechanisms to deal with internal and external stresses, but one of the most important is alternative mRNA processing and metabolism [20–23]. This term refers to the collection of phenomena that happen to an RNA molecule from its transcription to its degradation, to ensure correct regulation of gene expression. Newly transcribed mRNAs are processed to add a 5′ cap structure, undergo splicing to remove non-protein coding intronic sequences and are subject to the addition of a poly A tail prior to export from the nucleus. Following export, RNAs can be transported to specific subcellular localisations, stored and sequestered, or they may be translated (if they code for proteins). Their stability is regulated by post-transcriptional regulation by the action of microRNAs and other non-coding RNA (ncRNA) species, or RNA-binding proteins (RBPs). At the end of their lifecycle, they are then degraded by the exosome (Fig. 2). Ribosomal RNAs (rRNAs), transfer RNAs (tRNAs) and messenger RNAs (mRNAs) all undergo processing, and there is evidence that suggests that metabolism of all three RNA species are associated with ageing and/or cellular senescence [24,25].

Messenger RNA (mRNA) processing specifically is the collective set off phenomena that allow most eukaryotic genomes to adjust their transcriptomic output in response to internal and external environmental cues. It brings exceptional adaptability and plasticity to the human genome, and accordingly, over 95% of all human genes express more than one isoform [26]. Alternative isoforms may have differential spatial or expression patterns, and often confer alternative or antagonistic function. For example, the *VEGFA* gene produces two main classes of isoform, some of which promote angiogenesis, and some are anti-angiogenic [27]. There are seven primary forms of alternative mRNA isoform production. These are alternative promoter usage, alternative polyadenylation, cassette exon usage, mutually-exclusive exon usage, alternative 5′ splice site usage, alternative 3′ splice site usage and retained introns. Alternative mRNA splicing is regulated at two levels; at the level of DNA or pre-mRNA sequence, and by the binding of a series of splicing regulator proteins (Fig. 3). There are a number of sequence elements that regulate splicing; the core elements (the 5′ splice site, the 3′ splice site, the polypyrimidine tract and the branch point), which are binding platforms for elements of the spliceosomal machinery and are essential for splice site usage. Mutations in these regions are often causal for inherited genetic disease [28]. Then, there are a set of auxiliary binding sites termed exon and intron splicing silencers (ISS, ESS) and enhancers (ISE, ESE) [29]. These motifs are responsible for more regulated splicing, and are primarily responsible for the plasticity of splicing. These sequences are important because they bind important spliceosomal components or splicing regulatory proteins responsible for alternative splicing. Serine arginine-rich (SRSF) splicing factors bind to ESE and ISE elements and usually, but not exclusively, promote splice site usage. Conversely, heterogeneous nuclear ribonucleoprotein particles (hnRNPs) bind to ISE and ISS elements and usually, but again not exclusively, inhibit splice site usage. The combinatorial balance of splicing activators and inhibitors determines whether a given splice site is used and a given isoform expressed [30].

