



Caveolin-1, a master regulator of cellular senescence

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Abstract

Cellular senescence is a feature of most somatic cells. It is characterized by an irreversible cell cycle arrest and by the ability to secrete a plethora of mediators of inflammation and growth factors, which can alter the senescent cell's microenvironment. Senescent cells accumulate in tissues over time and contribute to both aging and the development of age-associated diseases. Senescent cells have antagonistic pleiotropic roles in cancer. Given the inability of senescent cells to proliferate, cellular senescence is a powerful tumor suppressor mechanism in young individuals. However, accumulation of senescent stromal cells during aging can fuel cancer cell growth in virtue of their capacity to release factors that stimulate cell proliferation. Caveolin-1 is a structural protein component of caveolae, invaginations of the plasma membrane involved in a variety of cellular processes, including signal transduction. Mounting evidence over the last 10–15 years has demonstrated a central role of caveolin-1 in the development of a senescent phenotype and the regulation of both the anti-tumorigenic and pro-tumorigenic properties of cellular senescence. In this review, we discuss the cellular mechanisms and functions of caveolin-1 in the context of cellular senescence and their relevance to the biology of cancer.

Keywords Caveolin · Stress-induced senescence · Oncogene-induced senescence · Cancer · Aging · p53

1 Introduction

Caveolae were first described as invaginations of the plasma membrane in the 1950s by electron microscopy [1, 2]. However, the functional significance and biological role of caveolae have emerged only after the discovery of caveolin-1 in the early 1990s. Since then, compelling *in vitro* and *in vivo* evidence, as well as human studies, has revealed a causal link between caveola/caveolin-mediated functions and a number of fundamental cellular processes and the pathogenesis of multiple human diseases. Cellular senescence has antagonistic pleiotropic properties in cancer biology [3–6]. On the one hand, cellular senescence prevents cells carrying potentially tumor-promoting genetic mutations from proliferating and forming a tumor in young organisms. On the other hand, the age-dependent accumulation of senescent cells has been linked to tumorigenesis given their ability to release

growth factors and mediators of inflammation that stimulate the growth of transformed cells. Interestingly, caveolin-1 has emerged as a key regulator of different forms of cellular senescence. Here, we provide a comprehensive description of the signaling pathways and molecular mechanisms through which caveolin-1 controls the development of a senescent phenotype and the role of caveolin-1-mediated cellular senescence in cancer.

2 Caveolin-1 and caveolae

Caveolin-1 is a ~20-kDa protein that is required for the formation of caveolae in most cell types [7]. Caveolae are 50–100-nm flask-shaped invaginations of the plasma membrane enriched in cholesterol and glycosphingolipids [8]. Caveolae can exist as individual invaginations or can be found in detached grape-like clusters and long tubular structures derived from the fusion of single caveola. The caveolin-1 protein sequence consists of four domains: (i) an NH₂-terminal domain, which includes both phosphorylation (Y14) and ubiquitination sites; (ii) a caveolin scaffolding domain (CSD; residues 82–101) carrying a cholesterol recognition/interaction consensus sequence (CRAC) and an ubiquitination site; (iii) a membrane domain, which interacts

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with membrane lipids; and (iv) a COOH-terminal domain, which possesses three palmitoylation sites. Oligomeric caveolin-1 complexes, embedded in cholesterol-enriched membranes, travel from the Golgi to the plasma membrane where they interact with cavins to form caveolae [9, 10]. The CSD mediates direct protein-protein interactions between caveolin-1 and a variety of signaling molecules carrying the caveolin-binding domain (CBD: $\Phi X\Phi XXXX\Phi$, $\Phi XXXX\Phi XX\Phi$, or $\Phi X\Phi XXXX\Phi XX\Phi$ where Φ represents an aromatic amino acid and X represents any amino acid) [11–13]. The direct interaction with caveolin-1 generally results in the sequestration of a given signaling molecule within caveolar membranes and modulation of its signaling activity. As such, caveolae are major regulators of signal transduction processes. Signaling transduction proteins that are associated with caveolae include the following: (i) kinases such as Src-family kinases, protein kinase A (PKA), protein kinase C (PKC), p38 and p42/44 mitogen-activated protein kinases; (ii) ion channels and ion exchangers, including Ca^{2+} -ATPase, L-type Ca^{2+} , Na^+/K^+ -ATPase, and voltage-gated K^+ ; (iii) receptors, including G protein-coupled receptors, receptor tyrosine kinases (RTKs), steroid hormone receptors, inositol 1,4,5-triphosphate receptor (IP_3R), and transforming growth factor- β receptors; and (iv) nitric oxide synthase (NOS) [14–28]. However, the role of caveolae in eukaryotic cells is not limited to signal transduction mechanisms. In fact, data show that caveolae mediate endocytosis, transcytosis, mechanoprotection, cellular metabolism, and lipid homeostasis [29–35].

Although caveolin-1 and caveolae regulate important and numerous cellular functions, the caveolin-1 null mouse is viable and fertile. However, a lack of caveolin-1 in mice has been linked to a variety of phenotypes such as pulmonary and cardiac defects, vascular abnormalities, atherosclerosis, metabolic disorders, and cancer [33, 36–39]. They are mostly the consequence of a lack of caveola-mediated functions in pulmonary, cardiac, vascular, and adipose tissues. In support of these data, caveolin-1 gene mutations have been linked to a number of pathologies in humans. They include pulmonary arterial hypertension [40], hypertriglyceridemia [41], and cancer [42–44]. Together, these animal and human data provide direct evidence that caveolae play key physiological roles.

3 Cellular senescence

3.1 Definitions and biochemical properties

Most cells cannot divide indefinitely due to a process termed cellular senescence [45–51]. Cellular senescence is a stable form of cell cycle arrest that occurs in somatic cells, with the exception of most tumor cells and certain stem cells. Cellular

senescence was originally described *in vitro* by Hayflick and colleagues when they observed that human diploid fibroblasts permanently stopped dividing after a finite number of cell divisions, becoming irreversibly arrested in the G1 phase of the cell cycle [52]. In contrast to quiescent cells, senescent cells do not re-enter the cell cycle in response to external stimuli, such as growth factors, and they are resistant to apoptotic stimuli [53, 54]. However, senescent cells remain viable and metabolically active despite their non-proliferative state [55].

Cellular senescence can be divided into two categories: replicative senescence (RS) and stress-induced premature senescence (SIPS). Replicative senescence is dependent on the number of divisions the cell has completed. This type of senescence is spontaneously achieved by somatic cells. It is now known that the cell division counting is controlled by telomere shortening, an unavoidable consequence of genome duplication [50, 56–59]. Senescence can be accelerated by a number of stressful stimuli such as oncogene activation, DNA damage, cytotoxic drugs, and oxidative stress [60–64]. This type of senescence is referred to as stress-induced premature senescence. SIPS is independent of telomere status but shares many molecular and functional features with replicative senescence.

