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Abstract

Ageing is a complex biological process characterized by the progressive loss of biological fitness due to the accumulation of macromolecular and cellular damage that affects most living organisms. Moreover, ageing is an important risk factor for many pathologies, including cardiovascular diseases, neurological disorders, and cancer. However, the ageing rate can be modulated by genetic, nutritional, and pharmacological factors, highlighting the concept of “ageing plasticity”. Progeroid syndromes are a group of rare genetic diseases that resemble many characteristics of physiological ageing. Accordingly, studies on these diseases have been very useful for gaining mechanistic insights in ageing biology. In recent years, a great effort has been made in ageing research and several works have confirmed that geromiRs, the growing subgroup of miRNAs implicated in ageing, are able to modulate organismal lifespan. However, very little is still known about the impact of miRNA in premature ageing. In this review, we will address the functional relevance of this class of small non-coding RNAs in the regulation of the hallmarks of progeroid syndromes. In addition, we will discuss the potential strategies for managing progeria based on geromiR modulation.

Keywords	Ageing, Progeroid syndromes, miRNAs
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FUNCTIONAL RELEVANCE OF miRNAS IN PREMATURE AGEING

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ABSTRACT

Ageing is a complex biological process characterized by the progressive loss of biological fitness due to the accumulation of macromolecular and cellular damage that affects most living organisms. Moreover, ageing is an important risk factor for many pathologies, including cardiovascular diseases, neurological disorders, and cancer. However, the ageing rate can be modulated by genetic, nutritional, and pharmacological factors, highlighting the concept of "ageing plasticity". Progeroid syndromes are a group of rare genetic diseases that resemble many characteristics of physiological ageing. Accordingly, studies on these diseases have been very useful for gaining mechanistic insights in ageing biology. In recent years, a great effort has been made in ageing research and several works have confirmed that geromiRs, the growing subgroup of miRNAs implicated in ageing, are able to modulate organismal lifespan. However, very little is still known about the impact of miRNA in premature ageing. In this review, we will address the functional relevance of this class of small non-coding RNAs in the regulation of the hallmarks of progeroid syndromes. In addition, we will discuss the potential strategies for managing progeria based on geromiR modulation.

KEY WORDS: Ageing, Progeroid syndromes, miRNAs

ABBREVIATIONS: CRP: C-reactive protein, DDR: DNA damage response, DSB: Double-strand breaks, HATs: Histone acetyltransferases, HDACs: Histone deacetylases, hMSCs: Human mesenchymal stem cells, HRR: Homologous recombination repair, MEFs: Mouse embryonic fibroblast, ROS: Reactive oxygen species, SASP: Senescence-associated secretory phenotype, shRNA: small hairpin RNA, and siRNA: small interference RNA.

INTRODUCTION

Ageing is a multifactorial biological process that is tightly regulated by several evolutionary conserved mechanisms, resulting in a progressive decay in systemic physiology due to the accumulation of macromolecular and cellular damage (Vijg and Campisi, 2008). One of the most important advances in ageing research was the discovery of ageing plasticity, which demonstrates that organismal lifespan can be modulated by altering certain biochemical pathways. In the 1980s, different studies showed that longevity could be significantly increased in several strains of *Caenorhabditis elegans* through mutations in certain genes (Klass, 1983; Friedman and Johnson, 1988). Afterwards, studies performed in *Drosophila melanogaster* and *Mus musculus* demonstrated that lifespan could also be extended in more complex organisms by modulating the expression of some ageing-related genes (Burtner and Kennedy, 2010). Nevertheless, when complexity increases, many conflicting synergies complicate the precise understanding of the ageing process. Despite this fact, and even though data in nonhuman primates seem somewhat contradictory in several aspects, caloric restriction without malnutrition has been an effective approach to increase lifespan in many organisms (Colman et al., 2009; Mattison et al., 2012; Mattison et al., 2017).

Recently, advances in the molecular characterization of mechanisms that underlie ageing have led to the identification of a subset of genes whose deregulation affects this complex biological process. Their study has been instrumental in defining the "Hallmarks of Ageing", nine biological features that are present in normal ageing and show the ability of accelerating or delaying this process by experimental aggravation or amelioration, respectively (López-Otín et al., 2013). These features can be divided into three groups: Primary hallmarks, antagonistic hallmarks and integrative hallmarks. Primary hallmarks include genomic instability, telomere attrition, epigenetic alterations and loss of proteostasis. Being undoubtedly detrimental, these processes are the main culprits of ageing-associated macromolecular damage. Mitochondrial dysfunction, cellular senescence and deregulated nutrient sensing fall into the second category of compensatory or antagonistic responses that counterbalance the primary hallmarks. These processes have an initial benign effect up to a point when they turn to be deleterious. Finally, the third category involves the integrative hallmarks and includes altered intercellular communication and stem

cell exhaustion. These hallmarks are ultimately responsible for the functional decline associated with ageing. More recently, the metabolic pathways which play a role in controlling ageing and longevity have also been defined, along with metabolism-related interventions that proved to extend longevity in several model organisms. Amongst these interventions are regular exercise, dietary restriction and its pharmacological mimetics (resveratrol and spermidine), the limited intake of amino acids, the administration of metformin (an antidiabetic drug) and the inhibition of trophic signal-transduction pathways (López-Otín et al., 2016).

Progeroid syndromes comprise a group of genetic disorders in which patients show ageing phenotypes in their youth (Ramírez et al., 2007). In spite of the many different progeroid diseases described to date, they can be categorized into two main subgroups that are defined by their etiology. Hence, the first type of progeroid syndromes – known as laminopathies – is caused by mutations in nuclear lamina components. This category includes Hutchinson-Gilford (HGPS) and Néstor-Guillermo (NGPS) progeria syndromes, other atypical progeria syndromes (APS), restrictive dermopathy (RD) and mandibuloacral dysplasia (MAD). The second group of progeroid syndromes stems from defects in DNA repair mechanisms. Here we can find Werner syndrome (WS), Bloom syndrome (BS), Rothmund-Thomson syndrome (RTS), Seckel syndrome (SS), Cockayne syndrome (CS), Hoyeraal-Hreidarsson syndrome (HHS), xeroderma pigmentosum (XP), trichothiodystrophy (TTD), Fanconi anemia (FA), ataxia telangiectasia (AT) and dyskeratosis congenita (DC). Recently, nine hallmarks which define the most prominent characteristics of progeroid syndromes and describe the mechanisms underlying their pathogenesis have been proposed (Carrero et al., 2016) (Fig. 1).