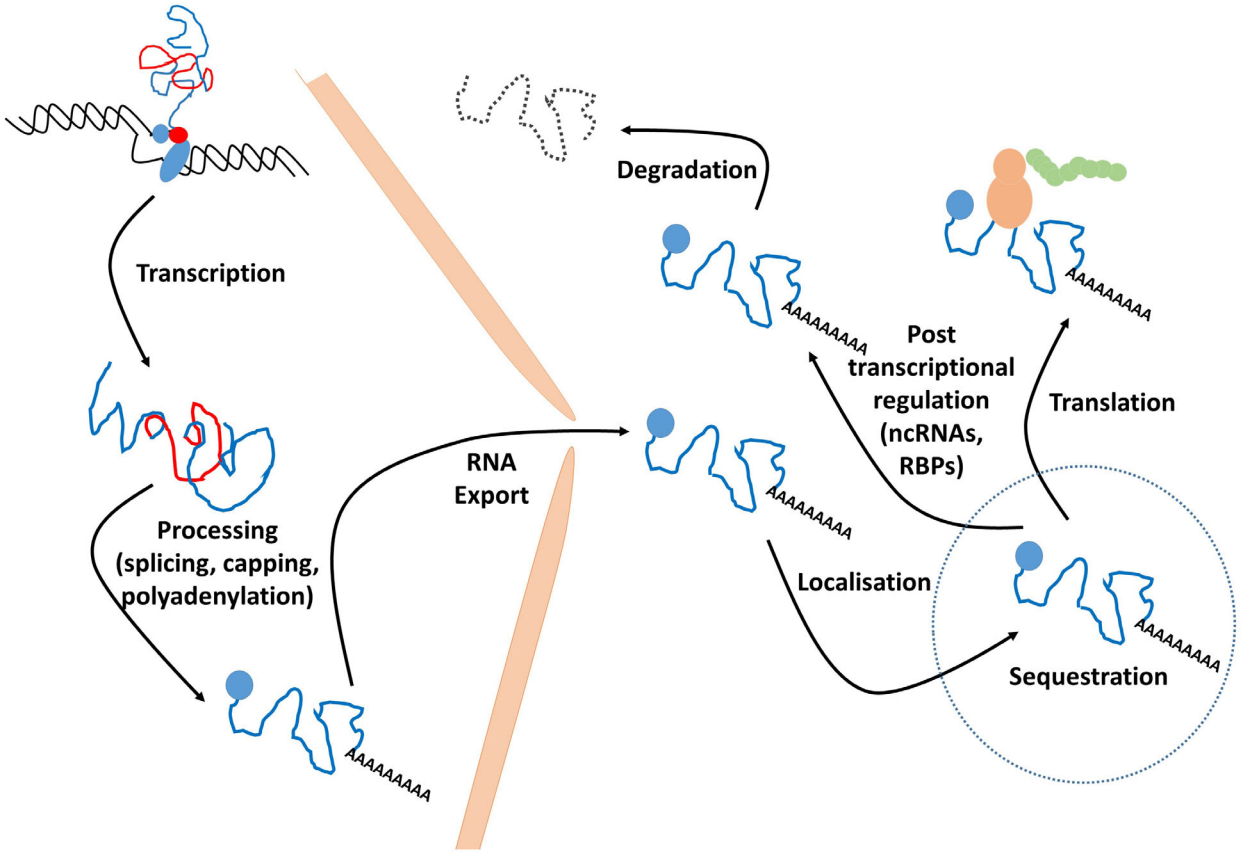

Fig. 2. The life cycle of an mRNA. This figure illustrates the lifecycle of an mRNA from birth to death. Gene expression is co-transcriptional, so these processes may occur simultaneously. Following transcription, messenger RNA transcripts are processed to add a 5′ cap structure, spliced to remove introns and undergo the addition of a poly A tail. Processed mRNAs are then exported from the nucleus to the cytoplasm, where they may be translated (if they code for proteins) or act as non-coding RNAs that regulate other genes. RNAs may be sequestered at specific cellular locations for later translation. RNA transcripts can be dynamically regulated by the action of non-coding RNAs or RNA binding proteins and are degraded at the end of their functional lifespan. Exonic sequences are given on blue, intrinsic sequences in red. The nuclear membrane and nuclear pore are indicated by a curved coral line. The cap structure is given by a blue circle and the ribosome is given in pale orange. The nascent polypeptide is given by green circles.

Regulation of splicing factors

Splicing factors themselves are regulated at multiple levels. They are regulated at the level of transcription by repeated and constitutive activation of cellular signalling pathways such as ERK and AKT [31], but also interface with other signalling pathways, including AMPK, FOXO1 and mTOR [32], due to crosstalk between pathways. They are thus influenced by a great many of the stimuli classically associated with ageing, including inflammation, DNA damage, mitochondrial dysfunction and dysregulated nutrient sensing [33–35]. Many SASP-associated cytokines associated with paracrine senescence are regulated by the NFκB pathway [36], which in turn is also influenced by ERK signalling. Splicing factor expression may initially increase in acute response to inflammatory factors, but chronic inflammation exerts a negative influence on their expression. Many splicing factors also feed forward and regulate the processing and stability of inflammatory genes [37,38]. Although individual splicing factors exert patterns of temporal and spatial specificity of expression, they are co-ordinately regulated at the level of transcription by 'master control genes' such as *FOXO1* and *ETV6*, which lie downstream of ERK and AKT [31], and also the DNA damage response gene *ATM* [39]. Splicing factors are also regulated by phosphorylation at the protein level; their subcellular localisation is controlled by kinases of the CLK and SPRK classes [40], and by the action of AKT [41]. Finally, splicing factors frequently regulate their own expression by the inclusion or exclusion of poison exons which promote degradation by the nonsense-mediated decay pathway [42,43].

