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MicroRegulators come of age in senescence

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Abstract

Cellular senescence was first reported five decades ago as a state of long-term growth inhibition in viable, metabolically active cells cultured *in vitro*. However, evidence that senescence occurs *in vivo* and underlies pathophysiologic processes has only emerged over the past few years. Coincident with this increased knowledge, our understanding of the mechanisms that control senescent-cell gene expression programs has also recently escalated. Such mechanisms include a prominent group of regulatory factors – microRNAs (miRNA), a family of small, noncoding RNAs that interact with select target mRNAs and typically repress their expression. Here, we review recent reports that miRNAs are key modulators of cellular senescence, and we examine their influence upon specific senescence regulatory proteins. We discuss evidence that dysregulation of miRNA-governed senescence programs underlies age-associated diseases, including cancer.

Senescence of cultured cells

Most somatic cells explanted from human tissue do not divide indefinitely in culture, but cease to proliferate after a finite number of divisions. As first described by Hayflick fifty years ago, cells then reach a state known as senescence, in which they remain viable and metabolically active [1,2]. Cells can become senescent via two distinct, but closely related mechanisms: through genetic programming (replicative senescence) and in response to damaging conditions (premature senescence) [3].

Replicative cellular senescence is achieved when the telomeres (the protective ends of chromosomes) in proliferating cells are progressively shortened because DNA polymerase cannot completely replicate the lagging strands. Ectopic expression of telomerase can prevent this form of senescence [4,5]. After decreasing to a critical length, the structure of the telomeres is lost, triggering a DNA damage response (DDR) that is associated with the appearance of nuclear foci enriched in DDR proteins [e.g., histone H2AX phosphorylated at serine 139 (γ -H2AX), the Nijmegen breakage syndrome 1 protein (NBS1), mediator of DNA-damage checkpoint 1 (MDC1), and the p53-binding protein 1 (53BP1)], and with the sequential activation of upstream kinases ataxia telangiectasia mutated (ATM) and ATM-related (ATR), downstream effectors (the checkpoint kinases CHK1 and CHK2), and cell cycle inhibitors that include cell division cycle 25 (CDC25) and the transcription factor and tumor suppressor p53. p53 and its transcriptional target p21^{Cip1} (hereafter p21), an inhibitor of cyclin-dependent kinases (cdks), are key components of one of the major senescence

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regulatory mechanisms, the p53/p21 senescence pathway. Replicative senescence can also be triggered directly via activation of the retinoblastoma (RB) tumor suppressor through its main upstream inducer, the cdk inhibitor p16^{INK4a} (hereafter p16) [6,7]; these proteins comprise the p16/RB senescence pathway, the second major mechanism to bring about senescence. Among the connections between both senescence pathways, the p53-induced p21 can also activate RB (Figure 1). Additional gene expression programs central to the senescent phenotype, some related to the p53/p21 and p16/RB pathways, some independent of these pathways (Figure 1), are described below.

Premature cellular senescence, also termed ‘stress-induced senescence’, can be triggered by a number of harmful stimuli, without apparent loss of telomere function. Explanted cells can undergo senescence rapidly due to a lack of optimal culture conditions [e.g., the absence of a proper extracellular matrix (ECM), neighboring cells, or a suitable culture medium] or due to their maintenance in the presence of supraphysiologic (ambient) oxygen that causes oxidative damage [8]. Premature senescence can also be induced in untransformed cells by oncoproteins such as Ras^{V12} and the v-Raf murine sarcoma viral oncogene homolog B1 (Braf)^{E600} [9,10], as well as by the loss of tumor suppressors like phosphatase and tensin homolog (PTEN), neurofibromatosis (NF)1 or the von Hippel-Lindau (pVHL) proteins [11–13]. These senescence triggering mechanisms typically require the presence of p16 or p53 and cannot be rescued by ectopic restoration of telomerase function [14].

Cells reaching senescence in culture, whether via replicative or premature senescence, can be identified by the presence of shorter telomeres and markers like senescence-associated β -galactosidase (SA- β gal) activity and DDR proteins [15–17] (see Box 1 for a list of senescence markers).

