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ABSTRACT

Cellular senescence is a state of permanent cell-cycle arrest triggered by different internal and external stimuli. This phenomenon is considered to be both beneficial and detrimental depending on the cell types and biological contexts. During normal embryonic development and after tissue injury, cellular senescence is critical for tissue remodeling. In addition, this process is useful for arresting growth of tumor cells, particularly during early onset of tumorigenesis. However, accumulation of senescent cells decreases tissue regenerative capabilities and induces inflammation, which is responsible for cancer and organismal aging. Therefore cellular senescence has to be tightly regulated, and dysregulation might lead to the aging and human diseases. Among many regulators of cellular senescence, in this review, I will focus on microRNAs, small non-coding RNAs playing critical roles in diverse biological events including cellular senescence.

INTRODUCTION

Over half a century ago, cellular senescence was first characterized as the finite replicative potential in cultured human fibroblasts by Leonard Hayflick (1, 2), which is known as replicative senescence. Since then, extensive studies have broadened the concept of cellular senescence, an irreversible cell-cycle arrest in response to a variety of internal and external stress signals (3-5). Along with the replicative senescence, senescence caused by diverse stressors is collectively known as premature senescence (5). However, depending on different stimuli the cells encounter, the premature and/or induced senescence can be classified into different types (6). Telomere shortening generated by repeated DNA replication is mainly responsible for replicative senescence (7, 8). DNA damage induced by ionizing radiation, ultraviolet (UV), and other oxidative agents resulting in a double-stranded DNA break are potent inducers of DNA damage-induced senescence (9, 10). Activation of oncogenes or inactivation of tumor suppressors is a major cause of oncogene-induced senescence (OIS) (7, 10, 11). In addition, oxidative stress and reactive oxygen species (ROS) can triggers oxidative stress-induced senescence (12). Recently, it has been reported that mitochondrial dysfunction-associated senescence with distinct secretory phenotypes is caused by dysfunctional mitochondria (13). However, in some cases, the classification of cellular senescence is ambiguous because of the complex causes of the events and overlapping effector pathways.

Phenotypically, senescent cells exhibit enlarged and flattened shapes that are in part determined by activation of the mTOR pathway (14). The cellular senescence is also characterized by increased lysosomal components, where the activity of the lysosomal enzyme, such as senescence-associated β galactosidase (SA-β gal), is commonly used as a marker for senescence (15). Alteration in chromatic structures, such as senescence-associated heterochromatin foci (SAHF), which are specialized domains of heterochromatin, is often

associated with senescent cells. At the molecular level, tumor-suppressor networks, namely, p53-p21 and $p16^{INK4A}$-retinoblastoma (p16-pRB) pathways, are commonly activated in the senescence program (16). However, no single characteristic is a specific hallmark of cellular senescence, and not all senescent cells show the aforementioned features of senescence.

Accumulation of evidence suggests that cellular senescence has both helpful and deleterious functions (10, 17). During normal embryogenesis, senescence participates in morphogenesis and tissue remodeling (10). In response to a fetal HLA-G signal, nearby natural killer (NK) cells enter senescent state which continuously secretes factors for maternal vascular remodeling (18). Developmentally programmed senescence responsible for morphogenesis is also found throughout embryonic development at multiple sites, such as mesonephros, endolymphatic sac (19), and the apical ectodermal ridge (20). In addition to developmental functions, senescent cells help restrict tumor progression (21) and fibrosis in the liver, heart, and kidneys (10) and promote wound healing (22, 23). However, accumulation of senescent cells has detrimental effects as well. They can trigger aging and age-related pathological processes, such as tumorigenesis (24) and metabolic diseases (10). For example, some senescence-associated secretory phenotype (SASP) factors promote invasion and metastasis of tumor cells by altering tissue structures (25), and others induce inflammatory phenotypes and cancer (26). In addition, it is reported that senescent cells contribute to increased vascularization of tumors (27). These findings all support that prolonged senescence can promote tumorigenesis. Senescence is also found in human mesenchymal stem cells (hMSCs), a major source of cell therapy, during extensive *in vitro* culture (28). Senescence in hMSCs leads to functional alterations, including differentiation defects (29-31), dysregulation of immunoregulatory activity (32), and decreased migratory capabilities (32, 33), which all reduce therapeutic potential. Therefore it is crucial to

elaborately regulate cellular senescence to achieve normal development and physiology.