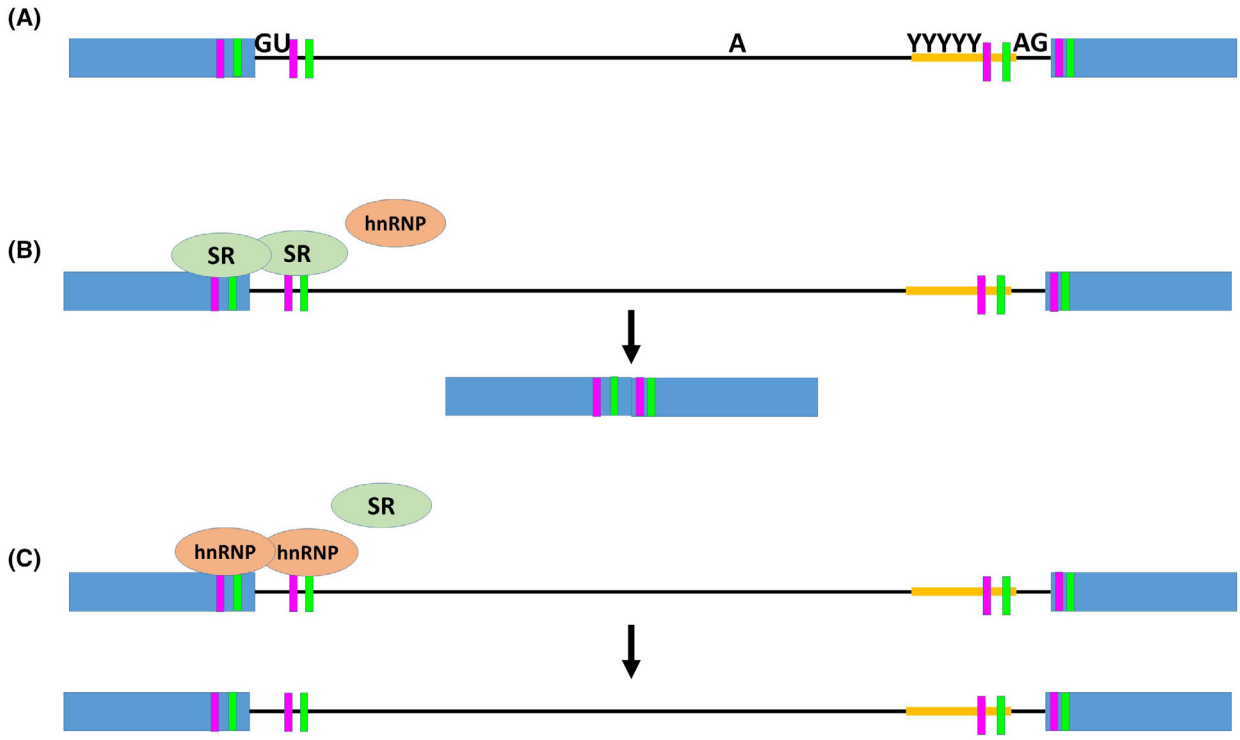

Fig. 3. The regulation of alternative splicing. (A) The schematic gives a representative intron (black line), flanked by its two exons (blue boxes), with the regulatory sequence elements and some of the proteins that bind to them marked. The splice donor site is given by GU. The branch site is marked by an A. The polypyrimidine tract is annotated by a gold bar annotated YYYYY. The splice acceptor site is given by AG. Intron and exon splicing enhancer sequences are given by green boxes, and intron and exon splicing silencer elements by pink boxes. (B) The binding of Serine Arginine rich splicing factor (SRSF) splicing activators (given in green and marked SR) activates splice site usage and results in spliced exons. (C) The binding of heterogeneous nuclear ribonucleoprotein particles (given in orange and marked hnRNP) inhibits splice site usage and results in lack of splicing and retention of intron.

Splicing factors, ageing and senescence

Gene set enrichment analyses (GSEA) of age-related gene expression signatures deriving from human peripheral blood indicates that the pathways that regulate alternative splicing are amongst the major pathways disrupted by age in multiple human populations [44]. Splicing factor genes are usually, but not exclusively, downregulated during ageing and senescence, but this may differ from tissue to tissue [45]. Each individual splicing event, however, is determined by combinatorial binding of splicing factors to individual splicing regulatory sequences [30], the directionality of effect for different splicing factors in different contexts may thus vary. Changes in splicing factor expression are manifested as changes to programs of alternative splicing in different organ systems in ageing people [44,46,47], and can be explained at least in part by upstream changes in splicing regulation [48]. Splicing factor changes have also been extensively reported by ourselves and others in senescent cells of multiple cell and tissue types [39,49–55]. Splicing factor expression has been shown to associate with lifespan in humans [56] and in other species [56,57], and similarly are causally involved in response to other lifespan-extending phenomena such as dietary restriction (DR) in humans [58] and in other species [57]. Interestingly, changes in splicing factor expression and the consequent downstream effects on splicing are *not* a feature of ageing in naked mole-rats, a species with exceptionally long life and negligible senescence [59].