Box 1

Detection of senescent cells in culture and *in vivo*

At present, there is no single definitive marker of senescence in culture or *in vivo*. Instead, several markers must be assessed together in order to identify senescent cells.

Long-term cell cycle arrest

Though not exclusive of senescence, the absence of proliferation is a key feature of the senescence phenotype. Growth arrest is imposed primarily by activation of an RB-dependent cell cycle barrier; therefore, hypophosphorylated (active) RB can serve to identify senescent cells. Contrary to earlier views, however, growth arrest during senescence may be reversed via inactivation of the p16 and p53 pathways [94,95].

Induction of senescence-associated β -galactosidase (SA- β gal) activity

Lysosomal β -galactosidase activity increases in senescent cells. Although SA- β gal activity is a popular marker of senescence [15], other cellular states can produce high SA- β gal-positive cells and there is no evidence that SA- β gal activity influences senescence. Thus, SA- β gal activity should be used in conjunction with other markers.

Markers of active tumor suppressor networks

Given the key roles of p53/p21 and p16/RB pathways in triggering senescence, several mediators in these pathways are used as senescence markers. Besides hypophosphorylated RB, high levels of cdk inhibitors (p16, p15, and p21, which block RB phosphorylation), Arf (an inducer of p53), and p53 are also informative senescence markers.

Changes in cellular morphology and organization

Depending on the cell type and senescence trigger, senescent cells often become flat and enlarged, but can also become refractile. In the cytoplasm, senescent cells can accumulate vacuoles and autophagosomes; in the nucleus, senescent cells show altered chromatin structure and senescence-associated heterochromatic foci (SAHF).

Senescence-associated secretory phenotype (SASP)

When cells reach senescence, they secrete numerous proteins, including many cytokines and chemokines [23] with broad implications in oncogenesis and other pathologies. SASP factors IL-6, IL-8, and CXCR2 are valid indicators of senescence in some instances, but it is unknown if they are broadly useful markers.

Oxidative and genotoxic damage

Damage from reactive oxygen species (ROS) directly affects cellular senescence, but little is known about the factors that elevate ROS or the cellular targets affected by ROS [96]. DNA damage caused by telomere attrition, ionizing radiation or chemotherapeutic drugs is an established trigger of senescence. Thus, DNA damage-response (DDR) proteins such as p53, γ -H2AX, NBS1, MDC1, and 53BP1 are important markers of senescence, although DNA damage may not always trigger senescence.

***In vivo* senescence: implication in carcinogenesis**

As it causes stable loss of proliferation, cellular senescence was proposed early on to serve as a tumor suppressor mechanism. This hypothesis was met with skepticism at first, since senescence of cultured cells could simply be an artifact of non-physiologic cell maintenance. However, these early concerns have largely been addressed over the past decade, through a wealth of evidence in human tissues and mouse models that senescence occurs *in vivo*, functions as an effective tumor suppressor mechanism, and underlies several physiologic and pathologic processes, as discussed below. Replicative senescent cells have been shown to accumulate in tissues from elderly persons and aged primates; they are identified by the presence of shorter telomeres, SA- β gal-positive cells, and DDR proteins [15–17]. Markers to identify senescent cells *in vivo* are also described in Box 1.

Premature senescence has also been observed *in vivo* and several genetic studies have independently provided strong support to the notion that senescence functioned as a tumor suppressor mechanism. Senescence repressed tumorigenesis in models of K-Ras^{V12}-triggered lung and pancreas malignancies, in lymphomagenesis triggered by N-Ras, and in melanoma, where benign melanocytic nevi express oncogenic Brat^{E600} [18–21]. Loss of PTEN, VHL, RB or NF1 can also precipitate senescence *in vivo* [11–13,22].

