HHS Public Access

Author manuscript

Ageing Res Rev. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Ageing Res Rev. ; 68: 101332. doi:10.1016/j.arr.2021.101332.

Altered endocytosis in cellular senescence

Eun-Young Shin[a], **Nak-Kyun Soung**[b], **Martin Alexander Schwartz**[c,d,**], **Eung-Gook Kim**[a,*]

[a]Department of Biochemistry, Chungbuk National University College of Medicine, Cheongju, 28644, South Korea

[b]World Class Institute, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Ochang-eup, Cheongju, 28116, South Korea

[c]Yale Cardiovascular Research Center, Section of Cardiovascular Medicine, Department of Internal Medicine, And Department of Cell Biology, Yale University School of Medicine, New Haven, CT, 06511, USA

[d]Wellcome Trust Centre for Cell-matrix Research, University of Manchester, Manchester, UK

Abstract

Cellular senescence occurs in response to diverse stresses (e.g., telomere shortening, DNA damage, oxidative stress, oncogene activation). A growing body of evidence indicates that alterations in multiple components of endocytic pathways contribute to cellular senescence. Clathrin-mediated endocytosis (CME) and caveolae-mediated endocytosis (CavME) represent major types of endocytosis that are implicated in senescence. More recent research has also identified a chromatin modifier and tumor suppressor that contributes to the induction of senescence via altered endocytosis. Here, molecular regulators of aberrant endocytosis-induced senescence are reviewed and discussed in the context of their capacity to serve as senescence-inducing stressors or modifiers.

Keywords

Endocytosis; Senescence; Caveolin-1; Amphiphysin; βPAK-interacting nucleotide exchange factor; (βPIX); ING1

1. Introduction

Cellular senescence is characterized by: i) irreversible cell cycle arrest, ii) altered metabolism iii) altered morphology, and iv) the senescence-associated secretory phenotype

*Corresponding author at: Department of Biochemistry, Chungbuk National University College of Medicine, 1 Chungdae-ro, Seowon-gu, Cheongju, Chungbuk, 28644, South Korea. egkim@chungbuk.ac.kr (E.-G. Kim). **Corresponding author at: Yale Cardiovascular Research Center, Section of Cardiovascular Medicine, Departments of Internal Medicine, Cell Biology and Biomedical Engineering, Yale University, New Haven, CT, 06511, USA. martin.schwartz@yale.edu (M.A. Schwartz).

Author contributions

E.G. Kim and M.A. Schwartz wrote the manuscript. E.Y. Shin and N.K. Soung provided intellectual input and material support. All authors discussed the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

(SASP) that involves secretion of multiple cytokines, chemokines and growth factors (Hernandez-Segura et al., 2018; Ito et al., 2017; Rodier and Campisi, 2011). Senescence is thought to be beneficial in tissue remodeling in wound healing and to suppress tumorigenesis (Rodier and Campisi, 2011). However, cellular senescence also contributes to aging-related diseases (Baker et al., 2011; Childs et al., 2015; He and Sharpless, 2017; Munoz-Espin and Serrano, 2014). Senescence can also promote tumor progression via SASP (Lee and Schmitt, 2019; Rodier and Campisi, 2011). Senescence thus seems to play distinct roles depending on biological contexts.

Depending on cell types and context, senescence is broadly classified into replicative senescence (RS) and damage- or stress-induced premature senescence (SIPS) (Ben-Porath and Weinberg, 2005; Toussaint et al., 2000). The observation on the growth arrest of long-term cultured fibroblasts led to the discovery of RS (Hayflick and Moorhead, 1961) in which telomere erosion is a triggering factor. By contrast, SIPS occurs relatively rapidly, typically a few days, in response to oncogenic or oxidative stress. Once induced, these two forms of senescence show similar features as described above. Senescence also occurs physiologically during embryonic development where it is induced by TGFβ/Smad2/3 signaling and leads to elimination of unneeded cells (Munoz-Espin et al., 2013). Senescence of endothelial cells is also seen during angiogenic remodeling of the vasculature, where it is important for pruning/normalization after robust angiogenesis (Binet et al., 2020).

Endocytosis is an essential biological process that transports a wide range of transmembrane receptors and their cargo from the cell surface to the interior, where they can be recycled to the plasma membrane, targeted to the lysosome for degradation, or targeted to other locations such as the Golgi or nucleus for specific functions (Sorkin and von Zastrow, 2009). Endocytosis has been extensively studied for signaling receptors such as epidermal growth factor (EGF) receptor, carriers such as transferrin receptors and adhesion receptors such as integrins and cadherins. Importantly, endocytosis of many receptors is an essential element of their signaling functions. For example, growth factor and cytokine receptors transduce distinct signals depending on their localization on the plasma membrane or endocytic vesicles (Sorkin and von Zastrow, 2009; Wang et al., 2002).

The two best characterized endocytic routes are clathrin- (CME) and caveolae-mediated (CavME) (Cremona and De Camilli, 1997; Doherty and McMahon, 2009; Nabi and Le, 2003; Vieira et al., 1996). Caveolin-1 (Cav1), the major structural protein of CavME in non-muscle cells, is upregulated in senescent cells (Park et al., 2000) whereas amphiphysin-1, a membrane curvature-generating protein critical in CME, was downregulated (Park et al., 2001). Disrupting endocytosis directly by treating with dynasore, a dynamin inhibitor that blocks multiple endocytic mechanisms, or by genetic approaches using dominant negative forms of dynamin1, Rab5 and Rab7, induced cellular senescence, suggesting a causal relationship between endocytosis and senescence (Kang et al., 2020; Olszewski et al., 2014; Rajarajacholan et al., 2013). This review highlights the role of endocytic components and their regulatory mechanisms in cellular senescence.

2. Clathrin-mediated endocytosis in senescence

CME occurs through recruitment and regulation of a group of adapter proteins that assemble in distinct steps and then mediate invagination and scission of the coated vesicle. These include amphiphysin 1 and 2, founding members of the N-BAR domain family that both sense and induce membrane curvature (Dawson et al., 2006; Peter et al., 2004). Amphiphysins recruit the dynamin GTPases that constrict the neck of the mature pit to induce scission (David et al., 1996), thus are essential for CME across a wide range of biological systems (Gallop, 2020; Wu et al., 2009).

Senescent fibroblasts specifically downregulate amphiphysin 1 among the CME components, coincident with reduced endocytosis of transferrin receptors (Park et al., 2001). Restoration of amphiphysin 1 rescued endocytic capacity in senescent cells (Park et al., 2001), though its contribution to senescence was not established. A recent study found that the reduction in amphiphysin was due to its cleavage by calpain and that blocking this step inhibited senescence (Shin et al., 2020). These results provided stronger support for reduced CME as a functionally important step in senescence. Amphiphysin cleavage in turn depended on events at integrin-mediated adhesions that involved the βPIX/GIT complex.

2.1. The βPIX/GIT complex in senescence

Beta PAK-interacting nucleotide exchange factor (βPIX/ARHGEF7) activates Rac1 and Cdc42 GTPases (Manser et al., 1998). βPIX binds tightly to G protein–coupled receptor kinase-interacting proteins 1 and 2 (GIT1/2) (Manser et al., 1998; Premont et al., 1998), adapter proteins with ArfGAP activity. These complexes principally localizes at cell-matrix adhesion sites, focal adhesions (FAs), via an association with paxillin, where it regulates cell signaling, FA turnover and cell motility (Frank and Hansen, 2008; Kuo et al., 2011; Premont et al., 2004; Zhao et al., 2000; Zhou et al., 2016).

Previous studies demonstrated that βPIX and GIT mainly control FA disassembly (Kuo et al., 2011; Wilson et al., 2014). This process also involves microtubule targeting (Ezratty et al., 2005), integrin endocytosis (Chao and Kunz, 2009; Ezratty et al., 2009) and calpain-mediated proteolysis of FA proteins (Chan et al., 2010; Cortesio et al., 2011; Franco et al., 2004). Analysis of these proteins in senescent cells revealed a new link between these events (Shin et al., 2020). First, to understand whether reduced βPIX expression contributes to the senescent phenotype, both loss-of-function and gain-of-function studies were performed. Silencing βPIX in both cultured human diploid fibroblasts and in mouse lung induced senescence; conversely, overexpressing βPIX attenuated age-dependent senescent characteristics (Shin et al., 2020). GIT knockdown also induced senescence in cultured fibroblasts, though this was not confirmed in vivo (Shin et al., 2020).

