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REVIEW

The emerging role of alternative splicing in senescence and aging

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Summary

Deregulation of precursor mRNA splicing is associated with many illnesses and has been linked to age-related chronic diseases. Here we review recent progress documenting how defects in the machinery that performs intron removal and controls splice site selection contribute to cellular senescence and organismal aging. We discuss the functional association linking *p53, IGF-1, SIRT1,* and *ING-1* splice variants with senescence and aging, and review a selection of splicing defects occurring in accelerated aging (progeria), vascular aging, and Alzheimer's disease. Overall, it is becoming increasingly clear that changes in the activity of splicing factors and in the production of key splice variants can impact cellular senescence and the aging phenotype.

Key words: aging; alternative splicing; pre-mRNA; RNA; RNA binding proteins; senescence; splice variants; splicing.

Introduction

Aging is defined as a progressive decline of fitness over time, ultimately leading to death (Kirkwood & Holliday, 1979; Kirkwood, 2005). This decline is associated with several changes such as tissue deterioration and disorganization, organ dysfunction, and loss of stem cell renewal, the latter contributing to age-associated immunodeficiency. At the cellular and molecular levels, the aging phenotype varies between tissues but can include common hallmarks such as genomic and epigenetic instability, mitochondrial dysfunction, telomere attrition, and the accumulation of senescent cells (Kirkwood & Holliday, 1979; Kirkwood, 2005; López-Otín *et al.*, 2013). Considered as one of the causes of age-related tissue degeneration, cellular senescence is an irreversible and programmed cell-cycle arrest that occurs in most diploid cell types (Hayflick & Moorhead, 1961). Senescence is associated with large-scale changes affecting a variety of processes such as cytokine secretion through the senescence-associated secretory phenotypes (SASPs), alterations in gene expression, and alternative splicing, as well as chromatin remodeling that includes senescence-associated heterochromatin foci (SAHF) (Narita *et al.*, 2003; Rodier *et al.*, 2009; Kuilman *et al.*, 2010; Campisi, 2013; Holly *et al.*, 2013). Senescence is also seen as a mechanism that prevents tumorigenesis (Serrano *et al.*, 1997). The upregulation of tumor suppressors, such as $p16^{INK4A}$, p21, and p53, as well as the activation of RB, is common in senescent cells, contributing to irreversible cell-cycle arrest (Beausejour, 2003; Sage *et al.*, 2003; Kuilman *et al.*, 2010; Campisi, 2013). Although replicative senescence is linked to telomere attrition (Allsopp *et al.*, 1992; Bodnar *et al.*, 1998), telomere shortening is not necessarily required for the onset of senescence, implying the existence of different senescent programs (van Deursen, 2014; Sharpless & Sherr, 2015). Consistent with this view, telomere-independent senescence can be controlled by pathways triggered by insults (stress-induced senescence), as well as by other intrinsic signals that occur during embryonic development and tissue repair (Von Zglinicki, 2002; Baker *et al.*, 2008; Krizhanovsky *et al.*, 2008; Schmidt *et al.*, 2010; Nardella *et al.*, 2011; Storer *et al.*, 2013). Notably, senescence can also be engaged by the hyperactivation of factors, such as RAS, that promote cell growth, a process known as oncogene-induced senescence that may be linked to telomere dysfunction (Courtois-Cox *et al.*, 2008; Günes & Rudolph, 2012). While the exact connection between senescence and organismal aging is still much debated (Sharpless & Sherr, 2015), it has become increasingly clear that cellular senescence plays a role in some age-related diseases and in tissue degeneration associated with aging (Baker *et al.*, 2008; Günes & Rudolph, 2012; van Deursen, 2014). Senescent cells progressively accumulate in the tissues and organs of aging mammals including humans (Herbig *et al.*, 2006; Ressler *et al.*, 2006; Jeyapalan *et al.*, 2007; Kreiling *et al.*, 2011). This accumulation of senescent cells may be due in part to a decreased ability of the immune system at removing them (Nikolich-Zugich, 2008; Wang *et al.*, 2011a). In addition, SASPs have been linked with aging organs and tissue degeneration (Parrinello *et al.*, 2005; Coppé *et al.*, 2008), where they are thought to enhance significantly the senescence of neighboring cells by a mechanism called paracrine senescence (Nelson *et al.*, 2012; Acosta *et al.*, 2013) (Fig. 1). Consistent with a role for senescence in aging, reducing the level of senescent cells is associated with a significant decrease in the incidence of age-related disorders (Baker *et al.*, 2008, 2011; Zhu *et al.*, 2015), and was shown recently to improve homeostasis and extend lifespan in mouse models (Baker *et al.*, 2016; Baar *et al.*, 2017). While nonreplicating cells in aging tissues such as muscle harbor senescent markers and can become senescent, they may, in general, be more resistant to senescence. However, muscle replicative stem cells and satellite cells likely undergo senescence and may contribute more importantly to aging. Altogether, the accumulation of senescent cells in tissues and organs suggests that this process contributes to the progressive deterioration that irremediably associates with aging (van Deursen, 2014).

As senescence and aging are characterized by global cellular and molecular changes, it is fair to expect that splicing control will also be subjected to alterations. The challenge is to determine whether these changes are collateral or direct effects, and how they contribute to senescence and aging. Several reviews have recently presented splicing defects linked to age-associated diseases, such as neurodegenerative disorders and cancer (Daguenet *et al.*, 2015; Scotti & Swanson, 2015; Chabot & Shkreta, 2016). Here, our discussion will

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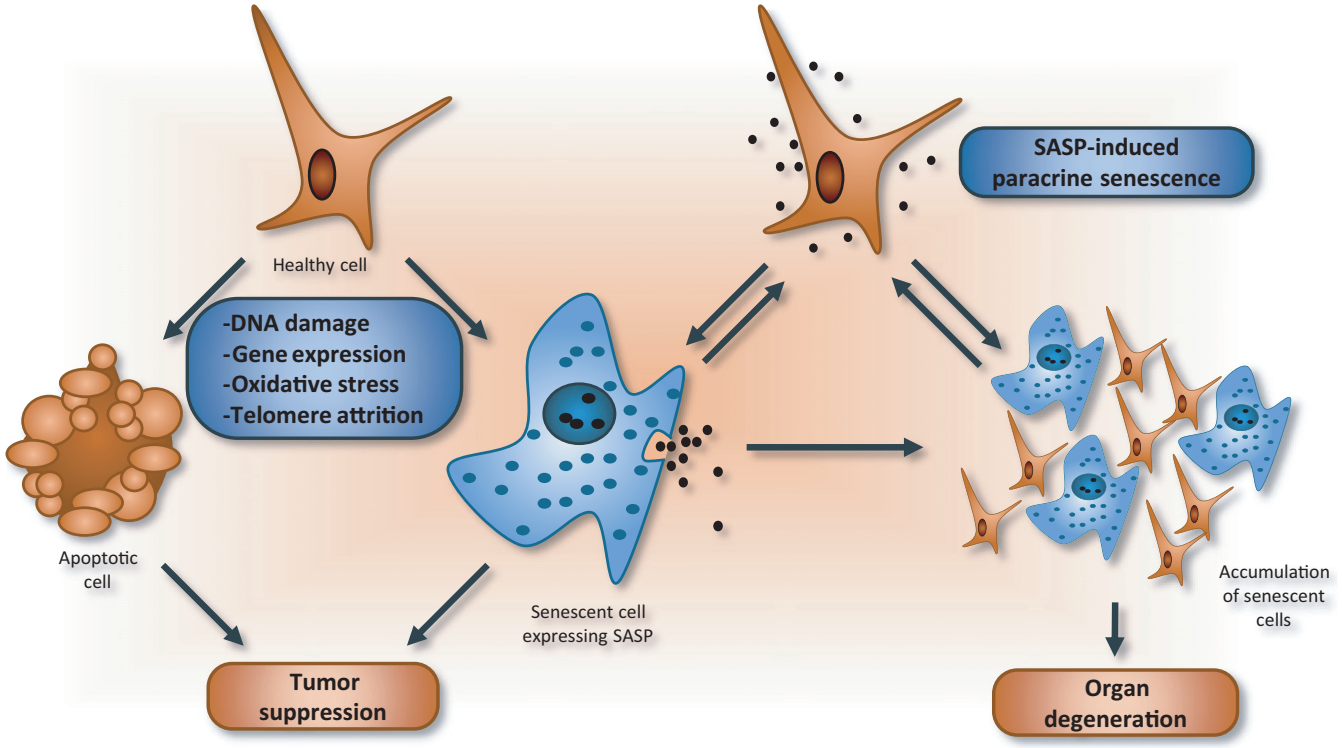

Fig. 1 Senescence leads to organ degeneration. The constant exposure of cells to intrinsic or extrinsic stresses may lead to senescence or apoptosis. Senescence-associated secretory phenotypes (SASP) trigger paracrine senescence in neighboring areas to enhance senescence in tissues. When an aging immune system fails to clear senescent cells, they accumulate in tissues over time, ultimately leading to organ dysfunction.

focus on recent progress achieved in documenting splicing alterations that directly contribute to cell senescence and aging, including accelerated aging (e.g., Hutchinson–Gilford progeria syndrome or HGPS).

