

Micromanaging aging with miRNAs

New messages from the nuclear envelope

Alejandro P. Ugalde, Yaiza Español and Carlos López-Otín*

Departamento de Bioquímica y Biología Molecular; Instituto Universitario de Oncología; Universidad de Oviedo; Oviedo, Spain

Over the last years, the discovery of microRNAs (miRNAs) has revolutionized the classic concepts of gene expression regulation and has introduced a new group of molecules that may contribute to the complex changes observed during aging. Although several *Caenorhabditis elegans* miRNAs have been proved to influence the nematode life span, the current knowledge about miRNA-mediated regulation of mammalian aging is still limited. Recently, we have analyzed the functional relevance of miRNAs in accelerate aging by using *Zmpste24*^{-/-} mice, a murine model that phenocopies Hutchinson-Gilford progeria syndrome. These studies have revealed that the nuclear abnormalities present in these mice affect the expression levels of several miRNAs, including a marked upregulation of miR-1 and miR-29. Furthermore, we have found that the altered expression of these miRNAs may contribute to the progeroid phenotype of mutant mice by modulating the levels of key components of the somatroph axis and DNA damage response pathways. Here, we discuss these recent discoveries and summarize the present evidences regarding the involvement of aging-associated miRNAs or *geromiRs* in senescence and longevity regulation.

proper understanding of the molecular basis of aging may contribute to prevent or attenuate these age-related alterations. Despite the strong research effort aimed to uncover the mechanisms that orchestrate aging, its multifactorial nature has hampered the acceptance of a consensus theory for organismal aging. Nevertheless, there is some agreement that a common feature of aging is the progressive accumulation of damaged cells, which compromises tissue homeostasis and function. Over the last years, several stressors contributing to aging have been identified, including oxidative reactions, telomere attrition and the decline of DNA repair and protein turnover systems.¹⁻³ At the molecular level, the identification of genetic mutations that cause accelerated aging syndromes in humans has allowed the characterization of several pathways that influence organism longevity.⁴ These discoveries have reinforced the role of DNA damage in the process of aging, since the vast majority of progeroid phenotypes are due to mutations in genes involved in genome maintenance or nuclear and chromatin organization. Thus, mutations in the nucleotide excision repair, homologous recombination or double strand break repair systems as well as in genes involved in DNA damage signaling, cause accelerate aging in humans.⁵ Similarly, alterations in genes that encode nuclear lamina proteins such as lamin A or BAF, are responsible for the development of different human progeroid laminopathies.⁶⁻¹⁰ Analogously, several evolutionary conserved stress-sensing circuits have been recognized as important modulators of aging, including the insulin/IGF-1 signaling, target of rapamycin (TOR),

Keywords: nuclear lamina, progeria, geromiRs, miR-1, miR-29, IGF-1, Ppm1d, DNA damage

Submitted: 08/22/11

Accepted: 09/05/11

<http://dx.doi.org/10.4161/nucl.2.6.17986>

*Correspondence to: Carlos López-Otín;
Email: clo@uniovi.es

Aging has been the objective of multiple works for many years. This complex and irremediable process associated with life has a special interest for human populations due to the growing incidence of age-associated chronic pathologies such as diabetes, arthritis, neurological disorders, cardiovascular diseases and cancer.^{1,2} The

sirtuins and AMP-activated protein kinase (AMPK) pathways.^{3,11} These stress response pathways measure changes in parameters such as energy status, nutrient availability, protein and DNA damage, and oxidative potential, orchestrating adaptive cellular responses aimed to preserve protein and DNA functionality during aging.

The Growing Relevance of miRNAs in Aging

The recent discovery and functional characterization of miRNAs has substantially changed the classic view of gene expression regulation and has unveiled a new group of molecules that contribute to the complex process of aging.¹²⁻¹⁴ miRNAs are

small RNA molecules that directly bind to the 3' untranslated regions (3'UTRs) of their target transcripts and inhibit protein production by mRNA degradation or translational blockage (Fig. 1).¹⁵ miRNA-mediated regulation is an extended phenomenon across animals and plants, which has the ability to control a considerable proportion of cellular transcripts. In humans, around 500 miRNAs have been validated and it has been estimated that more than 60% of transcripts are susceptible to miRNA regulation.¹⁶ Individually, each miRNA has the potential to repress tens to hundreds of transcripts, a feature that allows them to modulate the levels of several components of a single pathway or to affect the activity of related pathways through the regulation of different key

elements. In addition, their frequent organization in genomic clusters that coexpress different miRNAs further increases the range of potentially targeted pathways and functions. Thus, these molecules play important roles in almost every process of the cell, such as proliferation, differentiation, migration, apoptosis, senescence and autophagy. Likewise, miRNA activity is essential during embryonic development and in numerous physiological and pathological processes, including cancer.^{17,18}

On this basis, it was also tempting to speculate that miRNAs could be suitable candidates for aging modulation. The first studies aimed to address this question were performed in *C. elegans* and led to the finding that miRNAs may influence