Although senescent cells do not express one specific biomarker, a number of features and molecular markers, which represent hallmarks of senescence, can be used to identify senescent cells. Senescent cells can be experimentally identified by their enlarged and flattened morphology, as well as positive staining for β -galactosidase activity at pH 6 [46]. Their inability to proliferate in a pro-mitogenic environment is associated with increased expression of cell cycle inhibitors, including $p16^{INK4a}$, $p21^{WAF1/CIP1}$, and hypo-phosphorylated retinoblastoma gene protein (pRb) [45–49]. Senescent cells can also display elevated expression of $p19^{ARF}$, plasminogen activator inhibitor-1 (PAI-1), and p53 as well as a chronic DNA damage response with DNA damage foci [65] and senescence-associated heterochromatin foci [66] formation. Moreover, loss of lamin B1 [67], accumulation of lipofuscin [68], expression of decoy death receptor 2 (DCR2), and embryonic chondrocyte-expressed 1 (DEC1) [69] have been associated with cellular senescence. Importantly, a key feature of senescent cells is their ability to secrete a variety of factors, including growth factors, mediators of inflammation, and proteases, known as the senescence-associated secretory phenotype (SASP), which can alter the tissue microenvironment [5, 70] (Table 1).

3.2 The tumor suppressor role of cellular senescence

Cellular senescence is a basic cellular mechanism developed by organisms to prevent the propagation of cells with damaged DNA and potentially carrying oncogenic mutations. Therefore, cellular senescence is considered a powerful tumor

Table 1 Different forms of cellular senescence

RS	Replicative senescence	Senescence induced by telomere shortening
SIPS	Stress-induced premature senescence	Senescence induced by cellular stress
OIS	Oncogene-induced senescence	Senescence induced by oncogene expression
LTSIS	Loss of tumor suppressor gene-induced senescence	Senescence induced by loss of tumor suppressor gene expression
SASP	Senescent-associated secretory phenotype	Cytokines, chemokines, growth factors, and metalloproteinases secreted by senescent cells

suppressor mechanism. The anti-tumorigenic role of cellular senescence is evident from data showing how either (i) oncogene expression or (ii) loss of tumor suppressor genes triggers the development of a senescent phenotype.

i) Oncogene-induced senescence (OIS) was originally described in human fibroblasts in which over-expression of oncogenic H-Ras (H-Ras^{G12V}) induced a permanent cell cycle arrest [63]. K-Ras is a GTPase that plays an important role in normal tissue signaling. Wild-type K-Ras is activated by signals that promote the exchange of bound GDP to GTP. This is a transient activation due to the intrinsic ability of K-Ras to hydrolyze GTP and therefore turn itself off. However, single point mutations generate an oncogenic mutant form of K-Ras with a constitutively activated GTP-bound state. Oncogenic K-Ras can also promote oncogene-induced senescence through the elevation of reactive oxygen species [63, 71, 72]. The *in vivo* tumor suppressive role of OIS is supported by evidence showing that oncogenic K-Ras induces senescence in pre-malignant lung lesions in mice and that senescent cells are absent in malignant lung adenocarcinomas [63, 69, 73–75]. Tumor cell senescence is not restricted to mouse models, but has been reported in human premalignant lesions as well [69, 76–81]. Thus, oncogenic Ras over-expression promotes transformation and progression to higher grades of malignancy only when oncogenic Ras-expressing cells evade/bypass senescence checkpoints through either the expression of additional oncogenes or the additional loss of tumor suppressor genes. For example, co-expression of oncogenes such as c-Myc, E1A, or dead ringer-like-1 (DRIL1) bypasses K-Ras^{G12V}-induced cellular senescence [82], and oncogenic K-Ras-induced senescence is evaded by the inactivation of the tumor suppressors p19^{ARF}, p53, p16^{INK4a}, pRb, and pro-myelocytic leukemia (Pml) protein [63, 83]. Ras is not the only oncogene that induces OIS. For example, constitutively active forms of BRAF can also promote OIS in cells and induce the formation of pre-malignant skin lesions, i.e., melanocytic nevi, *in vivo*, which carry senescent cells [84]. Over-expression of oncogenes such as epidermal growth factor receptor

(EGF-R), phosphoinositide 3-kinase (PI3K), and human epidermal growth factor receptor 2 (HER2) can also induce senescence [85, 86], strengthening the concept that when the cell is hit by a sustained mitogenic stress, due to oncogene over-expression, it undergoes senescence to limit tumor growth.

ii) In addition to oncogene activation, cellular senescence can also be induced by the loss of tumor suppressor genes (i.e., loss of tumor suppressor gene-induced senescence [LTSIS]). A well-established example is loss of the tumor suppressor phosphatase and tensin homolog (PTEN). It leads to cellular senescence associated with increased expression of p16^{INK4A} as well as p53, which occurs in a mammalian target of rapamycin (mTOR)-dependent mechanism and through the inhibition of the oncogene mouse double minute 2 homolog (Mdm2) [77]. Loss of PTEN in a mouse model of prostate cancer promotes the formation of prostate intraepithelial neoplasia, pre-malignant prostate tumor lesions carrying senescent cells. Interestingly, senescence induced by loss of PTEN requires p53 function as these benign and senescent-positive lesions progress to senescence-negative invasive prostate cancer upon p53 inactivation [77, 87]. Finally, data show that inactivating mutations of neurofibromin 1 (NF1) [88], Rb [89], and von Hippel-Lindau [90] are associated with the development of cellular senescence and benign tumor lesions.

3.3 The tumor suppressor and tumor promoter roles of the SASP

The SASP acts as a double-edged sword in cancer. On the one hand, SASP factors, including interleukins such as IL-1 α [91], IL-6 [92], and IL-8 [93], as well as vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF- β), chemokine (C-C motif), ligand 2 (CCL2) and ligand 20 (CCL20) [94], can induce senescence in neighboring cells, including cancer cells, in a paracrine fashion, therefore inhibiting tumor growth. Interestingly, the release of IL-6 can also act in an autocrine manner to promote oncogene-induced senescence [92, 95] and the depletion of IL-6 causes

cells to bypass OIS [92]. Finally, chemokines and cytokines, which are secreted by senescent cells, inhibit tumor development by activating immune surveillance and promoting clearance of pre-neoplastic and cancer cells [96].

On the other hand, the release of pro-stimulatory SASP factors by senescent cells within the tumor microenvironment can promote tumor growth [97–100]. In fact, data show that senescent cells, which accumulate during the aging process [46, 101–104], can release factors that stimulate the proliferation of pre-malignant and malignant epithelial cells. The release of pro-tumorigenic SASP factors also occurs when chemotherapeutic drugs are used as cancer treatment. Studies in animals and humans have shown that although chemotherapy is initially advantageous in virtue of its ability to either kill cancer cells by apoptosis or induce permanent cell cycle arrest of cancer cells by inducing cancer cell senescence [105, 106], it can subsequently fuel cancer development given the capacity of chemotherapeutic drugs to induce senescence of immune, stromal, and cancer cells. Senescence of immune cells blunts the anti-tumorigenic properties of the immune system while senescent stromal and cancer cells can release SASP factors that stimulate the proliferation of non-senescent cancer cells that survived chemotherapy [107]. In addition, the SASP can attract immune-suppressive myeloid-derived suppressor cells (MDSCs) into the tumor [107].