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs that downregulate the expression of their mRNA targets (34). A single miRNA can simultaneously suppress hundreds of different target mRNAs, thereby effectively regulating a myriad of cellular processes (35, 36). Because they can control numerous target genes within key pathways, miRNAs can be used as tools to explore the multiple pathways and core networks that govern the specific cellular states (37). In this review, I will first describe the current understanding of miRNAs that are differentially expressed during cellular senescence. I will then review the miRNAs that regulate key nodes of the signaling pathways that are critical for driving and maintaining cellular senescence.

DIFFERENTIAL miRNA EXPRESSION DURING CELLULAR SENESCENCE

Several studies reported the miRNA expression profiles during cellular senescence by various profiling technologies (Table 1). Maes *et al.* reported the expression profiles of 462 miRNAs using a human miRNA microarray (MMchip) in replicative and premature senescent human fibroblasts along with quiescence cells (38). Depending on the growth-arrest conditions, a subset of miRNAs is specific or common in two or three states (38). Among those, miR-10b, miR-34a, miR-373, miR-377, miR-624, miR-633, miR-638, and miR-663 are commonly upregulated in three growth-arrested cells (38). Using miRNome arrays, which are based on qPCR analysis, the Abdelmohsen group validated that there are a subset of miRNAs notably up- or down-regulated in senescent human fibroblasts (39). Among those, miR-519, a tumor-suppressor miRNAs, is highly expressed in senescent cells, and when overexpressed in either young fibroblasts or HeLa cells, it indeed triggered senescence (39). The Wang group performed miRNA microarray analysis with replicative,

ionizing radiation (IR), or busulfan (BU)-induced senescent human fibroblasts (40). They showed that eight miRNAs are differentially expressed in both replicative and induced senescent cells: miR-152, miR-410, miR-431, and miR-493 are up-regulated, and miR-15a, miR-20a, miR-25, and miR-155 are down-regulated (40). Knockdown or overexpression of these miRNAs revealed their functions during senescence. Using deep sequencing analysis, Dhahbi *et al.* covered miRNAs differentially expressed in young and senescent human fibroblasts (41). They reported that 141 miRNAs were upregulated and 131 miRNAs were downregulated upon senescence. In addition to miRNAs already known to be associated with cellular senescence, there are novel miRNAs (e.g., miR-432 and miR-145) differentially expressed during senescence. The lists of miRNAs during senescence may disagree because of the different cell types or senescence models or technologies adopted.

miRNAs IMPLICATED IN KEY SENESCENCE PATHWAYS

In addition to the global profiling experiments, the role of individual miRNAs during senescence has been investigated by numerous functional studies. I will focus on two major senescence signaling pathways, namely, p53-p21 and/or p16-pRB to review the functions of senescence-associated miRNAs (Figure 1).

miRNAs associated with the p53-p21 axis

miRNAs directly regulate p53

The p53 protein, a key regulator of the G1/S and G2/M checkpoints, activates transcription of numerous genes participating in the control of the cell cycle, such as p21. There are miRNAs that directly and/or indirectly regulate p53, which indicates elaborate controls of a crucial tumor suppressor. miR-504 (42), miR-125b (43), miR-25, and miR-30d

(44) directly bind to and suppress p53. Different groups independently showed that ectopic expression of these miRNAs decreases p53 expression and several cellular functions, including p53-mediated cell-cycle arrest, suggesting their role in senescence suppression.