Since the first miRNA, *lin-4*, was identified in *C. elegans*, many studies have confirmed the regulatory roles of these small molecules in virtually all metabolic pathways and human diseases, including ageing (Boehm and Slack, 2005; Iorio and Croce, 2012). In fact, the term *geromiRs* has been proposed to name the group of miRNAs implicated in ageing (Ugalde et al., 2011a; Caravia and Lopez-Otin, 2015). However, the influence of miRNAs in accelerated ageing is currently poorly defined. In this review, we will discuss the most recent advances in the functional relevance of these important regulatory RNAs in the different progeroid syndromes in human and animal models.

Physiological *versus* pathological ageing

Both premature and physiological ageing share many common features. In fact, progeroid syndromes can be described as the expression of the aged phenotype at an early stage (Burtner and Kennedy, 2010). Beyond specific phenotypic features, both pathologies share many molecular characteristics. For example, HGPS is caused by point mutations in the *LMNA* gene, resulting in the activation of a cryptic donor splice site. As a consequence, aberrant mRNA splicing yields a toxic protein called progerin, a truncated form of prelamin A that triggers the functional decline associated with this pathology (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). Importantly, normal aged cells also accumulate significant amounts of progerin (Scaffidi and Misteli, 2006). Moreover, studies performed in human fibroblasts showed that caspases and mitochondrial apoptosis pathways are downregulated in both physiological ageing and HGPS compared with young individuals, whilst MAPK/ERK, IGF-1, FLT3, mTOR, PPAR, SMAD and TGF-β pathways are upregulated (Aliper et al., 2015).

Despite their molecular similarities, the fundamental causes underlying pathological and physiological ageing are quite different. Pathological ageing is induced by nuclear defects, be it in the nuclear lamina or in DNA repair mechanisms, which promote DNA damage accumulation (Carrero et al., 2016). Conversely, physiological ageing arises from the accumulation of genetic, epigenetic and protein alterations. Importantly, these are shared features with cancer, where miRNAs also have a relevant role (Hanahan et al., 2011; Lujambio and Lowe, 2012). Cancer and ageing appear to be unrelated conditions, but represent two sides of the same coin: cancer is defined by a gain in biological fitness while ageing implies a process of loss of fitness. However, both have a common origin: the accumulation of genomic damage. This genomic instability triggers senescence, apoptosis, and tissue degeneration, but sometimes leads cells to malignant transformation (López-Otín et al., 2013).

The study of progeroid syndromes can provide great benefits in addition to the improvement of current therapies for these dramatic diseases, given the similarities between physiological and pathological ageing. Thus, understanding progeroid syndromes can pave the ways for the definition of the cellular and

systemic molecular mechanisms present in physiological ageing and many associated-pathologies, such as cardiovascular diseases (Gordon et al., 2014).

Micro-managing premature ageing with miRNAs

As mentioned above, a set of hallmarks have been recently proposed that define the main features of progeroid syndromes (Carrero et al., 2016). These nine hallmarks of premature ageing are: Increased DNA damage and defective DNA repair, telomere dysfunction, changes in epigenetics and chromatin structure, aberrant nuclear architecture, defects in cell cycle and mitosis, cellular senescence, metabolic defects, inflammation and stem cell exhaustion. In this section, we will focus on the recent advances in understanding the regulatory roles of miRNAs in all these molecular hallmarks of premature ageing (Table 1).

1. Increased DNA damage and defective DNA repair

It is widely accepted that DNA damage, mutation accumulation, and loss of genomic integrity are common denominators of physiological and pathological ageing (Moskalev et al., 2013). Genetic lesions in DNA sequences caused by intrinsic or extrinsic genotoxic stresses are regular events through lifetime in most organisms. To maintain genomic integrity, cells trigger a complex network of signalling pathways, called DNA damage response (DDR), that involves signal transduction, cell cycle regulation, and DNA repair mechanisms (Jackson and Bartek, 2009). Over the last years, a large amount of knowledge in this field has been accumulated and, in this context, miRNAs have arisen as important regulators of DDR (Wan et al., 2011).

Transcription factor p53 is the main regulator of the DDR pathway. Activation of p53 induces apoptosis and cell cycle arrest, which can be transient or permanent, being the latter known as senescence. p53 regulates several genes, including miRNAs, being miR-34 the most prevalently regulated miRNA family (Raver-Shapira et al., 2007; Rokavec et al., 2014). An example of miR-34 modulation of DDR occurs during genomic DNA duplication, which is essential for cell proliferation. During chronic DNA replication stress, p53 transcriptionally activates miR-34, which downregulates a group of proteins required for DNA replication, the MCM2-7 factors, resulting in cell cycle arrest

and senescence (Bai et al., 2016). Due to its role in genome integrity maintenance, p53 is a paradigmatic tumour suppressor and over 80% of tumours display dysfunctional p53 signalling (Levine et al., 2004). Consequently, many lessons about the regulatory roles of miR-34 in p53-mediated DDR come from tumour cell biology. As an example, this miRNA represses *HDM4*, a negative regulator of p53, establishing a tumour suppressor feedback loop between p53, miR-34a and *HDM4*. However, some cancer cells express a short isoform of *HDM4,* which lacks the miR-34a binding site. The loss of this regulation allows cancer cells to evade control of proliferation mediated by p53/miR-34 (Okada et al., 2014). miR-34a also contributes to p53-independent elimination of distressed cells. This miRNA represses the essential component of the cellular response to genotoxic stress 53BP1, reducing its recruitment at DNA double strand breaks (DSB) sites. This effect counteracts DDR, driving to a mitotic catastrophe in damaged cells that impairs cell division. By these means, miR-34 contributes to genome surveillance and prevents the proliferation of aberrant cells (Kofman et al., 2013). In addition, p53 enhances the post-transcriptional maturation of several miRNAs and facilitates its processing from primary to precursor miRNAs (Suzuki et al., 2009).