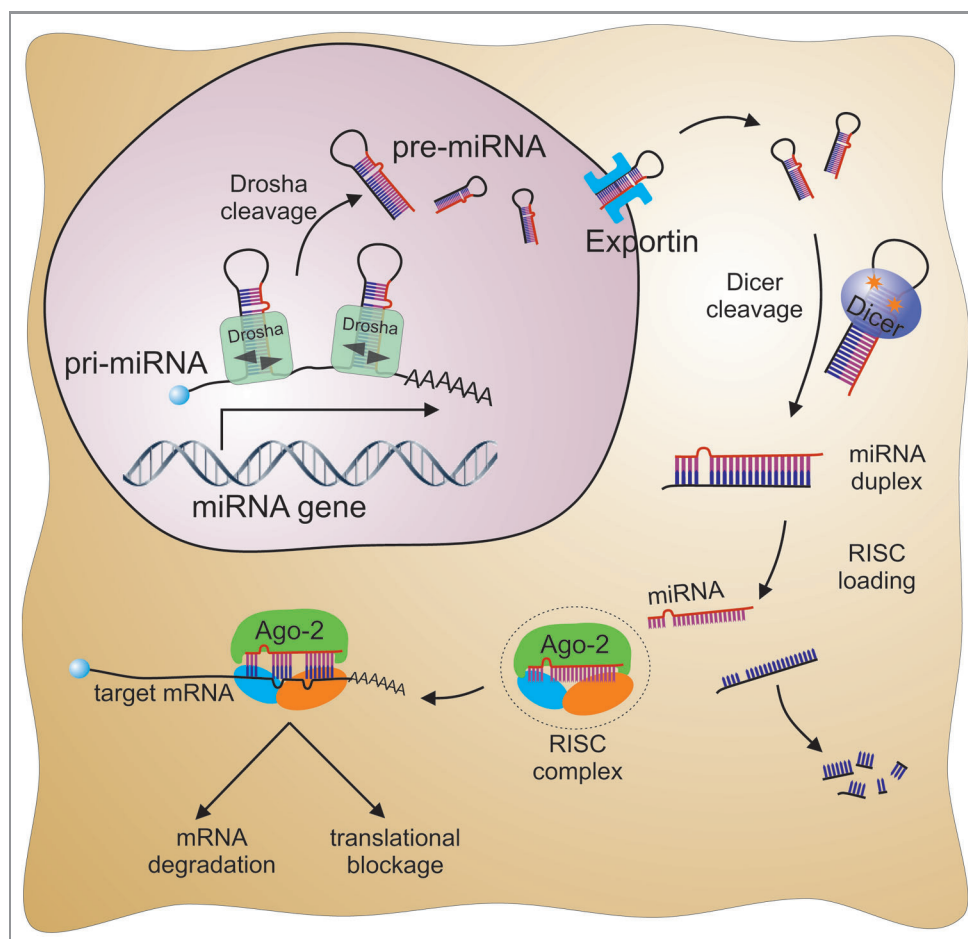


Figure 1. miRNA biogenesis and mechanism of action. miRNAs are transcribed in the nucleus as long primary transcripts (pri-miRNAs) that acquire a characteristic stem loop secondary structure. This structure is recognized by Drosha RNase III, which releases the ~70-nt precursor miRNAs (pre-miRNAs) that are then transported to the cytoplasm by exportin-5/Ran-GTP complexes. Once in the cytoplasm, they are processed by Dicer RNase III into 22-nt double stranded miRNAs (miRNA duplexes). Finally, one strand, which represents the mature miRNA, is incorporated into the RNA-induced silencing complex (RISC), which will mediate the repression of the target genes by direct cleavage of the mRNAs, by deadenylation and subsequent mRNA decay, or by translational blockage.

the longevity of this organism. Thus, Boehm et al.¹⁹ demonstrated that *lin-4* miRNA loss-of-function mutants have shortened lifespan when compared with wild-type animals. They also reported that *lin-4* overexpressing nematodes or the knockdown mutants of its target gene, *lin-14*, display the opposite effect. Likewise, de Lencastre et al.²⁰ found four miRNAs (miR-71, miR-238, miR-239 and miR-246) whose loss-of-function significantly extends or shortens *C. elegans* longevity. Further genetic analysis has revealed that some of these miRNAs act through the modulation of known aging circuits like the DNA damage response and the insulin-signaling pathways.²⁰ In mammals, however, the current knowledge about the implication of miRNAs in aging regulation is still very limited. Profiling studies in liver and brain from aged mice have revealed changes in miRNA expression during aging, whereas paradoxical results have been obtained in lung.²¹⁻²⁴ Similarly, differential miRNA expression levels have been found in human skeletal muscle and peripheral blood mononuclear aging cells.^{25,26} Hackl et al.²⁷ have also reported the down-regulation of several miRNAs in human foreskin, mesenchymal stem cells and CD8+ T cell populations from old and young donors. Likewise, Somel et al.²⁸ have recently analyzed the changes in miRNA, mRNA and protein levels in human and macaque brain and have found solid evidences of miRNA-mediated regulation of gene expression during aging. Additionally, several miRNAs are deregulated in the Ames dwarf mice, and miR-27 has been proposed to play a role in the delayed aging observed in these animals.²⁹

miRNAs, Nuclear Envelope and Premature Aging

Although the above evidences strongly suggest the implication of miRNAs in aging modulation, no studies in this regard had been performed in premature aging mice until our recent work demonstrating that mir-29 is overexpressed in *Zmpste24*-null mice.³⁰ These mutant animals exhibit profound nuclear envelope abnormalities and are an excellent model