4 Regulation of cellular senescence by caveolin-1

Mounting evidence over the last 10–15 years has shown that caveolin-1-mediated signaling is causally linked to the development of a senescent phenotype and regulates senescence-dependent physiological and pathological outcomes. In the section below, we describe the molecular mechanisms and signaling pathways through which caveolin-1 regulates cellular senescence with specific emphasis on the functional role of caveolin-1-mediated senescence in cancer.

4.1 Caveolin-1 and replicative senescence

Caveolin-1 expression and the number of caveolae are upregulated in replicative senescent human diploid fibroblasts, where the unresponsiveness of senescent cells to epidermal growth factor (EGF) stimulation is linked to the inhibitory role of caveolin-1 on EGF-R signaling [108, 109]. Interestingly, downregulation of caveolin-1 expression in senescent human diploid fibroblasts by siRNA and antisense oligonucleotides restores extracellular signal-regulated kinase (Erk) activation, DNA synthesis, and cell cycle progression in response to EGF stimulation, with the concomitant decrease of p53 and p21 expression [110]. Moreover, downregulation of caveolin-1 in senescent human diploid fibroblasts results in morphological

changes to a non-senescent-like shape possibly through altered focal adhesion and actin stress fiber formation due to focal adhesion kinase (FAK) and Rho family GTPase regulation [111]. It remains to be determined how downregulation of caveolin-1 can overcome/bypass the pro-senescent signaling initiated by telomere shortening. Consistent with these findings, caveolin-1 expression is upregulated in replicative senescent bone marrow mesenchymal ST2 cells [112] and overexpression of caveolin-1 in ST2 cells elevates the expression of the senescence markers p53 and p21 [112]. Similarly, caveolin-1 expression is upregulated in senescent human mesenchymal stem cells (hMSCs) and the over-expression of caveolin-1 in young hMSCs suppresses their adipogenic differentiation potential [113]. Finally, caveolin-1 expression is increased in replicative senescent bone marrow stromal cells [114], old macrophages [115], and senescent retinal pigment epithelium [116] and the upregulation of caveolin-1 positively correlates with shortening of chondrocyte replicative lifespan after treatment with interleukin (IL)-1 β [117]. The activity of phosphatidylcholine-specific phospholipase C (PC-PLC) is dramatically increased during replicative senescence of bone marrow stromal cells (BMSCs) [114]. Inhibition of PC-PLC activity by the specific inhibitor D609 reduces the upregulation of caveolin-1 expression and the number of replicative senescent BMSCs, suggesting that PC-PLC mediates senescence of BMSCs possibly with a mechanism involving caveolin-1-dependent signaling [114].

Senescent cells accumulate in mammalian tissues with age. Animal studies show that reduction of senescent cells in mice inhibits the development of age-associated phenotypes. Clearance of p16-expressing cells in the spindle assembly checkpoint protein BubR1 progeroid mouse background delays the development of aging-associated disorders [118]. Clearance of naturally occurring p16-positive cells in mice of two distinct genetic backgrounds attenuates age-related deterioration of several organs and extends lifespan [119]. Similarly, clearance of p16-positive cells in mice mitigates age-associated intervertebral disc degeneration [120]. Consistent with a pro-senescent role of caveolin-1, its expression is upregulated in the lung, spleen, and brain of old rats [108, 121] and in skeletal muscles of old mice [122]. Upregulation of caveolin-1 in skin fibroblasts in both chronological and UV-induced aging is linked to age-related alterations of the skin, including reduction of the dermal thickness, altered levels of hyaluronan, increase dermal white adipose tissue, reduced collagen expression, and increased inflammation [123, 124]. Treatment with methyl- β -cyclodextrin (M β CD) reduces caveolin-1 expression and upregulates collagen activity with increased skin thickness [124]. Caveolin-1 is upregulated in the old rat hippocampus and cerebral cortex, and data show a role of caveolin-1 in beta amyloid precursor protein (APP) processing by beta-secretase [121]. In *Caenorhabditis elegans*, the insulin/insulin-like growth

factor-1 (IGF-1) signaling upregulates caveolin-1 expression and insulin/IGF-1 signaling reduction downregulates caveolin-1 expression and neuronal caveolae [125]. Reduced caveolin-1 expression extends lifespan of *Caenorhabditis elegans* [125]. In humans, caveolin-1 expression is elevated with age in both the smooth muscle and epithelium of the prostate [126] and in the elderly cerebral cortex [121]. Moreover, increased caveolin-1 protein level in the hippocampus and caveolin-1 mRNA in the frontal cortex were described in patients with Alzheimer's disease [127]. Cavin-1 (PTRF) is a component of caveolae. Directly supporting a key role of caveolae in the signaling leading to cellular senescence, data show that PTRF expression is upregulated in replicative senescent human fibroblasts, over-expression of PTRF elevates the number of caveolae and promotes premature senescence, and downregulation of PTRF extends replicative lifespan [11].

4.2 Caveolin-1 and stress-induced premature senescence

Data linking caveolin-1 to the development of stress-induced premature senescence began to emerge in the early 2000s. Numerous independent reports, using both cell culture and animal models, provide compelling evidence causally linking caveolin-1-mediated signaling to the acquisition of a senescent phenotype when cells are hit with a stressful stimulus (Fig. 1). Our laboratory was the first to demonstrate that either subcytotoxic levels of hydrogen peroxide or UV-C light upregulate endogenous caveolin-1 expression and induce premature senescence in fibroblasts [128]. Oxidative stress induces premature senescence by stimulating caveolin-1 gene transcription through p38 mitogen-activated protein kinase/Sp1-mediated activation of two GC-rich promoter elements [129]. Treatment with the anti-oxidants quercetin and vitamin E prevents upregulation of caveolin-1 following hydrogen peroxide stimulation and the development of a premature senescent phenotype [128]. Importantly, oxidative stress-induced premature senescence is inhibited in NIH 3T3 cells harboring antisense caveolin-1 and in mouse embryonic fibroblasts (MEFs) derived from caveolin-1 null mice, which lack caveolin-1 expression [128, 130]. In support of these findings, studies show that over-expression of caveolin-1 is sufficient to block mouse embryonic fibroblasts (MEFs) in the G₀/G₁ phase of the cell cycle through modulation of the p53/p21^{waf1/Cip1} pathway [131]. MEFs transgenically over-expressing caveolin-1 have a shorter proliferative lifespan, as compared with MEFs derived from control littermate embryos, display a large, flat morphology, and express high levels of senescence-associated β -galactosidase activity [128]. Moreover, oxidative stress, following treatment with tert-butyl hydroperoxide, upregulates caveolin-1 mRNA and protein expression in nucleus pulposus cells while caveolin-1

downregulation by shRNA in these cells inhibits SIPS [132]. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a major source of oxidative stress. Data show that oxidized low-density lipoprotein (oxLDL) upregulates caveolin-1 expression and promotes translocation/activation of the NADPH oxidase subunit p47phox into caveolae in macrophages, resulting in the development of a ROS-dependent senescent phenotype [133]. Studies show that endothelial cells isolated from chronic smokers with premature atherosclerosis display senescent features, increased oxidative stress, and elevated caveolin-1 expression, as compared with endothelial cells isolated from non-smokers [134]. Consistent with these data, cellular senescence in endothelial cells isolated from patients with severe coronary artery disease is accelerated by oxidative stress associated with risk factors for cardiovascular diseases [135]. In these cells, caveolin-1 expression is elevated [135]. Induction of senescence in endothelial cells by either hydrogen peroxide or ARHGAP18/SENEX is associated with upregulation of caveolin-1 expression and an increased number of caveolae. The ability of ARHGAP18/SENEX to induce senescence in endothelial cells isolated from caveolin-1 null mice is significantly compromised [136].