When cellular βPIX and GIT are at normal levels, FA disassembly occurs at a regulated speed, which ensures physiological adhesion and migration (Fig. 1 left). However, βPIX decreases in senescent cells mediated by an unknown mechanism, which results in increased calpain cleavage of amphiphysin (Fig. 1 right panel, ①②). This occurs through decreased GIT1 levels, which destabilizes a paxillin-calpain complex that limits its activity toward amphiphysin. Declining βPIX in senescent cells thus inhibits CME by inactivating

amphiphysin. Active β1 integrin is a key cargo such that senescent cells retain surface active integrins (Fig. 1 right panel, ③). Senescent cells are thus hyper-adhesive, with persistent activation of the FAK, Rac1 and the NADPH oxidase (NOX) complex (Fig. 1 right panel, ④). Reactive oxygen species (ROS) generated through this pathway induce cellular senescence. This study thus provides new connections between the morphological and biochemical aspects of senescence.

2.2. The tumor suppressor ING1 in senescence

The ING family of tumor suppressor genes (*ING1-5*) regulates cell proliferation, DNA repair, apoptosis and senescence (Archambeau et al., 2019; Ludwig et al., 2011). All members exhibit tumor suppressor function via modulation of chromatin structure, however, *ING1* and *ING2* are the only two members found to induce senescence (Abad et al., 2011; Garkavtsev and Riabowol, 1997; Li et al., 2011; Pedeux et al., 2005; Rajarajacholan et al., 2013; Soliman et al., 2008). ING1 has four alternative splice isoforms with p47ING1a and p33ING1b the major forms in human tissues. Consistent with tumor suppressive function, ING1 expression is frequently decreased in tumors (Ohmori et al., 1999; Oki et al., 1999; Toyama et al., 1999) whereas both ING1a and ING1b are upregulated in senescent fibroblasts (Garkavtsev and Riabowol, 1997; Soliman et al., 2008) but the mechanism regulating their expression has not been described. Overexpression of ING1a or ING1b induced senescence in human fibroblasts (Abad et al., 2011; Goeman et al., 2005; Rajarajacholan et al., 2013; Soliman et al., 2008). Conversely, downregulation of ING1b by antisense RNA attenuated replicative senescence (Garkavtsev and Riabowol, 1997) and oncogene-induced senescence (Abad et al., 2011). It remains to be determined whether ING1a has a similar role. ING1b-induced senescence is p53 dependent (Fig. 2 ①) (Abad et al., 2011) whereas p53-dependence of ING2 varied between studies (Kumamoto et al., 2008; Pedeux et al., 2005). Isoform expression during replicative senescence also varies in different systems (Unoki et al., 2008). Downstream mediator(s) for ING2 are currently unknown.

Mice lacking both p31ING1 (p47ING1a in human) and p37ING1 (p33ING1b in human) displayed reduced cell proliferation and apoptosis, and increased susceptibility to tumorigenesis (Kichina et al., 2006). Unexpectedly, there was no difference in the lifespan of MEFs derived from ING1-deficient vs. wild type mice. In contrast, MEFs from ING1-deficient mice generated by gene trap technology demonstrated impaired oncogenic stress-induced senescence (Abad et al., 2007). The reason for the seemingly opposite findings from two different types of knockout mice is unclear.

The function of ING1a as an epigenetic regulator prompted analysis of genes whose expression is up- or downregulated. Several endocytosis-related genes were up-regulated (Rajarajacholan et al., 2013), including, most notably, rapid induction of intersectin 2 (ITSN2) by >25-fold (Fig. 2 ②). ITSN2 is a multidomain scaffolding protein that facilitates clathrin-coated pit assembly through an interaction with epsin, a clathrin pit component, and with AP2, a clathrin adaptor complex (Herrero-Garcia and O'Bryan, 2017; Okamoto et al., 1999). ITSN2 can also bind dynamin and synaptojanin, which are required to pinch off clathrin vesicles (Okamoto et al., 1999). Overexpression of ING1a drastically reduces

endocytosis of EGFRs in parallel with onset of the senescent phenotype through the Rb-E2F pathway (Rajarajacholan et al., 2013; Rajarajacholan and Riabowol, 2015) (Fig. 2 ③④), though other cargos remain to be tested. Other methods to block endocytosis yielded similar senescent features (Rajarajacholan et al., 2013), strengthening the link. How ING1a affects endocytosis is unknown but overexpression of ITSN2 seems a likely mediator, which might inhibit endocytosis via sequestering interacting proteins in inactive complexes or through its GEF activity for Cdc42 (Qualmann and Kessels, 2002) (Fig. 2 ③). Despite these limitations, results with ING1a offer a novel avenue for investigating the role of CME in cellular senescence.

3. Caveolae-mediated endocytosis in senescence

Caveolins are the major structural and functional constituents of caveolae, small flask-shaped invaginations enriched in lipid raft components (cholesterol and sphingolipids, and lipid modified proteins) (Rothberg et al., 1992; Thomas and Smart, 2008). Caveolin proteins have a central hydrophobic sequence that inserts into plasma membrane while the N- and C-termini are more hydrophilic; caveolin thus forms a hairpin-like structure with the central region buried in the membrane and the N- and C-termini in the cytoplasm (Williams and Lisanti, 2004). *CAV1* is widely expressed and essential for caveolae in non-muscle cells (Thomas and Smart, 2008); *CAV2* is a homologous gene that facilitates but is not sufficient for caveolae formation (Mora et al., 1999); *CAV3* resembles *CAV1* in sequence and function but is specific to cardiac and skeletal muscle (Tang et al., 1996). In addition to forming flask-shaped invaginations, caveolins can also form flat assemblies in the plasma membrane or exist in dispersed, non-caveolar states (Head and Insel, 2007; Pol A et al., 2020).

Caveolae mediate internalization of cargos such as virus particles, bacteria, toxins and lipid raft components (Nabi and Le, 2003; Parton and del Pozo, 2013). This process involves pinching off of vesicles by dynamin and delivery to endosomal compartments (Oh et al., 1998). As caveolae are enriched in cholesterol and glycosphingolipids, alterations in their intracellular uptake via caveolae can affect lipid metabolism. Consistently, cholesterol depletion with methyl-β-cyclodextrin inhibits CavME and loss or mutation of Cav1 causes severe lipidodystrophy (Parton and del Pozo, 2013). CavME is a highly regulated process, which varies depending on the clustering of cargo, tyrosine phosphorylation of Cav1 by Src family kinases (SFKs), and actin reorganization (Kiss, 2012). Interestingly, disruption of integrin-mediated adhesion by cellular detachment from the substrate strongly triggered caveolar endocytosis with resultant depletion of lipid raft components from the plasma membrane (del Pozo et al., 2004, 2005). This process inhibited multiple plasma membrane signaling pathways including Erk MAP kinase, Akt and small GTPases, thus, mediating anchorage-dependent growth of adherent cells. Conversely, *cav1*$^{-/-}$ cells showed constitutive activation of Rac1 and other pathways, with poor cell polarity and directional migration (Grande-Garcia et al., 2007).

Cav1, the most widely studied caveolin isoform, binds and modulates activity or function of a great many signaling proteins (Patel et al., 2008). Relevant targets include receptor tyrosine kinases (RTKs), Src family kinases (SFKs) and nitric oxide synthase 3 (eNOS) among many others (Couet et al., 1997; Li et al., 1996; Shaul et al., 1996). These

interactions are generally through the Cav1 scaffolding domain (amino acids 82–101) (Bucci et al., 2000). A hydrophobic consensus target sequence in these proteins that binds the Cav1 scaffolding domain has been identified but by no means accounts for all interactions. Deletion or overexpression of Cav1 thus alters a wide range of enzyme and signaling activities via direct effects as well as altered endocytosis (Boscher and Nabi, 2012).

An early study in long-term cultured HDFs revealed elevation of Cav1 and Cav2 protein levels as cells reached RS (Park et al., 2000). Cav1 was also elevated in multiple tissues of aged rats (Kang et al., 2006; Park et al., 2000). Despite the increased Cav1/2 protein, caveolae were reduced, as was the physical association of Cav1 with RTKs (Wheaton et al., 2001). Cav1 levels also increased in response to hydrogen peroxide and UV irradiation (Volonte et al., 2002) in association with SIPS. Forced expression of Cav1 induced senescence in young fibroblasts (Park et al., 2000; Volonte et al., 2002) and human mesenchymal stem cells (Park et al., 2005). Conversely, downregulation of Cav1 reversed senescent phenotypes (Cho et al., 2003). Taken together, these data identify Cav1 as an important modulator of senescence in these cell types.