of Hutchinson-Gilford progeria syndrome (Fig. 2).^{31,32} Previous work from our laboratory had already suggested that miRNAs could contribute to the progeroid phenotype characteristic of *Zmpste24*-deficient mice.³³ In fact, we had observed that the significant upregulation of miR-1 in the liver from these mutant mice could target the mRNA for insulin-like growth factor-1 (Igf-1) and be partially responsible for the marked deregulation of the somatotroph axis signaling found in these animals.³⁴ Based on these results, and considering that different murine models with accelerate aging have yielded important clues about molecular mechanisms of aging,^{4,5} we profiled the miRNA expression levels in *Zmpste24*-deficient mice.³⁰ This analysis

revealed that the three members of the miR-29 family of miRNAs displayed a significant upregulated expression in liver and muscle from these progeroid animals. Likewise, we found that miR-29 upregulation was also a characteristic feature of different tissue samples obtained from old mice. Interestingly, miR-29 levels also correlate with age in human and macaque brain cortex²⁸ and its expression is down-regulated in Ames dwarf mice, that display a delay in the onset of aging.²⁹ Collectively, these findings suggest a more general role for miR-29 family members in the regulation of processes associated with normal and pathological aging.

Taking into account that *Zmpste24*-null mice present nuclear abnormalities linked

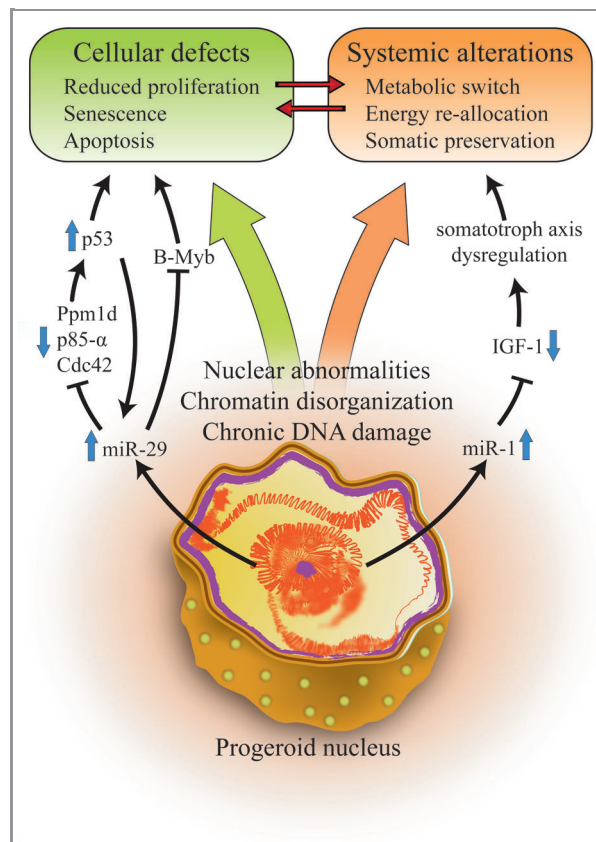


Figure 2. miRNA contribution to accelerate aging. The chronic DNA damage caused by the nuclear envelope abnormalities present in *Zmpste24*-deficient mice activates miR-29 transcription, which in turn represses several key regulators of cell proliferation and senescence. miR-29 targeting of Ppm1d, p85- α and Cdc42 converge in p53 signaling activation, which enhances the DNA damage response, reducing cell proliferation and triggering cellular senescence. In addition, B-Myb repression by this family of miRNAs could also be contributing to the senescence phenotype present in *Zmpste24*-deficient mice by controlling important cell cycle regulators. On the other hand, miR-1 upregulation in liver and other tissues participates in the somatotroph axis deregulation present in the mutant mice by repressing Igf-1 expression. This phenomenon is part of an adaptive response aimed to preserve somatic maintenance by re-allocating energy resources.

to altered chromatin architecture and p53 pathway activation,^{31,35} we also evaluated whether miR-29 upregulation was associated with a chronic response to genomic damage. In agreement with this hypothesis, we observed that miR-29 progressively accumulated during in vitro culture of both wild-type and *Zmpste24*-null adult fibroblasts, reaching the maximum expression at replicative senescence. Likewise, we confirmed that miR-29 is transcriptionally activated upon genotoxic treatment with doxorubicin and that this phenomenon is associated with the DNA damage response, since *p53*-deficient, *ATM*-deficient or *ATR-Seckel* fibroblasts fail to induce miR-29 expression upon this treatment.³⁰ Consistent with a role for miR-29 in the modulation of DNA damage response, functional studies demonstrated that transfection of miR-29 precursor molecules reduces cell proliferation and viability and induces senescence in wild-type primary fibroblasts.³⁰ Furthermore, miR-29 inhibition displayed the opposite effect and rescued the proliferative defects and the senescence phenotype of *Zmpste24*-null fibroblasts. Finally, we identified the Ppm1d/Wip1 phosphatase as a candidate target to mediate the effects of miR-29. Ppm1d is a key regulator of the DNA damage response that dephosphorylates a wide variety of proteins such as p53, Chk1, Chk2, p38, γ -H2AX and ATM.^{36,37} By using luciferase-based assays involving the 3' UTR of *Ppm1d*, we demonstrated that this phosphatase is a bona fide target of miR-29. Moreover, functional in vitro studies showed that these miRNAs are able to modulate p53 phosphorylation at Ser15, which is the residue targeted by Ppm1d.³⁰ According to these results, we propose that aging or chronic DNA damage activates a regulatory circuit involving Ppm1d and p53 which, in turn, enhances the DNA damage response and modulates cell proliferation, viability and senescence (Fig. 2).