Pro-inflammatory genes such as cyclooxygenase-2 (COX-2) have been proposed to play an important role in the aging process. Data show that NS-398, a selective COX-2 inhibitor, prevents the upregulation of caveolin-1 expression and the development of cellular senescence in human dermal fibroblasts [137]. Since a selective COX-2 inhibitor has been shown to prevent the Sp1 proteins from binding to SP1 sites [138], inhibition of COX-2 may inhibit the caveolin-1-mediated development of premature senescence following oxidative stress by limiting the Sp1-mediated activation of the caveolin-1 promoter. Endothelial-specific protein tyrosine phosphatase-1B (PTP1B) null mice display increased neointima formation. Genetic knockdown or pharmacological inhibition of PTP1B in endothelial cells induces oxidative stress, upregulation of caveolin-1, and cellular senescence, which can be inhibited by downregulation of caveolin-1 expression [139]. Together, these data support the idea that caveolin-1 may link oxidative stress-induced premature senescence to cardiovascular diseases.

Bleomycin and glucose are additional stimuli that can induce senescence. Bleomycin induces DNA strand breaks and is used to treat certain types of cancer [140]. Bleomycin upregulates caveolin-1 expression and downregulation of caveolin-1 by shRNA inhibits bleomycin-induced senescence in A549 human lung adenocarcinoma epithelial cells [141]. Epithelial cell senescence and pulmonary fibrosis induced by the intratracheal instillation of bleomycin are reduced in caveolin-1 null mice [142]. Treatment with high concentrations of glucose induces senescence in glomerular mesangial cells, which is abrogated by caveolin-1 siRNA [143]. Increased oxidative stress, upregulation of caveolin-1

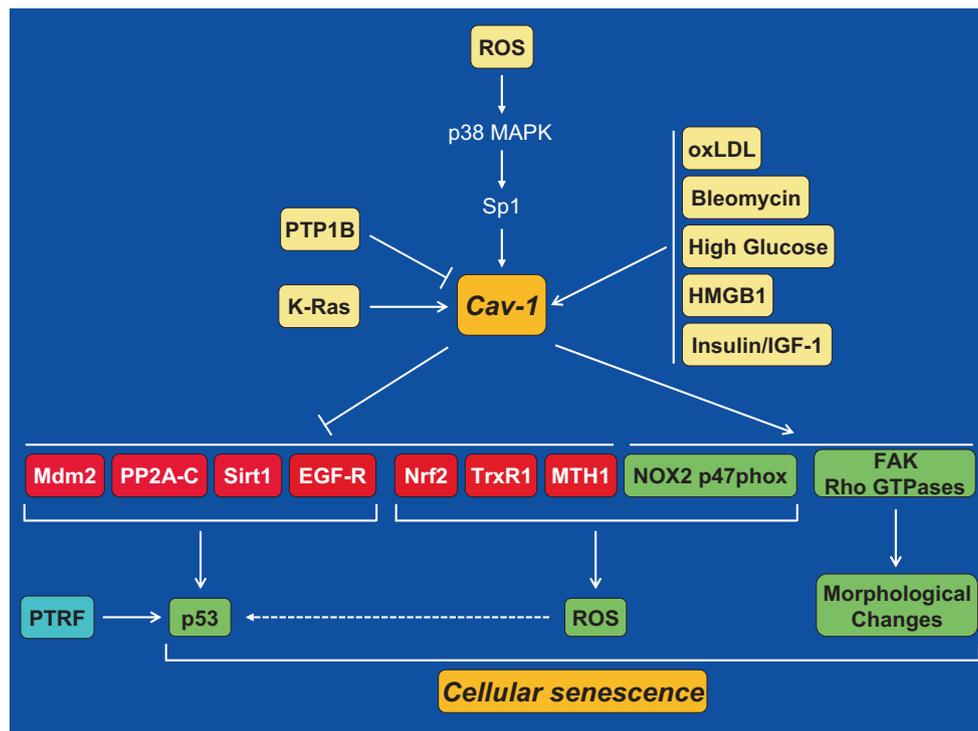


Fig. 1 Molecular mechanisms underlying caveolin-1-mediated cellular senescence. Oxidative stress activates the caveolin-1 gene promoter in a p38 MAPK-Sp1-dependent mechanism and upregulates caveolin-1 expression. Oncogenic K-Ras, oxLDL, bleomycin, high levels of glucose, HMGB1, the insulin/IGF-1 pathway, and downregulation of PTP1B induce senescence through caveolin-1-mediated signaling. Upregulation of caveolin-1 promotes cellular senescence by activating the p53/p21^{Waf1/Cip1} pathway. Activation of p53 occurs through multiple mechanisms: (1) sequestration of Mdm2 into caveolar membranes, away from p53, prevents the Mdm2-dependent degradation of p53, which stabilizes p53

expression; (2) sequestration of PP2A-C into caveolar membranes activates ATM, which in turn phosphorylates and activates p53; (3) the interaction of caveolin-1 with Sirt1 inhibits Sirt1 activity leading to increased acetylation/activation of p53; (4) inhibition of EGF-R by caveolin-1 has been linked to activation of p53; (5) inhibition of Nrf2, TrxR1, and MTH1, through direct interaction with caveolin-1, and activation of NOX2 p47phox activate p53 through ROS production. PTRF can also promote senescence through activation of p53. Finally, activation of FAK and Rho GTPases by caveolin-1 promotes morphological changes that are typical of senescent cells

expression, and induction of cellular senescence occur in fibroblasts derived from patients with type 2 diabetes [18]. Oxidative stress and cellular senescence are observed in diabetic wounds in mice, and downregulation of caveolin-1 inhibits diabetes/oxidative stress-induced senescence and accelerates tissue repair [144].

Finally, a causal link between caveola-mediated signaling and the development of SIPS is further strengthened by data showing that oxidative stress upregulates PTRF protein expression, promotes the interaction of PTRF with caveolin-1, and induces the formation of caveolae [145]. Downregulation of PTRF expression by shRNA inhibits SIPS [145].