In vivo, senescent cells may affect the malignant phenotype of surrounding cancer cells. Senescent human diploid fibroblasts (HDFs) and other senescent cells display dramatic changes in the patterns of secreted proteins, a phenomenon named senescence-associated secretory phenotype (SASP) [23,24]. The SASP includes secretion of numerous chemokines and cytokines, notably interleukin (IL)-6, IL-8, IL-1 α , granulocyte-macrophage colony stimulating factor (GM-CSF), the growth-regulated oncogene α (GRO α), monocyte chemotactic protein (MCP)-2, MCP-3, matrix metalloprotease (MMP)-1, MMP-3, and many insulin-like growth factor (IGF)-binding proteins [25]. In certain conditions, co-culture with senescent fibroblasts or with their conditioned medium containing these factors was shown to enhance oncogenesis [26–28]. Adding to the complex cross-influence of SASP and cancer cells, SASP components were shown to promote cancer cell migration by degrading the ECM, were pro- or anti-oncogenic depending on the tumor stage, could promote the clearance of tumor cells by immune cells, and were found to be necessary for maintenance of the senescent phenotype [29–32].

***In vivo* senescence linked to other diseases**

Besides cancer, senescence has been implicated in several other diseases. They include pathologies in tissues in which age-related diseases develop (e.g., atherosclerosis), in renewable tissues in which senescent cells accumulate in an age-related manner (e.g., the stroma, the epithelium of different organs, and the hematopoietic system), and in hyperproliferative lesions like nevi [15,33–35].

In some of these disease processes, senescence was proposed to play a protective role; for example, senescence of hepatic stellate cells following liver damage increased ECM deposition on fibrotic scars, preserving liver function [36]. However, in many instances, senescent cells can have detrimental consequences. Since SASP can lead to an elevated production of numerous chemokines and cytokines [25], the accumulation of senescent cells during aging can contribute to the chronic proinflammatory phenotype seen in the elderly; such chronic inflammation is believed to affect age-related pathologies such as diabetes, cancer, neurodegeneration, and cardiovascular disease [25]. SASP-dependent secretion of proinflammatory factors (by as-yet-unidentified senescent cells) could also cause chronic elevation of circulating factors leading to immunosenescence, an age-related decline of the adaptive immune system [37]. Aging also leads to the accumulation of senescent brain immune cells (microglia), a process linked to neurodegenerative pathologies such as Parkinson's and Alzheimer's diseases [38]. Senescent endothelial cells in atherosclerotic lesions were proposed to contribute to impaired vascular function in elderly patients [39]. In chronic obstructive pulmonary disease, p21-deficient mice showed an improvement of symptoms, suggesting that senescence contributed to this pathology [40]. Both p53 and p21 were proposed to enhance endothelial progenitor cell senescence, impairing vascularization in diabetes [41], while senescence of skeletal muscle cells and muscle cell precursors (satellite cells) were linked to age-related frailty and sarcopenia [42].

In sum, the past few years have firmly linked *in vivo* senescence to a growing number of physiologic processes and pathologies. Thus, understanding the molecular mechanisms that govern the senescent phenotype has become a very active area of pursuit.

Post-transcriptional gene regulation by miRNAs

Similarly to other cellular processes, such as proliferation, quiescence, apoptosis, or differentiation, cellular senescence is tightly controlled by specific gene expression programs [43]. Senescence gene regulation has an important transcriptional component, which includes transcription factors such as p53, activating protein (AP)-1, E2F, Id and Ets. In addition, two prominent classes of post-transcriptional regulators of senescence have emerged in recent years. The first is comprised by RNA-binding proteins like human antigen R (HuR), AU-binding factor (AUF1), and tristetraprolin (TTP), which associate with target mRNAs that encode senescence factors and influence cellular senescence [44–47]. The second is comprised by miRNAs, a family of small, non-coding RNAs synthesized in mammalian cells as explained in Box 2. The rising recognition that miRNAs potentially govern gene expression has stimulated efforts to identify senescence-regulatory miRNAs. Since 2007, a flurry of reports has begun to describe miRNAs that are differentially expressed during senescence [48–52] and to further implicate miRNAs in the implementation of the senescent phenotype. Below we review these studies, focusing on the specific senescence processes affected by miRNAs (Figure 1).