Interestingly, it has also been reported that miR-29 and miR-30 are upregulated upon induced and replicative senescence in an Rb-dependent manner and that both miRNAs regulate this process by direct repression of *B-Myb* expression.³⁸ In addition, Park et al.³⁹ have demonstrated that miR-29 targeting of p85 α and

CDC42 activates p53 and regulates cell viability in human cells. Taken together, these data suggest that the miR-29 family plays an essential role in the regulation of cell proliferation, viability and senescence by controlling multiple overlapping pathways. In agreement with these observations, numerous works have attributed a tumor suppressor function for miR-29 in a variety of human malignancies, including hepatocellular carcinoma,⁴⁰ cholangiocarcinoma,⁴¹ different types of leukemias,⁴²⁻⁴⁵ lung carcinoma,⁴⁶ rhabdomyosarcoma⁴⁷ and pleural mesothelioma.⁴⁸ These studies have also identified multiple miR-29 targets whose downregulation could contribute to explain the tumor-suppressive activity of this miRNA. These targets include *DNMT3A*, *DNMT3B*, *MCL1*, *BCL2*, *SKI*, *CDK6*, *TET1*, *YY1* and *TCL1*. Based on this information, it is tempting to speculate that the miR-29 family of miRNAs represents a novel example of molecules that support the antagonistic pleiotropy concept,¹ due to their ability to play a beneficial anti-tumor

role in the reproductive age that turns into a detrimental pro-senescent function later in life.

GeromiRs, a New and Expanding Group of miRNAs

In addition to the regulation of senescence by miR-29 and miR-30, a growing number of miRNAs are arising as important modulators of conserved pathways or processes that have been extensively linked to aging (Table 1). Although it will be necessary to generate animal models with loss- or gain-of-function in specific miRNA activities to uncover their real contribution to aging, these miRNAs—that we propose to call *geromiRs*—represent a new group of molecules which may be involved in the regulation of both normal and pathological aging processes.

Thus, several works have revealed a functional role for miRNAs in senescence modulation through different mechanisms. For example, miR-20a repression of LRF (leukemia/lymphoma related factor) in

Table 1. miRNAs associated with aging in different organisms

miRNA	Targets	Species	Pathway	Ref.
<i>lin-4</i>	<i>lin-14</i>	<i>C. elegans</i>	Insulin/Igf-1	19
miR-71, miR-238, miR-239, miR-246	Not validated	<i>C. elegans</i>	Insulin/Igf-1, DDR	20
miR-29	Ppm1d	Mouse	DDR-p53	30
	p85 α , CDC42	Human	DDR-p53	39
miR-29, miR-30	B-MYB	Human	E2F/Rb-B-Myb	38
miR-1	Igf-1	Mouse	Insulin/Igf-1	33
miR-20a	Lrf	Mouse	p19-p53	49
miR-24	p16INK4a	Human	p16-pRb	50
miR-106a	p21CDKN1a	Human	p53-p21	51
miR-146a/b	IRAK1	Human	IL-1-IRAK-NF κ B	52
	NOX4	Human	NOX4-ROS-senescence	53
miR-200c	ZEB1	Human	ZEB1-p21	54
	BMI	Human	BMI-1-p16/p21	68
miR-217	SIRT1	Human	SIRT1-p53	55
miR-34	SIRT1	Human	SIRT1-p53	57
miR-519	HuR	Human	HuR-SIRT1-p53	56
miR-22	SIRT1, CDK6, SP1	Human	p53/pRb	59
let-7b	HMGA2	Human	HMGA2-p16/p19	66
miR-23a, miR-26a, miR-30	HMGA2	Human	HDAC-HMGA2-p16/p21	67
miR-214	EZH2	Human	EZH2-p16/p21	68
miR-33	TP53	Human	p53-DDR	70

DDR, DNA damage response; ROS, reactive oxygen species

murine fibroblasts activates p19ARF and p16INKa and triggers cellular senescence,⁴⁹ whereas miR-24 decrease in senescent human fibroblasts is in part responsible for p16INKa up-modulation.⁵⁰ Similarly, the five members of the miR-106b family, including the aforementioned miR-20a, are downregulated in stress-induced senescence.⁵¹ miR-106a also contributes to this process by facilitating p21 upregulation.⁵¹ Moreover, miR-146a/b controls the senescence-associated secretory phenotype of human fibroblasts by a negative feedback loop that involves IRAK1 (IL-1 receptor-associated kinase 1) repression, thereby limiting IL-6 and IL-8 expression.⁵² Interestingly, miR-146a is downregulated in aging human endothelial cells and causes NOX4 upregulation, which in turn increases ROS production and induces senescence.⁵³ Similarly, ROS-induced senescence in human endothelial cells activates miR-200c expression, which further contributes to this process by targeting *ZEB1* expression, a key regulator of epithelial-mesenchymal transition that promotes p21 upregulation.⁵⁴