miRNAs indirectly regulate p53 through p53 suppressors

Several miRNAs negatively regulate the suppressors of p53, thereby inducing senescence. For instance, miR-192, miR-194, miR-215 (45), and miR-605 (46) indirectly upregulate p53 through downregulation of the murine double-minute clone 2 (MDM2), an oncogene suppressing p53 expression. Notably, this subset of miRNAs is regulated by p53, thereby constituting a positive feedback loop (45, 46). Another regulatory loop between miRNAs, a target gene, and p53 can be found in an example of miR-34a, one of the most-studied miRNAs in this network. Specifically, miR-34a promotes cellular senescence by suppressing the silent-mating type information regulation 2 homologue 1 (SIRT1), a deacetylase that negatively regulates p53 and stress-response pathways (47-52). Along with miR-34a, miR-22 (53, 54), miR-138 (55), miR-181a/b (55), miR-217 (56), and miR-449 (57) reduce SIRT1 expression, thereby increasing p53 expression and senescence in various cancers and normal cells.

The Ashraf group reported that miR-195 is overexpressed in senescent stem cells and that silencing miR-195 in old MSCs increases the expression of telomerase reverse transcriptase (TERT) and SIRT1 and increases p53 levels (58). The mediator of DNA damage checkpoint 1 (MDC1), a crucial component of the DNA damage response (DDR) machinery (59) is another regulatory point by miRNAs during senescence. It is reported that miR-22 directly suppresses MDC1 and hence promotes premature senescence (60).

miRNAs regulate p21

Multiple miRNAs directly downregulate p21 and therefore suppress senescence. Borgdorff *et al.* performed miRNA screening experiments and reported that 28 miRNAs prevented RAS^{G12V}-induced senescence by inhibiting p21 expression in human mammary epithelial cells: miR-106b family members, miR-130b, miR-302a/b/c/d, miR-512-3p, and miR-515-3p (61). This is consistent with the earlier finding that downregulation of miR-106a contributed to the upregulation of p21 in senescent human fibroblasts and trabecular meshwork cells (62). In a colon-carcinoma cell line, miR-20a downmodulates p21 expression and abrogates TGF-β-induced G1/S arrest (63). miR-663, which is more abundantly expressed in senescent cells (38, 39), directly targets p21 and inhibits the G1/S transition in nasopharyngeal carcinoma cells (64). As mentioned earlier, miR-519 is highly abundant in senescent cells (39). At the molecular level, miR-519 promotes growth inhibition partially by increasing DNA damage response and decreasing cytosolic calcium status, which all in turn elevate the p21 expression (65). Taken together, some miRNAs exert their effect on senescence by targeting multiple p21-inducing pathways.

miRNAs associated with the p16-pRB pathway

miRNAs directly regulate p16

The other core converged pathways during cellular senescence is p16-pRB (66, 67). The p16, the prototypical member of cyclin-dependent inhibitor, is encoded by the CDKN2A gene in humans (68, 69). Because of its crucial role in the cell cycle, regulation of p16 is complex and involves interactions with numerous factors. Lal *et al.* reported that decreased miR-24 expression is associated with increased p16 levels with replicative senescence (70). Several groups demonstrated that miR-24 directly binds to and suppresses p16 translation in human

cells, different tumor lines, and a disease model, such as osteoarthritis (70-72).