One of the most studied progerias caused by defects in DNA repair mechanisms is Werner syndrome (WS). WS is a human autosomal recessive ageing disorder caused by mutations in the RecQ-like DNA helicase gene *WRN* that drive to premature-ageing and high cancer incidence (Carrero et al., 2016). Cells lacking *WRN* exhibit chromosomal instability, telomere dysfunction, and a senescent phenotype. This progeroid syndrome can be partially reproduced in mice and worms through the deletion of the orthologous genes: *Wrn* and *wrn-1*, respectively. Surprisingly, miR-124 – an evolutionary conserved miRNA – is decreased in both animal models. More importantly, loss of miR-124 phenocopies the *wrn-1* deletion in *C. elegans* and results in reactive oxygen species (ROS) formation and shortened lifespan, which highlights its importance in the development of this progeroid phenotype (Fig. 2A). However, the mechanism by which the loss of *WRN* affects miR-124 expression in both mice and worms remains unknown (Dallaire et al., 2012).

Another progeroid syndrome, ataxia telangiectasia, is caused by mutations in *ATM,* a serine/threonine kinase which acts as the primary sensor and transducer of DNA damage signal. Patients affected by this pathology exhibit progressive

cerebellar degeneration, pigmentary abnormalities, hair greying and increased cancer susceptibility (Carrero et al., 2016). Several miRNAs regulate the ATM signalling pathway. For example, miR-335 downregulation after DNA damage allows the expression of its *bona fide* target *CTIP,* which controls DNA end resection in homologous recombination repair (HRR). Reduced miR-335 expression is controlled by CREB, which is in turn phosphorylated by ATM after DSB in order to reduce its transcriptional activity (Martin et al., 2013) (Fig. 2B).

2. Telomere dysfunction

Telomeres are specialized nucleoprotein structures with repetitive DNA sequences that are located at the end of chromosomes to prevent their degradation and their fusion with other chromosomes. Telomeric DNA plays a crucial role in telomere function and is particularly susceptible to age-related deterioration (Blackburn et al., 2006). It is well established that telomeric DNA damage triggers persistent DDR and cell senescence (Fumagalli et al., 2012). Telomere elongation and maintenance is driven by telomerase, an essential ribonucleoprotein complex which is active in germ and tumour cells, as well as in somatic stem cells and progenitors (Günes et al., 2013).

In mammals, a protein structure called shelterin ensures telomere integrity, and disruption of telomere/shelterin complex leads to cell senescence and death (Palm and de Lange, 2008). Telomeric repeat binding factor 2 (TRF2) is a double-stranded DNA binding protein belonging to the shelterin complex which plays important roles in telomere end protection. TRF2 interacts with lamins A and C from the nuclear envelope in order to form T-loops for telomeric DNA protection. Laminopathies such as HGPS, which are characterized by progerin accumulation and telomere attrition, exhibit an impaired association between mutant lamins and TRF2 (Wood et al., 2014). Moreover, shortening of telomeres activates progerin production, generating a feedback circuit that exacerbates the pathological features of HGPS patients (Cao et al., 2011). Importantly, TRF2 is a *bona-fide* miR-23a target and miR-23a overexpression reduces TRF2 levels, thus inducing telomere shortening and cell senescence (Luo et al., 2015). In addition, mutations in the telomerase components TERC and TERT lead to progeroid syndromes such as DC (Agarwal et al., 2010;

Batista et al., 2011). Moreover, TERT controls the expression of several miRNAs and its suppression results in the downregulation of these small regulatory elements, suggesting a potential role of miRNAs in DC (Lassmann et al., 2015).

3. Epigenetic changes and chromatin structure

There is a huge variety of epigenetic modifications that affect mammalian cells and tissues throughout life such as DNA methylation, histone modifications, and chromatin remodelling. These epigenetic changes influence gene expression and are important targets for many therapies, since they represent theoretically reversible alterations (Benayoun et al., 2015).

One of the most important epigenetic modifications is DNA methylation. It mainly leads to transcriptional silencing by methylating CpG islands on gene promoters, but is also gaining relevance as a defence against transposons (Suzuki and Bird, 2008). CpG islands are hypermethylated during ageing in both mice and humans, especially in genes involved in differentiation or development (Day et al., 2013). It has been hypothesized that these changes contribute to misregulation of gene expression during ageing, therefore altering lifespan (Sun et al., 2014). In *D. melanogaster*, overexpression of DNA methyltransferase *Dnmt2* increases longevity, while flies lacking this gene have a reduced lifespan (Lin et al., 2005). miRNAs are strongly involved in DNA methylation. For instance, *Dicer1* deficient mice have decreased DNA methylation levels caused by a low expression of *Dnmt1*, *Dnmt3a* and *Dnmt3b* DNA methyltransferases. The authors found miR-290 to be responsible, in a Rbl2-dependent manner, of this transcriptional silencing (Benetti et al., 2008). Moreover, miR-377 represses *DNMT1* and induces senescence in human skin fibroblasts (Xie et al., 2017).

Histone methylation can have either activating or repressing roles, depending on the residue affected. In physiological ageing, the reduction of heterochromatin and/or its inappropriate distribution due to interactions between chromatin elements and nuclear lamina may be the cause of cellular dysfunction (concept termed as the “loss of heterochromatin” model) (Dechat et al., 2008; Tsurumi et al., 2012). Moreover, expression changes in histone

methyltransferases and demethylases can alter lifespan (Greer and Shi, 2012). Histone methylation is also regulated by several miRNAs such as miR-101, whose deletion leads to histone methyltransferase *EZH2* overexpression in cancer (Varambally et al., 2008).

