



Emerging roles of extracellular vesicles in cellular senescence and aging

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Summary

Cellular senescence is a cellular program that prevents the proliferation of cells at risk of neoplastic transformation. On the other hand, age-related accumulation of senescent cells promotes aging at least partially due to the senescence-associated secretory phenotype, whereby cells secrete high levels of inflammatory cytokines, chemokines, and matrix metalloproteinases. Emerging evidence, however, indicates that extracellular vesicles (EVs) are important mediators of the effects of senescent cells on their microenvironment. Senescent cells secrete more EphA2 and DNA via EVs, which can promote cancer cell proliferation and inflammation, respectively. Extracellular vesicles secreted from DNA-damaged cells can also affect telomere regulation. Furthermore, it has now become clear that EVs actually play important roles in many aspects of aging. This review is intended to summarize these recent progresses, with emphasis on relationships between cellular senescence and EVs.

KEYWORDS

aging, cellular senescence, cytoplasmic DNA, EphA2, extracellular vesicles, telomere

1 | INTRODUCTION

1.1 | Cellular senescence and senescence-associated secretory phenotype (SASP)

Cellular senescence prevents the proliferation of cells exposed to potentially oncogenic stresses, such as DNA-damaging reagents, irradiation, telomere shortening, and oncogene activation (Kuilman, Michaloglou, Mooi & Peeper, 2010; Rodier & Campisi, 2011). Mutations in genes essential for the senescence-induced cell cycle arrest predispose cells to immortalization and shorten lifespan by increasing cancer incidence. However, cellular senescence not only arrests the cell cycle but also changes how the cell impacts its microenvironment. The way in which senescent cells influence their microenvironment is highly context dependent (Muñoz-Espín & Serrano, 2014). It promotes tumor development in many cases, but can also be tumor suppressive in certain circumstances. Removal of senescent cells that accumulated in the body during aging alleviates atherosclerosis,

hepatic steatosis, tumor development, and functional declines of heart, kidney, and fat tissues, resulting in prolonged healthspan and lifespan (Baar et al., 2017; Baker et al., 2016; Childs et al., 2016; Ogrodnik et al., 2017). These effects may be attributable to so-called senescence-associated secretory phenotype (SASP), whereby cells secrete high levels of inflammatory cytokines, chemokines, growth factors, and metalloproteinases (Coppé, Desprez, Krtolica & Campisi, 2010; Coppé et al., 2008). Among these SASP factors, for example, amphiregulin promotes cancer cell proliferation (Bavik et al., 2006); IL-6, IL-8, and CCL2 promote cancer cell invasion (Coppé et al., 2008; Ohanna et al., 2011); VEGF promotes angiogenesis (Coppé, Kauser, Campisi & Beauséjour, 2006); IL-6, IL-8, IGFBP7, and PAI-1 reinforce cellular senescence (Acosta et al., 2008; Kortlever, Higgins & Bernards, 2006; Kuilman et al., 2008; Wajapeyee, Serra, Zhu, Mahalingam & Green, 2008); TGF- β family ligands, CCL2, CCL20, and VEGF transmit cellular senescence (Acosta et al., 2013); and PDGF-AA promotes wound healing (Demaria et al., 2014). In senescent cells, these SASP factors are mostly activated at transcriptional

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level by NF- κ B and C/EBP β (Acosta et al., 2008; Chien et al., 2011; Kuilman et al., 2008).