One important unanswered question concerns the relative roles of caveolar vs. non-caveolar caveolin. Caveolar caveolin forms a structure that may respond to mechanical forces or mediate membrane trafficking. Caveolin, most likely both in and out of caveolae, interacts with and modulates activities of many signaling molecules through its scaffolding domain. These interactions are thought to contribute to the senescence phenotypes through cell cycle arrest, hypo-responsiveness and generation of ROS (Fig. 3). Non-caveolar caveolins can be seen outside of caveolae in the plasma membrane, focal adhesions (FAs), the endoplasmic reticulum and the Golgi apparatus even in endothelial cells and fibroblasts that have abundant caveolae (Pol et al., 2020) (Fig. 3). Non-caveolar caveolins can be generated when caveolae are disassembled in response to mechanical stress (Sinha et al., 2011), when caveolins are overexpressed or when Cavins are lacking (Hayer et al., 2010; Parton and Howes, 2010) (Fig. 3, ①②). Non-caveolar caveolins may contribute to the generation of ROS and senescent morphology through integrin signaling (Fig. 3). However, distinguishing the interactions and functions of caveolar vs non-caveolar Cav1 is challenging, and their relative contributions to senescence are largely unknown.

Transgenic mice in which Cav1 was overexpressed in hippocampal neurons, showed improved retention of hippocampal learning and memory during aging (Mandyam et al., 2017). These mice also showed extended survival in the $SOD1^{G93A}$ model of amyotrophic lateral sclerosis (Sawada et al., 2019). By contrast, endothelial-specific expression of Cav1 impaired microvascular permeability and angiogenesis, and accelerated progression of atherosclerosis, a senescence-associated disease (Bauer et al., 2005; Fernandez-Hernando et al., 2009). Initial interpretations ascribed these effects to Cav1 inhibition of eNOS. However, a recent study found that endothelial-specific Cav1 deletion potently inhibited atherosclerosis even in $eNOS^{-/-}$ mice (Ramirez et al., 2019). These effects correlated with reduced inflammatory activation of the endothelial cells and increased autophagy (also anti-inflammatory) (Zhang et al., 2020). However, endothelial senescence is also thought to contribute to atherosclerosis (Erusalimsky, 2009), thus, is another potential causative factor.

Several possibilities can be considered for the distinct outcomes in different systems. Fibroblasts and endothelial cells have abundant caveolae covering up to 50 % of the cell surface (Cho et al., 2003; Binet et al., 2020). By contrast, caveolae in neurons are barely detectible, though Cav1 is readily detected and increases with age (Kang et al., 2006). Neurons are in a soft, unstressed mechanical environment, whereas endothelial cells and fibroblasts are mechanically stressed, consistent with a role for caveolae in mechanotransduction (del Pozo et al., 2020). Differential expression of Cav1-interacting molecules is also a likely contributing factor. As described below, Cav1 inhibits EGF receptor signaling, but promotes signaling through the insulin receptor and the neuronal TrKB receptor that mediates the stimulation with brain-derived neurotrophic factor (Mandyam et al., 2017; Sawada et al., 2019). Which of these effects mediates distinct outcomes in different cell types and environments remains to be determined.

Cav1 has complex effects in many settings. It can suppress or promote tumors depending on cell type and cancer stage (Gupta et al., 2014; Quest et al., 2008). Its deletion activates or inhibits a wide range of pathways (Boscher and Nabi, 2012). Which of these effects are due to caveolae vs non-caveolar functions, and which caveolar functions are mediated by endocytosis is not well understood. Overexpression of Cav1 in mouse embryonic fibroblasts induced cell cycle arrest at G0/G1 through the p53/p21 pathway (Galbiati et al., 2001), a common effector pathway in senescence. This may involve Cav1-mediated binding to Mdm2 (Bartholomew et al., 2009) and Sirt1 (Volonte et al., 2015) (Fig. 3). Cav1 also directly binds the EGF receptor and sequesters it into caveolae, which may explain hypo-responsiveness to EGF in senescent cells (Park et al., 2000) (Fig. 3). Moreover, Cav1 interacts with and inhibits Nrf2 and TrxR1, thus, elevated Cav1 increases cellular ROS due to suppression of these anti-inflammatory mediators (Volonte and Galbiati, 2009; Volonte et al., 2013) (Fig. 3). Notably, Cav1 in senescent cells regulates FAK and Rho GTPases to generate the typical hyper-adhesive senescent morphology, which is reversed by Cav1 knockdown (Cho et al., 2004) (Fig. 3). The highly elevated activity of FAK and Rac1 results in elevated ROS production through the NADPH oxidase complex, which is causally implicated in senescence (Shin et al., 2020).

Together, these data clearly indicate a role for Cav1 in cellular senescence but to what extent it involves altered caveolar endocytosis is unknown. If anything, the data favor a role for non-caveolar functions. To our knowledge, the role of CavME *per se* in senescence, as opposed to other functions of Cav1 protein, has not been critically tested.

4. Signaling pathways linking endocytosis to senescence

Though the p16/Rb and p53 pathways are central for senescence of all types (Ben-Porath and Weinberg, 2005; Salama et al., 2014), additional signaling would seem to be required to explain the full senescent phenotype. Endocytosis impacts membrane receptor signaling, cell adhesion, migration and cytoskeletal organization, thus, is a good candidate. Senescent cells are typically large, flat, immobile cells with excessive actin stress fibers and focal adhesions (FAs). Bioinformatic analysis strongly implicated FA molecules in accelerated aging and aging-related diseases (Wolfson et al., 2009).

FAs are initiated by integrin binding to extracellular matrix, which then link to the actin cytoskeleton. Integrin trafficking, including internalization, recycling and degradation, are critical for their function and signaling (Caswell et al., 2009; Paul et al., 2015). Integrins are internalized via CME and clathrin-independent pathways, including CavME, macropinocytosis and clathrin-independent carrier-mediated endocytosis (Moreno-Layseca et al., 2019). Several lines of evidence suggest a role for integrins in senescence (Goddeeris et al., 2003; Jun and Lau, 2010; Rapisarda et al., 2017), in which altered integrin endocytosis may be central.

Cellular senescence is associated with downregulation of CME via several mechanisms. First, decreased expression of βPIX and GIT1 altered the assembly of protein complexes within the focal adhesions, resulting in calpain-mediated degradation of the key endocytic adapter amphiphysin 1 (Shin et al., 2020) (Fig. 1 right, ②). Decreased amphiphysin 1 then limits integrin endocytosis (Fig. 4, ①), leading to accumulation of active integrins at the plasma membrane (Shin et al., 2020). These changes resulted in elevated and abnormal integrin signaling that drove cellular senescence (Fig. 4). The tumor suppressor ING1a also controls receptor endocytosis. The effect of ING1a on CME is mediated at least in part through aberrant expression of ISTN2, a guanine nucleotide exchange factor for Cdc42 (Rajarajacholan et al., 2013) (Fig. 2); ISTN2 overexpression has been shown to dysregulate actin polymerization required for CME (Pucharcos et al., 2000). While these effects have not been linked specifically to integrin trafficking, the connection is intriguing.

Senescent cells have increased levels of Cav1 protein yet reduced or dysfunctional caveolae, suggesting diminished endocytic activity (Wheaton et al., 2001). Evidence indicates the inhibitory role of upregulated Cav1 in CavME (Lajoie and Nabi, 2007; Nabi and Le, 2003) and more directly in β1 integrin endocytosis (Vassilieva et al., 2008). It thus seems likely that upregulated Cav1 may extend the activation of integrin signaling, though the precise mechanism remains to be determined (Fig. 4, ③). Consistent with this, caveolar internalization of lipid raft components inhibits Cdc42 and Rac1 function; thus, *cav1*$^{-/-}$ fibroblasts, where this pathway is disabled, are very well spread but unpolarized and poorly migratory (del Pozo et al., 2005; Grande-Garcia et al., 2007), thus, sharing some features with senescent cells. The shift toward non-caveolar Cav1 may also contribute to the senescent phenotype (Fig. 3, ①②; Fig. 4, ④). Non-caveolar Cav1 has been reported to interact with integrins in association with the urokinase receptor (Wei et al., 1996, 1999), the SFK Fyn (Wary et al., 1996, 1998), galectin-3 (Goetz et al., 2008), and activated Rac1 (Nethe et al., 2010) (Fig. 3). In all of these cases, this non-caveolar pool promoted integrin signaling. These older studies thus fit well with recent findings on the importance of elevated integrin signaling in the senescent phenotype (Shin et al., 2020). We therefore favor a model in which a shift from caveolar to non-caveolar Cav1 contributes to aspects of the senescent phenotype through changes in both endocytic and non-endocytic mechanisms (Fig. 4).