Disrupted patterns of alternative splicing are a major characteristic of many common chronic age-related diseases, such as cancer [60], neurodegenerative disease [61], osteoarthritis and cardiovascular disease [62], and also more systemic diseases of the elderly, such as frailty and sarcopenia [63]. Most of these diseases are also characterised by senescence as described above. Splicing changes are also prevalent in rare diseases associated with premature ageing such as idiopathic pulmonary fibrosis [64] and Hutchinson Gilford Progeria Syndrome

(HGPS) [65]. There are also links between common chronic disease and dysregulation of splicing factors. Levels of the *HNRNPA0*, *HNRNPM* and *AKAP17A* are predictively associated with multiple ageing phenotypes in humans, where splicing factor levels measured at baseline are associated with later cognitive decline and hand grip strength, a measure of sarcopenia [66]. There is a well-characterised increase in transcriptional noise with ageing, but recent findings suggest that measures of expression at the level of mRNA processing may be a better determinant of the ageing process [67].

Potential mechanistic links between disrupted splicing regulation and senescence

The likely candidacy of disrupted mRNA processing regulators as a major driver of senescence stems from the multifunctional nature of these proteins (Fig. 4 and Table 2). The global nature of splicing-associated changes to the transcriptome means that effects are likely to be far reaching for the transcriptome as a whole. A total of 98% of genes undergo alternative splicing [26], and are represented in every cellular process. Progressive and irreversible dysregulation of splicing regulation will therefore inevitably lead to far-reaching consequences for cells and systems. Furthermore, the auto-regulatory nature of splicing factor regulation means that disruption to the homeostasis of splicing regulation is likely to result in ongoing and increasing disruption.

There are also isoforms of known senescence genes with altered functionality or expression. Isoforms of *CDKN1A* (which encodes p21) demonstrate different temporal dynamics in response to doxorubicin, with p21 variant 2 showing a slower, but more marked