Finally, histone acetylation is also an important epigenetic feature. Certain acetylations can activate DNA transcription, and are crucial in the ageing process. Global levels of H3K56 acetylation decrease during ageing, while acetylation of H4K16 increases in the course of this process (O'Sullivan et al., 2010). Therefore, histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a remarkable role in modulating lifespan. Amongst HDACs, we can highlight the sirtuins, especially SIRT1 and SIRT6. Brain-specific overexpression of deacetylase SIRT1 increases healthspan in mice through deacetylation of Nk2 homeobox 1 (Nkx2-1) and transcriptional activation of *Ox2r* (Satoh et al., 2013). Actually, this HDAC, which acts in a NAD^+-dependent manner, is essential in caloric restriction, and SIRT1 depletion abrogates its positive effects (Cohen, 2004; Verdin, 2015; Cunha-Santos et al., 2016). miRNAs play essential roles in epigenetic alterations, especially in *SIRT1* regulation. miR-217 is upregulated during ageing and promotes senescence by targeting SIRT1 (Menghini et al., 2009). miR-519 upregulation also favours senescence in human fibroblasts through repression of RNA binding protein HuR, which is a SIRT1 regulator (Abdelmohsen et al., 2007; Marasa et al., 2010).

4. Aberrant nuclear architecture

Nuclear lamina is a dense fibrillar network of intermediate filaments located in the inner nuclear membrane that contains lamins and other proteins involved in chromatin architecture, cell cycle control, and regulation of gene expression. There are two major classes of lamins: A-type, including lamins A and C encoded by *LMNA* gene, and B-type, encoded by *LMNB1* and *LMNB2* genes, that includes lamins B1, B2 and B3. Lamins require a complex post-translational processing including farnesylation, proteolytic cleavages and carboxyl methylation (Burke and Stewart, 2013). Mutations in genes that encode nuclear-lamina proteins cause progeroid syndromes such as HGPS,

NGPS, APS (including atypical neonatal progeria syndrome), RD and MAD (Carrero et al., 2016; Soria-Valles et al., 2016a).

Several miRNAs can regulate the expression of lamins. For example, miR-23 fine-tunes lamin B1 levels (Lin and Fu, 2009). More importantly, the products of *LMNA* gene, lamins A and C, are transcribed as two isoforms widely expressed in most tissues but in the brain. Lamin A is absent in neurons and glia because a brain-specific miRNA, miR-9, blocks the expression of prelamin A in these cells. Thus, the regulatory role of miR-9 could explain, at least in part, the absence of nervous system impairments in HGPS patients (Fig. 2C) (Jung et al., 2012; Nissan et al., 2012).

The *Zmpste24*-deficient mouse, which lacks the zinc metalloproteinase that cleaves the farnesylated C-terminal domain of prelamin A, was the first HGPS murine model developed (Pendás et al., 2002; Varela et al., 2005). In these mice, altered chromatin architecture due to defective lamin A assembly mediates the transcription of components of the miR-29 family in a p53-dependent manner, as a response to DNA damage during both pathological and physiological ageing. miR-29 represses Ppm1d, a phosphatase that regulates the DDR through inhibition of the activity of p53, causing senescence and reducing cell proliferation (Fig. 2D) (Ugalde et al., 2011b).

Patients with NGPS exhibit most HGPS symptoms, with the exception of cardiovascular defects. The absence of these alterations allows them to have a relatively longer lifespan (Cabanillas et al., 2011). This disease is caused by an A12T mutation in *BANF1* gene, which encodes BAF, a protein that links DNA to proteins from the nuclear envelope. As a consequence, BAF influences regulation of gene expression (Segura-Totten et al., 2002; Puente et al., 2011). *BANF1* is a well-known miR-203 target. Thus, miR-203 decreases BAF levels, reducing cancer risk but increasing nuclear structure instability and inducing cell senescence (Mao et al., 2015).

5. Defects in cell cycle and mitosis

Several alterations that arise in progeroid syndromes, such as nuclear envelope abnormalities, are closely related to alterations in cell cycle

progression due to deregulation of miRNAs (Harries, 2014). It has been described that patients with SS, a progeroid syndrome characterized by intrauterine growth retardation and postnatal dwarfism, exhibit an increased number of centrosomes during mitosis, as a result of an impaired G2/M checkpoint regulation (Griffith et al., 2008). In addition, patients with FA, a premature ageing disorder characterized by elevated cancer susceptibility and developmental alterations, accumulate DNA damage due to failure in arresting cell cycle until DNA is completely repaired (Bogliolo and Surralles, 2015).

Several studies have reported the miRNA-mediated regulation of cell proliferation in progeroid models. For example, transcriptomic analysis of miRNA expression performed in *Zmpste24*-deficient mouse embryonic fibroblast (MEFs) has revealed a total of 10 differentially expressed miRNAs between wild-type and mutant MEFs. Among then, miR-365, which is down-regulated in *Zmpste24*$^{-/-}$ MEFs, reduces cell proliferation and promotes senescence possibly due to its target *Rasd1* (Xiong et al., 2015). In addition, cells lacking the C-terminus of lamin B1 partially mimic the nuclear abnormalities typical from laminopathies. In these cells, miR-31 is overexpressed and regulates cell cycle progression by targeting p16/p19 (Malhas et al., 2010). Moreover, cell cycle arrest has been demonstrated to occur due to miR-34a, miR-34b and miR-34c participation. These miRNAs repress CDK4 and CCNE2 keeping the cell in G1 phase (He et al., 2007).

6. Cellular senescence

Senescence is a permanent state of cell cycle arrest that contributes to suppress cancer progression by enhancing tissue degeneration. However, the progressive accumulation of senescent cells is an ageing-promoting mechanism and contributes to the modulation of organismal lifespan (Rodier and Campisi, 2011). Significantly, senescent cells acquire a senescence-associated secretory phenotype (SASP) consisting in the release of proteins, especially cytokines and chemokines, that is involved in the pathogenesis of many diseases (Coppé et al., 2008).