4.3 Caveolin-1 promotes stress-induced senescence through activation of the p53-p21 signaling pathway

Activation of p53 can be a major contributor to the development of a senescent phenotype after cellular stress. Data demonstrate that activation of the p53-p21 pathway is a key signaling through which caveolin-1 promotes senescence. After our initial report that over-expression of caveolin-1 is

sufficient to induce premature senescence and activate the p53-p21 pathway [128, 131], we and others have shown that downregulation or a lack of caveolin-1 expression inhibits oxidative stress-induced activation of the p53-p21 signaling pathway and SIPS [130, 132, 141, 146, 147]. A number of studies have discovered various molecular mechanisms through which caveolin-1 activates p53 in the context of SIPS. More specifically, data show that caveolin-1 activates the p53/p21 pathway and induces premature senescence in virtue of its ability to directly interact with Mdm2, PP2A-C (the catalytic subunit of protein phosphatase PP2A), sirtuin 1 (Sirt1), nuclear erythroid 2 p45-related factor-2 (Nrf2), thioredoxin reductase 1 (TrxR1), and MutT homolog 1 (MTH1), and to sequester them into caveolar membranes after oxidative stress (Fig. 1).

i) Mdm2 is a negative regulator of p53 that promotes p53 degradation. Sequestration of Mdm2 into caveolae results in inhibition of Mdm2 activity, leading to stabilization of p53 expression and induction of premature senescence [130]. In support of these findings,

upregulation of caveolin-1 and PTRF expression in fibroblasts from patients with type 2 diabetes results in sequestration of Mdm2 away from p53 and activation of the p53-p21 pathway [144]. Moreover, replicative senescent bone marrow mesenchymal ST2 cells display a strong association between caveolin-1 and Mdm2 and over-expression of caveolin-1 in these cells upregulates p53 and p21 [112]. Consistent with these data, PTRF expression is necessary for the oxidant-induced sequestration of Mdm2 into caveolar membranes, away from p53, and activation of the p53-p21-senescence pathway [145]. Expression of a mutant form of PTRF, which fails to localize to caveolar membranes after oxidative stress, inhibits oxidative stress-induced activation of p53 [145].

- ii) Ataxia telangiectasia-mutated (ATM) is a positive regulator of p53. PP2A is a negative regulator of ATM autophosphorylation and activity *in vivo* [148]. Caveolin-1-mediated localization of PP2A-C into caveolae, after oxidative stress, inhibits PP2A and therefore promotes the ATM-dependent phosphorylation/activation of p53 and induction of cellular senescence [146].
- iii) Sirt1 is a class III histone deacetylase that regulates a variety of cellular processes, including cellular senescence. Deacetylation of p53 by Sirt1 inactivates p53. Oxidative stress promotes the interaction of Sirt1 with caveolin-1, which leads to inactivation of Sirt1 activity and the consequent acetylation/activation of p53 and induction of premature senescence [149]. Data show that phosphorylation of caveolin-1 on tyrosine 14 is a post-translational modification, induced by oxidative stress in a p38 MAPK-dependent manner, which promotes the oxidant-induced localization of Sirt1 in caveolae resulting in the activation of the p53/senescence pathway in fibroblasts [149]. Consistent with these data, expression of a phosphomimetic mutant form of caveolin-1 (Caveolin-1 Y14D) in MDA-MB-435 cancer cells activates p53, inhibits cellular proliferation, and inhibits tumor growth in xenograft studies *in vivo* [150]. One can speculate that the phospho-caveolin-1-dependent sequestration of Sirt1 in caveolae might contribute to the activation of p53 and the development of the phenotypes observed in these cancer cells. Finally, downregulation of caveolin-1 in rats fed high-fat diet supplemented with soy protein isolate results in Sirt1 activation and deacetylation of p53 [151].
- iv) Nrf2 is a transcription factor that mediates cytoprotective responses against stress. Under resting conditions, Nrf2 localizes with caveolin-1 in caveolar membranes [152]. After oxidative stress, caveolin-1 limits the translocation of Nrf2 to the nucleus and therefore inhibits the activation of an anti-oxidant

response [152]. As a result, the p53-p21 pathway is activated and cells undergo cellular senescence [152]. A mutant form of Nrf2 that fails to interact with caveolin-1 hyperactivates Nrf2 target genes and inhibits both oxidant-induced activation of the p53-p21 pathway and induction of SIPS [152]. In epithelial Beas-2B cells, caveolin-1 inhibits Nrf2-mediated transcription by promoting the interaction of Nrf2 with its inhibitor Kelch-like ECH-associated protein 1 (Keap1) [153]. Downregulation of caveolin-1 in vascular endothelial cells decreases Keap1 protein levels and increases Nrf2 activity [154]. In breast cancer cells, caveolin-1 loss correlates with activation of Nrf2 and rescue of caveolin-1 expression in these cells inhibits Nrf2-mediated transcription [155].

- v) TrxR1 is an essential anti-oxidant enzyme that controls cellular redox homeostasis [156]. It reduces thioredoxin by using nicotinamide adenine dinucleotide phosphate [157]. TrxR1 is a caveolar membrane-resident protein [158] and caveolin-1 is an endogenous inhibitor of TrxR1 [158]. Data show that oxidative stress fails to activate the p53/p21^{Waf1/Cip1} pathway and induce premature senescence in cells expressing a mutant form of TrxR1 that cannot bind to caveolin-1 and is constitutively active [158].
- vi) MTH1 is the major mammalian detoxifier of the oxidized DNA precursor 8-oxo-dGTP. MTH1 prevents the incorporation of 8-oxoguanine into the DNA, by removing it from the dNTP pool, and therefore inhibits the initiation of a DNA damage/senescence response [159–164]. Interestingly, oncogenic K-Ras promotes the interaction between caveolin-1 and MTH1, which results in MTH1 inhibition, activation of the p53/p21 pathway, and induction of cellular senescence [165].

It is worth mentioning that a few studies have also linked caveolin-1-mediated regulation of epidermal growth factor receptor (EGF-R), NOX2 p47phox, focal adhesion kinase (FAK), and small Rho GTPase pathways to the development of replicative cellular senescence (Fig. 1), as discussed elsewhere in this review.

4.4 Caveolin-1-induced senescence and age-related diseases

Pulmonary emphysema is an age-related lung disease mostly caused by chronic exposure to cigarette smoking. Cigarette smoke is enriched in oxidants and oxidative stress is believed to play a major role in the pathogenesis of emphysema [166]. Cell culture studies show that cigarette smoke induces senescence of lung fibroblasts, which can be inhibited by anti-oxidant treatments [146, 167]. Interestingly, lung fibroblasts obtained from patients with emphysema are positive for the