These findings raise the possibility that multiple mechanisms of cellular senescence may converge on receptor endocytosis, with integrins emerging as potentially central regulators. Whether this speculation will be borne out by the data remains to be determined, but the tools to address these questions are in hand and answers are likely in the near future.

5. Conclusion and future prospects

Re-examination of the literature on senescence and endocytosis in light of recent findings supports the concept that aberrant endocytosis contributes to cellular senescence via multiple mechanisms. Notably, the data implicating integrins fits well with bioinformatic analysis of senescence and aging-related diseases (Wolfson et al., 2009), and with the well-known hyper-adhesive senescent phenotype. Persistently activated integrin signaling can explain many features of senescent cells, including Rho GTPase-dependent morphological changes, prominent actin stress fibers and focal adhesions, and ROS-mediated oxidative stress. Key questions for future research include the roles of Cav1 in both caveolar and non-caveolar forms, the mechanism of βPIX downregulation during senescence; the role of reduced endocytosis in ING1a-induced senescence; and whether any of these events offer opportunities for intervention in age-related diseases.

Considering the enormous complexity of endocytosis and senescence, the identification of additional components contributing to the aberrant endocytosis-induced senescence pathway will provide more insights into these events. These findings may also facilitate the discovery of therapeutic targets for senescence-related diseases.

Acknowledgements

This work was supported, in part, by grants from the National Research Foundation of Korea (2020R1A5A2017476) and the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2017M3A9D8063627), and USPHS grant RO1 GM47214 to M.A.S.

References

Abad M, Menendez C, Fuchtbauer A, Serrano M, Fuchtbauer EM, Palmero I, 2007. Ing1 mediates p53 accumulation and chromatin modification in response to oncogenic stress. J. Biol. Chem 282, 31060–31067. [PubMed: 17693408]

Abad M, Moreno A, Palacios A, Narita M, Blanco F, Moreno-Bueno G, Narita M, Palmero I, 2011. The tumor suppressor ING1 contributes to epigenetic control of cellular senescence. Aging Cell 10, 158–171. [PubMed: 21078114]

Archambeau J, Blondel A, Pedeux R, 2019. Focus-ING on DNA integrity: implication of ING proteins in cell cycle regulation and DNA repair modulation. Cancers (Basel) 12.

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM, 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236. [PubMed: 22048312]

Bartholomew JN, Volonte D, Galbiati F, 2009. Caveolin-1 regulates the antagonistic pleiotropic properties of cellular senescence through a novel Mdm2/p53-mediated pathway. Cancer Res. 69, 2878–2886. [PubMed: 19318577]

Bauer PM, Yu J, Chen Y, Hickey R, Bernatchez PN, Looft-Wilson R, Huang Y, Giordano F, Stan RV, Sessa WC, 2005. Endothelial-specific expression of caveolin-1 impairs microvascular permeability and angiogenesis. Proc. Natl. Acad. Sci. U. S. A 102, 204–209. [PubMed: 15615855]

Ben-Porath I, Weinberg RA, 2005. The signals and pathways activating cellular senescence. Int. J. Biochem. Cell Biol 37, 961–976. [PubMed: 15743671]

Binet F, Cagnone G, Crespo-Garcia S, Hata M, Neault M, Dejda A, Wilson AM, Buscarlet M, Mawambo GT, Howard JP, Diaz-Marin R, Parinot C, Guber V, Pilon F, Juneau R, Laflamme R, Sawchyn C, Boulay K, Leclerc S, Abu-Thuraia A, Cote JF, Andelfinger G, Rezende FA, Sennlaub F, Joyal JS, Mallette FA, Sapieha P, 2020. Neutrophil extracellular traps target senescent vasculature for tissue remodeling in retinopathy. Science 369.

Boscher C, Nabi IR, 2012. Caveolin-1: role in cell signaling. Adv. Exp. Med. Biol 729, 29–50. [PubMed: 22411312]

Bucci M, Gratton JP, Rudic RD, Acevedo L, Roviezzo F, Cirino G, Sessa WC, 2000. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. Nat. Med 6, 1362–1367. [PubMed: 11100121]

Caswell PT, Vadrevu S, Norman JC, 2009. Integrins: masters and slaves of endocytic transport. Nat. Rev. Mol. Cell Biol 10, 843–853. [PubMed: 19904298]

Chan KT, Bennin DA, Huttenlocher A, 2010. Regulation of adhesion dynamics by calpain-mediated proteolysis of focal adhesion kinase (FAK). J. Biol. Chem 285, 11418–11426. [PubMed: 20150423]

Chao WT, Kunz J, 2009. Focal adhesion disassembly requires clathrin-dependent endocytosis of integrins. FEBS Lett. 583, 1337–1343. [PubMed: 19306879]

Childs BG, Durik M, Baker DJ, van Deursen JM, 2015. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat. Med 21, 1424–1435. [PubMed: 26646499]

Cho KA, Ryu SJ, Park JS, Jang IS, Ahn JS, Kim KT, Park SC, 2003. Senescent phenotype can be reversed by reduction of caveolin status. J. Biol. Chem 278, 27789–27795. [PubMed: 12730243]

Cho KA, Ryu SJ, Oh YS, Park JH, Lee JW, Kim HP, Kim KT, Jang IS, Park SC, 2004. Morphological adjustment of senescent cells by modulating caveolin-1 status. J. Biol. Chem 279, 42270–42278. [PubMed: 15263006]

Cortesio CL, Boateng LR, Piazza TM, Bennin DA, Huttenlocher A, 2011. Calpain-mediated proteolysis of paxillin negatively regulates focal adhesion dynamics and cell migration. J. Biol. Chem 286, 9998–10006. [PubMed: 21270128]

Couet J, Sargiacomo M, Lisanti MP, 1997. Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. J. Biol. Chem 272, 30429–30438. [PubMed: 9374534]

Cremona O, De Camilli P, 1997. Synaptic vesicle endocytosis. Curr. Opin. Neurobiol 7, 323–330. [PubMed: 9232811]

David C, McPherson PS, Mundigl O, de Camilli P, 1996. A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. Proc. Natl. Acad. Sci. U. S. A 93, 331–335. [PubMed: 8552632]

Dawson JC, Legg JA, Machesky LM, 2006. Bar domain proteins: a role in tubulation, scission and actin assembly in clathrin-mediated endocytosis. Trends Cell Biol. 16, 493–498. [PubMed: 16949824]

del Pozo MA, Alderson NB, Kiosses WB, Chiang HH, Anderson RG, Schwartz MA, 2004. Integrins regulate Rac targeting by internalization of membrane domains. Science 303, 839–842. [PubMed: 14764880]

del Pozo MA, Balasubramanian N, Alderson NB, Kiosses WB, Grande-Garcia A, Anderson RG, Schwartz MA, 2005. Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. Nat. Cell Biol 7, 901–908. [PubMed: 16113676]

del Pozo MA, Lolo FN, Echarri A, 2020. Caveolae: mechanosensing and mechanotransduction devices linking membrane trafficking to mechanoadaptation. Curr. Opin. Cell Biol 68, 113–123. [PubMed: 33188985]

Doherty GJ, McMahon HT, 2009. Mechanisms of endocytosis. Annu. Rev. Biochem 78, 857–902. [PubMed: 19317650]

Erusalimsky JD, 2009. Vascular endothelial senescence: from mechanisms to pathophysiology. J. Appl. Physiol 106 (1985), 326–332. [PubMed: 19036896]

Ezratty EJ, Partridge MA, Gundersen GG, 2005. Microtubule-induced focal adhesion disassembly is mediated by dynamin and focal adhesion kinase. Nat. Cell Biol 7, 581–590. [PubMed: 15895076]