Basic concepts of alternative splicing

The vast majority of precursor mRNAs (pre-mRNAs) produced by mammalian cells are made of exons separated by introns. Introns are normally removed leaving joined exons that form the mature mRNA. Splicing is carried out by the spliceosome, a massive complex that includes hundreds of proteins and five small nuclear ribonucleoproteins (snRNPs) named U1, U2, U4, U5, and U6 (Matera & Wang, 2014). The U1 and U2 snRNPs, respectively, recognize the 5′ splice site and the branch site near the 3′ splice site, making these two snRNPs important in defining intron borders. While many introns are removed constitutively, a large fraction of splicing signals are not always used leading to alternative splicing (Fig. 2). Alternative splicing occurs in transcripts produced by more than 95% of human genes (Pan *et al.*, 2008; Wang *et al.*, 2008), including ones implicated in senescence, apoptosis, and DNA repair (Schwerk & Schulze-Osthoff, 2005; Kelemen *et al.*, 2013; Tang *et al.*, 2013).

Alternative splicing is a complex and tightly regulated process. Many RNA binding proteins act as splicing regulators to facilitate or inhibit splice site recognition by spliceosome components (Fu & Ares, 2014). Splicing regulators include, but are not limited to, members of the hnRNP and SR families of proteins (Busch & Hertel, 2012; Giulietti *et al.*, 2013). The combinatorial cooperation or antagonism between distinct RNA binding proteins is thought to play a major role in conferring specificity to splicing decisions (Fu & Ares, 2014). Their interactions and their cellular localization can be modulated by post-translational modifications (Howard & Sanford, 2015; Shkreta *et al.*, 2016) that are often tightly integrated to environmental cues and homeostatic imbalances. Many splicing regulatory factors recognize short sequences within the pre-mRNA, and these elements can be classified in four groups termed Intronic and Exonic Splicing Silencers (ISS and ESS, respectively) and Intronic and Exonic Splicing Enhancers (ISE and ESE, respectively). The availability of these elements and of the splice sites will vary depending on the secondary structure adopted by the pre-mRNA (Jin *et al.*, 2011). The position of these elements relative to splicing signals is often critical to determine whether they will have a positive or negative impact on splicing. For example, a RBFOX binding site positioned downstream of a 5′ splice site usually stimulates splicing, whereas the same site positioned in the upstream intron may promote exon skipping (Yeo *et al.*, 2009) (Fig. 3).

In addition, the control of splice site selection is often coupled with transcription (Naftelberg *et al.*, 2015). In this context, at least two mechanisms of control can exist. First, RNA polymerase II, transcription factors, and chromatin components can recruit generic and regulatory splicing factors. Second, change in the speed of transcription elongation and the presence of pausing sites linked to chromatin structure will affect the time given to splicing regulatory complexes to assemble and affect the use of a splice site in a competitive environment (Lee & Rio, 2015; Naftelberg *et al.*, 2015; Nieto Moreno *et al.*, 2015). One notable example of this coupling involves the DNA binding protein CTCF, which pauses the elongating polymerase to favor inclusion of exon 5 in the nascent *CD45* pre-mRNA (Shukla *et al.*, 2011). A developmentally induced DNA methylation event located in exon 5 inhibits the binding of CTCF to promote exon 5 skipping (Shukla *et al.*, 2011).

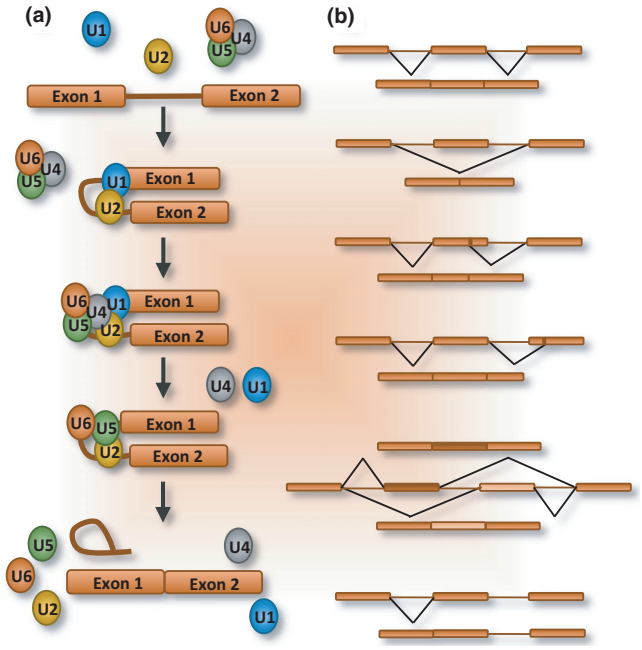

Fig. 2 Constitutive and alternative splicing. (a) The U1 snRNP recognizes the 5′ splice site on the pre-mRNA, while U2 snRNP interacts with the branchsite near the 3′ splice site. The U4/U5/U6 tri-snRNP complex is then recruited. After the release of U1 and U4, the spliceosome first executes 5′ splice site cleavage coupled with branch formation. The second step (3′ splice site cleavage and exon ligation) then occurs, producing the mRNA and the excised intron. (b) Alternative splicing differentially combines exons or portions thereof to increase transcriptome diversity. In the case of exon skipping, the splice sites flanking the exon are not recognized leading it to be considered as part of an intron. Different modes of alternative splicing exist. From top to bottom: constitutive splicing, exon skipping, alternative 5′ splice site use, alternative 3′ splice site use, mutually exclusive exon inclusion, and intron retention.

It must also be pointed out that alternative splicing defects can arise when the levels of generic spliceosome components are altered. For example, the reduced expression of the protein SMN, that is implicated in snRNP assembly, leads to a wide-range of alternative splicing alterations (Zhang *et al.*, 2008). Likewise, mutations in the snRNP components PRPF8, PRPF3, U2AF35, and SF3B1 cause diseases (e.g., retinitis pigmentosa for PRPF proteins, and myelodysplasia for U2AF35 and SF3B1) that are associated with broad changes in the production of splice variants (Chabot & Shkreta, 2016). Splicing defects may occur because splice site selection and spliceosome kinetics are highly dependent on the concentration of core spliceosomal components, and that alternative splicing units often harbor weaker splice sites that may be more sensitive to drops in the levels of generic splicing factors.

Aging and alternative splicing control

With such a complex regulatory machinery controlling splicing decisions, the molecular changes that occur with aging are therefore likely to impact the activity of factors that control splicing. A gene ontology analysis in both human and mouse reported that changes in pathways such as mRNA binding, RNA processing, and RNA splicing are strongly associated with age (Southworth *et al.*, 2009; Harries *et al.*, 2011). Age-related splicing changes in the human brain affect pathways such as sugar metabolism and DNA repair (Tollervey *et al.*, 2011), both relevant to aging (Colman *et al.*, 2009; López-Otín *et al.*, 2013).

It is estimated that more than 50% of all age-associated alterations in alternative splicing are due to changes in the expression of splicing factors (Mazin *et al.*, 2013) (Table 1). Through microarray analysis, splicing changes have been linked to several splicing factors, including PTB (aka hnRNP I), ESRP1/ESRP2, NOVA1, and hnRNP K, whose expression also decreases with age (Tollervey *et al.*, 2011). Recently, the expression of splicing regulators hnRNP A1 and A2 was linked positively to parental longevity in humans (Lee *et al.*, 2016). Alterations in RBP expression may be caused by changes in the activity of transcription factors (Table 1). For instance, the activity of transcription factors such as STAT, IRF, GATA, and NF-κB is presumed to vary with age (Stilling *et al.*, 2014). Moreover, transcription factors can themselves be subject to age-dependent alternative splicing that in turn may affect the expression of target genes. For example, microarray analysis showed that *STAT1* splicing was significantly disrupted in aging human peripheral blood cells (Harries *et al.*, 2011), although it is unclear whether STAT1 splice variants display differences in their ability to activate the expression of target genes.

In the worm *Caenorhabditis elegans*, the enhanced longevity elicited by caloric restriction is compromised by depleting SFA-1, which is the homolog of the mammalian splicing factor SF1, known to interact with the branchsite region (Heintz *et al.*, 2016). Notably, overexpression of SFA-1 is sufficient to increase worm longevity. The depletion of SFA-1 preferentially promotes splicing defects in transcripts involved in metabolic processes including lipid catabolism and carbohydrate transport, and affects the activity of components of the TORC1 pathway (Heintz *et al.*, 2016). Whether overexpression of SFA-1 corrected the noted splicing defects has not been investigated. An important component of the TORC1 pathway is mTOR whose alternative splicing is controlled by Sam68 (Huot *et al.*, 2012). Although overexpression of the splice variant mTORβ can promote tumorigenesis (Panasyuk *et al.*, 2009), it is not known whether its production is affected during aging. However, ablation of Sam68 protects mice from age-related loss of bone mass (Richard *et al.*, 2005).