1.2 | Extracellular vesicles (EVs)

Although the involvement of typical secretory proteins in the non-cell-autonomous effects of senescent cells has been well studied, the functions of membrane-enclosed vesicles secreted by senescent cells have not been studied until recently. These extracellular vesicles (EVs) were once thought to be cellular trash, but now it is clear that they are critical mediators in intercellular communication (Raposo & Stoorvogel, 2013). Emerging evidence indicates that EVs also play important roles in cellular senescence and aging (Figure 1). This field is rapidly advancing especially since Valadi et al. (2007) reported that EVs deliver functional RNA to the recipient cells. Extracellular vesicles contain a huge variety of proteins and nucleic acids in a cell type-dependent manner. Extracellular vesicles are actually heterogeneous population (Bobrie, Colombo, Krumeich, Raposo & Théry, 2012; Kowal et al., 2016; Raposo & Stoorvogel, 2013) that can be classified based on their origin. The most well-studied EVs are exosomes (Colombo, Raposo & Théry, 2014), which originate as intraluminal vesicles (ILVs) in the late endosomal compartment through inward budding of the endosomal membranes. Exosomes are secreted from the cells once the vesicle containing ILVs, namely multivesicular body (MVB), fuse with the plasma membrane. Biogenesis of ILVs involves ESCRT complexes (Colombo et al., 2013), tetraspanins (Andreu & Yáñez-Mó, 2014), ceramide (Trajkovic et al., 2008), or their combination. In the ESCRT-dependent pathway, ESCRT-0 complex first recruits cargos and ESCRT-I/-II complexes to the endosomal membrane, then ESCRT-I/-II complexes induce bud formation, and ESCRT-III drives scission of ILVs. Ubiquitinated proteins are recognized by the ESCRT-0/-I/-II complexes during these processes and are enriched in exosomes (Hanson & Cashikar, 2012). In what way tetraspanins and ceramide induce ILV formation is not well understood. However, it has been shown that tetraspanin CD81 is required for sorting its interacting proteins into exosomes (Perez-Hernandez et al., 2013). There are also some mechanisms that target specific miRNAs to exosomes. RNA-binding proteins such as hnRNP A2B1, YBX1, SYNCRIP, and MEX3C are at least a few involved in this process (Lu et al., 2017; Santangelo et al., 2016; Shurtleff, Temoche-Díaz, Karfilis, Ri & Schekman, 2016; Villarroja-Beltri et al., 2013). The Rab family proteins that regulate intracellular vesicular trafficking required for exosome secretion include at least Rab7 (Baietti et al., 2012), Rab11 (Savina, Vidal & Colombo, 2002), Rab27A/B (Ostrowski et al., 2010), and Rab35 (Hsu et al., 2010). Based on their localization, it is proposed that Rab27A/B regulates the plasma membrane trafficking of more matured MVB compared to Rab11 and Rab35 (Kowal, Tkach & Théry, 2014). The exosome biogenesis pathway that predominates depends on the cell type. Once secreted, exosomes can be taken up by recipient cells through endocytosis, phagocytosis, macropinocytosis, or membrane fusion (Mulcahy, Pink & Carter, 2014). Exosomal membrane proteins have the same

topology as the cells and can target exosomes to specific cell type. Other classes of EVs include, but are not limited to, microvesicles and apoptotic bodies. Although many proteins such as ESCRT proteins and tetraspanins are enriched in exosomes and are used as exosome markers, they are not necessarily strictly exosome specific (Witwer et al., 2013). It is therefore difficult to attribute the given function of EVs exclusively to specific EV subpopulation. For this reason, although most of the references cited herein are focused on exosomes or at least exosome-like EVs, the general term “EVs” is used in this review instead of referring to specific EV subpopulations.