Box 2

miRNA biogenesis

MiRNA genes are initially transcribed as long, primary miRNA (pri-miRNA) transcripts by RNA polymerases II and III [97]. These transcripts are processed by a complex known as microprocessor that includes the ribonuclease Drosha and DiGeorge critical region 8 (DGCR8) to generate microRNA precursors (pre-miRNA); pre-miRNAs are then exported to the cytoplasm by exportin 5. The exported pre-miRNAs are cleaved by the ribonuclease Dicer, yielding duplex RNAs ~22-nucleotides long; one strand of each duplex is loaded into the microRNA-containing ribonucleoprotein complex (RISC), which contains Argonaute (Ago) proteins [99,100]. Each of these complexes then targets a specific mRNA, typically at the mRNA 3'-untranslated region (UTR), forming a partial hybrid through the miRNA 'seed' region (nucleotides 2–7). MiRNAs associate with mRNAs with varying degrees of complementarity, and either reduce mRNA stability or suppress mRNA translation, although under specific circumstances (cellular quiescence) they can also promote translation [46,47,101]. Several miRNAs often work in concert to repress expression of a shared target mRNA.

MiRNAs targeting the p53/p21 senescence pathway

Given the central role of p53 in aging and senescence [53], it was little surprise to find that p53 controls the expression of senescence-regulatory miRNAs. In 2007, miR-34 was shown to be a potent trigger of senescence in colon cancer cells and in HDFs [54,55]. Five additional groups almost simultaneously identified the miR-34 family as a key effector of p53 actions, including growth arrest and apoptosis [56–60]. MiR-34a is now a well-established tumor suppressor and a potent trigger of senescence; these functions are elicited through the growing number of identified mRNAs repressed by miR-34a, including those that encode E2F, c-Myc, sirtuin 1 (SIRT1), Cdk4, Cdk6, B-cell leukemia (Bcl)-2, Met, and cyclins D1 and E2 [61–65]. miR-34a can also be upregulated in a p53-independent manner, as shown for Braf-triggered HDF senescence, where miR-34a was upregulated via the transcription factor Elk1 [66], and granulocyte senescence, where it was upregulated by the transcription factor CCAAT enhancer-binding protein (C/EBP) α [67]. In agreement with the notion that miR-34 triggers senescence and thereby elicits tumor suppression, hypermethylation (and thereby silencing) of the miR-34a locus was proposed to represent an oncogenic alteration in many different cancer cell types (e.g., lung, breast, colon, kidney) [68]. Table 1 lists the miRNAs reported to-date that influence expression of senescence-regulatory proteins and thereby affect senescence *in vitro* or *in vivo*.

In addition to suppressing tumorigenesis, elevated levels of miR-34a were linked to endothelial cell senescence associated with a reduction in SIRT1 expression and inhibition of cell proliferation, likely contributing to atherosclerosis [69]. In senescent endothelial progenitor cells (EPCs), elevated levels of miR-34a lowered SIRT1 production and reduced EPC-mediated angiogenesis [70]. In human umbilical vein vascular endothelial cells (HUVEC), aortic endothelial cells, and human coronary artery endothelial cells, miR-217 also lowered SIRT1 expression, triggering premature senescence and impairing angiogenesis [71]. Very recently, overexpression of miR-885-5p in neuroblastoma cells was shown to trigger cellular senescence; although the *CDK2* and *MCM5* mRNAs were identified as direct targets, miR-885-5p potently activated the p53 pathway and induced p53-regulated genes [72].

MiRNAs that modulate the p16/RB pathway

Another increasingly recognized group of senescence-regulatory miRNAs target the cell division cycle, particularly the RB pathway. Although the RB pathway is closely interconnected with the p53 pathway, RB function is distinctly controlled by cdk activity. Translation of p16, an inhibitor of Cdk4 and Cdk6, was repressed by miR-24 in HDFs [48].

Although proliferating HDFs expressed high levels of miR-24, leading to low p16 expression, and late-passage (near-senescent) HDFs expressed low miR-24, which allowed p16 expression, ectopic overexpression of miR-24 alone did not prevent senescence, nor did ectopic antagonization of miR-24 accelerate senescence, likely because miR-24 also reduces expression of several proliferative proteins [73]. Only in combination with other miRNAs (miR15b, miR-25, miR-141, as explained below, [74]), did miR-24 contribute to enhancing senescence. Expression of cyclin D1 and Cdk6 was also repressed by miR-34 [75].