Age-related splicing changes in mice can also be regulated in an unexpected manner. For instance, the age-dependent nuclear translocation of the ion channel subunit protein P2X6 in mouse allows it to interact with and sequester the U2 snRNP protein SF3A1, decreasing overall splicing activity (Díaz-Hernández *et al.*, 2015). However, this phenomenon appears to saturate when mice reach adult age, suggesting that this process may be part of normal maturation and development rather than being associated with aging *per se*.

Most transcripts encoding splicing regulatory factors are themselves subject to alternative splicing. High-throughput analyses revealed that the splicing profiles of *Hnrnph1, Hnrnpll, Hnrnpa1, Srsf5, Srsf6,* and *Srsf11* change through aging in mice, with human *SRSF6* and *SRSF1* being among the most altered by aging (Harries *et al.*, 2011; Stilling *et al.*, 2014). These changes in turn possibly alter the alternative splicing of numerous transcripts. Altogether, there is growing evidence documenting that the transcription and splicing of splicing regulators are altered in aging tissues. The accumulation of such alterations is likely to have a profound effect on the transcriptome of aging cells and the aging phenotype.

Age-related splicing alterations in growth regulators

Several genes produce transcripts whose alternative splicing changes in aging tissues or during prolonged cell passages in culture. An important question is whether those changes are the causes or the consequences of aging. Although recent studies have reported splicing changes in transcripts encoding proteins involved in processes that are intimately

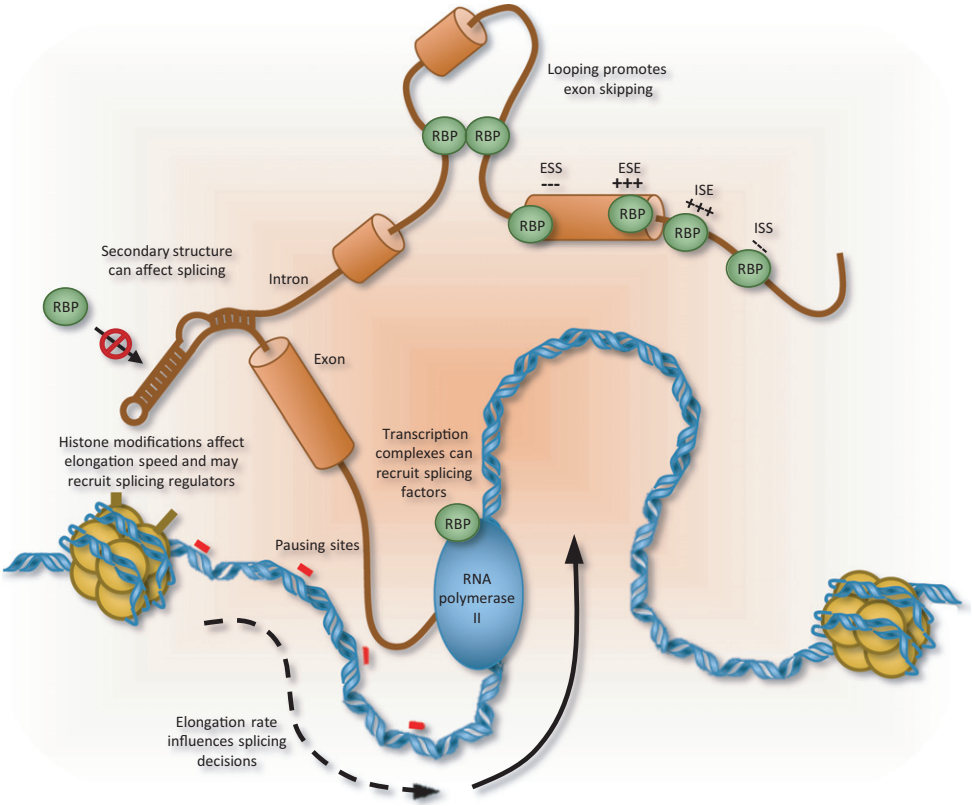

Fig. 3 Control of alternative splicing. Exonic (ESS and ESE) and intronic (ISS and ISE) elements recruit RNA binding proteins (RBPs) to silence or enhance the use of splice sites. Interactions between RNA binding proteins may also reconfigure the architecture of the pre-mRNA to affect splice site selection (Martinez-Contreras *et al.*, 2007). In addition, pre-mRNA secondary structure may be inhibitory or may be used to approximate a regulatory element near a target splice site. Splice site selection can also be influenced by transcription. The speed of transcription will determine the time given for a complex to be assembled and influence selection when splice sites are in competition. In other cases, splicing regulators may be interacting with the polymerase complex or with chromatin to be deposited on the pre-mRNA that emerges from the transcription complex. The presence of specific modifications on histones will impact the speed of transcription and the recruitment of adapters that in turn interact with splicing regulators.

associated with aging, such as DNA damage sensing, DNA repair, and telomere biogenesis (Tollervey *et al.*, 2011; Rodríguez *et al.*, 2016), we currently do not know if these switches produce splice variants with distinct functional properties. Here we will restrict our discussion to splice variants for which experimental evidence support a role in aging or senescence.

p53

One of the best known and possibly most important growth regulators is the tumor suppressor protein p53 that is implicated in cell-cycle arrest, senescence, and apoptosis (Vogelstein *et al.*, 2000; Lowe *et al.*, 2004). Mice lacking p53 develop normally but are highly predisposed to spontaneous tumor formation (Donehower *et al.*, 1992). Most human cancers have a mutated p53 or express low level of wild-type p53 (Kamijo *et al.*, 1998; Onel & Cordon-Cardo, 2004). The role of p53 in human aging is supported by epidemiological studies (van Heemst *et al.*, 2005; Ørsted *et al.*, 2007), and the fact that p53 regulates several processes relevant to organismal aging (Rufini *et al.*, 2013); p53 can promote apoptosis, the DNA damage response, autophagy, and mitophagy (Eisenberg *et al.*, 2009; Liang, 2010; Gao *et al.*, 2011; Wang *et al.*, 2011b; Tucci, 2012). Although p53 contributes to senescence-associated replicative arrest, it can restrain SASP (Coppé *et al.*, 2008; Raj & Attardi, 2013).

The alternative splicing of *p53* transcripts generates the truncated variant p44 that lacks the transactivation domain (Fig. 4a), and whose production increases in aging mice (Pehar *et al.*, 2014). Notably, p44 can also be produced by an internal ribosome entry site (IRES) in the p53 mRNA. This mechanism occurs preferentially at the G1-S cell-cycle transition and is not inducible by stress (Courtois *et al.*, 2002; Ray *et al.*, 2006). The respective contribution of the splicing-mediated and the IRES-mediated processes to the production of p44 in aging cells is unclear. What is clear however is that overexpression of p44 in transgenic mice ($p44^{+/+}$) leads to an accelerated age-associated phenotype, suppresses cell proliferation, and increases senescence (Maier *et al.*, 2004) (Fig. 4b). These effects do not occur in p44 mice lacking p53, indicating that full-length p53 is necessary to elicit the aging phenotype (Maier *et al.*, 2004). Notably, p47 (the human orthologue of p44) interacts with full-length p53 to modulate p53 activity (Courtois *et al.*, 2002; Ghosh *et al.*, 2004). Compared to the p53 homodimer, the p44/p53 complex displays distinct half-life, post-translational modifications, and promoter affinity (Campisi, 2004; Scrable *et al.*, 2005). Interestingly, the accelerated aging and growth arrest caused by disrupting the balance between p53 and p44 is functionally linked to the pathway initiated by the Insulin-like Growth Factor-1 (IGF-1) (Maier *et al.*, 2004), which extends lifespan in *C. elegans, D. melanogaster,* and mice when inhibited. p53 modulates the IGF-1 pathway by decreasing the expression of the IGF-1 receptor (IGF-1R) (Werner *et al.*, 1996). In contrast, mice overexpressing p44 display high levels of IGF-1/IGF-1R (Pehar *et al.*, 2010). Notably, the oncogenic MEK protein kinase has been associated with senescence when it is hyperactivated in a mouse model (Lin *et al.*, 1998). Hyperactivation of the RAF-MEK-ERK pathway by high levels of IGF-1 induces cell-cycle arrest (Maier *et al.*, 2004). This effect is considered a fail-safe mechanism that increases senescence, and it may explain at least in part the aging defect of $p44^{+/+}$ mice (Campisi, 2004; Maier *et al.*, 2004). $p44^{+/+}$ transgenic mice also display premature synaptic deficit, cognitive decline as well as Alzheimer's disease-like features, possibly associated with the abnormal phosphorylation of the microtubule-binding protein tau (Pehar *et al.*, 2010). In fact, p44 is reported to bind, independently of p53, to the

Table 1 Age-related alteration of notable transcription (in bold) and splicing factors