Senescence-associated (SA)-miRNAs critically regulate this complex cellular process and the overexpression of some of them can trigger senescence in

proliferating cells (Rivetti di Val Cervo et al., 2012). Metformin and caloric restriction − two pro-longevity strategies − upregulate the miRNA-processing enzyme *DICER1,* that in turn increases the expression of miR-20a, miR-34a, miR-130a, miR-106b, miR-125, and let-7. Importantly, metformin treatment decreases cellular senescence in a DICER1-dependent manner, lowering p16 and p21 levels (Noren Hooten et al., 2016). Additionally, synthetic delivery of miR-22 is sufficient to induce cell senescence in a mouse model of breast carcinoma (Xu et al., 2011). Conversely, senescence can also be triggered by the transcriptional gene silencing exerted by another miRNA, let-7. This miRNA represses cell proliferation inducing RB1/E2F-target genes and therefore, inhibition of let-7 hampers entry of cells into senescence (Benhamed et al., 2012). miR-34 targets multiple senescent-associated pathways. As an example, p53 transcriptionally activates miR-34a, which represses SIRT1, preventing SIRT1-mediated p53 deacetylation and inhibition. This regulatory feedback loop enhances p53 activity and results in a cellular senescent phenotype (Tazawa et al., 2007).

Additionally, many groups have reported the deregulated expression of miRNAs in progeroid syndromes. Patients suffering from WS, a premature ageing disorder largely characterized by a senescence phenotype, show 19 differentially-expressed miRNAs compared with normal controls (Tang et al., 2016). In addition, several SA-miRNAs have been reported to be deregulated in a mouse model of premature ageing (Nidadavolu et al., 2013).

7. Metabolic defects

The relationship between deregulated nutrient sensing and ageing has been widely demonstrated (López-Otín et al., 2016). In fact, dietary restriction is a proved lifespan-increasing intervention in mice (Fontana et al., 2010). In addition, autophagy, the process that cells use to self-degrade cytoplasmic substrates, decreases with ageing (Rubinsztein et al., 2011). However, among all the metabolic pathways that interfere in nutrient sensing, the evolutionary conserved somatotrophic axis that encompasses insulin-like growth factor (IGF-1) and growth hormone (GH), has a predominant impact on ageing modulation (Barzilai et al., 2012). GH is produced by pituitary gland and stimulates many cell types to produce IGF-1, which shares a common pathway with insulin,

called “insulin and IGF-1 signalling” (IIS). Among its multiple targets, we can mention FOXO and mTOR. Repression of these pathways have been linked to longevity (Saxton and Sabatini, 2017).

Some miRNAs are important in the deregulation of somatotrophic axis and autophagy. *Zmpste24*-deficient mice also exhibit considerable alterations in mTOR, which induce autophagy, and in the GH/IGF-1 pathways. The latter is caused by miR-1 upregulation (Mariño et al., 2008; Mariño et al., 2010) (Fig. 2E). In addition, miR-496 regulates *mTOR* and their expression levels are inversely correlated in peripheral blood mononuclear cells. Importantly, old individuals show high expression of miR-496 while *mTOR* is repressed (Rubie et al., 2016). On the other hand, miR-27a and miR-27b are pivotal elements in the control of mitophagy through PTEN-induced putative kinase 1 (PINK1) regulation, especially during Parkinson disease pathogenesis (Kim et al., 2016).

8. Inflammation

In mammals, ageing is accompanied by a mild inflammatory phenotype (Franceschi et al., 2000; Osorio et al., 2016; Soria-Valles et al., 2016b). This phenomenon, called “inflammaging”, is caused by multiple factors such as the accumulation of proinflammatory tissue damage, deficient immune surveillance, the SASP response, the enhanced activation of NF-κB transcription factor, and a defective autophagic response. All these alterations trigger the activation of intracellular damage sensors called inflammasomes which regulate the activity of several inflammatory caspases, especially caspase 1 (Salminen et al., 2012).

Progeroid syndromes also display a systemic inflammatory phenotype (Carrero et al., 2016)**.** NF-κB is activated in an ATM-dependent manner in mouse models of laminopathies (Osorio et al., 2012). Similarly, WS patients show high levels of pro-inflammatory cytokines and C-reactive protein (CRP), and inflammation-linked protein present in plasma (Goto et al., 2012). In addition, fibroblasts derived from these patients have the p38 signalling pathway activated (Davis and Kipling, 2006)**.** Moreover, FA patients show inflammatory-stress activation of Notch, thereby compromising hematopoietic stem cells self-renewal (Du et al., 2013).

Given the relevance of miRNAs in a great number of physiological processes, it is not unexpected that several of them are intimately involved in the inflammation process. High levels of miR-21, which triggers the expression of anti-inflammatory molecules like IL-10 and TGF-β, have been identified as inflammaging biomarkers (Olivieri et al., 2012). Other three miRNAs, miR-34a, miR-181a and miR146a, are importantly upregulated during ageing. These three miRNAs are mitochondrial miRNAs (mitomiRs) and control the expression of important mitochondrial proteins like Bcl-2 and CHUK. Bcl-2, apart from its anti-apoptotic function, controls mitochondrial fusion/fission dynamics and inhibits NLRP1, a core component of inflammasomes; CHUK, on the other hand, is a key inhibitor of NF-κB, highlighting the impact of the dysregulation of these miRNAs in the ageing process (Rippo et al., 2014). miR-146a, along with miR-146b, also targets IL-6 and IL-8, both secreted during stress-induced cellular senescence (Bhaumik et al., 2009) and it has also been related to age-related pathologies such as Alzheimer disease (Lukiw et al., 2008).

9. Stem cell exhaustion

Stem cells are characterized by a balance between pluripotency and quiescence, given that a defective proliferation triggers senescence whilst hyperproliferation threatens tissue regeneration. Stem cell exhaustion is the major responsible for ageing phenotype (Oh et al., 2014).