Ezratty EJ, Bertaux C, Marcantonio EE, Gundersen GG, 2009. Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. J. Cell Biol 187, 733–747. [PubMed: 19951918]

Fernandez-Hernando C, Yu J, Suarez Y, Rahner C, Davalos A, Lasuncion MA, Sessa WC, 2009. Genetic evidence supporting a critical role of endothelial caveolin-1 during the progression of atherosclerosis. Cell Metab. 10, 48–54. [PubMed: 19583953]

Franco SJ, Rodgers MA, Perrin BJ, Han J, Bennin DA, Critchley DR, Huttenlocher A, 2004. Calpain-mediated proteolysis of talin regulates adhesion dynamics. Nat. Cell Biol 6, 977–983. [PubMed: 15448700]

Frank SR, Hansen SH, 2008. The PIX-GIT complex: a G protein signaling cassette in control of cell shape. Semin. Cell Dev. Biol 19, 234–244. [PubMed: 18299239]

Galbiati F, Razani B, Lisanti MP, 2001. Caveolae and caveolin-3 in muscular dystrophy. Trends Mol. Med 7, 435–441. [PubMed: 11597517]

Gallop JL, 2020. Filopodia and their links with membrane traffic and cell adhesion. Semin. Cell Dev. Biol 102, 81–89. [PubMed: 31843255]

Garkavtsev I, Riabowol K, 1997. Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33ING1 candidate tumor suppressor. Mol. Cell. Biol 17, 2014–2019. [PubMed: 9121449]

Goddeeris MM, Cook-Wiens E, Horton WJ, Wolf H, Stoltzfus JR, Borrusch M, Grotewiel MS, 2003. Delayed behavioural aging and altered mortality in Drosophila beta integrin mutants. Aging Cell 2, 257–264. [PubMed: 14570233]

Goeman F, Thormeyer D, Abad M, Serrano M, Schmidt O, Palmero I, Baniahmad A, 2005. Growth inhibition by the tumor suppressor p33ING1 in immortalized and primary cells: involvement of two silencing domains and effect of Ras. Mol. Cell. Biol 25, 422–431. [PubMed: 15601862]

Goetz JG, Joshi B, Lajoie P, Strugnell SS, Scudamore T, Kojic LD, Nabi IR, 2008. Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. J. Cell Biol 180, 1261–1275. [PubMed: 18347068]

Grande-Garcia A, Echarri A, de Rooij J, Alderson NB, Waterman-Storer CM, Valdivielso JM, del Pozo MA, 2007. Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. J. Cell Biol 177, 683–694. [PubMed: 17517963]

Gupta R, Toufaily C, Annabi B, 2014. Caveolin and cavin family members: dual roles in cancer. Biochimie 107 (Pt B), 188–202. [PubMed: 25241255]

Hayer A, Stoeber M, Bissig C, Helenius A, 2010. Biogenesis of caveolae: stepwise assembly of large caveolin and cavin complexes. Traffic 11, 361–382. [PubMed: 20070607]

Hayflick L, Moorhead PS, 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res 25, 585–621. [PubMed: 13905658]

He S, Sharpless NE, 2017. Senescence in health and disease. Cell 169, 1000–1011. [PubMed: 28575665]

Head BP, Insel PA, 2007. Do caveolins regulate cells by actions outside of caveolae? Trends Cell Biol. 17, 51–57. [PubMed: 17150359]

Hernandez-Segura A, Nehme J, Demaria M, 2018. Hallmarks of cellular senescence. Trends Cell Biol. 28, 436–453. [PubMed: 29477613]

Herrero-Garcia E, O'Bryan JP, 2017. Intersectin scaffold proteins and their role in cell signaling and endocytosis. Biochim. Biophys. Acta Mol. Cell. Res 1864, 23–30.

Ito Y, Hoare M, Narita M, 2017. Spatial and temporal control of senescence. Trends Cell Biol. 27, 820–832. [PubMed: 28822679]

Jun JI, Lau LF, 2010. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat. Cell Biol 12, 676–685. [PubMed: 20526329]

Kang MJ, Chung YH, Hwang CI, Murata M, Fujimoto T, Mook-Jung IH, Cha CI, Park WY, 2006. Caveolin-1 upregulation in senescent neurons alters amyloid precursor protein processing. Exp. Mol. Med 38, 126–133. [PubMed: 16672766]

Kang D, Jung SH, Lee GH, Lee S, Park HJ, Ko YG, Kim YN, Lee JS, 2020. Sulfated syndecan 1 is critical to preventing cellular senescence by modulating fibroblast growth factor receptor endocytosis. FASEB J.

Kawabe J, Okumura S, Lee MC, Sadoshima J, Ishikawa Y, 2004. Translocation of caveolin regulates stretch-induced ERK activity in vascular smooth muscle cells. Am. J. Physiol. Heart Circ. Physiol 286, H1845–1852. [PubMed: 15072971]

Kichina JV, Zeremski M, Aris L, Gurova KV, Walker E, Franks R, Nikitin AY, Kiyokawa H, Gudkov AV, 2006. Targeted disruption of the mouse ing1 locus results in reduced body size,

hypersensitivity to radiation and elevated incidence of lymphomas. Oncogene 25, 857–866. [PubMed: 16170338]

Kiss AL, 2012. Caveolae and the regulation of endocytosis. Adv. Exp. Med. Biol 729, 14–28. [PubMed: 22411311]

Kumamoto K, Spillare EA, Fujita K, Horikawa I, Yamashita T, Appella E, Nagashima M, Takenoshita S, Yokota J, Harris CC, 2008. Nutlin-3a activates p53 to both down-regulate inhibitor of growth 2 and up-regulate mir-34a, mir-34b, and mir-34c expression, and induce senescence. Cancer Res. 68, 3193–3203. [PubMed: 18451145]

Kuo JC, Han X, Hsiao CT, Yates JR 3rd, Waterman CM, 2011. Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. Nat. Cell Biol 13, 383–393. [PubMed: 21423176]

Lajoie P, Nabi IR, 2007. Regulation of raft-dependent endocytosis. J. Cell. Mol. Med 11, 644–653. [PubMed: 17760830]

Lee S, Schmitt CA, 2019. The dynamic nature of senescence in cancer. Nat. Cell Biol 21, 94–101. [PubMed: 30602768]

Li S, Seitz R, Lisanti MP, 1996. Phosphorylation of caveolin by src tyrosine kinases. The alpha-isoform of caveolin is selectively phosphorylated by v-Src in vivo. J. Biol. Chem 271, 3863–3868. [PubMed: 8632005]

Li N, Li Q, Cao X, Zhao G, Xue L, Tong T, 2011. The tumor suppressor p33ING1b upregulates p16INK4a expression and induces cellular senescence. FEBS Lett. 585, 3106–3112. [PubMed: 21896275]

Ludwig S, Klitzsch A, Baniahmad A, 2011. The ING tumor suppressors in cellular senescence and chromatin. Cell Biosci. 1, 25. [PubMed: 21767350]

Mandyam CD, Schilling JM, Cui W, Egawa J, Niesman IR, Kellerhals SE, Staples MC, Busija AR, Risbrough VB, Posadas E, Grogman GC, Chang JW, Roth DM, Patel PM, Patel HH, Head BP, 2017. Neuron-targeted Caveolin-1 improves molecular signaling, plasticity, and behavior dependent on the Hippocampus in adult and aged mice. Biol. Psychiatry 81, 101–110. [PubMed: 26592463]

Manser E, Loo TH, Koh CG, Zhao ZS, Chen XQ, Tan L, Tan I, Leung T, Lim L, 1998. PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. Mol. Cell 1, 183–192. [PubMed: 9659915]

Mora R, Bonilha VL, Marmorstein A, Scherer PE, Brown D, Lisanti MP, Rodriguez-Boulan E, 1999. Caveolin-2 localizes to the golgi complex but redistributes to plasma membrane, caveolae, and rafts when co-expressed with caveolin-1. J. Biol. Chem 274, 25708–25717. [PubMed: 10464308]

Moreno-Layseca P, Icha J, Hamidi H, Ivaska J, 2019. Integrin trafficking in cells and tissues. Nat. Cell Biol 21, 122–132. [PubMed: 30602723]

Munoz-Espin D, Serrano M, 2014. Cellular senescence: from physiology to pathology. Nat. Rev. Mol. Cell Biol 15, 482–496. [PubMed: 24954210]