Genes	Function	Age-related alteration	Direction	Notes	References
Ap1	**Transcription**	**Expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
Eftud2	Splicing	Alternative splicing	N/A	Mice	Rodríguez *et al.* (2016)
ESRP1	Splicing	Expression	Up	Human	Tollervey *et al.* (2011)
ESRP2	Splicing	Expression	Up	Human	Tollervey *et al.* (2011)
Ets	**Transcription**	**Expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
Gata	**Transcription**	**Expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
HnrnpH1	Splicing	Alternative splicing	N/A	Mice	Stilling *et al.* (2014)
HnrnpII	Splicing	Alternative splicing	N/A	Mice	Stilling *et al.* (2014)
HNRNPK	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
HrnrpA1	Splicing	Alternative splicing	N/A	Mice	Stilling *et al.* (2014)
Irf	**Transcription**	**Expression, expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
Nfkb1	**Transcription**	**Expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
NOVA1	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
Prpf3	Splicing	Alternative splicing	N/A	Mice	Rodríguez *et al.* (2016)
Prpf8	Splicing	Alternative splicing	N/A	Mice	Rodríguez *et al.* (2016)
PTBP1	Splicing	Expression	Up	Human	Tollervey *et al.* (2011)
PTBP2	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
RBFOX1	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
Sf3b1	Splicing	Alternative splicing	N/A	Mice	Rodríguez *et al.* (2016)
SLU7	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
SNRPB2	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
Spl	**Transcription**	**Expression of target genes**	**Up**	**Mice**	**Stilling *et al.* (2014)**
SRSF1	Splicing	Alternative splicing	N/A	Human and mice	Stilling *et al.* (2014), Harries *et al.* (2011)
Srsf11	Splicing	Alternative splicing	N/A	Mice	Stilling *et al.* (2014)
SRSF2	Splicing	Expression	Down	Human	Tollervey *et al.* (2011)
Srsf5	Splicing	Alternative splicing	N/A	Mice	Stilling *et al.* (2014)
RSF6	Splicing	Alternative splicing	N/A	Human and mice	Stilling *et al.* (2014), Harries *et al.* (2011)
STATs	**Transcription**	**Alternative splicing, expression, expression of target genes**	**Up**	**Human and mice**	**Stilling *et al.* (2014), Harries *et al.* (2011)**

promoter of several tau kinases genes resulting in their overexpression (Pehar *et al.*, 2014). Thus, altering the balance between p53 variants can affect aging in mice (Campisi, 2004; Maier *et al.*, 2004; Pehar *et al.*, 2014), although it is unclear if the above conclusions apply to human aging.

In addition to p44, other p53 variants participate in the onset of senescence. In contrast to p53, whose level remained unchanged, the Δ133p53 and p53β splice variants (Fig. 4a) are, respectively, downregulated and upregulated in human senescent fibroblasts (Fujita *et al.*, 2009). This functional link is supported by the fact that the knockdown of Δ133p53 induces robust senescent phenotypes, such as flattened morphology, an increase in β-galactosidase activity, and p21 overexpression. In contrast, upregulation of Δ133p53 induces cell proliferation, as well as repression of p21 and the E3 ubiquitin ligase MDM2 involved in p53 degradation; while expression of p53β has the opposite effects (Fujita *et al.*, 2009). Overexpressing either Δ133p53 or p53β in p53-null fibroblasts had no effect, indicating that these variants need to interact with full-length p53 to impact the senescent program (Fujita *et al.*, 2009).

Despite clear differences in the function of p53 splice variants, little is known about what regulates their production by alternative splicing. The SR protein SRSF3 interacts with the *p53* pre-mRNA (Tang *et al.*, 2013) and downregulates the production of p53β (Tang *et al.*, 2013). Moreover, senescent human fibroblasts and p53β overexpressing cells display reduced levels of SRSF3 mRNA. A drop in SRSF3 is not seen in oncogenic RAS-induced senescence, suggesting that the role of SRSF3 is specific to replicative senescence. The knockdown of SRSF3 also induces a senescent phenotype, p53 phosphorylation and an increase in p53β (Tang *et al.*, 2013). These findings suggest a crucial role for SRSF3 in preventing senescence, but additional analyses in tissues are needed to confirm whether this observation is relevant to normal aging.

IGF-1

While IGF-1 is expressed in nearly every tissue, it is mostly produced by the liver, from which it enters the general circulation (Flier *et al.*, 1997; Sjögren *et al.*, 1999). IGF-1 is an important player in normal growth and development. Mutations that reduce IGF-1 signaling increase the lifespan of *D. melanogaster* and *C. elegans* (Altintas *et al.*, 2016). Caloric restriction, a condition that increases longevity in several organisms, promotes a drop in IGF-1 synthesis and signaling (Sjögren *et al.*, 1999; Campisi, 2004; Tucci, 2012; Higashi *et al.*, 2014). The *IGF-1* transcript undergoes alternative splicing to produce at least three mRNA variants: IGF-1Ea is the most abundant variant lacking exon 5; IGF-1Eb is only found in humans and lacks exon 6; and IGF-1Ec, also known as the Mechano Growth Factor (MGF), contains both exons 5 and 6, and is associated with satellite cell activation, proliferation, and skeletal muscle repair (Yang & Goldspink, 2002; Goldspink, 2005). Exon 5 of *IGF-1* contains a regulatory ESE that recruits SRSF1 to promote exon 5 inclusion (Smith *et al.*, 2002).

The splicing variants IGF-1Ea and MGF affect different pathways. IGF-1Ea through its receptor IGF-1R activates both the RAF-MEK-ERK and the

PI3K-AKT pathways that are respectively associated with proliferation and differentiation (Coolican *et al.*, 1997; Stavropoulou *et al.*, 2009). MGF activates in an IGF-1R-independent manner the RAF-MEK-ERK pathway only, supporting its role in proliferation and skeletal muscle repair (Philippou *et al.*, 2009; Stavropoulou *et al.*, 2009).

Early studies on *IGF-1* splice variants revealed that their relative production did not significantly change with age. However, in contrast to IGF-1Ea, MGF levels are significantly induced after exercise in young compared to older rats, as well as in humans (Owino *et al.*, 2001; Hameed *et al.*, 2003). Based on these results, it was suggested that the age-associated decline in skeletal muscle repair and maintenance (Brooks & Faulkner, 1990) may be related to a progressive loss of MGF production after exercise.

SIRT1

The sirtuin SIRT1 is the mammalian orthologue of yeast sir2 and is an NAD^+-dependent histone deacetylase (HDAC). SIRT1 has important functions in gametogenesis, embryonic development, skeletal muscle differentiation, and homeostasis (Cheng *et al.*, 2003; Fulco *et al.*, 2003; McBurney *et al.*, 2003; Feige & Johan, 2008). SIRT1 also regulates inflammation by interfering with the NF-κB pathway (Salminen *et al.*, 2008; Shinozaki *et al.*, 2014) and represses p53 activity to reduce apoptosis during stress (Luo *et al.*, 2001; Cheng *et al.*, 2003).

Sir2/SIRT1 activation extends lifespan in yeast, *C. elegans*, *Drosophila*, as well as in mice (Kaeberlein *et al.*, 1999; Tissenbaum & Guarente, 2001; Whitaker *et al.*, 2013; Mitchell *et al.*, 2014). Although sirtuins are considered anti-aging proteins (Guarente, 2007), the role of SIRT1 in human aging remains controversial (Ledford, 2011; Lombard *et al.*, 2011). For instance, while resveratrol stimulates the interaction between SIRT1 and lamin A, whose importance in aging defects is well documented (Liu *et al.*, 2012), activation of SIRT1 by resveratrol has no demonstrated impact on health or longevity (Semba *et al.*, 2014). In mammals, the activation of SIRT1 reduces the level of circulating IGF-1, also consistent with the caloric restriction model (Brown *et al.*, 2010). Sir2 can activate autophagy (Morselli *et al.*, 2010) and may enhance longevity by producing more homogeneously healthy mitochondria (Nemoto *et al.*, 2005). It has been proposed that the drop in NAD^+ level that occurs in aging tissues (Braidy *et al.*, 2011; Massudi *et al.*, 2012) decreases the activity of SIRT1 leading to a progressive accumulation of