miRNAs indirectly regulate p16

Some miRNAs indirectly regulate p16 and hence affect cellular senescence. Interestingly, several miRNAs control polycomb repressive complexes (PRC1 and PRC2). Amongst other diverse cellular processes, these epigenetic regulators influence senescence in part by silencing the INK4/ARF locus, where p16 is located. For instance, the miR-9, miR-125, and miR-181 families modulate CBX7 (chromobox homologue 7), one of the components of PRC1, which in turn induces senescence in a p16-dependent manner (73). Other polycomb group (PcG) proteins, such as BMI1 (B cell-specific Moloney murine leukemia virus integration site 1, polycomb ring finger oncogene) and EZH2 (enhancer of zeste homologue 2) are also targeted by miRNAs, thereby affecting the senescence state. BMI1 is repressed by miR-141, which in turn promotes senescence in human fibroblasts (74). The same protein is also regulated by miR-128a with increased intracellular ROS level and senescence in medulloblastoma cancer cells (75). miR-138 induces senescence through EZH2 repression in renal-cell carcinoma (76). This type of regulation between miRNAs with PcG and senescence are all dependent on p16 overexpression. More recently, a systematic approach combining miRNA screening and miRNA profiling revealed a more complex association of miRNAs, epigenetic regulators, and a p16 pathway (77). miR-26b, miR-181a, miR-210, and miR-424 directly suppress diverse PcG proteins, such as CBX7, EED (embryonic ectoderm development), EZH2, and Suz12 (suppressor of zeste 12 homologue), following increased levels of p16 and senescence (77).

Additionally, forced expression of miR-335 is associated with senescence phenotypes, including augmented p16 levels in hMSCs, with reduction of therapeutic potential (78).

Interestingly, a loss-of-function screening assay identified miR-335 as a tumor suppressor involved in senescence by targeting p16, pRB, p21, and CARF (collaborate of ARF) (79). Not surprisingly, in some cases, a battery of miRNAs (e.g., miR-15b, miR-24, miR-25, and miR-141) all together represses MKK4 (mitogen-activated protein kinase (MAPK) kinase 4) and decreases p16 protein levels and senescence in human fibroblasts (80).

miRNAs regulate pRB

It has been reported that expression of two miRNA families, miR-29 and miR-30, is induced during senescence in a pRB-dependent mode (81). Martinez *et al.* demonstrated that these miRNAs exert their effect by directly targeting the B-Myb oncogene, which indicates their role in Rb-driven cellular senescence (81). Interestingly, downregulation of B-Myb is also associated with senescence through the ROS-mediated p53/p21 axis, both *in vivo* and *in vitro* (82), which suggests the integration of senescence regulation.

In prostate cancer cells, miR-449a directly represses the cyclin D1 (CCND1) gene, a regulator of Rb activity, which sequentially modulates growth and senescence in an Rb-dependent mechanism (83). Similar regulation of miR-449a within the Rb regulatory network and senescence has been shown in human lung-cancer cells through targeting E2F3, a key regulator of G1/S transition (84, 85). In addition, the E2F3 is a downstream target of miR-203 in human melanoma cells (86). Interestingly, miR-203 represses ZBP-89 as well but silencing of E2F3, not ZBP-89, contributes to the induction of senescence phenotypes. Consistent with this result, E2F3 overexpression rescued melanoma cells from senescence induced by miR-203 (86).

CONCLUSIONS AND PERSPECTIVES

Senescence is a highly heterogeneous cellular process. It is becoming increasingly evident that regulation of a single factor by an individual regulator can hardly define how senescence is initiated and maintained. Therefore, it is crucial to understand specific and more general regulatory mechanisms at many levels. miRNAs are one of the suitable regulators in this process, because they can simultaneously alter levels of multiple genes and pathways. Analyzing global miRNA expression profiles of different senescence states or comparing other growth-arrest conditions, such as quiescence, would be a primary approach to understanding the molecular constitutions of cellular senescence. Alternatively, miRNA functions can be studied more globally by removing all miRNAs in the system, by deleting genes involved in miRNA biogenesis, namely, Dicer or DGCR8. Loss of miRNA biogenesis by ablating the Dicer gene in mouse fibroblasts induces $p19^{Arf}$-p53 levels and senescence (87). Similarly, DGCR8 loss triggers cellular senescence in both murine and human fibroblasts in a p21-dependent manner (88). Finally, as described above, numerous studies performed on individual miRNAs also greatly expand our knowledge of senescence controls. In many cases, the feedback loop between miRNAs and key nodes of regulatory pathways are reported, which further indicate the complex regulation of this process. Additional studies are now needed to develop strategies to manipulate and deliver therapeutic miRNA to reinforce or prevent the senescent state, depending on the physiological outcome one might expect.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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FIGURE LEGENDS

Figure 1. miRNAs regulate key signaling pathways critical for cellular senescence.