The sirtuin pathway, which extends longevity in yeast, worms and flies,³ is also influenced by miRNAs. Thus, miR-217 upregulation in human endothelial cells during aging reduces Sirt1 activity and promotes senescence,⁵⁵ whereas miR-519 targeting of the RNA binding protein HuR decreases Sirt1 protein levels and contributes to human fibroblast senescence.⁵⁶ Likewise, members of the miR-34 family are transcriptionally activated by p53 and facilitate a positive feedback loop by targeting Sirt1 expression and

influencing cell cycle progression, senescence and apoptosis.^{57,58} Additionally, miR-22 also targets Sirt1 as well as CDK6 and SP1, and promotes senescence in both normal and tumor cells.⁵⁹ Similarly to the sirtuin pathway, the IGF-1/insulin signaling is also regulated by miRNAs. As discussed above, several miRNAs have been shown to modulate longevity in *C. elegans* through this pathway,^{19,20} whereas we have recently described the anomalous upregulation of miR-1, which targets Igf-1, in premature aging mice.³³ In addition, miR-206 and miR-320 also target this somatotroph axis protein in rats,^{60,61} and human miR-145 represses the expression of IGF-1 receptor and its substrate, IRS-1.⁶²

Finally, the decay of adult stem cells self-renewal and pluripotency abilities is considered a key determinant in the age-associated decline of tissue homeostasis and maintenance and there are evidences that miRNAs could regulate some aspects of these processes.⁶³ Thus, recent reports have described senescence or age-related changes in miRNAs of human or rhesus macaque mesenchymal stem cells.^{64,65} Furthermore, the loss of self-renewal potential in old neural stem cells has been linked to age-dependent upregulation of *let-7b*, which in turn inhibits HMGA2 expression, a repressor of the INK4a/ARF locus.⁶⁶ In relation to this work, Lee et al.⁶⁷ have identified miR-23a, miR-26a and miR-30 as HMGA2-targeting miRNAs that accelerate senescence in human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs).⁶⁷ Likewise, miR-200c and miR-214

upregulation in hUCB-MSCs senescence contributes to this process by targeting BMI1 and EZH2, respectively,⁶⁸ and the miR-106b-25 cluster regulates adult neural stem cell proliferation and neuronal differentiation in mice.⁶⁹ In this regard, it is also remarkable that miR-33 repression of *TP53* expression has been shown to regulate hematopoietic stem cell self-renewal.⁷⁰

In conclusion, the discovery of miRNAs has opened a new chapter in aging research that could help to achieve a deeper knowledge of the molecular network underlying this complex process. Although we are far from understanding the precise involvement of these molecules in the multiple age-related cell and tissue changes, solid evidences from the literature support an important role for the growing group of *geromiRs* in aging modulation. Moreover, the recent advances in strategies to effectively block specific miRNA activities in vivo may also facilitate new therapeutic opportunities to delay or ameliorate age-related alterations as well as premature aging syndromes including those caused by alterations in the nuclear envelope.

Acknowledgments

We apologize for all those works that were not discussed because of limited space. This work has been supported by grants from Ministerio de Ciencia e Innovación-Spain and European Union (FP7 Micro-EnviMet). The Instituto Universitario de Oncología is supported by Obra Social Cajastur and Acción Transversal del Cáncer-RTICC. C.L.O. is an Investigator of the Botín Foundation.

References

- Kirkwood TB. Understanding the odd science of aging. *Cell* 2005; 120:437-47; PMID:15734677; <http://dx.doi.org/10.1016/j.cell.2005.01.027>
- Vijg J, Campisi J. Puzzles, promises and a cure for ageing. *Nature* 2008; 454:1065-71; PMID:18756247; <http://dx.doi.org/10.1038/nature07216>
- Kenyon CJ. The genetics of ageing. *Nature* 2010; 464:504-12; PMID:20336132; <http://dx.doi.org/10.1038/nature08980>
- Burtner CR, Kennedy BK. Progeria syndromes and ageing: what is the connection? *Nat Rev Mol Cell Biol* 2010; 11:567-78; PMID:20651707; <http://dx.doi.org/10.1038/nrm2944>
- Garinis GA, van der Horst GT, Vijg J, Hoeijmakers JH. DNA damage and ageing: new-age ideas for an age-old problem. *Nat Cell Biol* 2008; 10:1241-7; PMID:18978832; <http://dx.doi.org/10.1038/ncb1108-1241>
- Puente XS, Quesada V, Osorio FG, Cabanillas R, Cadinanos J, Fraile JM, et al. Exome sequencing and functional analysis identifies BANF1 mutation as the cause of a hereditary progeroid syndrome. *Am J Hum Genet* 2011; 88:650-6; PMID:21549337; <http://dx.doi.org/10.1016/j.ajhg.2011.04.010>
- Vlcek S, Foisner R. Lamins and lamin-associated proteins in aging and disease. *Curr Opin Cell Biol* 2007; 19:298-304; PMID:17466505; <http://dx.doi.org/10.1016/j.ceb.2007.04.001>
- Gonzalez-Suarez I, Gonzalo S. Nurturing the genome: A-type lamins preserve genomic stability. *Nucleus* 2010; 1:129-35; PMID:21326943; <http://dx.doi.org/10.4161/nucl.1.2.10797>
- De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, et al. Lamin A truncation in Hutchinson-Gilford progeria. *Science* 2003; 300:2055; PMID:12702809; <http://dx.doi.org/10.1126/science.1084125>
- Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 2003; 423:293-8; PMID:12714972; <http://dx.doi.org/10.1038/nature01629>
- Haigis MC, Yankner BA. The aging stress response. *Mol Cell* 2010; 40:333-44; PMID:20965426; <http://dx.doi.org/10.1016/j.molcel.2010.10.002>
- Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* 2001; 294:862-4; PMID:11679672; <http://dx.doi.org/10.1126/science.1065329>
- Chen LH, Chiou GY, Chen YW, Li HY, Chiou SH. microRNA and aging: a novel modulator in regulating the aging network. *Ageing Res Rev* 2010; 9(Suppl 1): S59-66; PMID:20708718; <http://dx.doi.org/10.1016/j.arr.2010.08.002>