Expression of p21, a broad-spectrum cdk inhibitor, was repressed by miRNAs of the miR-106b family and others with similar seed sequences (miR-130b, miR-302a-d, miR-512-3p and miR-515-3p) in human mammary epithelial cells [76]. In this model system, p21 was necessary for inducing senescence and reducing p21 expression rescued cells from Ras^{V12}-induced senescence [76]. The increased levels of p21 in senescent HDFs and human trabecular meshwork (HTM) cells was attributed, at least in part, to the reduced levels of miR-106b [74]; the lower abundance of miR-15 family members was proposed to allow the accumulation of Bcl-2, in keeping with the increased resistance to apoptosis of senescent cells [77,78].

MiRNAs targeting transcriptional and post-transcriptional factors in senescence

Yet another subset of senescence-controlling miRNAs affects the expression of transcriptional and post-transcriptional regulators. Ectopic overexpression of miR-128a triggered senescence and growth inhibition associated with the downregulation of the Bmi-1 polycomb repressor [79]. The enhanced senescence was believed to result from increased p16 levels when its transcriptional repressor Bmi-1 was less abundant [79]. Senescence of human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs) was triggered by inhibition of histone deacetylase (HDAC) activity. This intervention increased the levels of miR-23a, miR-26a and miR-30a, which in turn repressed high mobility group A2 (HMGA2) proteins. In this model system, the increased expression of p21 in cells with reduced HMGA2 appeared particularly important for the implementation of hUCB-MSC senescence [80]. Senescent HDFs and HTMs showed increased miR-182 abundance, which likely contributed to reducing the levels of target mRNA encoding retinoic acid receptor γ (RAR γ), with possible implications in skin aging [77]. Very recently, HeLa cell senescence was shown to cause the RB-dependent accumulation of miR-29 and miR-30 [81]. A prominent target of miR-29 and miR-30 was B-Myb, a transcription factor whose overexpression can overcome Ras-induced senescence and whose reduction leads to cellular senescence [82].

Post-transcriptional regulators of senescence are also important targets of miRNAs. The alternative splicing factor/splicing factor 2 (ASF/SF2) controls the splicing of many genes, including oncogenes and tumor suppressor genes. In mouse embryo fibroblasts (MEFs), the pro-oncogenic transcriptional repressor LRF (leukemia/lymphoma-related factor) prevented senescence by decreasing the levels miR-28 and miR-505, two miRNAs that selectively lowered the abundance of ASF/SF2 [83]. Another RNA-binding protein, HuR, promoted the stabilization and translational upregulation of mRNAs encoding cell cycle regulatory factors (e.g., cyclin B1 and cyclin A), and the AP-1 component c-Fos, and delayed cellular senescence [44,84]. Ectopic overexpression of miR-519, which inhibits HuR translation, suppressed cell proliferation, and elicited senescence in HeLa cells and HDFs [50,85,86].

MiRNAs modulating SASP-related pathways

As mentioned above, the SASP is characterized by elevated secretion of numerous growth factors, enzymes that degrade the ECM, and cytokines [23]. The latter group includes two

critical mediators of inflammation, IL-6 and IL-8. It was recently reported that in HDFs, miR-146a/b reduced expression of IRAK1, a key component of the IL-1 signal transduction pathway; in turn, reduction of signaling via IRAK blocked the secretion of IL-6 and IL-8 and thus avoided excessive SASP [87]. Expression of miR-146a (and suppression of SASP) was also elevated after long-term culture of BJ fibroblasts ectopically overexpressing telomerase, indicating that SASP could be regulated independently of senescence [51]. Expression of integrin β 1, a cell surface protein that interacts dynamically with the ECM and affects HDF senescence, was reduced by miR-183 in a model of H₂O₂-triggered senescence [88].