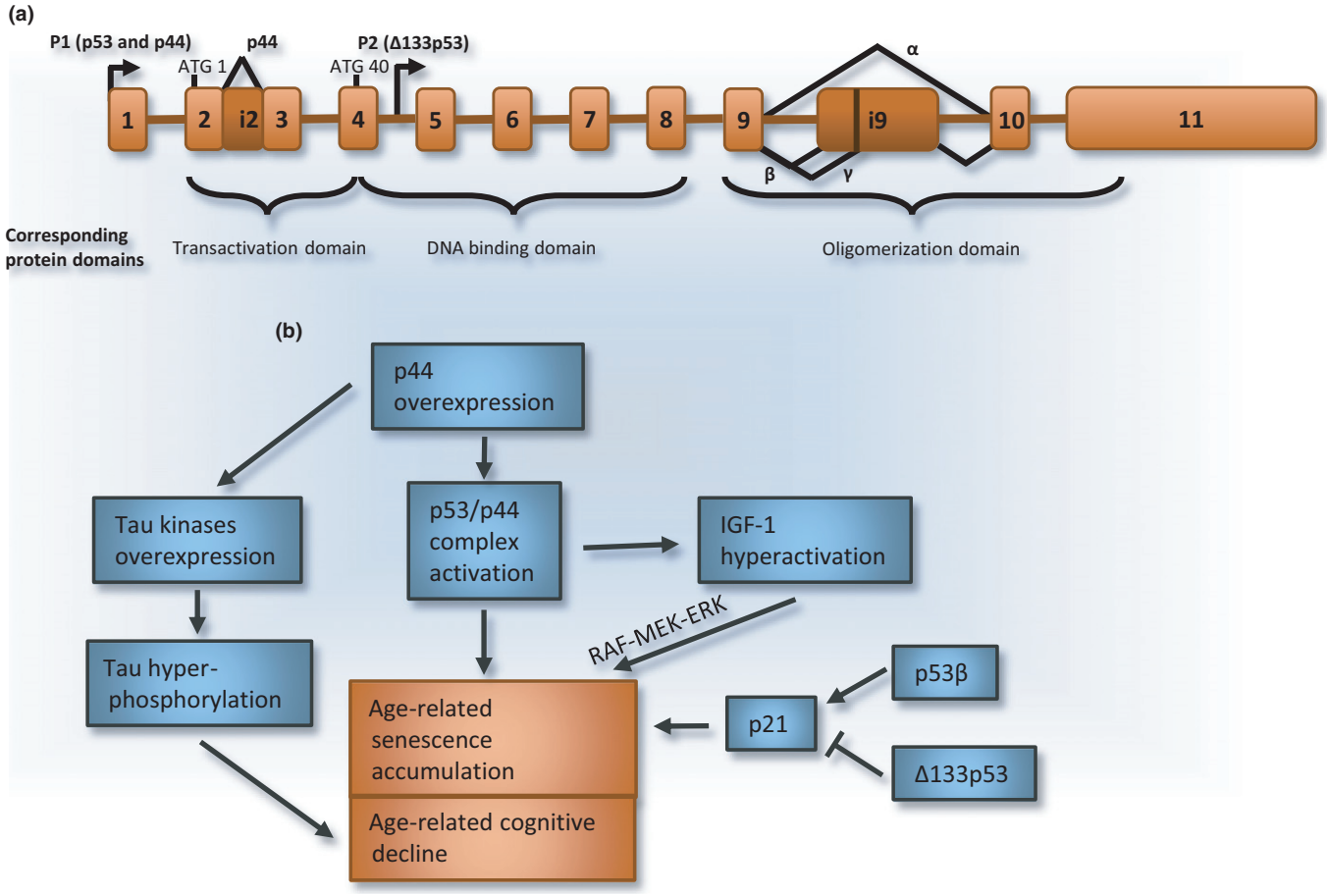

Fig. 4 p53 splice variants and aging. (a) p53 alternative splicing and the production of splice variants. The structure of the *p53* gene is illustrated with exons and introns, alternative start sites of transcription (P1 and P2 arrows), and translation initiation codons (ATG). p44 is produced when intron i2 is retained, eliciting the use of a downstream initiation codon in exon 4. The use of the alternative start site (P2) leads to the production of the Δ133p53 isoform. Complex alternative splicing can occur within intron 9 to produce β or γ (α is indicated here as the simplest case where the whole intron i9 is removed). Both p44 and Δ133p53 lack the transactivation domain. (b) p44 forms a complex with p53 to regulate its activity. When overexpressed, p44 leads to senescence and hyperactivates the IGF-1 pathway that in turn promotes cell-cycle arrest through the RAF-MEK-ERK pathway. p44 is also linked to age-related cognitive decline as its overexpression upregulates tau kinases. Variants p53β and Δ133p53 also regulate senescence by, respectively, activating and inhibiting the cell-cycle arresting protein p21.

reactive oxygen species (ROS) and mitochondrial dysfunction (López-Otín *et al.*, 2013; Kim *et al.*, 2014).

SIRT1 undergoes alternative splicing of exon 8 to produce SIRT1Δ8 which lacks a portion of the deacetylase domain. SIRT1Δ8 expression rises after stresses, and while this variant by itself displays reduced p53 deacetylation activity, it exerts an additive deacetylation effect on p53 when expressed with full-length SIRT1 (Lynch *et al.*, 2010). Combining stresses with the depletion of p53 also leads to an overexpression of *SIRT1Δ8* mRNA and protein, while activating p53 has the opposite effect (Lynch *et al.*, 2010). In stress conditions, the depletion of SIRT1Δ8 leads to an abnormal accumulation of p53, accompanied by a significant increase in apoptosis, consistent with a repressive role for SIRT1Δ8 in p53 regulation after stress (Lynch *et al.*, 2010).

A stress-induced drop in the expression of SRSF2 has been implicated in the production of the SIRT1Δ8 variant (Lynch *et al.*, 2010). *SIRT1* splicing implicates RNA binding proteins that are also linked to p53 activity. Overexpression of RNA binding proteins TIA1 or TIAL1 promotes *SIRT1* exon 8 inclusion and inhibits cell growth (Zhao *et al.*, 2014), while their knockdown has the opposite effect. A gene ontology analysis reveals that the tumor suppressor phenotype triggered by TIA1/TIAL1 overexpression involves the upregulation of p53 targets (Sánchez-Jiménez *et al.*, 2015). Conversely, the RNA binding protein HuR, which enhances p53 mRNA translation by binding to its 3′ UTR (Mazan-Mamczarz *et al.*, 2003; Abdelmohsen *et al.*, 2014), also promotes *SIRT1* exon 8 skipping when overexpressed, while the opposite effect is observed following HuR knockdown (Zhao *et al.*, 2014). Taken together, the p53-dependent alternative splicing of *SIRT1* exon 8 and the reciprocal regulatory relationship with p53 suggest a negative feedback loop (Lynch *et al.*, 2010) (Fig. 5) that may be important to prevent abnormal cell growth. Notably, both suboptimal and excess levels of SIRT1 have a tumor suppressive impact in rodents (Firestein *et al.*, 2008; Kabra *et al.*, 2009). Thus, while SIRT1 may play an important role in preventing tumor formation, the role of SIRT1 and its splice variant in senescence and apoptosis remains to be better understood.

ING1

ING1 functions in apoptosis, senescence, and DNA repair (Kichina *et al.*, 2006; Coles *et al.*, 2007; Peña *et al.*, 2008; Abad *et al.*, 2011). The expression of ING1 is ubiquitous, p53-independent and is frequently reduced in human cancers, supporting its role as a tumor suppressor (Toyama *et al.*, 1999; Cheung *et al.*, 2000). Consistently, knocking out *ING1* provokes a high frequency of spontaneous tumor development in mice (Kichina *et al.*, 2006). *ING1* produces at least four mRNA isoforms of which two are splice variants: *ING1a* and *ING1b* (Feng *et al.*, 2002). INGb, but not ING1a, interact with HDAC complexes, and only overexpression of ING1b leads to increased acetylation of histones H3 and H4 (Skowyra *et al.*, 2001; Vieyra *et al.*, 2002a).

The level of ING1a increases at the expense of ING1b when cells approach senescence, suggesting a role for ING1a in cell-cycle arrest (Soliman *et al.*, 2008). Consistent with this view, cells overexpressing ING1a display senescence phenotypes such as formation of SAHF, flat morphology, and expression of senescence-associated β-galactosidase and p16 (Soliman *et al.*, 2008). While ING1b also participates in senescence through upregulation of p16 (Li *et al.*, 2011), it sensitizes young cells to apoptosis (Scott *et al.*, 2001; Vieyra *et al.*, 2002b; Guérillon *et al.*, 2013) (Fig. 6). ING1b-mediated apoptosis may occur by stabilization of p53, possibly by inhibiting deacetylation mediated by SIRT1 (Vaziri *et al.*, 2001; Kataoka *et al.*, 2003; Binda *et al.*, 2008; Guérillon *et al.*, 2013). The splicing shift toward ING1a may help to maintain the senescence status and thus may prevent apoptosis. Overall, the fact that splice variants of *p53* or *ING1* can differentially trigger senescence *in vitro* or age-related phenotypes *in vivo* suggests that their alternative splicing control is directly implicated in aging.

Alternative splicing and age-related diseases

A number of human diseases known as progeroid syndromes provoke phenotypic alterations that resemble those noted during normal aging. These include Hutchinson–Gilford Progeria syndrome (HGPS or progeria), Werner syndrome, and Cockayne syndrome. The study of HGPS has revealed that alternative splicing defects are responsible for some of its distinctive features.