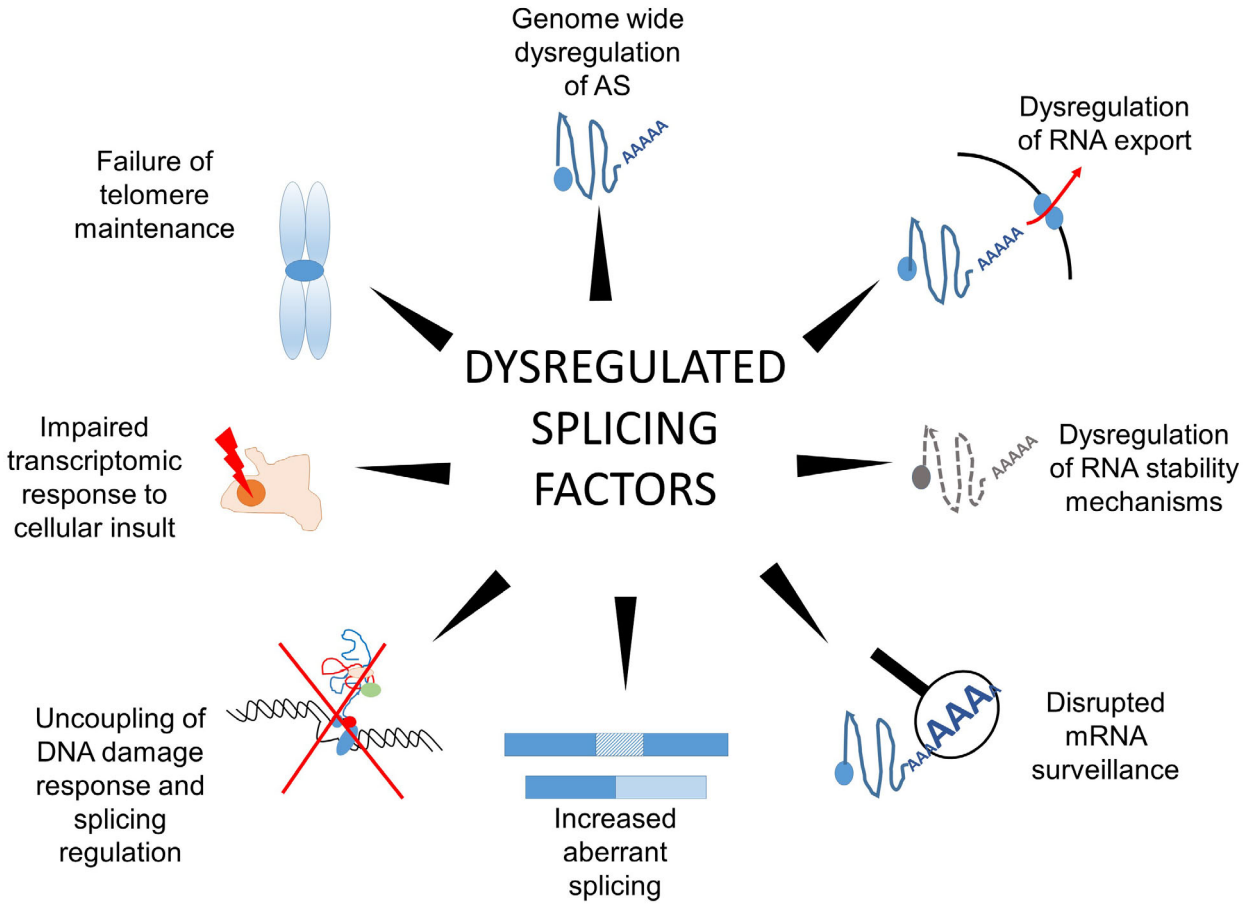

Fig. 4. The mechanisms by which dysregulated splicing factor expression could induce and maintain cellular senescence. These include genome-wide disruption of alternative splicing, dysregulation of mRNA export, dysregulation of RNA stability, disruption of mRNA surveillance pathways such as nonsense-mediated decay, increase in aberrant splice products, stabilisation of mRNAs encoding inflammatory factors, impaired transcriptomic response to cellular insult resulting in reduced molecular resilience, failure of telomere maintenance and uncoupling of transcription-coupled DNA damage and splicing interactions.

Table 2. Mechanisms by which dysregulation of splicing factor expression or activity can modulate senescence phenotypes. The progressive dysregulation of splicing factor gene expression and activity over time is predicted to interface with many of the molecular mechanisms underpinning cellular homeostasis and cellular health, by virtue of their multifunctional nature. Some of the pathways that may be affected by dysregulated expression of splicing factors are given below.

Mechanism	Consequence	References
Changes to patterns of canonical splicing	Expression of pro-senescence isoforms	[68,69,71]
Increase in aberrant splicing	Increase in transcriptional noise and production of isoforms with altered functionality	[73,74]
Disruption to RNA surveillance	Failure of RNA quality control and fine tuning of gene expression	[77,115]
Altered dynamics of RNA turnover	Enhanced stability of SASP mRNAs	[81,82]
Defective RNA export	Altered subcellular localisation of mRNAs, nuclear accumulation and impaired translation of mRNAs	[88,116]
Compromised telomere maintenance	Telomere shortening and initiation of replicating senescence	[89–91]
Decreased plasticity and adaptability of transcriptome	Decreased molecular stress resilience	[117]
Uncoupling of DNA damage response and splicing regulation	Accumulation and faulty repair of DNA damage	[70,95]

response to genotoxic stimuli [68]. Isoforms of *ANRIL*, derived from the *CDKN2A* locus which also encodes p14, p15 and p16, also demonstrate altered functionality in response to different senescence-inducing provocations [69]. Splicing response is also tightly coupled to DNA damage [70]. Many genes with important roles in damage repair have alternatively spliced isoforms. The cyclin D1 gene encodes several isoforms with different abilities to initiate DNA damage response (DDR); with the cyclin D 1a isoform able to initiate DDR, but the cyclin D 1b isoform lacking this ability [71]. Similarly, an age-related increase in the expression of the truncated *Tp53* isoform Δ40p53 is associated with an accelerated ageing phenotype and increased levels of senescence [72].

Disrupted splicing is likely to result not only in changes to the abundance of canonical isoforms, but also result in the occurrence of aberrant splicing events. Recent evidence has also suggested that dynamic retained intron events occur in senescence and in aged tissues, and were negatively correlated with the expression of their host genes [73]. Accumulation of retained introns has also been described in the pre-symptomatic stage of ageing in wild-type mouse models [74]. Aberrant isoforms may have dominant negative properties, altered functional characteristics or misdirected subcellular localisation which may have profound implications for cells, tissues and systems and contribute to ageing and senescence phenotypes.