14. Grillari J, Grillari-Voglauer R. Novel modulators of senescence, aging, and longevity: Small non-coding RNAs enter the stage. *Exp Gerontol* 2010; 45:302-11; PMID:20080172; <http://dx.doi.org/10.1016/j.exger.2010.01.007>
15. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136:215-33; PMID:19167326; <http://dx.doi.org/10.1016/j.cell.2009.01.002>
16. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2008; 19:92-105; PMID:18955434; <http://dx.doi.org/10.1101/gr.082701.108>
17. Lu M, Zhang Q, Deng M, Miao J, Guo Y, Gao W, et al. An analysis of human microRNA and disease associations. *PLoS ONE* 2008; 3:e3420; PMID:18923704; <http://dx.doi.org/10.1371/journal.pone.0003420>
18. Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. *J Pathol* 2011; 223:102-15; PMID:21125669; <http://dx.doi.org/10.1002/path.2806>
19. Boehm M, Slack F. A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science* 2005; 310:1954-7; PMID:16373574; <http://dx.doi.org/10.1126/science.1115596>
20. de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ. MicroRNAs both promote and antagonize longevity in *C. elegans*. *Curr Biol* 2010; 20:2159-68; PMID:21129974; <http://dx.doi.org/10.1016/j.cub.2010.11.015>
21. Maes OC, An J, Sarojini H, Wang E. Murine microRNAs implicated in liver functions and aging process. *Mech Ageing Dev* 2008; 129:534-41; PMID:18561983; <http://dx.doi.org/10.1016/j.mad.2008.05.004>
22. Williams AE, Perry MM, Moschos SA, Lindsay MA. microRNA expression in the aging mouse lung. *BMC Genomics* 2007; 8:172; PMID:17573962; <http://dx.doi.org/10.1186/1471-2164-8-172>
23. Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S. Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. *FASEB J* 2009; 23:3243-50; PMID:19465468; <http://dx.doi.org/10.1096/fj.09-135251>
24. Li N, Bates DJ, An J, Terry DA, Wang E. Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. *Neurobiol Aging* 2011; 32:944-55; PMID:19487051; <http://dx.doi.org/10.1016/j.neurobiolaging.2009.04.020>
25. Drummond MJ, McCarthy JJ, Fry CS, Esser KA, Rasmussen BB. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. *Am J Physiol Endocrinol Metab* 2008; 295:E1333-40; PMID:18827171; <http://dx.doi.org/10.1152/ajpendo.90562.2008>
26. Noren Hooten N, Abdelmohsen K, Gorospe M, Ejiogu N, Zonderman AB, Evans MK. microRNA expression patterns reveal differential expression of target genes with age. *PLoS ONE* 2010; 5:e10724; PMID:20505758; <http://dx.doi.org/10.1371/journal.pone.0010724>
27. Hackl M, Brunner S, Fortschegger K, Schreiner C, Muckova L, Muck C, et al. miR-17, miR-19b, miR-20a, and miR-106a are down-regulated in human aging. *Aging Cell* 2010; 9:291-6; PMID:20089119; <http://dx.doi.org/10.1111/j.1474-9726.2010.00549.x>
28. Somel M, Guo S, Fu N, Yan Z, Hu HY, Xu Y, et al. MicroRNA, mRNA, and protein expression link development and aging in human and macaque brain. *Genome Res* 2010; 20:1207-18; PMID:20647238; <http://dx.doi.org/10.1101/gr.106849.110>
29. Bates DJ, Li N, Liang R, Sarojini H, An J, Masternak MM, et al. MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. *Aging Cell* 2010; 9:1-18; PMID:19878148; <http://dx.doi.org/10.1111/j.1474-9726.2009.00529.x>
30. Ugalde AP, Ramsay AJ, de la Rosa J, Varela I, Marino G, Cadinanos J, et al. Aging and chronic DNA damage response activate a regulatory pathway involving miR-29 and p53. *EMBO J* 2011; 30:2219-32; PMID:21522133; <http://dx.doi.org/10.1038/emboj.2011.124>
31. Varela I, Cadiñanos J, Pendas AM, Gutierrez-Fernández A, Folgueras AR, Sánchez LM, et al. Accelerated ageing in mice deficient in Zmpstc24 protease is linked to p53 signalling activation. *Nature* 2005; 437:564-8; PMID:16079796; <http://dx.doi.org/10.1038/nature04019>
32. Osorio FG, Obaya AJ, Lopez-Otin C, Freije JM. Accelerated ageing: from mechanism to therapy through animal models. *Transgenic Res* 2009; 18:7-15; PMID:19015945; <http://dx.doi.org/10.1007/s11248-008-9226-z>
33. Marino G, Ugalde AP, Fernandez AF, Osorio FG, Fueyo A, Freije JM, et al. Insulin-like growth factor 1 treatment extends longevity in a mouse model of human premature aging by restoring somatotrophic axis function. *Proc Natl Acad Sci USA* 2010; 107:16268-73; PMID:20805469; <http://dx.doi.org/10.1073/pnas.1002696107>
34. Ugalde AP, Mariño G, Lopez-Otin C. Rejuvenating somatotrophic signaling: a therapeutic opportunity for premature aging? *Aging* 2010; 2:1017-22; PMID:21212467
35. Espada J, Varela I, Flores I, Ugalde AP, Cadinanos J, Pendas AM, et al. Nuclear envelope defects cause stem cell dysfunction in premature-aging mice. *J Cell Biol* 2008; 181:27-35; PMID:18378773; <http://dx.doi.org/10.1083/jcb.200801096>