senescence marker senescence-associated β -galactosidase activity and display a reduced proliferation capacity [168, 169]. Data show that caveolin-1 plays a central role linking cigarette smoke-induced oxidative stress to the development of cellular senescence. More precisely, cigarette smoke treatment upregulates endogenous caveolin-1 expression in lung fibroblasts in cell culture studies and in mice [146]. In addition, development of cellular senescence is inhibited in caveolin-1-lacking lung fibroblasts after exposure to cigarette smoke extracts in cell culture studies or when caveolin-1 null mice are chronically exposed to cigarette smoke [146]. A very intriguing finding is that the development of pulmonary emphysema is inhibited in caveolin-1 null mice after 6 months of exposure to cigarette smoking [146], possibly linking oxidative stress-induced and caveolin-1-mediated cellular senescence to the pathogenesis of pulmonary emphysema. Data suggest that cigarette smoke-initiated and caveolin-1-mediated signaling leads to senescence by enforcing an ATM-dependent DNA damage response. In fact, cigarette smoke exposure, a well-known cause of DNA damage, activates ATM and upregulates both p53 and p21 protein expression in wild-type lung fibroblasts but to a much lesser extent in caveolin-1-lacking lung fibroblasts [146]. Mechanistically, caveolin-1 activates ATM by sequestering PP2A-C into caveolar membranes [146]. EGF-R is known to promote the resolution of γ H2AX damage foci [170]. Since caveolin-1 can act as an inhibitor of EGF-R [108], an additional mechanism through which caveolin-1 promotes a DNA damage response is by blocking EGF-R signaling, which would support the chronic DNA damage response that is seen in senescent cells. Interestingly, caveolin-1 might also enhance DNA damage itself by elevating the levels of free radicals through inhibition of anti-oxidants such as TrxR1 [158] and Nrf2 [152].

Age-related intervertebral disc (IVD) degeneration is a cause of chronic low back pain [171]. Cellular senescence contributes to IVD degeneration [120, 172–174]. Human cells from the nucleus pulposus (NP) of degenerate discs show evidence of cellular senescence and express high levels of caveolin-1 [173, 175], suggesting that caveolin-1-mediated cellular senescence may be involved in the pathogenesis of IVD degeneration. Treatment of articular chondrocytes with IL-1 β and hydrogen peroxide upregulates caveolin-1 mRNA and protein expression and induces cellular senescence [147]. Downregulation of caveolin-1 expression with antisense oligonucleotides prevents the development of chondrocyte senescence induced by these stimuli [147]. Thus, caveolin-1 may play a role in the pathogenesis of osteoarthritis by mediating chondrocyte senescence.

4.5 Downregulation of caveolin-1 can induce premature senescence

Although abundant data causally link increased caveolin-1 expression and caveolin-1-mediated signaling to replicative

senescence and SIPS, reports exist showing that downregulation of caveolin-1 in unstimulated cells can also induce a senescent phenotype. For example, either knockout or knockdown of caveolin-1 induces senescence in resting human diploid fibroblasts, HCT116, A549, and MEFs, which correlates with mitochondrial dysfunction [176]. Consistent with these data, caveolin-1 maintains mitochondrial integrity and function by promoting the mitochondrial localization of matrix-oriented AAA (*m*-AAA) protease and its quality control functions [177]. Moreover, downregulation of caveolin-1 promotes cellular senescence in resting human diploid fibroblasts through formation of the primary cilium following the proteasomal-dependent degradation of aurora kinase A [178]. These results are consistent with data showing accumulation of senescence markers in human breast cancer-associated fibroblasts (CAFs) in which caveolin-1 expression is significantly downregulated [179, 180]. Thus, caveolin-1 may represent a pleiotropic regulator of cellular senescence: elevated caveolin-1-mediated signaling contributes to the development of replicative senescence and premature senescence following cellular stress while caveolin-1 deficiency promotes premature senescence in resting cells by promoting primary cilium formation and mitochondrial dysfunctions. This pleiotropic function is not unique to caveolin-1, as other signaling molecules, such as members of the DNA damage signaling pathway [76, 181] and E2F transcription factors [182, 183], have been shown to either promote or inhibit senescence depending on their expression levels.

5 Caveolin-1-mediated senescence as an anti-tumorigenic mechanism

Senescent cells are characterized by an irreversible exit from the cell cycle. As such, cellular senescence is a powerful tumor suppressor mechanism. Given the key role that caveolin-1 plays in the development of a senescent phenotype, studies have addressed caveolin-1-mediated senescence as a potential anti-tumorigenic mechanism. Over-expression of caveolin-1 in A549 human lung carcinoma epithelial cells, in which caveolin-1 expression is reduced but not lost [184], enhances SIPS [158]. Consistent with these data, over-expression of caveolin-1 in hydrogen peroxide-treated A549 cells inhibits tumor formation in nude mice [158]. Upon cell transformation, TrxR1 supports tumor cell growth, as shown by elevated TrxR protein levels and activity in many cancer cells [185]. Caveolin-1 is an endogenous inhibitor of TrxR1 [158]. Interestingly, over-expression of caveolin-1 inhibits the ability of TrxR1 to increase the transformed phenotype of A549 cells. Moreover, A549 cells maintained their ability to grow in soft agar when caveolin-1 expression was transiently downregulated by siRNA at the time of oxidative stress, in contrast to control siRNA-transfected A549 cells, which formed only a

few small foci in soft agar assays [158]. Over-expression of caveolin-1 induces premature senescence in A549 and H460 lung cancer cells and inhibits their growth in soft agar even in the absence of oxidative stress [165]. Thus, inhibition of TrxR1 by caveolin-1 induces premature senescence and acts in a tumor-suppressor manner.

Nrf2 signaling has been linked to tumor growth in HCT116 colon cancer cells, which are known to express caveolin-1. Downregulation of caveolin-1 expression by siRNA in HCT116 cells activates anti-oxidant-responsive elements (AREs) while the over-expression of caveolin-1 inhibits the oxidant-induced upregulation of heme oxygenase 1 (HO1) [152], an Nrf2 target [186]. Thus, caveolin-1 acts as an inhibitor of Nrf2 signaling in colon cancer cells. Importantly, the stable over-expression of caveolin-1 in HCT116 cells potentiates oxidative stress-induced premature senescence [152]. Moreover, over-expression of caveolin-1 inhibits the transformed phenotype of HCT116 cells, as assessed by growth in soft agar, after oxidative stress [152]. These data support findings showing that Nrf2 signaling may be beneficial to cancer cells and suggest that caveolin-1-mediated suppression of Nrf2 inhibits cell transformation by promoting premature senescence. Similarly, over-expression of caveolin-1 induces premature senescence in MCF-7 breast cancer cells following oxidative stress [129]. Thus, caveolin-1-mediated signaling promotes the tumor suppressor functions of premature senescence in lung, breast, and colon cancer cell lines.

Induction of oncogene-induced senescence is a well-established function of certain oncogenes, including K-Ras [63, 71, 72]. Cellular senescence has been reported in human premalignant lesions but not in the tumor itself [69, 76, 77, 81, 187], suggesting that oncogene-transformed cells need to either evade or bypass the OIS barrier in order to proliferate and progress to higher grades of malignancy [63, 69, 73, 74]. Data show a direct and causal role of caveolin-1 in the development of OIS. Over-expression of K-Ras^{G12V} induces senescence in wild-type MEFs [165]. In contrast, K-Ras^{G12V}-induced senescence is inhibited in MEFs lacking caveolin-1 expression [165]. In support of these data, oncogenic K-Ras induces senescence in normal human bronchial epithelial cells; downregulation of caveolin-1 in these cells inhibits K-Ras^{G12V}-induced senescence [165]. Thus, K-Ras^{G12V} induces cellular senescence in a caveolin-1-dependent manner. Oncogenic K-Ras promotes OIS through oxidative DNA damage [63, 71, 72]. Data show that caveolin-1 promotes K-Ras-induced premature senescence by inhibiting the detoxification function of MTH1. The scaffold kinase suppressor of Ras 1 (KSR1) regulates the activation of the Raf/MEK/ERK pathway. KSR1 interacts with caveolin-1 and promotes the localization of MEK and ERK in caveolin-1-rich domains, which is required for optimal ERK activation [188]. When the interaction between

caveolin-1 and KSR1 is prevented, H-Ras^{G12V}-mediated senescence is inhibited, suggesting that caveolin-1 can also promote OIS by facilitating the assembly at the plasma membrane of key signaling molecules that transduce Ras signaling, at least when the pathway is activated by H-Ras [188].