Altered levels of splicing factors may also compromise the ability of an organism to react to challenging stimuli in its environment and reduce transcriptomic resilience, leading to multiple forms of cellular stress. As described above, cellular stress is a major provocation for senescence. Many genes use mRNA surveillance pathways such as nonsense-mediated decay (NMD) as part of their normal regulation, in addition to its role in mRNA quality control [75]. Proper splicing is essential for NMD, as the deposition of the exon-junction-complex (EJC) at the site of spliced exons is a signal for initiation of degradation [76]. Without effective NMD, RNA quality control mechanisms may be compromised, as has been observed in nematode models [77] and in humans [78].

Many splicing factors also have roles in RNA stabilisation or destabilisation [79,80]. In addition to its role on splicing, splicing factors such as hnRNPD (also known as AUF1) act to destabilise their targets through binding to A-rich elements (AREs) in their 3′ untranslated regions [81]. Other splicing factors such as hnRNPA1 can stabilise, rather than destabilise their targets [82]. The aberrant production of transcripts with alternative 3′ untranslated regions can have consequences for their regulation by microRNAs or RNA-binding proteins, another stress responsive gene regulatory mechanism [83]. This has particular relevance when the role of splicing factors in regulation of multiple components of the SASP is considered. Many pro-inflammatory cytokines are regulated by A-rich elements in their 3′ untranslated regions [84,85]. Dysregulated expression of splicing factors can thus alter the negative regulation of SASP factors, leading to induction of chronic levels of inflammation.

Splicing factors also have additional roles in RNA export [86,87]. The ability to shuttle processed RNAs to the cytoplasm for translation or storage is a key component of the quality control of gene expression. Defects in this process lead to the accumulation of aberrant or mislocalised RNAs in the cell and are commonly observed in neurodegenerative disease [88].

Many splicing factors have roles in telomere maintenance [89–91]. HNRNPF and H associate with the G quadruplex elements in the hTERC component of the

telomerase holoenzyme and modulate telomerase activity and telomere length [92]. Other splicing factors can promote recruitment of telomerase to telomeres [93] or modulate the accessibility to the telomerase promoter to its transcription factors [91]. Telomerase itself is also regulated during development by alternative splicing [94].

Finally, there are known interactions between mRNA processing factors and transcription-coupled DNA damage response. ATM, an initiator of DNA damage response, is known to be a negative regulator of splicing factor expression [39], but the mRNA processing pathway is however co-transcriptional, not linear. RNA molecules may exist in a state where there may be interactions between not only RNA and mRNA processing factors, but also between nascent transcripts and DNA. Evidence is mounting that dysregulated coordination between different RNA processing steps may facilitate introduce defects in genome stability by disrupting the interactions between nascent RNA and the DNA template [95]. DNA damage is also able to directly modify splicing proteins via PARylation, arginine methylation, acetylation, ubiquitination/sumoylation or phosphorylation [70].

Splicing factor dysregulation may thus act at many points in the development of senescence, and contribute to multiple senescence phenotypes. These proteins play multiple and interlinked roles in many aspects of the initiation and persistence of the senescence phenotypes, including DNA repair, telomere shortening, decreased molecular stress resilience and secretion of the SASP. As such, this phenomenon is poised to interface with multiple hallmarks of ageing, with influence on many aspects of ageing biology.