Munoz-Espin D, Canamero M, Maraver A, Gomez-Lopez G, Contreras J, Murillo-Cuesta S, Rodriguez-Baeza A, Varela-Nieto I, Ruberte J, Collado M, Serrano M, 2013. Programmed cell senescence during mammalian embryonic development. Cell 155, 1104–1118. [PubMed: 24238962]

Nabi IR, Le PU, 2003. Caveolae/raft-dependent endocytosis. J. Cell Biol 161, 673–677. [PubMed: 12771123]

Nethe M, Anthony EC, Fernandez-Borja M, Dee R, Geerts D, Hensbergen PJ, Deelder AM, Schmidt G, Hordijk PL, 2010. Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. J. Cell. Sci 123, 1948–1958. [PubMed: 20460433]

Oh P, McIntosh DP, Schnitzer JE, 1998. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. J. Cell Biol 141, 101–114. [PubMed: 9531551]

Ohmori M, Nagai M, Tasaka T, Koeffler HP, Toyama T, Riabowol K, Takahara J, 1999. Decreased expression of p33ING1 mRNA in lymphoid malignancies. Am. J. Hematol 62, 118–119. [PubMed: 10577281]

Okamoto M, Schoch S, Sudhof TC, 1999. EHSH1/intersectin, a protein that contains EH and SH3 domains and binds to dynamin and SNAP-25. A protein connection between exocytosis and endocytosis? J. Biol. Chem 274, 18446–18454. [PubMed: 10373452]

Oki E, Maehara Y, Tokunaga E, Kakeji Y, Sugimachi K, 1999. Reduced expression of p33(ING1) and the relationship with p53 expression in human gastric cancer. Cancer Lett. 147, 157–162. [PubMed: 10660101]

Olszewski MB, Chandris P, Park BC, Eisenberg E, Greene LE, 2014. Disruption of clathrin-mediated trafficking causes centrosome overduplication and senescence. Traffic 15, 60–77. [PubMed: 24138026]

Park WY, Park JS, Cho KA, Kim DI, Ko YG, Seo JS, Park SC, 2000. Upregulation of caveolin attenuates epidermal growth factor signaling in senescent cells. J. Biol. Chem 275, 20847–20852. [PubMed: 10781609]

Park JS, Park WY, Cho KA, Kim DI, Jhun BH, Kim SR, Park SC, 2001. Downregulation of amphiphysin-1 is responsible for reduced receptor-mediated endocytosis in the senescent cells. FASEB J. 15, 1625–1627. [PubMed: 11427507]

Park JS, Kim HY, Kim HW, Chae GN, Oh HT, Park JY, Shim H, Seo M, Shin EY, Kim EG, Park SC, Kwak SJ, 2005. Increased caveolin-1, a cause for the declined adipogenic potential of senescent human mesenchymal stem cells. Mech. Ageing Dev 126, 551–559. [PubMed: 15811424]

Parton RG, del Pozo MA, 2013. Caveolae as plasma membrane sensors, protectors and organizers. Nat. Rev. Mol. Cell Biol 14, 98–112. [PubMed: 23340574]

Parton RG, Howes MT, 2010. Revisiting caveolin trafficking: the end of the caveosome. J. Cell Biol 191, 439–441. [PubMed: 21041440]

Patel HH, Murray F, Insel PA, 2008. Caveolae as organizers of pharmacologically relevant signal transduction molecules. Annu. Rev. Pharmacol. Toxicol 48, 359–391. [PubMed: 17914930]

Paul NR, Jacquemet G, Caswell PT, 2015. Endocytic trafficking of integrins in cell migration. Curr. Biol 25, R1092–1105. [PubMed: 26583903]

Pedeux R, Sengupta S, Shen JC, Demidov ON, Saito S, Onogi H, Kumamoto K, Wincovitch S, Garfield SH, McMenamin M, Nagashima M, Grossman SR, Appella E, Harris CC, 2005. ING2 regulates the onset of replicative senescence by induction of p300-dependent p53 acetylation. Mol. Cell. Biol 25, 6639–6648. [PubMed: 16024799]

Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT, 2004. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science 303, 495–499. [PubMed: 14645856]

Pol A, Morales-Paytuvi F, Bosch M, Parton RG, 2020. Non-caveolar caveolins - duties outside the caves. J. Cell. Sci 133.

Premont RT, Claing A, Vitale N, Freeman JL, Pitcher JA, Patton WA, Moss J, Vaughan M, Lefkowitz RJ, 1998. beta2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein. Proc. Natl. Acad. Sci. U. S. A 95, 14082–14087. [PubMed: 9826657]

Premont RT, Perry SJ, Schmalzigaug R, Roseman JT, Xing Y, Claing A, 2004. The GIT/PIX complex: an oligomeric assembly of GIT family ARF GTPase-activating proteins and PIX family Rac1/Cdc42 guanine nucleotide exchange factors. Cell. Signal 16, 1001–1011. [PubMed: 15212761]

Pucharcos C, Estivill X, de la Luna S, 2000. Intersectin 2, a new multimodular protein involved in clathrin-mediated endocytosis. FEBS Lett. 478, 43–51. [PubMed: 10922467]

Qualmann B, Kessels MM, 2002. Endocytosis and the cytoskeleton. Int. Rev. Cytol 220, 93–144. [PubMed: 12224553]

Quest AF, Gutierrez-Pajares JL, Torres VA, 2008. Caveolin-1: an ambiguous partner in cell signalling and cancer. J. Cell. Mol. Med 12, 1130–1150. [PubMed: 18400052]

Rajarajacholan UK, Riabowol K, 2015. Aging with ING: a comparative study of different forms of stress induced premature senescence. Oncotarget 6, 34118–34127. [PubMed: 26439691]

Rajarajacholan UK, Thalappilly S, Riabowol K, 2013. The ING1a tumor suppressor regulates endocytosis to induce cellular senescence via the Rb-E2F pathway. PLoS Biol. 11 e1001502. [PubMed: 23472054]

Ramirez CM, Zhang X, Bandyopadhyay C, Rotllan N, Sugiyama MG, Aryal B, Liu X, He S, Kraehling JR, Ulrich V, Lin CS, Velazquez H, Lasuncion MA, Li G, Suarez Y, Tellides G, Swirski FK, Lee WL, Schwartz MA, Sessa WC, Fernandez-Hernando C, 2019. Caveolin-1 regulates atherogenesis by attenuating low-density lipoprotein transcytosis and vascular inflammation independently of endothelial nitric oxide synthase activation. Circulation 140, 225–239. [PubMed: 31154825]

Rapisarda V, Borghesan M, Miguela V, Encheva V, Snijders AP, Lujambio A, O'Loghlen A, 2017. Integrin Beta 3 regulates cellular senescence by activating the TGF-beta pathway. Cell Rep. 18, 2480–2493. [PubMed: 28273461]

Rodier F, Campisi J, 2011. Four faces of cellular senescence. J. Cell Biol 192, 547–556. [PubMed: 21321098]

Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG, 1992. Caveolin, a protein component of caveolae membrane coats. Cell 68, 673–682. [PubMed: 1739974]

Salama R, Sadaie M, Hoare M, Narita M, 2014. Cellular senescence and its effector programs. Genes Dev. 28, 99–114. [PubMed: 24449267]

Sawada A, Wang S, Jian M, Leem J, Wackerbarth J, Egawa J, Schilling JM, Platoshyn O, Zemljic-Harpf A, Roth DM, Patel HH, Patel PM, Marsala M, Head BP, 2019. Neuron-targeted caveolin-1 improves neuromuscular function and extends survival in SOD1(G93A) mice. FASEB J. 33, 7545–7554. [PubMed: 30894019]

Shaul PW, Smart EJ, Robinson LJ, German Z, Yuhanna IS, Ying Y, Anderson RG, Michel T, 1996. Acylation targets emdothelial nitric-oxide synthase to plasmalemmal caveolae. J. Biol. Chem 271, 6518–6522. [PubMed: 8626455]

Shin EY, Park JH, You ST, Lee CS, Won SY, Park JJ, Kim HB, Shim J, Soung NK, Lee OJ, Schwartz MA, Kim EG, 2020. Integrin-mediated adhesions in regulation of cellular senescence. Sci. Adv 6 eaay3909. [PubMed: 32494696]

Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Vedie B, Johannes L, Morone N, Parton RG, Raposo G, Sens P, Lamaze C, Nassoy P, 2011. Cells respond to mechanical stress by rapid disassembly of caveolae. Cell 144, 402–413. [PubMed: 21295700]