K-Ras^{LA2-G12D} mice are heterozygous for the K-Ras G12D mutation and develop lung tumors with a histopathology very similar to human disease [189]. The role of caveolin-1 in OIS is supported by *in vivo* data obtained using the K-Ras^{LA2-G12D}/Cav-1^{-/-} mouse, which lacks caveolin-1 expression [165]. The number of cells positive for SA- β -gal activity and the expression of p21 and p16 are reduced in the lung of K-Ras^{LA2-G12D}/Cav-1^{-/-} mice, as compared with control K-Ras^{LA2-G12D}/Cav-1^{+/+} mice [165]. Consistent with the tumor suppressor role of OIS, the number of microscopic malignant lesions in the lung of K-Ras^{LA2-G12D}/Cav-1^{-/-} mice is increased by 3-fold, as compared with that of K-Ras^{LA2-G12D}/Cav-1^{+/+} mice. Moreover, the total number of surface lung tumors is 2.5-fold higher in K-Ras^{LA2-G12D}/Cav-1^{-/-} than in K-Ras^{LA2-G12D}/Cav-1^{+/+} mice. Overall survival and mean age of death of K-Ras^{LA2-G12D}/Cav-1^{-/-} mice are lower than those of K-Ras^{LA2-G12D}/Cav-1^{+/+} mice [165]. Thus, caveolin-1 promotes the tumor suppressive function of cellular senescence during lung cancer development in mice.

As mentioned above, K-Ras^{G12D} mice (wild type for caveolin-1) show abundant lung cell senescence and only marginal lung tumor formation. However, they do develop lung tumors as they age, albeit to a lesser extent than their caveolin-1-lacking counterparts [165]. Interestingly, endogenous caveolin-1 expression is downregulated during aging in K-Ras^{G12D} mice. Similarly, caveolin-1 expression is downregulated in K-Ras^{G12V}-infected MEFs that escape OIS [165]. Thus, a selective pressure appears to exist in cells expressing oncogenic K-Ras, which allows them to escape senescence, possibly through the downregulation of caveolin-1, and promote tumor development. These cell culture and animal data are supported by human studies using lung adenocarcinoma tissue microarrays (TMAs) showing that 86% of human adenocarcinomas have caveolin-1-negative cancer epithelial cells and that 91% of adenocarcinoma cases carrying mutant K-Ras are caveolin-1-negative [165]. Moreover, a significant correlation exists between increased methylation of the caveolin-1 gene and reduced caveolin-1 expression in human lung adenocarcinomas [165]. Finally, gene expression analysis using RNA derived from lung adenocarcinoma patients shows that the rate of death was 60% lower for patients with high caveolin-1 expression [165]. These human data strengthen the notion that caveolin-1 has tumor suppressive properties in cancer. Consistent with a tumor suppressor role of caveolin-1, its expression is downregulated in other human cancers, such as breast [43, 190], colon [191, 192], ovarian [193], glioblastoma [194], and sarcoma [195] cancer. Moreover, a lack of caveolin-1

increases tumorigenesis in certain mouse models of cancer, such as breast [196] and skin cancer [36].

6 Caveolin-1-mediated senescence as a pro-tumorigenic mechanism

Stromal cells, including fibroblasts, are known to have a profound effect on tumor development. As described in Section 3.3, senescent stromal cells, through the secretion of SASP factors, can stimulate the growth of pre-neoplastic and neoplastic cells. Since caveolin-1 promotes both replicative and stress-induced senescence in fibroblasts, studies have been performed to determine whether caveolin-1-mediated senescence can also represent a pro-tumorigenic mechanism. Wild-type MEFs, which are induced to senesce by oxidative stress, but not caveolin-1 null MEFs, enhance the growth of both H-Ras^{G12V}-transformed NIH 3T3 and MDA-MB-231 breast cancer cells in co-culture experiments [130]. The caveolin-1-mediated growth-promoting action of senescent fibroblasts is due to their ability to release pro-stimulatory factors since conditioned medium derived from wild-type senescent MEFs, but not caveolin-1 null MEFs, is sufficient to stimulate the growth of Ras^{G12V}-transformed NIH 3T3, MDA-MB-231 [130], and PC3 prostate cancer cells [149]. This effect occurs *in vivo* as well, as shown by the enhanced tumor growth when either H-Ras^{G12V}-transformed NIH 3T3 or MDA-MB-231 cells are co-injected with senescent wild-type MEFs, but not caveolin-1 null MEFs, in immunodeficient mice, as compared with injection of cancer cells alone [130].

Data show that IL-6 is a caveolin-1-specific pro-tumorigenic factor. In fact, both IL-6 mRNA expression level in senescent wild-type MEFs and IL-6 protein level in conditioned medium derived from senescent wild-type MEFs are upregulated following oxidative stress, but not in MEFs obtained from caveolin-1 null mice [149]. Downregulation of caveolin-1 by siRNA in fibroblasts inhibits oxidant-induced activation of the IL-6 promoter [149]. Re-expression of caveolin-1 in caveolin-1 null MEFs rescues oxidant-induced upregulation of IL-6 mRNA. The caveolin-1-mediated upregulation of IL-6 is dependent on the ability of caveolin-1 to inhibit Sirt1 since the free radical-induced upregulation of IL-6 mRNA and activation of the IL-6 promoter can be restored in caveolin-1-lacking fibroblasts following downregulation of Sirt1 by siRNA [149]. Importantly, the enhancement of Ras^{G12V}-transformed NIH 3T3, MDA-MB-231, and PC3 prostate cancer cell proliferation by the conditioned medium derived from senescent wild-type fibroblasts is inhibited by an IL-6 neutralizing antibody [149]. Thus, the pro-stimulatory properties of IL-6, which is secreted by senescent fibroblasts, are caveolin-1-dependent.