Table 1. miRNAs differentially expressed in senescent cells.

Profiling technology	Differentially expressed miRNAs in senescent cells		Reference
miRNA microarray (MMchip)	Up	let-7c/f/g, miR-10b, miR-26a, miR-34a, miR-106b, miR-136, miR-137, miR-144, miR-195, miR-200b, miR-363, miR-373, miR-377, miR-432, miR-485-5p, miR-517, miR-609, miR-624, miR-633, miR-638, miR-663	38
	Down	miR-32, miR-147, miR-196b, miR-197, miR-218, miR-365, miR-425, miR-512-5p, miR-517a, miR-619	
miRNome array	Up	miR-34c-3p, miR-122, miR-124, miR-129-3p, miR-146b-3p, miR-203, miR-216b, miR-219-1-3p, miR-372, miR-431, miR-432, miR-451, miR-492, miR-499-3p, miR-513a-5p, miR-513b, miR-519a, miR-519b-3p, miR-519c-3p, miR-548b-3p, miR-548k, miR-548p, miR-561, miR-584, miR-600, miR-641, miR-658, miR-663, miR-874, miR-890, miR-944, miR-1180, miR-1185, miR-1204, miR-1225-5p, miR-1244, miR-1248, miR-1250, miR-1255b, miR-1259, miR-1270, miR-1271, miR-1273, miR-1279, miR-1282, miR-1284, miR-1288, miR-1289, miR-1291, miR-1303, miR-1305, miR-1323, miR-1537	39
	Down	let-7a/b/c/d/e/f/g/i, miR-7, miR-10a/b, miR-15a, miR-18a/b, miR-20a, miR-30b, miR-96, miR-100, miR-101, miR-103, miR-106a, miR-107, miR-125a-5p, miR-125b, miR-127-3p, miR-140-3p, miR-140-5p, miR-141, miR-155, miR-194, miR-221, miR-411, miR-450a, miR-503, miR-506, miR-520e, miR-543, miR-545, miR-548c-5p, miR-548d-5p, miR-548e, miR-569, miR-572, miR-576-3p, miR-625, miR-628-5p, miR-649, miR-1181, miR-1182, miR-1200, miR-1201, miR-1203, miR-1228, miR-1234, miR-1238, miR-1246,	

		miR-1247, miR-1254, miR-1257, miR-1258, miR-1260, miR-1265, miR-1274a, miR-1280, miR-1283, miR-1287	
miRNA array	Up	miR-22, miR-27, miR-29b, miR-30a/c, miR-34a, miR-101b, miR-103, miR-106a, miR-123, miR-127, miR-128a, miR-129, miR-134, miR-152, miR-190, miR-219, miR-296, miR-323, miR-337, miR-340, miR-376a, miR-376b, miR-379, miR-380-3p, miR-382, miR-410, miR-431, miR-432, miR-433 miR-486, miR-493, miR-494, miR-496, miR-516-35p	40
	Down	miR-19b, miR-20a/b, miR-25, miR-29b, miR-30c-1, miR-32, miR-92-1a/b, miR-93a, miR-106a/b, miR-123b, miR-135b, miR-143, miR-145, miR-155, miR-195, miR-217b, miR-218a, miR-224, miR-321, miR-424-2, miR-450-2b, miR-483	
Deep sequencing[(a)]	Up	miR-122, miR-126, miR-129-3p, miR-129-5p, miR-184, miR-217, miR-323b-3p, miR-375, miR-432, miR-449a, miR-449b/c, miR-491-5p, miR-496, miR-539, miR-584, miR-668, miR-765, miR-1197, miR-1246, miR-1274a/b, miR-1275, miR-1290, miR-3656, miR-3911	41
	Down	miR-15a/b, miR-16, miR-17, miR-18a/b, miR-19a/b, miR-20a, miR-33b, miR-106a, miR-145, miR-146a, miR-146b-3p, miR-148a, miR-155, miR-195, miR-196a, miR-199b-5p, miR-218, miR-296-3p, miR-296-5p, miR-345, miR-490-5p, miR-497, miR-548u, miR-549, miR-551b, miR-576-5p, miR-766, miR-887, miR-1245, miR-1261, miR-1270, miR-1271, miR-3154, miR-3187, miR-3622a-5p, miR-3912	