2 | CELLULAR SENESCENCE INCREASES EV SECRETION

Senescence-associated increase in EV secretion was first described by Lehmann et al. (2008). This increase seems to be a general feature of cellular senescence and has been observed in fibroblasts, epithelial cells, and cancer cells. Cellular senescence triggered by serial passaging, irradiation, DNA-damaging reagents, and oncogenic Ras expression all enhance EV secretion, with an increase of more than 10-fold in most cases (Lehmann et al., 2008; Takahashi et al., 2017; Takasugi et al., 2017). This increase is at least partially mediated by p53 (Lehmann et al., 2008) and one of its targets, TSAP6 (Lespagnol et al., 2008; Yu, Harris & Levine, 2006), although the mechanism whereby TSAP6 regulates EV secretion is not well understood. In addition, p53 induces several genes involved in endosome regulation, such as Caveolin-1 and the ESCRT-III subunit Chmp4 (Yu, Riley & Levine, 2009). It has also been reported that cellular senescence of cervical cancer cells induces expression of several Rab genes including Rab5B, which plays important roles in endosome maturation (Wells et al., 2003). Another report showed that expression of Rab27B, which promotes the secretion of EVs, is enhanced in senescent fibroblasts (Fujii et al., 2006). It is known that EV secretion contributes to the clearance of harmful molecules in the cells (Desdín-Micó & Mittelbrunn, 2017), such as cytoplasmic DNA. As described later in this review, EV-mediated removal of cytoplasmic DNA is essential for the survival of senescent cells (Takahashi et al., 2017), which may explain why EV secretion is increased in senescent cells.

3 | SENESCENT CELLS STIMULATE MITOGENIC PATHWAY IN CANCER CELLS THROUGH EV-ASSOCIATED EPHA2

A recent study has shown that EVs are important mediators of the protumorigenic effect of the senescent cell secretome (Takasugi et al., 2017). In this study, the authors first showed that conditioned media prepared from senescent cells lose their proproliferative effect on MCF-7 breast cancer cells when EVs are removed from the conditioned media. Conversely, EVs purified from senescent cells

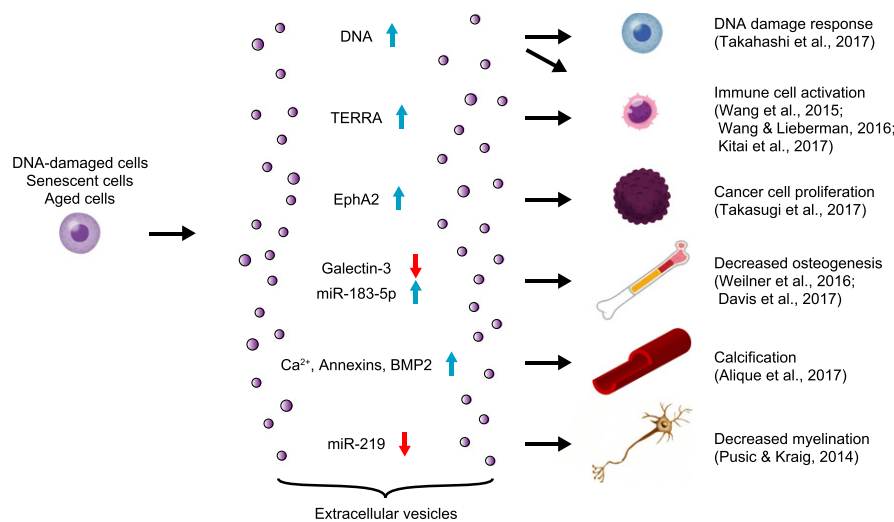


FIGURE 1 The functions of extracellular vesicles (EVs) secreted from DNA-damaged, senescent, or aged cells

promote the proliferation of several types of cancer cells. Proteomic profiling of senescent and nonsenescent cell EVs revealed drastic differences in their contents, including the enrichment of receptor tyrosine kinase EphA2 in senescent cell EVs. The binding of EphA2 to ephrin ligands can activate Erk pathway through so-called reverse signals in ephrin-expressing cells. Indeed, EphA2 was found to be responsible for the proproliferative effect of senescent cell EVs. Remarkably, an antibody targeted to the extracellular region of ephrin-A1 significantly suppresses the proproliferative effect of conditioned medium of senescent cells regardless of whether cellular senescence was induced by serial passaging, oncogenic Ras, or doxorubicin. Interestingly, EphA2 tends to be upregulated in breast and ovary cancer stroma. It is also known that ephrin-A1 is upregulated in many types of cancer cells (Beauchamp & Debinski, 2012). Moreover, a recent study showed that the plasma levels of EV-associated EphA2 are increased in pancreatic cancer patients (Liang et al., 2017). These facts suggest the contribution of EV-associated EphA2 to cancer development.