Progeria

Progeria or HGPS is defined by a premature aging phenotype associated with a high incidence of age-related disorders, such as cardiovascular impairment and atherosclerosis (Merideth *et al.*, 2008; Hisama *et al.*, 2011). Premature cell senescence has also been reported, consistent with the notion that senescence is associated with aging (Huang *et al.*, 2005). While the relationship between HGPS and normal physiological aging is still debated (Burtner & Kennedy, 2010), tissues from HGPS patients are considered a good model for research on vascular aging as HGPS shares common mechanisms with age-related vascular dysfunction (Brassard *et al.*, 2016). Lamin proteins are components of the nuclear lamina and play key roles in nuclear structure and in processes such as transcription and DNA replication (Goldman *et al.*, 2002; Dechat *et al.*, 2008, 2009). Lamin A interacts with and activates SIRT1 (Liu *et al.*, 2012). The *LMNA* gene normally encodes both lamin A and C proteins. A silent C to T mutation in exon 11 has been implicated in the most common form of HGPS (Hutchison, 2002; Eriksson *et al.*, 2003). The mutation increases by 50-fold the use of an alternative 5′ splice site situated 5 nucleotides upstream of the mutation, producing a variant lacking 150 nucleotides and a truncated protein called progerin lacking the corresponding 50 amino acids (Fig. 7). Progerin is abnormally processed and becomes sequestered in the nuclear membrane, decreasing the acetylase activity of SIRT1. Notably, progerin is detected in trace amounts in healthy tissues that lack the mutation. This observation and the fact that progerin is also produced in other animals (Lopez-Mejia *et al.*, 2011) suggest that progerin may have a normal physiological function (Hutchison, 2002; De Sandre-Giovannoli *et al.*, 2003; Eriksson *et al.*, 2003; Scaffidi & Misteli, 2006; Liu *et al.*, 2012). Lamins, including progerin, may regulate senescence pathways in patients with HGPS and healthy individuals (Karlseder, 2002; Freund *et al.*, 2012; Wood *et al.*, 2014). Notably, physical insults like UVA, but not UVB, drastically change the splicing profile of normal *LMNA* to favor the production of progerin in cultured human fibroblasts (Ikehata & Ono, 2011; Takeuchi & Rünger, 2013). In contrast to UVB, which preferentially produce pyrimidine dimers, the indirect generation of oxidative DNA damage through ROS induction by UVA (Cadet *et al.*, 2009) may explain the upregulation of progerin. Thus, the UVA-induced increase in progerin production may accelerate skin aging (Takeuchi & Rünger, 2013). It has also been noted that the production of progerin in an HGPS mouse model associates with impaired developmental splicing and an altered production of splice variants in the skin (Rodríguez *et al.*, 2016).

Although the mRNA level of progerin does not significantly increase in aging biopsies (Scaffidi & Misteli, 2006; Rodriguez *et al.*, 2009; Cao *et al.*, 2011), the accumulation of progerin in the nuclear membrane, as reported in HGPS and healthy tissues, is believed to contribute to

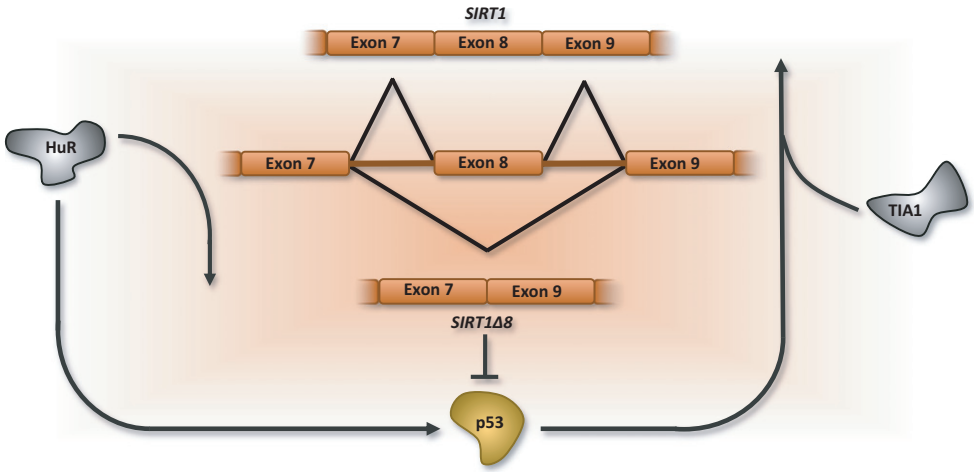

Fig. 5 Regulating the alternative splicing of *SIRT1*. Skipping of exon 8 in the *SIRT1* pre-mRNA generates SIRT1Δ8. The HuR splicing factor enforces the production of SIRT1Δ8, while TIA1 and p53 (by an unknown mechanism) show the opposite effect. However, the upregulation of SIRT1Δ8 inhibits p53 leading to reciprocal regulation and a negative feedback loop.

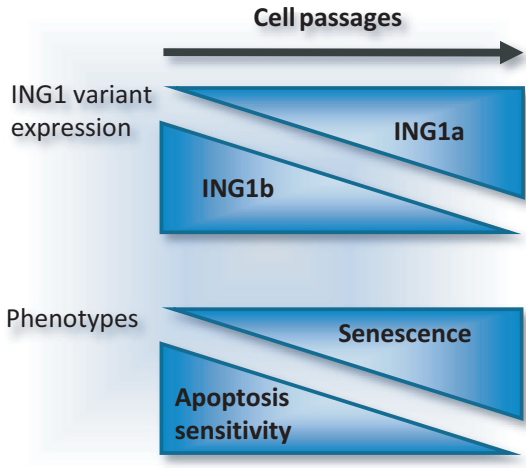

Fig. 6 ING1 splice variants and senescence. *ING1* pre-mRNA alternative splicing generates two variants termed ING1b and ING1a. In young cultured cells, ING1b is predominant and, when overexpressed, can trigger apoptosis through p53 acetylation. ING1a production increases when cells approach senescence in culture. ING1a elicits several senescence phenotypes when ectopically overexpressed.

senescence. Consistent with this view, ectopic expression of progerin can induce progeroid phenotypes, such as nuclear misshape, premature senescence (Candelario *et al.*, 2008), and telomere aggregation and dysfunction independently of telomere attrition (Benson *et al.*, 2010; Cao *et al.*, 2011). Also, the depletion of progerin downregulates expression of *p21*, as well as that of growth regulators IGFBP3 and GADD45B (Varela *et al.*, 2005; Scaffidi & Misteli, 2006). Moreover, shortened telomeres induce the production of progerin, suggesting a positive feedback loop that ultimately converges into activation of senescence in normal tissues (Cao *et al.*, 2011).

The above results suggest that the abnormal accumulation of progerin contributes to senescence and accelerated aging. As this accumulation also occurs during the normal aging process, the same senescence pathway may be activated in healthy individuals (Scaffidi & Misteli, 2006; McClintock *et al.*, 2007). Despite several aging phenotypes displayed by patients with HGPS, an increase in the incidence of cancer is not one of them (Hennekam, 2006; Coppedè & Migliore, 2010). Based on these observations, it is tempting to suggest that progerin, while protecting the organism from tumorigenesis, accelerates the accumulation of senescence leading to degenerative phenotypes. Notably, patients with HGPS also do not show any increase in the incidence of Alzheimer's disease, potentially providing insight into protective mechanisms (Nelson *et al.*, 2011).

The splicing regulatory proteins SRSF1 and SRSF6 bind close to the progerin-producing splice site and elicit mutually antagonistic effects in both HeLa cells and primary HGPS fibroblasts; SRSF6 decreases the use of the progerin-specific 5′ splice site, while SRSF1 enhances it (Lopez-Mejia *et al.*, 2011). Consistent with this result, the depletion of SRSF6 increases progerin accumulation in the nucleus of HGPS fibroblasts, while depletion of SRSF1 reduces the level of progerin and nuclear misshape (Lopez-Mejia *et al.*, 2011). Notably, the C to T mutation in exon 11 of *LMNA* increases the accessibility of the progerin 5′ splice site, which is normally sequestered in a conserved duplex structure (Lopez-Mejia *et al.*, 2011).

Vascular aging

Vascular aging is characterized by a decline of endothelial function leading to a progressive deficiency in cardiovascular repair mechanisms (Ribera-Casado, 1999) and vascular walls remodeling (Brandes *et al.*, 2005; Minamino & Komuro, 2007). The decreasing proliferative potential of endothelial cells and the accumulation of senescent cells also deleteriously affect angiogenesis, nutrient trafficking, homeostasis, and the response to TGF-β (Dimri *et al.*, 1995; Foreman & Tang, 2003). Alternative splicing plays a crucial role in vascular integrity, and defective splicing leads to cardiovascular diseases such as aortic aneurysm and arrhythmias (Martin *et al.*, 2014; Dlamini *et al.*, 2015; Rizzacasa *et al.*, 2017). For instance, the proportion of titin splice variants, which contribute to cardiac myocytes stiffness, is altered in heart diseases (LeWinter & Granzier, 2013). Alternative splicing of the titin transcript is controlled by RBM20, which acts on other pre-mRNAs whose splicing is linked to dilated cardiomyopathy and other vascular diseases (Guo *et al.*, 2012; Li *et al.*, 2013; Maatz *et al.*, 2014). Splicing regulators like hnRNP A1 and PTBP1 also contribute to cardiovascular risk factors including the metabolism of cholesterol, omega-3, and omega-6 (Medina *et al.*, 2011; Mozaffarian & Wu, 2011; Reardon *et al.*, 2011; Yu *et al.*, 2014). Given that aging is a risk factor to vascular diseases, it is likely that age-dependent alterations in at least some of the above splicing events and regulators contribute to cardiovascular diseases.