Influence of miRNAs in senescence *in vivo*

With accumulating knowledge of the impact of miRNAs upon senescence in cultured cells, the stage is set for exploring the function of miRNAs in senescence and senescence-regulated processes *in vivo*. For example, assessment of the levels of miR-34a in cancer cells revealed CpG hypermethylation of the miR-34a promoter in primary melanoma samples [68], consistent with lower transcription of miR-34a in this malignancy. These findings indicated that p53 was capable of upregulating miR-34a production *in vivo*, and further supported the notion that miR-34a elicited senescence *in vivo*.

The development of genetic models to study this process is only now beginning. After a mouse lacking both alleles of the miRNA synthesis enzyme Dicer showed early embryonic lethality, a conditional Dicer-null mouse was developed [89,90]. Dicer ablation in MEFs triggered a premature senescence phenotype associated with growth arrest, upregulation of Arf (which elevates p53 by blocking the ubiquitin-mediated degradation of p53), increased levels of p53 and p21, elevated SA- β gal activity, and the appearance of senescence-associated heterochromatic foci [90]. When Dicer-conditional mice were crossed with ARF/p16-null or p53-null mice, the resulting MEFs were rescued from senescence after Dicer ablation, indicating that impaired miRNA biogenesis triggered senescence via Arf/p16- and p53-dependent pathways [90]. In developing mouse limbs, where Dicer regulates morphogenesis, conditional ablation of Dicer also led to senescence, as seen on day E16 [90,91]. In adult mice, where hair follicle development was Dicer-dependent, Dicer ablation caused hair loss and rough skin associated with morphological and biochemical signs of senescence [90,92]. The specific miRNAs that prevent premature senescence in these two developmental scenarios remain to be identified. Dicer downregulation in HDFs also induces senescence [93].

In human tissues, expression of the mitogen-activated protein kinase (MAPK) kinase MKK4, an upstream activator of stress-response and senescence-associated response MAPKs p38 and JNK (c-Jun N-terminal kinase) was found to be higher in tissues from older individual donors than from younger donors [74]. This expression pattern mirrored the elevated MKK4 expression in senescent HDFs relative to proliferating, early-passage HDFs. In culture, miR-15b, miR-24, miR-25, and miR-141 were elevated in early-passage HDFs and functioned in concert to downregulate MKK4 expression levels, while in senescent HDFs, the reduced levels of these miRNAs contributed to the upregulation of MKK4. In tissues from older donors, these four miRNAs were also significantly lower, suggesting that they may contribute to diminishing MKK4 production in old tissues *in vivo*. It is interesting to note that activation of p38 and the ensuing phosphorylation of MK2 can lead to the transcriptional upregulation of miR-34 expression via p53-independent mechanisms [62,66]. Whether the senescent phenotype observed in cells with elevated MKK4 is due to the secondary elevation of miR-34a levels remains to be established.

Concluding remarks and future perspectives

The past ten years have yielded a wealth of knowledge of the triggers, markers, and consequences of cellular senescence. Accordingly, it is now solidly accepted that senescence occurs *in vivo* and that it underlies many physiologic and pathologic processes – not only cancer, but also conditions such as atherosclerosis, diabetes, sarcopenia, neurodegeneration, and cardiovascular and pulmonary disease. The past five years have also uncovered that miRNAs are pivotal regulators of senescence. The tiny RNAs have become powerful players in many aspects of senescence. As discussed in this review and represented in Figure 1, key senescence-associated processes are modulated by microRNAs. Activation of p53 increases the transcription of miR-34a, a repressor of numerous senescence-regulatory proteins, and p21. Several miRNAs that diminish p21 and p16 levels show reduced abundance in senescent cells, allowing p21 and p16 to accumulate, inhibit cdks, and activate RB. Senescent cells also display altered levels of transcriptional and post-transcriptional factors (e.g., transcription factors, RNA-binding proteins), some of which influence key proteins in the p53/p21 and p16/RB senescence pathways. Acting together, these three groups of proteins halt cell cycle progression and broadly alter senescence-associated gene expression programs. Finally, microRNAs can also promote SASP, facilitating the secretion of IL-6 and IL-8 and in turn increasing local and systemic inflammation and reducing ECM integrity.