Selective increase of EphA2 in senescent cell EVs is regulated by both expression level and post-translational modification of EphA2. It is known that activated p53 increases EphA2 expression (Dohn, Jiang & Chen, 2001). In some types of senescent cells, however, EphA2 is increased in EVs without any changes in its expression level, such as in Ras-induced senescent cells. PTP1B phosphatase, which suppresses autophosphorylation of EphA2 (Sabet et al., 2015), is oxidatively inactivated in senescent cells due to elevated reactive oxygen species (ROS) levels, resulting in endosomal internalization of EphA2. Endosomal internalization is the first step to sort EphA2 into exosomes, and its inhibition through PTP1B overexpression abrogates EV-mediated secretion of EphA2. Therefore, non-cell-autonomous protumorigenic effects of senescent cells are not only regulated by known mechanisms of SASP regulation, but also by ROS-regulated cargo sorting into EVs.

4 | EV-MEDIATED SECRETION OF CYTOPLASMIC DNA: IMPLICATIONS IN INFLAMMATION

Extracellular vesicles contain various lengths of genomic DNA fragments without any noticeable sequence specificity (Kahlert et al., 2014; Thakur et al., 2014) and seem to be one of the major routes of DNA secretion (Fernando, Jiang, Krzyzanowski & Ryan, 2017). DNA associated with the outer membrane of EVs is larger and mostly double-stranded, whereas both single-stranded DNA and double-stranded DNA are abundant inside the EVs (Thakur et al., 2014). Extracellular vesicle-mediated DNA secretion increases upon cellular senescence (Takahashi et al., 2017). Intriguingly, γ H2AX-positive cytoplasmic chromatin fragments appear in senescent cells, suggesting that damaged DNA may be the major source of EV-associated DNA in senescent cells (Ivanov et al., 2013). Apoptotic DNA fragments, however, are unlikely to be the major source of EV-associated DNA (Takahashi et al., 2017). Inhibition of EV-mediated DNA secretion induces apoptosis in senescent cells (Takahashi et al., 2017), although the autophagy-lysosomal pathway also removes cytosolic DNA (Ivanov et al., 2013; Lan, Londoño, Bouley, Rooney & Hacohen, 2014). Even in nonsenescent cells, inhibition of EV secretion increases cytosolic DNA and induces apoptosis and/or growth arrest in cGAS-STING-dependent manner (Takahashi et al., 2017). cGAS directly binds to cytosolic DNA and thereby activates and synthesizes cyclic GMP-AMP (cGAMP) from ATP and GTP. Cyclic GMP-AMP binds and induces a conformational change in the endoplasmic reticulum-membrane adaptor STING. STING then activates transcription factors such as IRF3, NF- κ B, and STAT6 through TBK1 and IKK (Chen, Sun & Chen, 2016). Emerging studies revealed that STING is critical for the induction of SASP genes and establishment of cellular senescence in vitro and in vivo (Dou et al., 2017; Glück et al., 2017; Yang, Wang, Ren, Chen & Chen, 2017). Therefore, elevated cytosolic DNA is important for the senescent