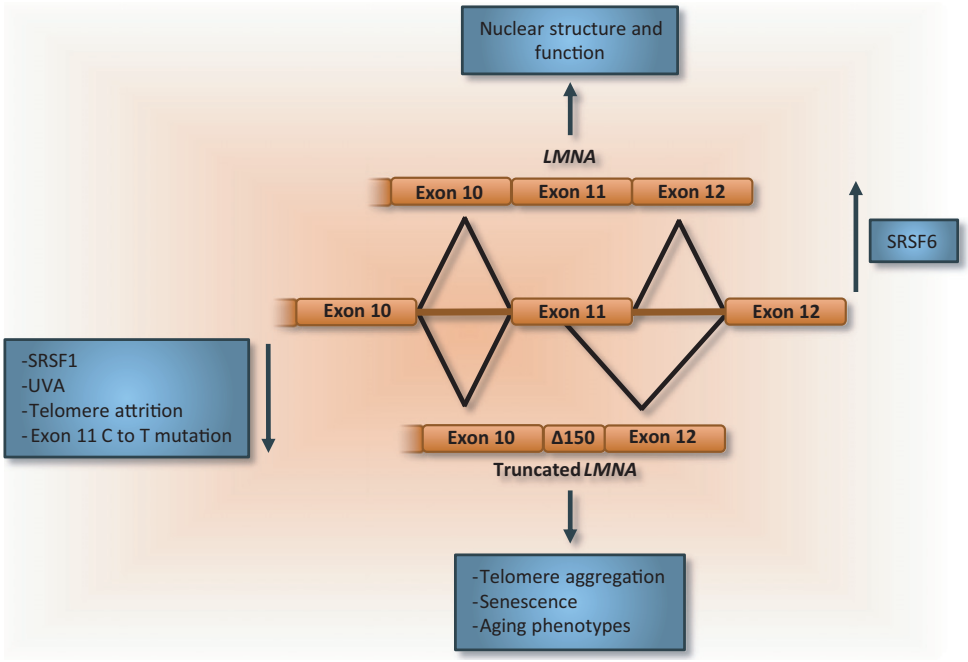

Fig. 7 The alternative splicing of *LMNA* produces the progerin variant that is associated with aging and age-related diseases. The use of an alternative 5′ splice site in exon 11 of the *LMNA* pre-mRNA produces a truncated mRNA variant encoding progerin. In the primary form of progeria, a silent C to T mutation in exon 11 increases the use of this cryptic site by 50-fold. Ectopic expression of progerin causes senescence, telomere aggregation, and a progeroid phenotype in animal models. The expression of progerin can also be triggered by UVA, telomere attrition, overexpression of SRSF1, or the depletion of SRSF6.

One example of age-related splicing change in the vascular context concerns the *ENG* gene which codes for the glycoprotein endoglin, a transmembrane co-receptor of TGF-β that functions in endothelial cell growth, angiogenesis, differentiation, and senescence. The TGF-β receptor complex includes endoglin and the TGF-β type I and II receptors. The type I receptor associates with two antagonistic proteins, ALK1 and ALK5, that regulate the signal induced by TGF-β (Bernabeu *et al.*, 2007; ten Dijke & Arthur, 2007). The *ENG* pre-mRNA is alternatively spliced to produce at least two mRNA variants: the long (L) and the short (S) version. The S variant has a retained intron that provides a premature stop codon before the last exon (Bellón *et al.*, 1993; Pérez-Gómez *et al.*, 2005). While L-endoglin is predominantly produced in young vascular endothelial cells, a shift toward the S form occurs during normal aging, as well as at in late passages in cell culture (Blanco *et al.*, 2008; Blanco & Bernabeu, 2011). S-endoglin is more likely to interact with ALK5 than ALK1, whereas L-endoglin has more affinity for ALK1. Thus, the cellular responses mediated by TGF-β signaling may depend on the alternative splicing of endoglin as the levels of ALK1 and ALK5 do not significantly change during senescence (ten Dijke & Arthur, 2007). L-endoglin has a proliferative and pro-angiogenic effect on endothelial cells through its interaction with ALK1, while S-endoglin is more likely to induce a senescence phenotype (Lebrin *et al.*, 2004; Düwel *et al.*, 2006). Endothelial cells of transgenic mice overexpressing S-endoglin show a lower proliferative rate, suggesting that S-endoglin may contribute to senescence and age-associated vascular pathologies (Blanco *et al.*, 2008).

SRSF1 has been implicated in regulating the production of S- and L-endoglins. Overexpressing SRSF1 increases S-endoglin mRNA, possibly by interfering with branch site usage to promote intron 2 retention in *ENG* transcripts (Blanco & Bernabeu, 2011).

Alzheimer's disease (AD)

Among age-related disorders, AD is characterized by progressive neurodegenerative phenotypes leading to memory loss and dementia (Burns & Iliffe, 2009), possibly caused by aggregation of amyloid beta (Aβ) in the brain (Zheng & Koo, 2011; Kandalepas & Vassar, 2014). Aβ is the product of two subsequent enzymatic digestions driven, respectively, by the β-secretase (BACE) and the γ-secretase; the initial substrate for these enzymes is the amyloid precursor protein (APP) (Citron *et al.*, 1995; Koffie *et al.*, 2011; Zheng & Koo, 2011; Chami & Checler, 2012; Kandalepas & Vassar, 2014). BACE turns out to be a more desirable target against AD as γ-secretase is believed to be more critical for normal function (Mowrer & Wolfe, 2008). The complex alternative splicing profile of BACE exons 3 and 4 generates at least four splice variants: I-501, I-476, I-457, and I-432 (Mowrer & Wolfe, 2008; Fisette *et al.*, 2012). While total level of *BACE* transcripts remains unchanged when comparing young and old mice, the level of full-length I-501 increases in an age-dependent manner (Zohar *et al.*, 2005). Considering the strong correlation between BACE activity and Aβ accumulation in the brain (Hsiao *et al.*, 1996; Tanahashi & Tabira, 2001), a rise in BACE level and the consequent accumulation of Aβ may increase the predisposition to AD as well as to other age-associated cognitive impairments.

In addition, age-related expression and splicing changes have been noted both in *BACE* and *APP* in the mice hippocampus (Stilling *et al.*, 2014). Interestingly, overexpression of APP in AD mice model alters *BACE* alternative splicing in the brain, suggesting a feedback role for APP on Aβ production through *BACE* splicing (Zohar *et al.*, 2005).

Little is known about the control of alternative splicing of *BACE1* transcripts. An ESE potentially recognized by SRSF2 positively regulates the inclusion of exon 4 (Mowrer & Wolfe, 2009). The exon 3 and exon 4 contain several alternative splice sites leading to a complex splicing profile whose regulation is not understood. Notably, a G-rich sequence within exon 3 controls 5′ splice site selection to enhance production of the full-length mRNA. This G-rich sequence forms a G-quadruplex structure that interacts with the splicing factor hnRNP H (Fisette *et al.*, 2012). Decreasing the level of hnRNP H by RNA interference significantly reduces production of full-length *BACE,* hence decreasing Aβ production (Zheng & Koo, 2011; Chami & Checler, 2012; Fisette *et al.*, 2012). These findings identify potential targets for therapies that may help reduce the damaging effects of the Aβ accumulation in the brain observed in AD and normal aging, and show the essential role that alternative splicing and its regulation play in such diseases. It is worth restating that patients

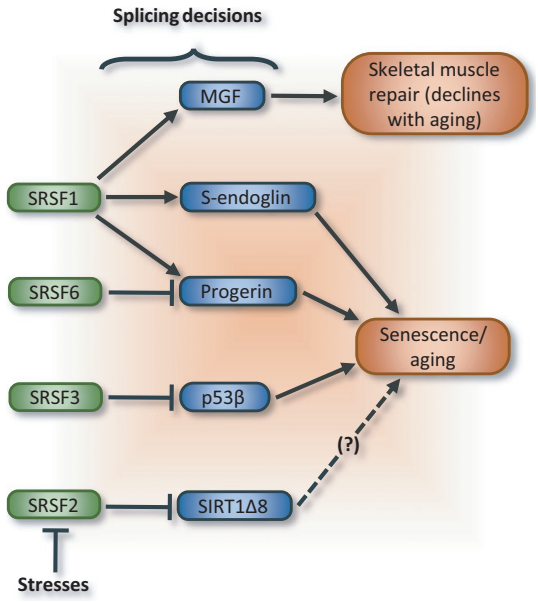

Fig. 8 Contribution of SR proteins to senescence and the aging phenotype. As indicated, SRSF1, SRSF2, SRSF3, and SRSF6 enhance or repress the production of specific splice variants implicated in age-related phenotype, aging, and senescence.

with HGPS are largely spared from Alzheimer's disease and that they may represent a unique cohort to study the underlying protective mechanisms.