36. Lu X, Nguyen TA, Moon SH, Darlington Y, Sommer M, Donehower LA. The type 2C phosphatase Wip1: an oncogenic regulator of tumor suppressor and DNA damage response pathways. *Cancer Metastasis Rev* 2008; 27:123-35; PMID:18265945; <http://dx.doi.org/10.1007/s10555-008-9127-x>
37. Cha H, Lowe JM, Li H, Lee JS, Belova GI, Bulavin DV, et al. Wip1 directly dephosphorylates gamma-H2AX and attenuates the DNA damage response. *Cancer Res* 2010; 70:4112-22; PMID:20460517; <http://dx.doi.org/10.1158/0008-5472.CAN-09-4244>
38. Martínez I, Cazalla D, Almstead LL, Steitz JA, DiMaio D. miR-29 and miR-30 regulate B-Myb expression during cellular senescence. *Proc Natl Acad Sci USA* 2011; 108:5222-7; PMID:21187425; <http://dx.doi.org/10.1073/pnas.1017346108>
39. Park SY, Lee JH, Ha M, Nam JW, Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. *Nat Struct Mol Biol* 2009; 16:23-9; PMID:19079265; <http://dx.doi.org/10.1038/nsmb.1533>
40. Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, et al. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology* 2010; 51:836-45; PMID:20041405
41. Mott JL, Kobayashi S, Bronk SF, Gores GJ. miR-29 regulates Mcl-1 protein expression and apoptosis. *Oncogene* 2007; 26:6133-40; PMID:17404574; <http://dx.doi.org/10.1038/sj.onc.1210436>
42. Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* 2009; 113:6411-8; PMID:19211935; <http://dx.doi.org/10.1182/blood-2008-07-170589>
43. Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, et al. Tc1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. *Cancer Res* 2006; 66:11590-3; PMID:17178851; <http://dx.doi.org/10.1158/0008-5472.CAN-06-3613>
44. Garzon R, Heaphy CE, Havelange V, Fabbri M, Volinia S, Tsao T, et al. MicroRNA 29b functions in acute myeloid leukemia. *Blood* 2009; 114:5331-41; PMID:19850741; <http://dx.doi.org/10.1182/blood-2009-03-211938>
45. Teichler S, Illmer T, Roemhild J, Ovcharenko D, Stiewe T, Neubauer A. MicroRNA29a regulates the expression of the nuclear oncogene Ski. *Blood* 2011; PMID:21685371.
46. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanoni N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci USA* 2007; 104:15805-10; PMID:17890317; <http://dx.doi.org/10.1073/pnas.0706281104>
47. Wang H, Garzon R, Sun H, Ladner KJ, Singh R, Dahlman J, et al. NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. *Cancer Cell* 2008; 14:369-81; PMID:18977326; <http://dx.doi.org/10.1016/j.ccr.2008.10.006>
48. Pass HI, Goparaju C, Ivanov S, Donington J, Carbone M, Hoshen M, et al. hsa-miR-29c* is linked to the prognosis of malignant pleural mesothelioma. *Cancer Res* 2010; 70:1916-24; PMID:20160038; <http://dx.doi.org/10.1158/0008-5472.CAN-09-3993>
49. Polisenio L, Pitto L, Simili M, Mariani L, Riccardi L, Ciucci A, et al. The proto-oncogene LRF is under post-transcriptional control of MiR-20a: implications for senescence. *PLoS ONE* 2008; 3:e2542; PMID:18596985; <http://dx.doi.org/10.1371/journal.pone.0002542>
50. Lal A, Kim HH, Abdelmohsen K, Kuwano Y, Pullmann R, Jr., Srikantan S, et al. p16(INK4a) translation suppressed by miR-24. *PLoS One* 2008; 3:e1864; PMID:18365017.
51. Li G, Luna C, Qiu J, Epstein DL, Gonzalez P. Alterations in microRNA expression in stress-induced cellular senescence. *Mech Ageing Dev* 2009; 130:731-41; PMID:19782699; <http://dx.doi.org/10.1016/j.mad.2009.09.002>
52. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Orjalo AV, Rodier F, et al. MicroRNAs miR-146a/b negatively modulate the senescence-associated inflammatory mediators IL-6 and IL-8. *Aging* 2009; 1:402-11; PMID:20148189
53. Vasa-Nicotera M, Chen H, Tucci P, Yang AL, Saintigny G, Menghini R, et al. miR-146a is modulated in human endothelial cell with aging. *Atherosclerosis* 2011; 217:326-30; PMID:21511256; <http://dx.doi.org/10.1016/j.atherosclerosis.2011.03.034>
54. Magenta A, Cencioni C, Fasanaro P, Zaccagnini G, Greco S, Sarra-Ferraris G, et al. miR-200c is up-regulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. *Cell Death Differ* 2011; PMID:21527937.
55. Menghini R, Casagrande V, Cardellini M, Martelli E, Terrinoni A, Amati F, et al. MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. *Circulation* 2009; 120:1524-32; PMID:19786632; <http://dx.doi.org/10.1161/CIRCULATIONAHA.109.864629>
56. Marasa BS, Srikantan S, Martindale JL, Kim MM, Lee EK, Gorospe M, et al. MicroRNA profiling in human diploid fibroblasts uncovers miR-519 role in replicative senescence. *Aging* 2010; 2:333-43; PMID:20606251

57. Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. *Proc Natl Acad Sci USA* 2008; 105:13421-6; PMID:18755897; <http://dx.doi.org/10.1073/pnas.0801613105>
58. Yamakuchi M, Lowenstein CJ. MiR-34, SIRT1 and p53: the feedback loop. *Cell Cycle* 2009; 8:712-5; PMID:19221490; <http://dx.doi.org/10.4161/cc.8.5.7753>
59. Xu D, Takeshita F, Hino Y, Fukunaga S, Kudo Y, Tamaki A, et al. miR-22 represses cancer progression by inducing cellular senescence. *J Cell Biol* 2011; 193:409-24; PMID:21502362; <http://dx.doi.org/10.1083/jcb.201010100>
60. Shan ZX, Lin QX, Fu YH, Deng CY, Zhou ZL, Zhu JN, et al. Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. *Biochem Biophys Res Commun* 2009; 381:597-601; PMID:19245789; <http://dx.doi.org/10.1016/j.bbrc.2009.02.097>
61. Wang XH, Qian RZ, Zhang W, Chen SF, Jin HM, Hu RM. MicroRNA-320 expression in myocardial microvascular endothelial cells and its relationship with insulin-like growth factor-1 in type 2 diabetic rats. *Clin Exp Pharmacol Physiol* 2009; 36:181-8; PMID:18986336; <http://dx.doi.org/10.1111/j.1440-1681.2008.05057.x>
62. La Rocca G, Badin M, Shi B, Xu SQ, Deangelis T, Sepp-Lorenzino L, et al. Mechanism of growth inhibition by MicroRNA 145: the role of the IGF-I receptor signaling pathway. *J Cell Physiol* 2009; 220:485-91; PMID:19391107; <http://dx.doi.org/10.1002/jcp.21796>
63. Rossi DJ, Jamieson CH, Weissman IL. Stems cells and the pathways to aging and cancer. *Cell* 2008; 132:681-96; PMID:18295583; <http://dx.doi.org/10.1016/j.cell.2008.01.036>
64. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS ONE* 2008; 3:e2213; PMID:18493317; <http://dx.doi.org/10.1371/journal.pone.0002213>
65. Yu JM, Wu X, Gimble JM, Guan X, Freitas MA, Bunnell BA. Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow. *Aging Cell* 2011; 10:66-79; PMID:20969724; <http://dx.doi.org/10.1111/j.1474-9726.2010.00646.x>
66. Nishino J, Kim I, Chada K, Morrison SJ. Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf Expression. *Cell* 2008; 135:227-39; PMID:18957199; <http://dx.doi.org/10.1016/j.cell.2008.09.017>
67. Lee S, Jung JW, Park SB, Roh K, Lee SY, Kim JH, et al. Histone deacetylase regulates high mobility group A2-targeting microRNAs in human cord blood-derived multipotent stem cell aging. *Cell Mol Life Sci* 2011; 68:325-36; PMID:20652617; <http://dx.doi.org/10.1007/s00018-010-0457-9>
68. So AY, Jung JW, Lee S, Kim HS, Kang KS. DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. *PLoS ONE* 2011; 6:e19503; PMID:21572997; <http://dx.doi.org/10.1371/journal.pone.0019503>
69. Brett JO, Renault VM, Rafalski VA, Webb AE, Brunet A. The microRNA cluster miR-106b-25 regulates adult neural stem/progenitor cell proliferation and neuronal differentiation. *Aging* 2011; 3:108-24; PMID:21386132
70. Herrera-Merchan A, Cerrato C, Luengo G, Dominguez O, Piris MA, Serrano M, et al. miR-33-mediated downregulation of p53 controls hematopoietic stem cell self-renewal. *Cell Cycle* 2010; 9:3277-85; PMID:20703086; <http://dx.doi.org/10.4161/cc.9.16.12598>