[a] miRNAs which show more than five-fold changes in senescent cells are listed. A complete list of differentially expressed miRNAs is found in Ref 41.

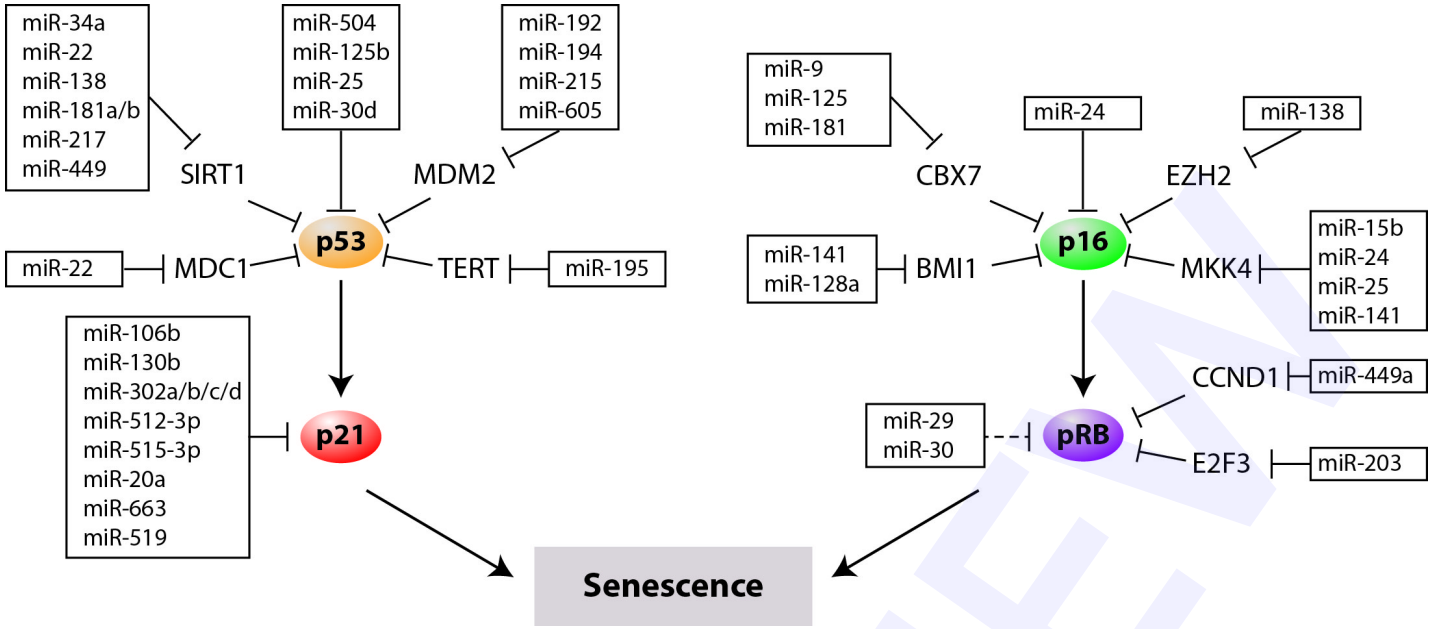

Fig. 1.