Several genes that play important roles in stem cell maintenance are regulated by miRNAs. For example, the self-renewal factor Upd is remarkably decreased during *Drosophila* ageing, causing a dramatic loss of testis stem cells due to let-7 repression (Toledano et al., 2012). In addition, let-7a1, let-7d, let-7f1, miR-26a, and miR-30a levels are increased during human umbilical cord blood-derived multipotent stem cells senescence, which is mediated by a HDAC inhibitor (Lee et al., 2011). High levels of miR-335 were also observed in human mesenchymal stem cells (hMSCs) from aged donors, and its overexpression triggers senescence-like alterations in these cells (Tomé et al., 2014).

The molecular mechanisms controlling hMSC ageing in the context of prelamin A accumulation, which could be considered as the main cause of

progeroid laminopathies, are under miRNA control (Rosengardten et al., 2011; Pacheco et al., 2014). For example, miR-141, which binds to the 3'UTR of *Zmpste24*, is overexpressed during senescence as a result of epigenetic dysregulation, inducing a reduction of *Zmpste24* expression levels (Yu et al., 2013) (Fig. 2F). However, miRNA targeting could also be a way of regulating tissue homeostasis. For example, mouse muscle stem cells exhibit a remarkable overexpression of miR-489, which targets the oncogene *Dek*, responsible for transient proliferative expansion of myogenic progenitors. This overexpression decreases during cell differentiation, which suggests that it has a key role in maintaining adult stem-cell population quiescence (Cheung et al., 2012).

Clinical scenario

The development of effective therapies against premature ageing disorders appears as a daunting task due to both disease diversity and the plethora of molecular mechanisms involved. Hence, only a limited number of clinical approaches are really effective nowadays, and most of them are aimed at palliating particular symptoms of the disease instead of targeting the molecular basis of the pathology.

As it happens in most human pathologies, the role of animal models has been pivotal in the study of premature ageing. Several therapeutic approaches have derived from studies performed in mice, particularly *Wrn*$^{-/-}$, *Lmna*^{G609G}, and *Zmpste24*$^{-/-}$ mice (Kenyon, 2010). For example, treatment with statins and aminobisphosphonates proved to extend longevity in the *Zmpste24*$^{-/-}$ model (Varela et al., 2008). In addition, *Lmna*^{G609G} mice, which harbor the same mutation as HGPS patients, show increased lifespan after being treated with an antisense morpholino-based therapy that prevents the pathogenic *Lmna* splicing (Osorio et al., 2011). It has been also shown that NF-kB hyperactivation in HGPS, NGPS and aged cells impairs cell rejuvenation through DOT1L, a reprogramming inhibitor. Importantly, downregulation of DOT1L extends lifespan in the *Zmpste24*$^{-/-}$ mouse model (Soria-Valles et al., 2015). Recombinant IGF-1 also increases lifespan in this HGPS model by rescuing the IGF-1/GH imbalance, which is largely caused by miR-1 upregulation (Mariño et al., 2010).

The discovery of miRNAs has opened new avenues for therapeutic approaches targeting many human diseases. Both miRNA mimics and antagomiRs – modified antisense oligonucleotides that target miRNAs – have been used in a wide range of disease models. Remarkably, two clinical trials in this regard are now taking place. In one of them, antisense miR-122 is being used to sequester this miRNA, thus preventing its stabilizing interaction with the hepatitis C virus (Janssen et al., 2013). In the second trial, a mimic miR-34a (a known tumour suppressor miRNA) loaded into liposomal nanoparticles is being tested in liver cancer (Bouchie, 2013; Hayes et al., 2014).

The primary cause of the pathological features developed by HGPS is the accumulation of mutant farnesylated lamin A (progerin) in their cells. Consequently, the reduction of progerin levels could improve the lifespan of these patients. Some interventions using small interference RNA (siRNA) have proved to be useful to treat progeroid syndromes. In this case, a short hairpin RNA (shRNA) designed to target mutated *LMNA* gene was expressed in HGPS fibroblasts. This experimental approach reduced progerin levels to 26% when compared to untreated HGPS cells, ameliorated aberrant nuclear morphology, improved proliferation and impaired senescence triggering (Huang et al., 2005). Actually, HGPS patients do not show any signs of neurologic degeneration because miR-9, a miRNA specifically expressed in neurons, targets lamin A and progerin expression (Nissan et al., 2012). This remarkable mechanism is now trying to be replicated by packaging mimic miR-9 into adeno-associated virus in order to infect cells from HGPS patients. However, further studies are needed in order to translate miRNA-based therapies in clinical diagnostics and therapeutics (www.progeriaresearch.org).

Conclusions and future perspectives

The biological phenomenon of ageing has largely concerned scientists and, given the extraordinary increment in global population and human lifespan, there is an urgent need to find new approaches to improve healthspan. Accordingly, it is essential to progress in the study of the molecular basis of ageing-associated alterations. The discovery of miRNAs has revolutionized the classical view of gene expression control and has unveiled a novel layer of

regulation in cell biology. This class of small non-coding RNAs affects virtually all biological processes within cells and organisms, acting at multiple steps. Accordingly, miRNAs also influence ageing and ageing-associated diseases. A solid evidence from the literature supports the regulatory roles of geromiRs – the growing subgroup of miRNAs implicated in ageing – on organismal lifespan. In this scenario, these molecules appear as putative relevant targets for clinical intervention in physiological and pathological ageing. Moreover, we have discussed here how geromiRs can actually act on every hallmark of ageing, meaning that any ageing-related pathology could be treated with a miRNA targeting strategy no matter its nature or its underlying molecular cause. However, we are far from understanding the specific functions of miRNAs in premature ageing disorders. Most of these rare genetic diseases lack effective treatments, and further studies are needed to precisely elucidate the potential therapeutic role of miRNA in progeroid syndromes. Importantly, cellular and animal models represent a unique opportunity to achieve a deeper knowledge in this field and offer different alternatives for implementing new therapeutic approaches into clinics.

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FIGURE LEGENDS:

Figure 1: Hallmarks of progeroid syndromes and premature ageing disorders. Schematic representation of molecular and cellular hallmarks of progeroid syndromes and the two groups of premature ageing disorders.