phenotype, while some of it has to be removed through EVs or autophagy–lysosomal pathway for cell survival. Cancer cells, on the other hand, are more tolerant to cytosolic DNA. It has been shown that inhibition of EV secretion does not affect proliferation of HEK293T, HeLa, and U2OS cells (Takahashi et al., 2017). Nonetheless, inflammatory gene expressions in breast, pancreatic, prostate cancers, and melanoma correlate with STING expression (Dou et al., 2017). Its impact on prognosis, however, depends largely on the type of cancer (Yang et al., 2017). The function of EV-associated DNA is also context dependent. In hormone receptor-positive breast cancer cells, cell-free DNA including EV-associated DNA promotes cell proliferation (Wang et al., 2017). On the other hand, it has been suggested that EV-associated DNA activates antitumor immunity. Antitumor agent topotecan (TPT), an inhibitor of topoisomerase I, induces EV-associated DNA secretion in cancer cells, which in turn activates dendritic cells in a STING-dependent manner. Notably, TPT requires STING to activate immune cells and inhibit tumor growth in mice bearing E0771 breast cancer cells (Kitai et al., 2017). It has also been reported that topoisomerase I inhibitor CPT11 induces EV-associated DNA secretion from cells proliferating in the intestine, which then activates immune cells and causes diarrhea (Lian et al., 2017). In this case, another DNA sensor, AIM2, primarily mediates the effects of EV-associated DNA. In spite of these findings, how EV-associated DNA secreted from senescent cells impacts their microenvironment is still largely unknown. It has been suggested that EV-associated DNAs secreted from senescent cells trigger a DNA damage response in recipient cells (Takahashi et al., 2017), and therefore, it may contribute to reinforce or transmit cellular senescence.

5 | EVS SECRETED FROM DNA-DAMAGED CELLS INDUCE TELOMERE DYSREGULATION

Emerging evidence suggests the involvement of EVs in telomere regulation. It has been shown that EVs secreted from irradiated breast cancer cells suppress telomerase activity and induce telomere shortening in recipient cells (Al-Mayah et al., 2017). Both RNA and protein contents of EVs seem to be contributing to this effect. Another recent paper showed the enrichment of telomeric repeat-containing RNA (TERRA) in EVs (Wang & Lieberman, 2016; Wang et al., 2015). In cells, TERRA is known to have diverse functions in telomere regulation such as repression of telomerase activity (Azzalin & Lingner, 2015). Impairment of telomere integrity by TRF2 inhibition results in increased TERRA levels in cells and EVs (Wang & Lieberman, 2016). Although it is still unclear whether EV-mediated TERRA secretion is enhanced in the context of cellular senescence or aging, it has been shown that telomere shortening, which is involved in replicative senescence and aging, increases expression level of TERRA (Cusanelli, Romero & Chartrand, 2013). Extracellular vesicle-associated TERRA was found to induce inflammatory gene expressions in recipient cells (Wang & Lieberman, 2016; Wang et al., 2015). However, EV-associated TERRA is significantly shorter than intracellular

TERRA, and thus, whether it also affects telomere regulation is not clear.

6 | AGE-RELATED CHANGES IN EVS

A cross-sectional and longitudinal study by Eitan et al. (2017) showed that plasma EV concentration decreases with human age, at least from early 30s to late 60s. They showed that monocyte and B cells but not T cells internalize plasma EVs and that B cells from the elderly uptake more EVs. Interestingly, smoking and higher BMI are both positively correlated with plasma EV concentration. Considering that smoking and obesity trigger cellular senescence in specific tissues (Tsuji, Aoshiba & Nagai, 2004; Yoshimoto et al., 2013), this finding may suggest that EVs secreted from accumulated senescent cells can account for substantial portions of circulating EVs. In addition to the concentration, aging alters RNA and protein composition of EVs. Galectin-3, which plays a role in osteoblast maturation, is reduced in the plasma EVs of elderly people. Cellular senescence decreases EV-mediated galectin-3 secretion of endothelial cells and may be responsible for its age-related reduction. Plasma EVs isolated from young but not elderly donors promote the osteogenic differentiation of mesenchymal stem cells in a galectin-3-dependent manner (Weilner et al., 2016). These findings suggest that reduced EV-mediated secretion of galectin-3 is one of the causes of the age-related decline in bone formation. Another paper showed that large EVs isolated from elderly subjects' plasma or senescent endothelial cells promote the calcification of human aortic smooth muscle cells (Alique et al., 2017). These large EVs secreted from senescent endothelial cells contain increased amounts of calcium, calcium-binding annexins, and BMP2, all of which are involved in vascular calcification. In addition to the plasma EVs, bone marrow EVs of the elderly also show anti-osteogenic effect. Extracellular vesicles purified from the elderly suppress cell proliferation and osteogenic differentiation of bone marrow stromal cells (Davis et al., 2017). The amount of miR-183-5p is increased in aged bone marrow EVs, and the overexpression of miR-183-5p in bone marrow stromal cells mimics the effects of aged bone marrow EVs. This increase in EV-associated miR-183-5p is at least partially attributable to enhanced ROS levels in the aged bone marrow (Davis et al., 2017). Consistently, it has been shown that ROS-induced senescence enhances miR-183 expression (Li, Luna, Qiu, Epstein & Gonzalez, 2009). EV-associated miRNAs-associated miRNAs are also involved in brain aging. Serum EVs isolated from young rats promote oligodendrocyte precursor cell (OPC) differentiation and remyelination in slice cultures. Moreover, nasal administration of young EVs increases myelination in aged rat brain. Mechanistically, it has been shown that young EVs contain high levels of miR-219, which reduces the expression of inhibitory regulators of OPC differentiation. Indeed, transfection of miR-219 inhibitor along with the administration of young EVs abolishes the beneficial effects of EVs (Pusic & Kraig, 2014). Finally, a recent report by Zhang et al. (2017) demonstrated that administration of