Conclusions and perspectives

Given the challenges associated with maintaining homeostasis in cells and tissues subjected to constant internal and external insults, we can anticipate that a subset of mutations and epigenetic changes may alter the expression or activity of spliceosome components and splicing regulatory factors. These changes may, in turn, alter the splicing profile in several transcripts, resulting in a cascade of alterations that may either activate senescence, promote apoptosis, or elicit tumor formation. Although senescence and apoptosis may protect against tumor formation, the gradual accumulation of senescent cells will elicit tissue degeneration and organ dysfunction. While progressive age-related disturbances in homeostasis do indeed correlate with a broad range of alterations in alternative splicing, the current challenge is to determine whether a specific splicing change contributes to the aging phenotype or is simply a consequence with little or no functional impact. In this review, we have focused on altered alternative splicing events whose contributions to age-related phenotypes are experimentally supported. These events occur in genes known for their implication in mechanisms that are relevant to cell senescence and organismal aging. Although we reviewed the impact of selected splice variants on aging, regulatory networks likely coordinate the production of splice variants from different genes to maximize functional outcomes that determine cell fate, and ultimately the aging phenotype. Consistent with this proposition, the activity of p53 in senescence and apoptosis can be modulated by SIRT1 and ING1, in turn affecting ING1 signaling and SIRT1 activity. Extending these relationships to the full repertoire of splice variants for all the components of the extended p53 regulatory network may be required to determine how important is the level of coordination and feedback involved in the production of splice variants contributing to aging. Already, the splicing regulatory proteins SRSF1, SRSF2, SRSF3, and SRSF6 are emerging as central players coordinating multiple splicing decisions in age-relevant and senescent transcripts (Fig. 8). Future studies will likely investigate in more details how defects in the expression or activity of these proteins, as well as hnRNP proteins and core spliceosomal components, affect senescence and aging. Another group of potentially relevant regulatory molecules are noncoding RNAs. Some display age-dependent changes in expression (e.g., see Gruner *et al.*, 2016), and recent studies have implicated microRNAs, large noncoding RNAs, and circular RNAs in senescence and SASP (Abdelmohsen & Gorospe, 2015; Panda *et al.*, 2017a). Specifically, the circular RNA CircPVT1, whose

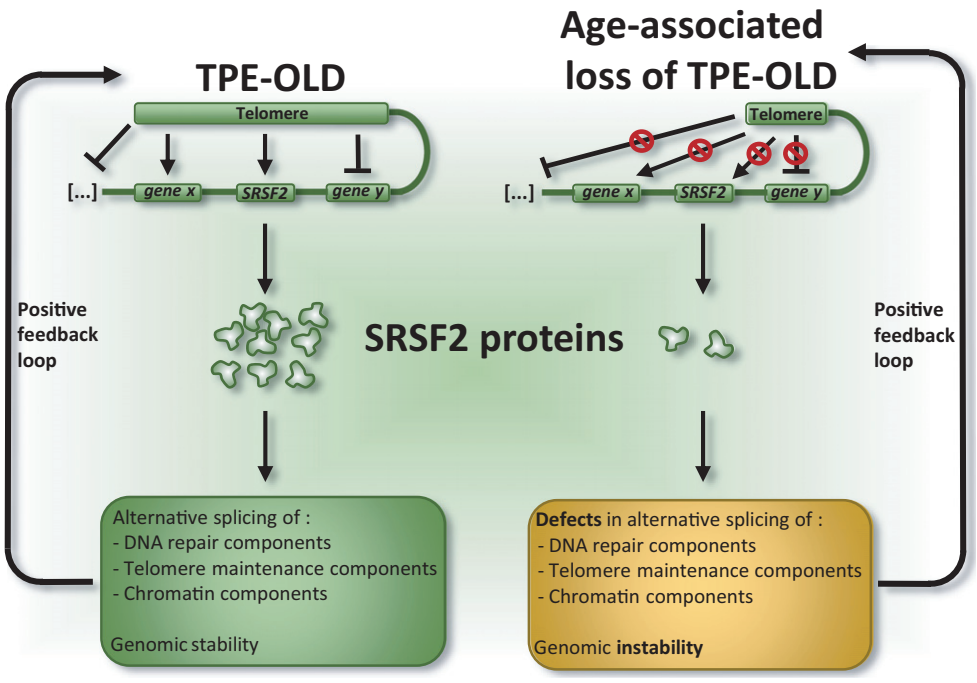

Fig. 9 A model that links telomere function with splicing control. Telomere erosion would abrogate TPE-OLD to alter the expression/activity of splicing regulators such as SRSF2, in turn promoting a cascade of splicing alterations to affect the maintenance of genomic, chromatin, and telomere integrity (see text for details).

expression is reduced in senescent cells, is produced by circularization of an exon of the *PVT* gene through RNA splicing. CircPVT sequesters *let-7* RNA and its depletion triggers senescence (Panda *et al.*, 2017b). There is clearly a need to investigate further how splicing regulation is altered to affect the production of circular RNA molecules during senescence and aging. To help clarify the contribution of an expanding list of splice variants and regulators associated with aging, it would be useful to combine expression assays with the monitoring of phenotypes like cell growth and the production of senescent markers. Likewise, it would be informative to determine whether and how SASP components produced by senescent cells reprogram the splicing profiles of neighboring cells.

While alterations in alternative splicing are likely contributing to aging, it is unclear what events initially trigger the change in activity of splicing regulators. Relevant to this question are recent studies in human cells that have uncovered links between telomere function and gene expression. Specifically, chromosome looping allows long telomeres to extend their effect on transcription as far as 10 Mb, a process termed Telomere Position-Effect On Long Distance (TPE-OLD) (Robin *et al.*, 2014). As telomeres shorten during aging, TPE-OLD regulation becomes compromised, directly altering gene expression (Robin *et al.*, 2015). If the looped-out chromosome portions host genes encoding splicing regulators or factors that control their activity, the loss of TPE-OLD may have a global impact on alternative splicing. A link between telomere function and the activity of splicing factors is supported by the observation that telomere dysfunction promotes the production of the *LMNA* splice variant progerin, which elicits senescence (Cao *et al.*, 2011). In addition, mouse hematopoietic progenitor cells from telomerase-deficient mice have reduced levels of U2AF2, SF3B1, and SRSF2 (Colla *et al.*, 2015). Moreover, telomerase-deficient and SRSF2-deficient mice share defects in the splicing of transcripts encoding components involved in DNA repair, chromatin structure, and telomere maintenance (Colla *et al.*, 2015). These observations can be integrated into a model whereby TPE-OLD would be important for the activity of splicing factors such as SRSF2 (Fig. 9). Notably, the human and mouse *Srsf2* and *U2af2* genes are located less than 10 Mb from the telomeres on their respective chromosomes. In young cells, optimal telomere function would insure optimal SRSF2 expression, allowing correct splicing of transcripts encoding telomere maintenance, DNA repair, and chromatin factors. In cells of aging animals however, telomere erosion would lead to a disruption of TPE-OLD and decreased levels of SRSF2 that would elicit aberrant splicing of components required for genome integrity. It is also notable that age-related splicing changes occur in the same categories of transcripts that are affected by DNA damaging agents (i.e., DNA repair, chromatin, and RNA splicing) (Southworth *et al.*, 2009; Harries *et al.*, 2011; Tollervey *et al.*, 2011; Shkreta & Chabot, 2015). While short telomeres not engaged in TPE-OLD may activate the DDR, this pathway may be further stimulated by the downstream pre-mRNA splicing changes. The resulting amplification of genomic stress would lead to senescence (or cancer, if the senescent or apoptotic programs are subfunctional). The stochastic nature of telomere erosion (Martin-Ruiz *et al.*, 2004) may create variation in TPE-OLD between cells, possibly contributing to the age-dependent increase in transcriptional variability observed between individual cells (Martinez-Jimenez *et al.*, 2017), a process that may also apply to alternative splicing control.

Although senescence and aging affect a wide-range of biological processes, interventions that prevent or encourage the production of specific splice variants may help counteract the emergence of age-related phenotypes and disorders. Splice-switching antisense oligonucleotides (SSOs) that block or stimulate the use of a splice site are now used with success in animal models of human diseases and are being tested in clinical trials (Brosseau *et al.*, 2014; Havens & Hastings, 2016). In principle, they could be used to prevent the production of pro-senescence variants in p53, ING1, and other transcripts. A recent encouraging study used an SSO to shift the splicing of *ApoER2* and explore its contribution to AD in a mouse model: correction of *ApoER2* splicing improved synaptic function, learning, and memory (Hinrich *et al.*, 2016). Alternatively, given that a large collection of apoptotic regulators produce functionally antagonistic splice variants, and that the apoptotic pathway is repressed in senescent cells (Wang, 1995), SSOs may serve to encourage the production of pro-death variants in senescent cells to favor their elimination and thus prevent their deleterious contribution to aging tissues. Other anti-aging tools may emerge from the screening of small molecules capable of modulating splicing (Salton & Misteli, 2016). Compounds targeting generic splicing factors, such as SF3B1, may find a use as anticancer agents. As compounds that control the activity of SR proteins are being identified (Shkreta *et al.*, 2017; Sigala *et al.*, 2017), their pro-apoptotic or antisenescent activities will need to be investigated. Overall, while additional links between splicing and different facets of aging will continue to be uncovered, compounds that can modulate specific splicing events or relevant splicing programs may offer innovative approaches to correct or postpone age-related disorders.

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Conflict of interest

None declared.

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