Soliman MA, Berardi P, Pastyryeva S, Bonnefin P, Feng X, Colina A, Young D, Riabowol K, 2008. ING1a expression increases during replicative senescence and induces a senescent phenotype. Aging Cell 7, 783–794. [PubMed: 18691180]

Sorkin A, von Zastrow M, 2009. Endocytosis and signalling: intertwining molecular networks. Nat. Rev. Mol. Cell Biol 10, 609–622. [PubMed: 19696798]

Tang Z, Scherer PE, Okamoto T, Song K, Chu C, Kohtz DS, Nishimoto I, Lodish HF, Lisanti MP, 1996. Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. J. Biol. Chem 271, 2255–2261. [PubMed: 8567687]

Thomas CM, Smart EJ, 2008. Caveolae structure and function. J. Cell. Mol. Med 12, 796–809. [PubMed: 18315571]

Toussaint O, Medrano EE, von Zglinicki T, 2000. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp. Gerontol 35, 927–945. [PubMed: 11121681]

Toyama T, Iwase H, Watson P, Muzik H, Saettler E, Magliocco A, DiFrancesco L, Forsyth P, Garkavtsev I, Kobayashi S, Riabowol K, 1999. Suppression of ING1 expression in sporadic breast cancer. Oncogene 18, 5187–5193. [PubMed: 10498868]

Unoki M, Kumamoto K, Robles AI, Shen JC, Zheng ZM, Harris CC, 2008. A novel ING2 isoform, ING2b, synergizes with ING2a to prevent cell cycle arrest and apoptosis. FEBS Lett. 582, 3868–3874. [PubMed: 18951897]

Vassilieva EV, Gerner-Smidt K, Ivanov AI, Nusrat A, 2008. Lipid rafts mediate internalization of beta1-integrin in migrating intestinal epithelial cells. Am. J. Physiol. Gastrointest. Liver Physiol 295, G965–976. [PubMed: 18755811]

Vieira AV, Lamaze C, Schmid SL, 1996. Control of EGF receptor signaling by clathrin-mediated endocytosis. Science 274, 2086–2089. [PubMed: 8953040]

Volonte D, Galbiati F, 2009. Inhibition of thioredoxin reductase 1 by caveolin 1 promotes stress-induced premature senescence. EMBO Rep. 10, 1334–1340. [PubMed: 19820694]

Volonte D, Zhang K, Lisanti MP, Galbiati F, 2002. Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. Mol. Biol. Cell 13, 2502–2517. [PubMed: 12134086]

Volonte D, Liu Z, Musille PM, Stoppani E, Wakabayashi N, Di YP, Lisanti MP, Kensler TW, Galbiati F, 2013. Inhibition of nuclear factor-erythroid 2-related factor (Nrf2) by caveolin-1 promotes stress-induced premature senescence. Mol. Biol. Cell 24, 1852–1862. [PubMed: 23637463]

Volonte D, Zou H, Bartholomew JN, Liu Z, Morel PA, Galbiati F, 2015. Oxidative stress-induced inhibition of Sirt1 by caveolin-1 promotes p53-dependent premature senescence and stimulates the secretion of interleukin 6 (IL-6). J. Biol. Chem 290, 4202–4214. [PubMed: 25512378]

Wang Y, Pennock S, Chen X, Wang Z, 2002. Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival. Mol. Cell. Biol 22, 7279–7290. [PubMed: 12242303]

Wary KK, Mainiero F, Isakoff SJ, Marcantonio EE, Giancotti FG, 1996. The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. Cell 87, 733–743. [PubMed: 8929541]

Wary KK, Mariotti A, Zurzolo C, Giancotti FG, 1998. A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. Cell 94, 625–634. [PubMed: 9741627]

Wei Y, Lukashev M, Simon DI, Bodary SC, Rosenberg S, Doyle MV, Chapman HA, 1996. Regulation of integrin function by the urokinase receptor. Science 273, 1551–1555. [PubMed: 8703217]

Wei Y, Yang X, Liu Q, Wilkins JA, Chapman HA, 1999. A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. J. Cell Biol 144, 1285–1294. [PubMed: 10087270]

Wheaton K, Sampsel K, Boisvert FM, Davy A, Robbins S, Riabowol K, 2001. Loss of functional caveolae during senescence of human fibroblasts. J. Cell. Physiol 187, 226–235. [PubMed: 11268002]

Williams TM, Lisanti MP, 2004. The caveolin proteins. Genome Biol. 5, 214. [PubMed: 15003112]

Wilson E, Leszczynska K, Poulter NS, Edelmann F, Salisbury VA, Noy PJ, Bacon A, Rappoport JZ, Heath JK, Bicknell R, Heath VL, 2014. RhoJ interacts with the GIT-PIX complex and regulates focal adhesion disassembly. J. Cell. Sci 127, 3039–3051. [PubMed: 24928894]

Wolfson M, Budovsky A, Tacutu R, Fraifeld V, 2009. The signaling hubs at the crossroad of longevity and age-related disease networks. Int. J. Biochem. Cell Biol 41, 516–520. [PubMed: 18793745]

Wu Y, Matsui H, Tomizawa K, 2009. Amphiphysin I and regulation of synaptic vesicle endocytosis. Acta Med. Okayama 63, 305–323. [PubMed: 20035287]

Zhang X, Ramirez CM, Aryal B, Madrigal-Matute J, Liu X, Diaz A, Torrecilla-Parra M, Suarez Y, Cuervo AM, Sessa WC, Fernandez-Hernando C, 2020. Cav-1 (Caveolin-1) deficiency increases autophagy in the endothelium and attenuates vascular inflammation and atherosclerosis. Arterioscler. Thromb. Vasc. Biol 40, 1510–1522. [PubMed: 32349535]

Zhao ZS, Manser E, Loo TH, Lim L, 2000. Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. Mol. Cell. Biol 20, 6354–6363. [PubMed: 10938112]

Zhou W, Li X, Premont RT, 2016. Expanding functions of GIT Arf GTPase-activating proteins, PIX Rho guanine nucleotide exchange factors and GIT-PIX complexes. J. Cell. Sci 129, 1963–1974. [PubMed: 27182061]

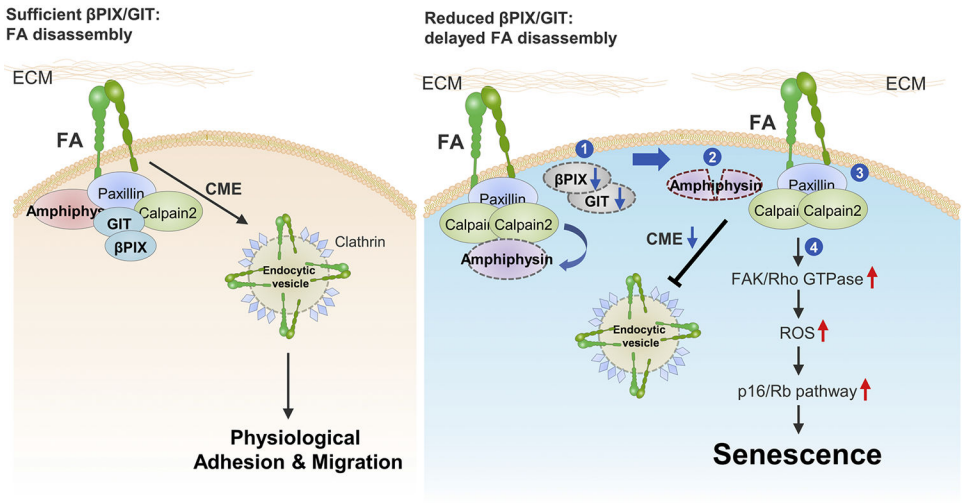

Fig. 1.

A model for the role of βPIX/GIT complex and CME in senescence. βPIX/GIT complex and calpain 2 localize to focal adhesions by binding to paxillin, which inhibits calpain 2 binding to paxillin, thus preventing calpain 2-mediated cleavage of amphiphysin 1 (left panel). Internalization of β1 integrin via CME maintains normal integrin signaling (left panel). Reduced levels of the βPIX/GIT complex in senescent cells allow calpain 2 binding to paxillin, resulting in cleavage of amphiphysin 1 (right panel, ①②). Excessive accumulation of cell surface active β1 integrin results in persistent and abnormal signaling, including excessive ROS, which accelerates senescence (right panel,③④).