Figure 2: miRNA-mediated regulation in premature ageing. (A) Mouse and worm models of WS show miR-124 downregulation, which results in ROS formation, low ATP levels and shortened lifespan. (B) After DSB, ATM phosphorylates CREB, which in turn reduces miR-335 expression. Lower levels of this miRNA cause an upregulation of its target *CTIP*, which controls DNA end resection in HRR. (C) In neurons and glia, miR-9 targets prelamin A mRNA and inhibits its translation, preventing the occurrence of neurological disorders in HGPS patients. (D) In HGPS cells, the accumulation of progerin upregulates miR-29, which represses the phosphatase *Ppm1d*, enhancing p53 and causing cell senescence. (E) In *Zmpste24*$^{-/-}$ *cells*, GH/IGF-1 imbalance is caused by miR-1 overexpression. (F) In aged hMSCs, a decrease in HDAC activity increases histone acetylation and miR-141 upregulation. High miR-141 levels lead to a reduction of Zmpste24 and, therefore, the accumulation of prelamin A.

Epigenetic changes

Nuclear lamina alterations

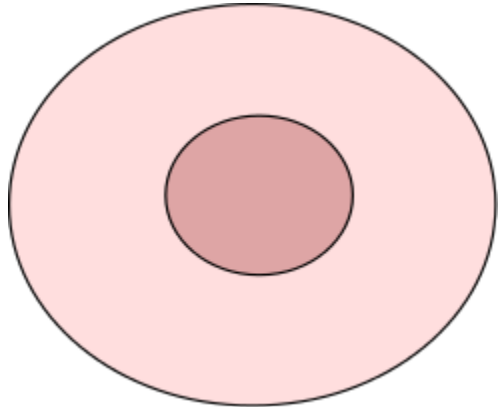
Cell cycle deregulation

Senescence

Metabolic defects

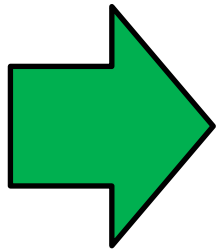
Telomere dysfunction

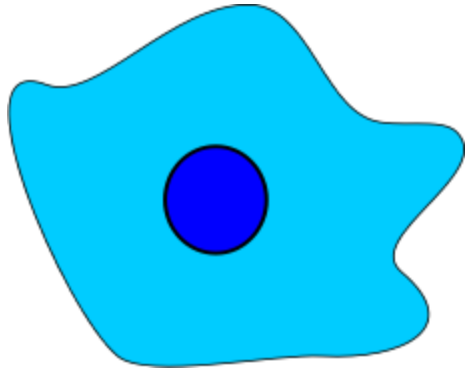
Inflammation



Increased DNA damage

Normal cell





Progeroid cell

Stem cell exhaustion

LAMINOPATHIES

- Hutchinson-Gilford progeria syndrome
- Néstor-Guillermo progeria syndrome
- Atypical progeria syndromes
- Restrictive dermopathy
- Mandibuloacral dysplasia

DEFECTS IN DNA-REPAIR MECHANISMS

- Werner syndrome
- Bloom syndrome
- Seckel syndrome
- Rothmund-Thomson syndrome
- Cockayne syndrome
- Xeroderma pigmentosum
- Hoyeraal-Hreidarsson syndrome
- Dysketatosis congenita
- Trichothiodystrophy
- Ataxia telangiectasia
- Fanconi anemia