Splicing factors as potential therapeutic targets for age-related disease

Many important gene regulatory proteins are tightly regulated to maintain correct homeostatic levels, and splicing factors are no exception. Splicing factors are normally held in a narrow homeostatic range by a combination of autoregulation and transcriptional control via cellular signalling pathways, many of which are responsive to age-associated stimuli [31,39]. During the ageing process, repeated and constitutive activation of signalling pathways such as AKT and ERK exerts negative regulatory pressures on splicing factor expression via the transcription factors ETV6 and FOXO1 [31]. Splicing factors also commonly auto-regulate via the inclusion or exclusion of a poison exon, which promotes mRNA degradation [96]. The expression of most splicing factors declines with age [44]. It therefore follows that restoration of splicing factor levels back within their normal homeostatic constraints may confer an advantage to the cell. Early studies suggested that some splicing factors may be upregulated by treating cells with the polyphenol resveratrol [97], a molecule long associated with healthspan benefits [98–100]. Subsequent studies demonstrated that the effect of resveratrol and associated analogues produced a global restoration of splicing factor levels, and was associated with reversal of multiple aspects of cellular senescence in primary human dermal fibroblasts [101]. Similar results were obtained in studies where primary human dermal fibroblasts were treated with specific inhibitors of the negative upstream signalling pathways ERK and AKT, or their downstream effectors FOXO1 or ETV6 [31]. Other studies demonstrated that it was possible to uncouple reversal of different aspects of the senescence phenotype and produce reversal and attenuation of SASP, without re-entry to cell cycle in primary human endothelial cells by treatment of cells with mitochondria-targeted hydrogen sulphide (H_2S) donors [102]. These data clearly demonstrate the potential utility of small molecule or genetic modulation of splicing factor expression for attenuation of cellular senescence, but challenges still remain in terms of specific delivery to senescent cells, translation to systemic models and precise dosing to maintain homeostasis. In this respect, the presence of auto-regulatory feedback loops may actually be an advantage, as overstimulation will elicit a negative regulatory response and attenuation of splicing factor mRNA levels. The development of splicing regulatory modulators is in its infancy, but nevertheless holds great potential for future senotherapeutics.

Dysregulated mRNA processing: the 10th hallmark of ageing?

Dysregulation of mRNA processing fulfils all the criteria for categorisation as a new hallmark of ageing. The first criteria is that a hallmark must occur during normal ageing. Data suggest that splicing factor dysregulation does occur during human ageing, both at the level of populations [44] but also in multiple cell types at the individual cellular level in terms of senescence [31,39,49,50,101,102]. This is echoed by disruption to patterns of alternative splicing in multiple tissues in multiple species during ageing [48,56,103,104], except in animals that have negligible senescence [59]. The second criteria is that its experimental induction of defects in RNA processing should result in accelerated ageing. Data on this aspect are

harder to find as complete knockout of many splicing factors is lethal and mimicking transcriptome wide patterns of splicing defects consistent with those found in ageing experimentally is difficult. Mice where the *HNRNPD* gene has been ablated do show evidence of accelerated ageing, however, including kyphosis (hunched back), reduced subcutaneous fat and reproductive organ atrophy [91]. Experimental depletion of *HNRNPD* or *SRSF2* expression also yields cellular senescence [102]. Similarly, genetic perturbation of *HNRNPA3*, *SRSF7* and *SRSF4* expression levels was sufficient to provoke senescence, as was disruption of transcriptome wide splicing patterns using a pharmacological inhibitor of SF3B1, an important component of the U2 snRNP involved in branch site recognition [53]. Similarly, depletion of the pre-mRNA processing factor *Prp19* promotes cellular senescence and premature ageing in mouse skin [95]. The third criteria of a hallmark of ageing is that its experimental abrogation should bring about improvement to aspects of the ageing phenotype. Restoration of splicing factor expression using small molecule or genetic means has been shown to be capable of rescuing multiple aspects of the senescent cell phenotype [31,101,102]. Similarly, overexpression of the RNA processing factor *PRP19* has been shown to extend human endothelial cell lifespan *in vitro* by increasing stress resilience and DNA repair capacity [105]. Examples of the beneficial effect of systemic restoration of splicing regulation on *in vivo* ageing models are not so well-documented, but, overexpression of *Prp19* has been shown to increase lifespan in a *Drosophila* systemic model [106]. It should also be noted that the original description of the hallmarks of ageing states that not all the hallmarks are fully supported yet by interventions that succeed in ameliorating ageing [1].

Conclusion and open questions

Given the evidence presented here, it should be considered that dysregulated mRNA processing fulfils at