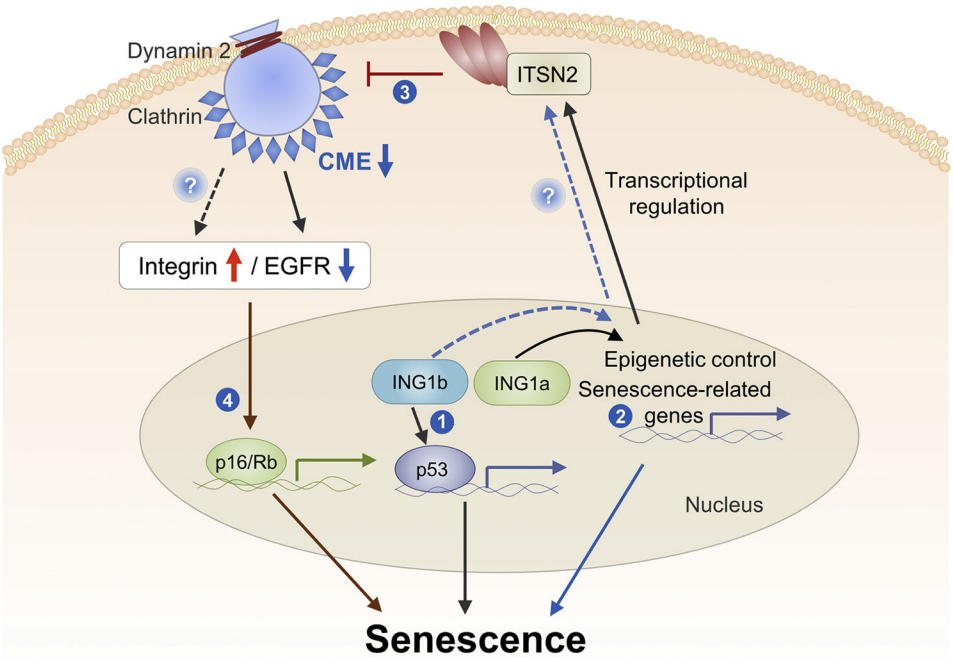

Fig. 2.

A model for ING1-induced senescence. ING1b regulates p53 function and epigenetic control by modulating chromatin structure (①②). ING1a upregulates ITSN2 through epigenetic control (②), which in turn inhibits CME (③). This may result in activation of integrin signaling, while inhibiting EGFR signaling. Downstream of these signaling events, the p16/Rb pathway induces cell cycle arrest and senescence (③④). It is yet unclear whether ING1b also blocks CME via ITSN2.

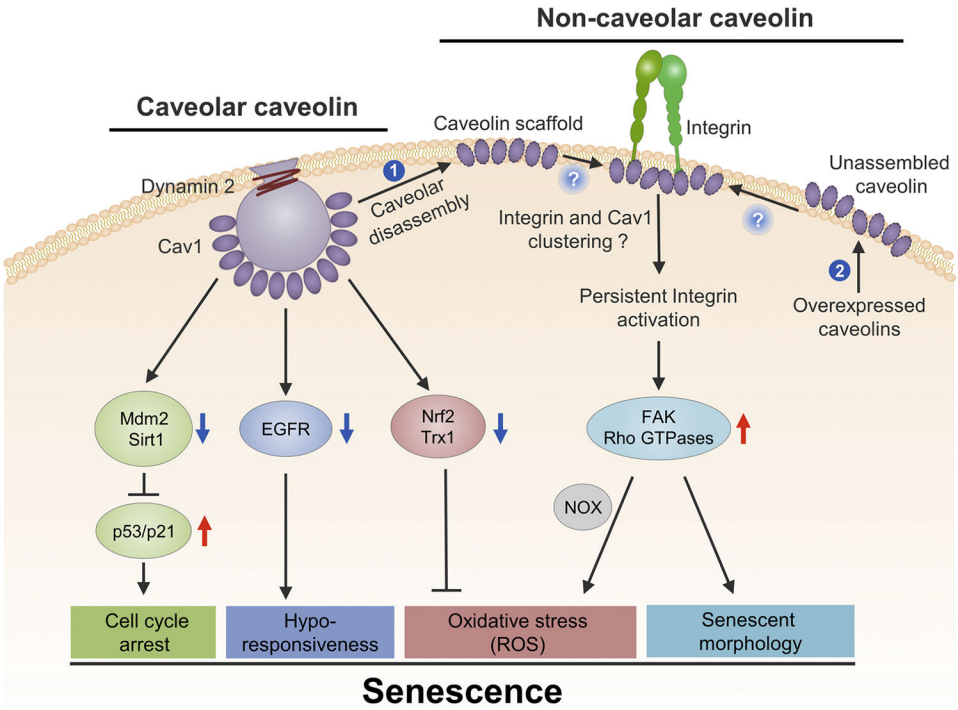

Fig. 3.

A model for Cav1-induced senescence. In general, caveolar Cav1 recruits signaling molecules mediated by interactions with its scaffolding domain and sequesters them into an inactive state. Non-caveolar Cav1 can be produced by dissociation of caveolae in response to mechanical stress (Sinha et al., 2011) (①), or when caveolins are overexpressed (Hayer et al., 2010; Parton and Howes, 2010) (②). This pool can translocate to focal adhesions where it can form oligomers with β1 integrin, which results in integrin activation (Kawabe et al., 2004). Non-caveolar Cav1 is also present in diverse cellular compartments where it regulates distinct pathways including integrin activation and function (Head and Insel, 2007; Pol et al., 2020).

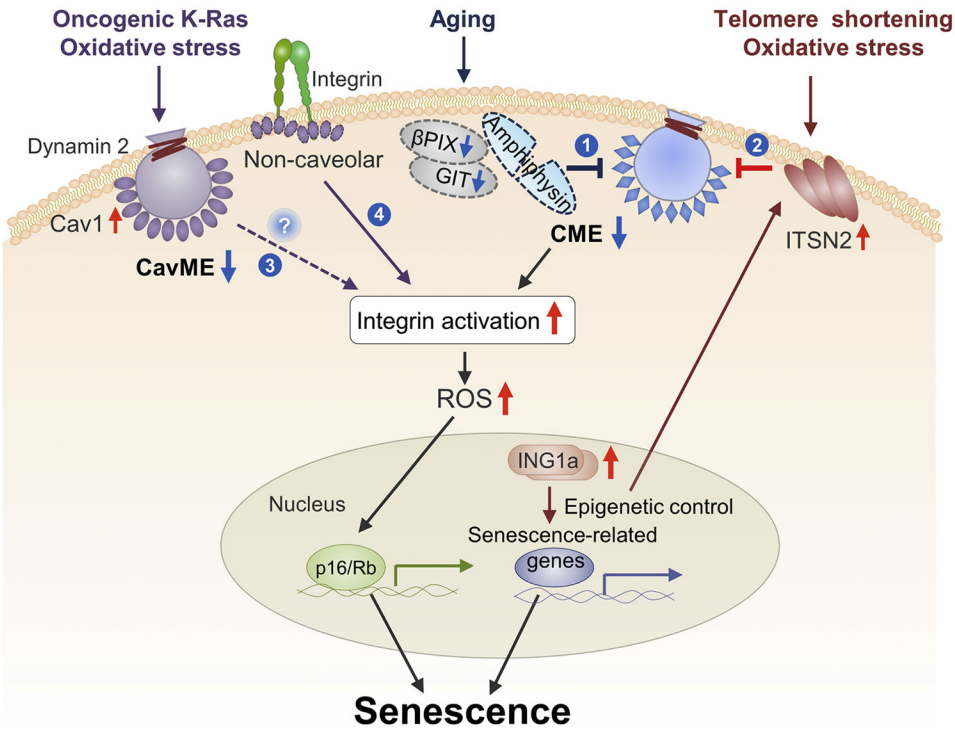

Fig. 4.

A model for the central role of integrin activation in aberrant endocytosis-induced senescence. Reduced βPIX/GIT complex and upregulated ING1a inhibit CME, which results in activation of integrin signaling (①②). Caveolar and non-caveolar Cav1 contribute to integrin activation, though it is unclear to what extent they do (③④). This model highlights the complex interplay between key endocytic proteins and senescence regulators. Overall, reduced endocytosis that deregulates signaling of membrane receptors may be a common mediator for multiple senescence pathways.