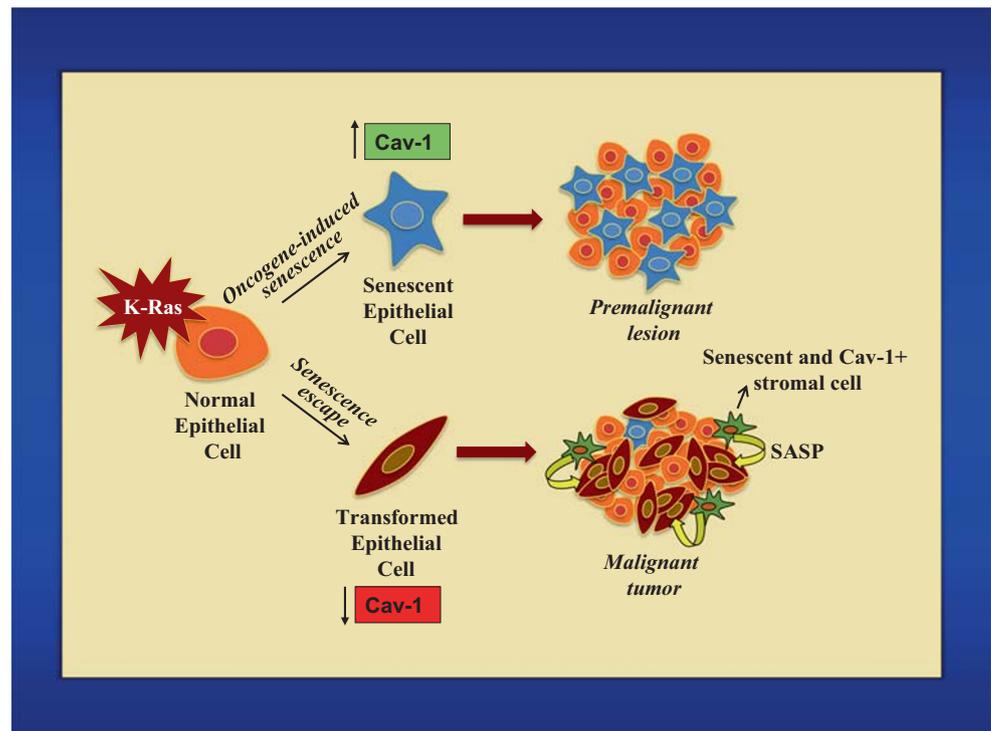
In support of a pro-tumorigenic role of caveolin-1 in stromal fibroblasts, downregulation of caveolin-1 in cancer-associated fibroblasts (CAFs), which were derived from pancreatic cancer patients, reduces the ability of their conditioned medium to stimulate invasiveness of MIAPaCa-2 pancreatic cancer cells [197]. Moreover, caveolin-1 expression in CAFs is associated with pancreatic cancer patients' poor prognosis [197]. In support of these findings, although caveolin-1 expression is reduced in lung adenocarcinoma epithelial cells, caveolin-1 remains highly expressed in the lung tumor stroma, which is caveolin-1-positive in 82% of cases [165]. These data are in apparent contrast with data showing accumulation of senescence markers in human breast cancer-associated fibroblasts in which caveolin-1 expression is significantly downregulated [179, 180]. However, as discussed above in Section 4.5, downregulation of caveolin-1 expression can also induce, under certain conditions, cellular senescence. Thus, one can speculate that depending on the cancer type, either excessively high or low caveolin-1 levels in CAFs can have pro-tumorigenic functions through induction of senescence and the release of SASP factors.

7 Conclusive remarks

Senescent cells accumulate with aging and contribute to the development of age-related deterioration of organ functions. An additional detrimental effect of cellular senescence relates to the accumulation of senescent stromal cells within the tumor microenvironment, where they secrete pro-tumorigenic SASP factors that stimulate the growth of pre-neoplastic and transformed epithelial cells. However, cellular senescence represents a natural tumor suppressor mechanism given the permanent exit from the cell cycle of senescent cells. Cancer cells need to either escape or bypass the barrier represented by cellular senescence in order to produce a clinically relevant tumor mass. Thus, the identification of the molecular mechanisms underlying the development and evasion of cellular senescence is key to a better understanding of the pathogenesis of age-related diseases, including cancer.

Multiple and independent findings from a number of laboratories have identified caveolin-1 as a major contributor to the acquisition of a senescent phenotype. Upregulation of caveolin-1 expression has been causally linked to the development of both replicative senescence and stress-induced premature senescence. Cellular stressors that promote caveolin-1-mediated SIPS include oxidative stress, UV-C light, bleomycin, and oncogenic K-Ras. Mechanistically, activation of the p53/p21 pathway is pivotal to the pro-senescent action of caveolin-1. Modulation of EGF-, focal adhesion-, and small Rho GTPase-dependent signaling represents additional regulatory mechanisms that have been linked to caveolin-1-mediated senescence. Downregulation of caveolin-1 in resting

Fig. 2 Proposed pleiotropic role of caveolin-1-mediated senescence in cancer. Expression of oncogenic K-Ras induces oncogene-induced senescence in epithelial cells in a caveolin-1-dependent manner. This is an anti-tumorigenic event that prevents premalignant lesions from progressing to cancer. Downregulation of caveolin-1 in oncogenic K-Ras-expressing cells is a pro-tumorigenic event that allows the escape/bypass of OIS and the development of a malignant tumor. Moreover, accumulation of caveolin-1-positive senescent stromal cells in the tumor microenvironment has pro-tumorigenic properties in virtue of their capacity to release growth-promoting factors that fuel the progression and possibly the metastatic features of cancer cells



cells has also been causally linked to the development of premature senescence through mitochondrial dysfunction and the forced maintenance of the primary cilium, supporting the notion that both “too much” and “too little” of caveolin-1-mediated signaling promote a senescent response.

Oncogenic K-Ras (K-Ras^{G12D})-induced senescence is inhibited in caveolin-1 null mice, which display enhanced lung tumorigenesis after expression of K-Ras^{G12D}. Also, both downregulation of endogenous caveolin-1 expression and lung tumor development are age-dependent in K-Ras^{G12D}-expressing mice (wild type for caveolin-1). Moreover, caveolin-1 expression is downregulated in MEFs expressing oncogenic K-Ras that escape OIS. As such, downregulation of caveolin-1 expression might represent an early molecular event in lung tumorigenesis through which oncogene-expressing epithelial cells evade/bypass senescence to progress to cancer (Fig. 2). In contrast, caveolin-1-mediated senescence in stromal cells might, through the release of pro-tumorigenic SASP factors, fuel the growth of oncogene-expressing epithelial cells that have already evaded senescence (Fig. 2). This would be consistent with data suggesting that caveolin-1 functions predominantly as a tumor suppressor in early stages of disease while it is linked more to tumor progression and metastasis at later stages [198, 199] and with data showing that caveolin-1 expression in lung cancer patients is lost in cancer epithelial cells but maintained in cancer stromal cells. Thus, caveolin-1-mediated functions contribute to explain the pleiotropic role of senescence in cancer (Fig. 2). Further studies are

necessary to determine whether targeted therapeutic interventions aimed at preventing downregulation of caveolin-1 expression in epithelial cells can limit the pro-tumorigenic evasion from oncogene-induced senescence during the early phases of tumorigenesis while those aimed at promoting the downregulation of caveolin-1 in stromal cells can restrain the accumulation of pro-tumorigenic senescent stromal cells in the tumor microenvironment during the later stages of tumorigenesis.

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