While our understanding of the influence of miRNAs on gene expression in senescence is rapidly advancing, we know much less about the mechanisms by which senescence alters miRNA levels. The senescence-associated increase of miR-34 transcription by p53 and miR-29/miR-30 transcription by RB are the best-documented examples thus far. Future studies to uncover the transcriptional and post-transcriptional mechanisms that underlie senescence-associated miRNA changes are warranted.

As we witness the convergence of the two fields – *in vivo* senescence and miRNA control of senescence – the challenges and questions ahead begin to come into view. How do miRNAs influence senescence in the context of the organism? What senescence-regulatory miRNAs are needed to maintain physiologic balance? Which miRNAs modulate pathologies with aberrant senescence? How are the levels of these miRNAs regulated at the transcriptional and post-transcriptional levels? Can we increase or antagonize miRNAs to modulate senescence towards therapeutic goals?

To answer these questions, it will be essential to fully understand the process of senescence *in vivo*, in the context of the tissue, the organ, and the organism. For this, it will be particularly helpful to identify universal, specific, and sensitive markers of *in vivo* senescent cells. It will also be critical to use animal models to investigate the cellular, molecular, genetic, and biochemical characteristics of senescent cells *in vivo*. The use of transgenic mice that overexpress or lack certain miRNAs (for example, miR-34a), constitutively or conditionally, will provide vital information of senescence-regulatory miRNAs within the framework of the entire animal. As much as possible, human studies should follow closely behind, so that we can fully understand the impact of senescence miRNAs in physiologically relevant situations. A more complete understanding of specific miRNAs on senescence will also enable us to consider the therapeutic potential of miRNA-based interventions in age-related pathologies. In sum, miRNAs have become promising molecular tools and targets in our efforts to restore age-dependent losses in body homeostasis.

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Abbreviations

Cdk	cyclin-dependent kinase
CR	coding region
DDR	DNA damage response
ECM	extracellular matrix
HDFs	human diploid fibroblasts
RB	retinoblastoma
RBP	RNA-binding protein
RISC	RNA-induced silencing complex
SASP	senescence-associated secretory phenotype
UTR	untranslated region

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Figure 1. MiRNAs that influence senescence-relevant pathways

Cells progressing to senescence acquire several prominent phenotypes and the levels of many proteins governing each of these phenotypes are influenced by senescence-associated miRNAs. As shown in the light gray boxes, senescent cells have elevated levels of p53 (subject to negative regulation by MDM2, a protein repressed by miR-885-5p), which transcriptionally upregulates miR-34 expression; in turn, miR-34 represses the levels of several proliferative proteins (E2F, c-Myc, cyclins and cdk) and anti-apoptotic proteins (Bcl-2, SIRT1). Senescent cells also have active (hypophosphorylated) RB, resulting from elevated levels of two potent inhibitors of cdk (the RB kinases), p21 (upregulated in part via p53) and p16; a number of miRNAs that reduce p21 and p16 abundance have been reported. Senescence cell-specific gene expression programs are controlled via changes in proteins that modulate gene transcription (via promoter elements or chromatin remodeling) like Bmi-1, HMGA2, RAR γ , and B-Myb, and proteins that affect gene expression post-transcriptionally like the splicing factor ASF/SF2 and the mRNA-stabilizer and translation regulator HuR; each of these proteins is also subject to control by senescence-associated miRNAs and some of them in turn cross-talk with the p53/p21 pathway (HMGA2 represses p21 transcription) and with the p16/RB pathway (Bmi-1 represses p16 transcription). Importantly, not only are we learning how miRNAs affect senescence, but we are also discovering how senescence factors directly modulate the expression of miRNAs; for example, p53 transcriptionally upregulates miR-34 and RB transcriptionally upregulates miR-29/miR-30. In addition, senescent cells also secrete a distinctive subset of proteins, a feature known as SASP; secretion of some of these factors (e.g., IL-6 and IL-8) is under control of IRAK1, a protein whose levels are reduced by the senescence-associated miR-146. Factors secreted through the SASP, as well as lower integrin β 1 activity resulting from higher miR-183 affect ECM function.