EVs isolated from young cells ameliorates age-related functional declines in mice. This study focused on hypothalamic neural stem cells (htNSCs) and showed that the number of htNSC and thus htNSC-derived EVs decrease with age. Selective ablation of htNSCs accelerates age-related functional declines such as decreases in treadmill performance and cognitive functions, resulting in a shorter lifespan. Administration of EVs isolated from newborn-derived htNSCs suppresses this acceleration of aging to at least some extent. The number and miRNA content of htNSC-derived EVs are much higher than those of astrocyte-derived EVs. Indeed, htNSC-derived EVs significantly contribute to the miRNA profile in the cerebrospinal fluid, suggesting that miRNA may be mediating the anti-aging effects of htNSC-derived EVs. As summarized above, EVs have been implicated in many aspects of aging in the past few years. At least some of these age-related changes in EVs could be attributed to cellular senescence, further supporting the usefulness of targeting senescent cells as a treatment for aging and age-related diseases.

7 | CONCLUSION

The importance of cellular senescence in aging and age-related diseases is increasingly appreciated, and methods to remove senescent cells or suppress SASP are currently under intensive studies. A better understanding of how senescent cells impact their microenvironment is necessary for efficient drug development. Notably, recent findings implicate senescent cell EVs in cancer development (Takasugi et al., 2017), vascular calcification (Alique et al., 2017), and age-related decline in bone formation (Weilner et al., 2016). Increased secretion of EV-associated DNA from senescent cells (Takahashi et al., 2017) is likely to be pro-inflammatory (Kitai et al., 2017; Lian et al., 2017) and may contribute to age-related chronic inflammation. Whether senescent cell EVs promote or suppress cancer development may be context dependent. Extracellular vesicle-associated EphA2 secreted from senescent cells stimulates mitogenic pathway in cancer cells (Takasugi et al., 2017). Extracellular vesicle-associated DNA can also promote cancer cell proliferation through unknown mechanisms (Wang et al., 2017). On the other hand, EV-associated DNA can induce antitumor immunity (Kitai et al., 2017). Despite these progresses, it should be noted that the functions of senescent cell EVs are still understudied, at least partially due to inadequate understanding of EVs themselves. This research field is immature and the methods used are not sufficiently standardized yet. In this regard, guidelines provided by the International Society for Extracellular Vesicles would be useful for researchers (Mateescu et al., 2017; Witwer et al., 2013). Nevertheless, EVs have now shown to be critical players in cellular senescence and aging, and more functions will be revealed in the future as the EV research field matures. As there seem to be substantial differences between regulation of EVs and known SASP factors in senescent cells, further investigation of EVs will enable more specific modulation of the senescent cell secretome.

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CONFLICT OF INTEREST

None declared.

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