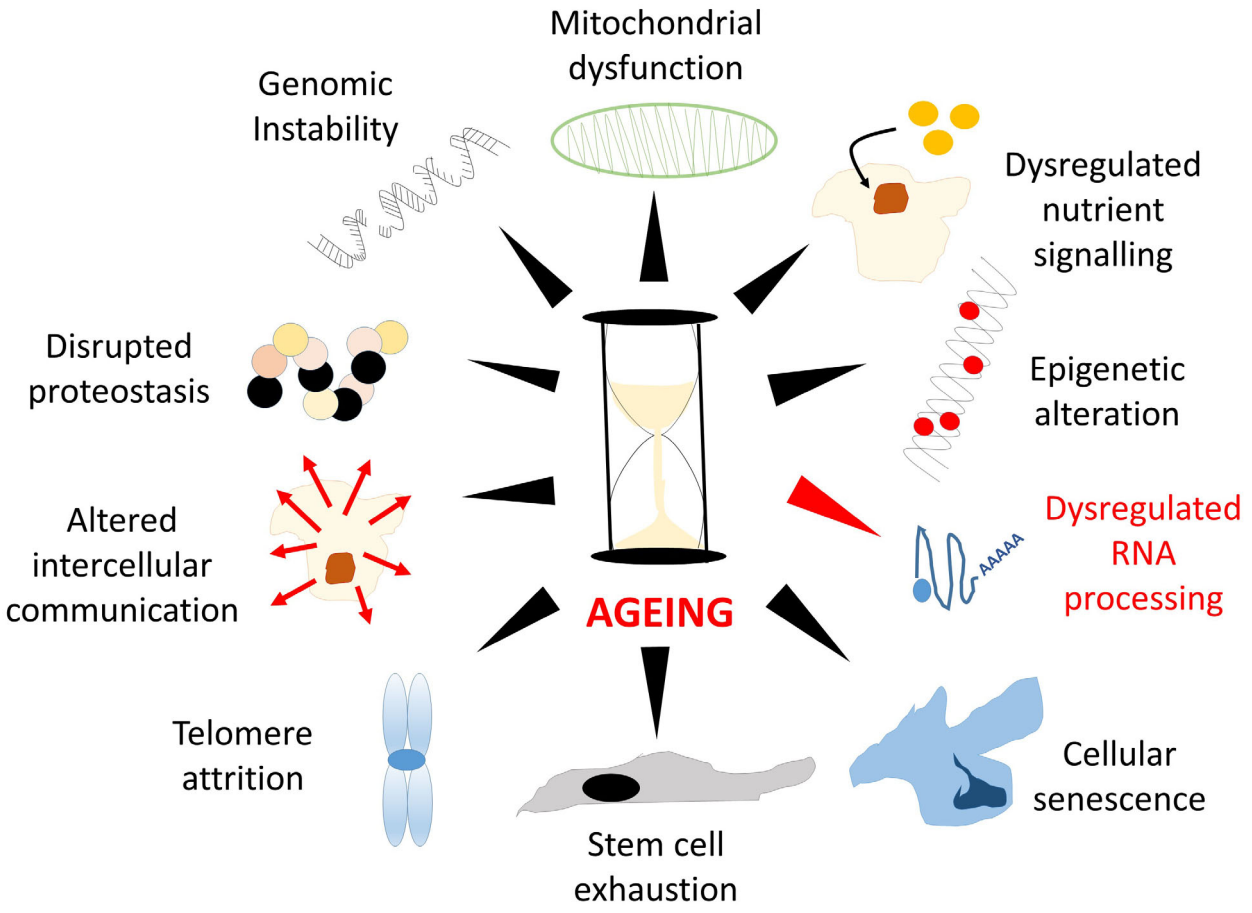

Fig. 5. The 10 hallmarks of ageing. The addition of dysregulated mRNA processing as the 10th hallmark of ageing, joining mitochondrial dysfunction, dysregulated nutrient sensing, epigenetic alteration, cellular senescence, stem cell exhaustion, telomere attrition, altered cellular communication, disrupted proteostasis and genomic instability.

least as many of the criteria as some of the phenomena already designated as hallmarks of ageing, and as such should be designated as such (Fig. 5). The links between dysregulated mRNA processing, cellular senescence and ageing are now beginning to coalesce into a clearer picture, which is perhaps unsurprising given that correct alternative splicing is a fundamental pre-requisite to cellular health. Approaches to restore splicing regulation and restore transcriptomic plasticity may therefore represent a useful new tools in our senotherapeutic armoury, although there are still questions to answer. First, what is the most facile and effective methodology to restore them to their correct homeostatic positions? Is it sufficient to do this systemically, or do we need to restrict our interventions to target organs and cell types? The answer to this will largely depend upon the desired outcome; restoring splicing regulation in accessible target organs in the context of relieving aspects of age-related disease is likely lower hanging fruit than tackling bigger and more systemic issues. A second question is regarding the impact of splicing-targeted senotherapies on the non-senescent cells resident in the same organs. In this scenario, it may be advantageous to consider senescent cell targeting, or strategies to attenuate only aspects of the senescent cell phenotype such as the SASP. There is still work to do here and this work is in its infancy, but represents an exciting new future therapeutic avenue.

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Conflict of interest

Lorna Harries is co-founder, director and CSO of SENISCA Ltd.

Author contributions

LWH wrote and reviewed the manuscript.

Data availability statement

There are no data associated with this publication.

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