These pathways and regulatory processes influence key aspects of the senescence phenotype (dark gray). Major downstream consequences of activation of p53/p21 and p16/RB and gene regulatory factors are the inhibition of the cell division machinery and the implementation of senescence-associated gene expression patterns. The p53/p21 and p16/RB pathways elicit cell responses to cell damage by genotoxins and oxidants. The main consequences of the SASP are the secretion of factors that cause inflammation and compromise the integrity of the ECM.

Table 1

MiRNAs affecting specific senescence pathways

Although several *en masse* studies have identified large subsets of miRNAs upregulated and downregulated [48–52], only a few of these have been experimentally shown to modulate senescence. Listed in the Table are several miRNAs showing altered levels during senescence (column 1) and the specific senescence-regulatory proteins that they target (column 2). The specific cultured cells and *in vivo* systems in which these miRNAs have been shown to affect senescence (column 3) and the consequences of these regulatory interactions upon senescence are also noted (column 4; n.d., not determined).

Changes in microRNAs	Target senescence regulatory factors	Cultured / <i>in vivo</i> model	Consequences	References
p53/p21 pathway				
↓ miR-885-5p	↑ MCM5	Neuroblastoma cells	↓ Senescence	[71]
↑ miR-34	↓ E2F	Neuroblastoma cells	↑ Senescence	[54]
		AML cells (C/EBP α induces miR-34a)	n.d.	[67]
	↓ c-Myc	Braf HDFs (Elk1 induces miR-34a)	↑ Senescence	[66]
	↓ SIRT1	Colon carcinoma, endothelial cells	↑ Senescence	[69,70]
	↓ Cyclin D1, Cdk6	Non-small cell lung carcinoma cells	↑ Senescence	[75]
	↓ Cdk4, Met, Cyclin E2	MEFs	↑ Senescence	[55]
	↓ Bcl-2	Chronic lymphocytic leukemia cells	n.d.	[56]
	(–)	Breast, lung, colon, kidney, bladder, pancreatic carcinoma, melanoma cell lines, and primary melanoma	↓ Senescence	[68]
↑ CpG-methylated miR-34a				
p16/RB pathway				
↓ miR-106b (and related miR-130b, miR-302a-d, miR-512-3p, miR-515-3p)	↑ p21	Mammary epithelial cells (Ras ^{V12} -triggered senescence)	↑ Senescence	[76,77]
↓ miR-24	↑ p16	HFDs	n.d.	[48]
Gene regulation				
↓ miR-128	↑ Bmi-1	Medulloblastoma cells	↓ Senescence	[79]
↑ miR-23a, miR-26a, miR-30a	↓ HMG A2	hUCB-MSCs	↑ Senescence	[80]
↑ miR-182a	↓ RAR γ	HDFs, HTMs	↑ Senescence	[77]
↑ miR-29, miR-30	↓ B-Myb	Cervical carcinoma cells	↑ Senescence	[81]
↑ miR-28, miR-505	↓ ASF/SF2	MEFs	↑ Senescence	[83]
↑ miR-519a-c	↓ HuR	Cervical carcinoma cells, HDFs	↑ Senescence	[50]
SASP				
↑ miR-146a,b	↓ IRAK1	HDFs	Blocked secretion of IL-6 IL-8	[87]
↑ miR-183	↓ Integrin β 1	HDFs, HTMs (after H ₂ O ₂)	↑ Senescence	[88]
Other				
↓ miR-155, miR-20a	n.d.	HDFs (after ionizing radiation or busulfan)	↑ Senescence	[52]

Changes in microRNAs	Target senescence regulatory factors	Cultured / <i>in vivo</i> model	Consequences	References
↓ miR-15b, miR-24, miR-25, miR-141	↑ MKK4	HDFs, tissues from old donors	↑ Senescence	[74]
↓ microRNAs (global)	(multiple)	Conditional Dicer-null MEFs, developing limb, skin	↑ Senescence	[90]