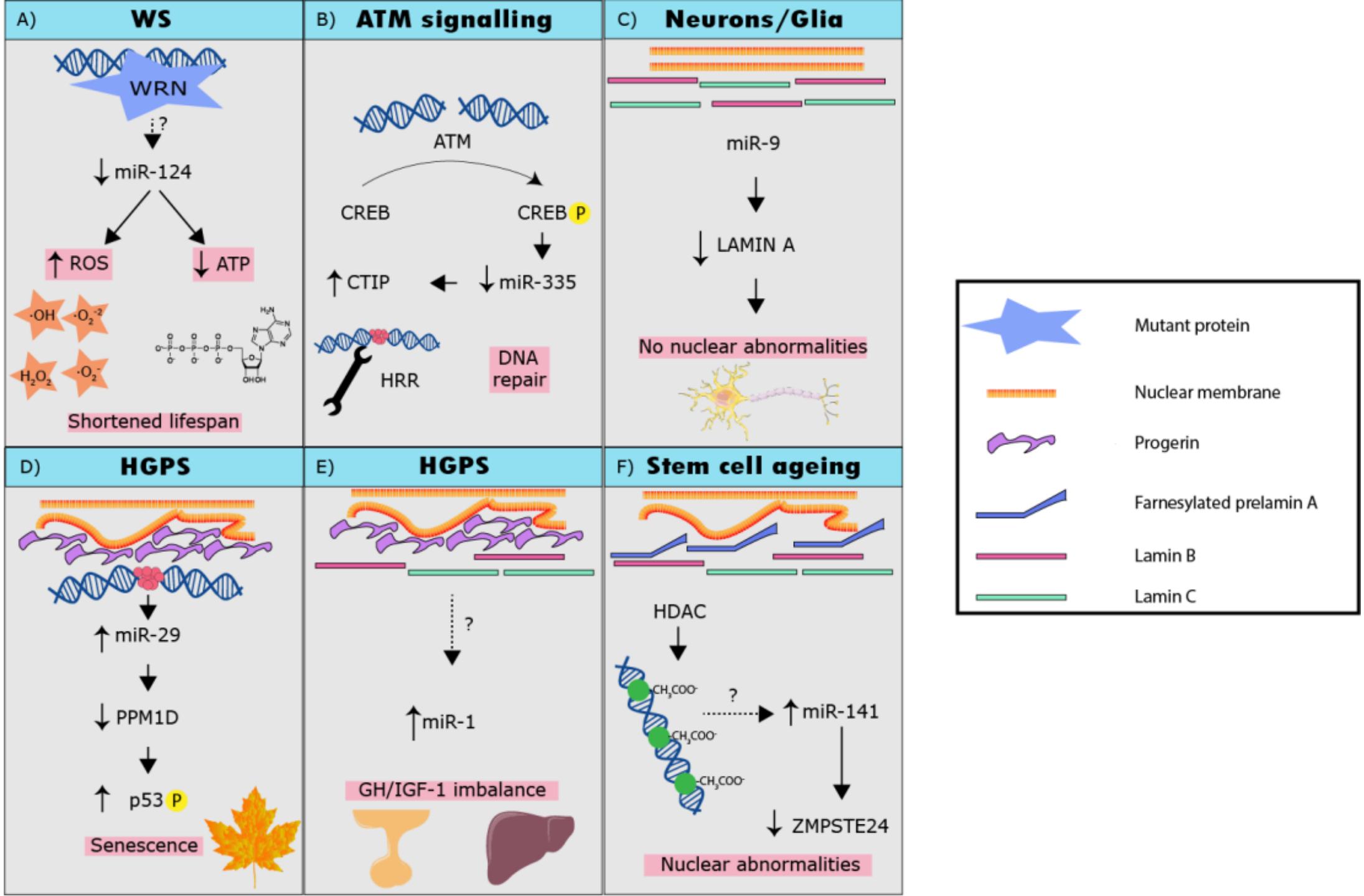

A) WS
WRN
?
miR-124
ROS
ATP
·OH
·O2-2
H2O2
·O2-
Shortened lifespan
B) ATM signalling
ATM
CREB
CREB P
CTIP
miR-335
HRR
DNA repair
C) Neurons/Glia
miR-9
LAMIN A
No nuclear abnormalities
D) HGPS
miR-29
PPM1D
p53 P
Senescence
E) HGPS
?
miR-1
GH/IGF-1 imbalance
F) Stem cell ageing
HDAC
CH3COO-
?
miR-141
ZMPSTE24
Nuclear abnormalities
Mutant protein
Nuclear membrane
Progerin
Farnesylated prelamin A
Lamin B
Lamin C

Table 1. Summary of currently known miRNAs implicated in the regulation of hallmarks of progeroid syndromes.

Hallmark	miRNA	Targets	Model	Organism	References
Increased DNA damage	miR-34	Mcm2-7	Embryonic fibroblasts	Mouse	(Bai et al., 2016)
	miR-34	*HDM4*	Lung adenocarcinoma	Human	(Okada et al., 2014)
	miR-34	*53BP1*	Glioblastoma cell line	Human	(Kofman et al., 2013)
	miR-124	Unknown	Werner syndrome	*Mouse* and *C.elegans*	(Dallaire et al., 2012)
	miR-335	*CTIP*	HeLa and patient-derived cells	Human	(Martin et al., 2013)
Telomere dysfunction	miR-23a	*TRF2*	Fibroblasts	Human	(Luo et al., 2015)
Epigenetic changes	miR-290	*Rbl2*	ES cells	Mouse	(Benetti et al., 2008)
	miR-377	*DNMT1*	Skin fibroblast	Human	(Xie et al., 2017)
	miR-101	*EZH2*	Prostate cancer cells	Human	(Varambally et al., 2008)
	miR-217	*SIRT1*	Endothelial cells	Human	(Menghini et al., 2009)
	miR-519	*ELAVL1* (HuR)	Fibroblasts	Human	(Abdelmohsen et al., 2007; Marasa et al., 2010).
Nuclear lamina alterations	miR-23	*LMNB1*	HEK293 and oligodendrocytes	Human	(Lin and Fu, 2009)
	miR-9	*LMNA*	HGPS (neural cells)	Human	(Jung et al., 2012; Nissan et al., 2012)
	miR-29	*Ppm1d*	*Zmpste24*$^{-/-}$	Mouse	(Ugalde et al., 2011b)
	miR-203	BANF	Cervical cancer	Human	(Mao et al., 2015)
Cell cycle deregulation	miR-365	*Rasd1*	*Zmpste24*-deficient MEFs	Mouse	(Xiong et al., 2015)
	miR-31	*P16, P19*	Laminopathies	Human	(Malhas et al., 2010)
	miR-34	*CDK4, CCNE2*	Tumor cell lines	Human	(He et al., 2007)
Senescence	miR-22	*Cdk6, Sirt1, Sp1*	Breast carcinoma	Mouse	(Xu et al., 2011)
	let-7	RB1/E2F target genes	Fibroblasts	Human	(Benhamed et al., 2012)
	miR-34a	SIRT1	Colon cancer cell lines	Human	(Tazawa et al., 2007)
Metabolic defects	miR-1	*Igf-1*	*Zmpste24*$^{-/-}$	Mouse	(Mariño et al., 2008; Mariño et al., 2010)
	miR-27	*PINK1*	Parkinson's disease	Human	(Kim et al., 2016)
Inflammation	miR-21	*IL-10, TGF- β*	Blood	Human	(Olivieri et al., 2012)
	miR-34a, miR-181a and miR-146a	*Bcl2, IKKα* (CHUK)	HUVECs	Human	(Rippo et al., 2014)
	miR-146	*IL6, IL8*	Fibroblasts	Human	(Bhaumik et al., 2009)
Stem cell exhaustion	let-7	*Upd*	Testis stem cells	*D. melanogaster*	(Toledano et al., 2012)
	miR-141	*ZMPSTE24*	hMSCs	Human	(Yu et al., 2013)
	miR-489	*Dek*	Muscle stem cells	Mouse	(Cheung et al., 2012)

ES, Embryonic stem; HGPS, Hutchinson-Gilford progeria syndrome; HUVECs: Human Umbilical Vein Endothelial Cells; hMSCs, Human mesenchymal stem cells.

HIGHLIGHTS

- The ageing rate can be modulated by genetic, nutritional and pharmacological factors.
- geromiRs, the growing subgroup of miRNAs implicated in ageing, are essential regulators of both physiological or pathological ageing.
- miRNAs are able to regulate all the molecular and cellular hallmarks of progeroid syndromes.
- Therapeutic interventions based on geromiR modulation are promising approaches to treat progeroid syndromes and extend longevity.