Molecules and Cells

Minireview

The Role of Extracellular Vesicles in Senescence

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Cells can communicate in a variety of ways, such as by contacting each other or by secreting certain factors. Recently, extracellular vesicles (EVs) have been proposed to be mediators of cell communication. EVs are small vesicles with a lipid bilayer membrane that are secreted by cells and contain DNA, RNAs, lipids, and proteins. These EVs are secreted from various cell types and can migrate and be internalized by recipient cells that are the same or different than those that secrete them. EVs harboring various components are involved in regulating gene expression in recipient cells. These EVs may also play important roles in the senescence of cells and the accumulation of senescent cells in the body. Studies on the function of EVs in senescent cells and the mechanisms through which nonsenescent and senescent cells communicate through EVs are being actively conducted. Here, we summarize studies suggesting that EVs secreted from senescent cells can promote the senescence of other cells and that EVs secreted from nonsenescent cells can rejuvenate senescent cells. In addition, we discuss the functional components (proteins, RNAs, and other molecules) enclosed in EVs that enter recipient cells.

Keywords: cellular senescence, circular RNA, exosome, extracellular vesicle, long noncoding RNA, microRNA

INTRODUCTION

In the early 1960s, Hayflick discovered that the number of times that human cells can divide before senescence is limited (Hayflick and Moorhead, 1961). Cellular senescence is a key feature of aging, and senescent cells accumulate in aging human tissues (Dimri et al., 1995). Senescent cells, which are enlarged and flattened, express senescence-associated β-galactosidase as well as distinct patterns of transcripts and proteins, including $p16^{INK4a}$ (CDKN2A), p21 (CDKN1A), DPP4, and SCAMP4 (Casella et al., 2019; Dimri et al., 1995; Kim and Kim, 2021; Kim et al., 2017, 2018; Munk et al., 2021). Senescent cells also produce and secrete inflammatory proteins, including interleukins (ILs), chemokines, and growth factors. This characteristic production and secretion pattern in senescent cells is defined as the senescence-associated secretory phenotype (SASP), which affects neighboring cells (Kuilman and Peeper, 2009; Malaquin et al., 2016; Young and Narita, 2009).

In a multicellular organism, cells communicate with each other through various signals, such as pressure, voltage, small molecules, or peptides. Recently, extracellular vesicles (EVs) have been identified as mediators of intercellular communication. EVs have a lipid bilayer and are classified as exosomes (30-150 nm in diameter), microvesicles (100-1,000 nm in diameter), or apoptotic bodies (50-5,000 nm in diameter), depending on their size and origin. Exosomes are derived from multivesicular bodies (MVBs) that fuse to the plasma membrane, while microvesicles are directly produced by evagination of the plasma membrane, and apoptotic bodies are produced by the membrane of apoptotic cells (Borges et al., 2013; Doyle and Wang, 2019; Yáñez-Mó et al., 2015; Zaborowski et al., 2015).

EVs encapsulate and carry components such as proteins, lipids, DNA, and various RNAs (e.g., messenger RNA [mRNA], microRNAs [miRNAs], long noncoding RNAs [lncRNAs],

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and circular RNAs [circRNAs]) and can affect recipient cells (Raposo and Stoorvogel, 2013; Zhang et al., 2017). Both mRNAs and noncoding RNAs (ncRNAs) (miRNAs, lncRNAs, circRNAs, etc.) are transcribed and processed, but ncRNAs do not produce protein (Guttman et al., 2013). miRNAs are small ncRNAs (~22 nt) that interact with the 3' untranslated regions (UTRs) of target mRNAs to inhibit translation (Fabian and Sonenberg, 2012). LncRNAs are longer than 200 nt and play important roles in various biological processes, such as the development of the immune response (Noh et al., 2018). CircRNAs are functional molecules in which the 3' and 5' ends are joined to form a circular structure. Some common circRNAs may block miRNA function by binding to miRNAs, thereby acting as miRNA sponges (Hsiao et al., 2017).

Recently, many studies have demonstrated the function of EVs in senescent cells. EVs are secreted at higher levels by senescent cells than by proliferating cells (Hernandez-Segura et al., 2018; Jakhar and Crasta, 2019; Riquelme et al., 2020). Circulating EVs derived from senescent cells have the potential to affect other cells (Jakhar and Crasta, 2019). Therefore, recent studies have suggested that senescent cell-derived EVs are part of the SASP (Misawa et al., 2020). In addition, interesting reports have suggested that EVs secreted from cells under certain conditions can affect rejuvenation by affecting senescent cells (Zhang et al., 2019; 2020; Zhao et al., 2021). Since many studies have reported the relationship between senescence and EVs, it is necessary to clarify their functions. In this review, we summarize the effects of EV components derived from senescent cells and, conversely, the regulatory effects of EV components secreted by other cells on senescent cells.

THE EFFECTS OF EV COMPONENTS DERIVED FROM SENESCENT CELLS

Proteins

Increasing evidence suggests that proteins in senescent cell-derived EVs contribute to diverse cellular functions. For example, in 2017, Takasugi et al. (2017) performed mass spectrometry using control and doxorubicin-treated senescent RPE-1 cells and found that EphA2 was abundant in EVs secreted from senescent cells but not in EVs secreted from control cells. They demonstrated that EV-associated EphA2 promoted cancer cell proliferation through interaction with Ephrin-A1, which is highly expressed in multiple types of cancer cells. These findings support the notion that senescent cell-derived EVs promote the growth of cancer cells (Takasugi et al., 2017). Another study revealed that the accumulation of growth factors in EVs derived from senescent cholangiocytes contributed to changes in targeted cells. Many growth factors, including epidermal growth factor (EGF), are enriched in EVs derived from senescent cholangiocytes but not in normal human cholangiocytes (NHCs)-derived EVs. To investigate the effects of senescence-associated EVs on target cells, Al Suraih et al. (2020) treated NHCs and malignant human cholangiocytes (MHCs) with EVs secreted from senescent cholangiocytes. They observed that NRAS (neuroblastoma-ras) and ERK1/2 (extracellular signal-regulated kinase 1/2) activation in NHCs was promoted in an EGF-dependent manner, which in turn promoted cell proliferation. In addition, they showed that senescence-associated EVs increased the proliferation and migration of MHCs. Moreover, EVs isolated from senescent cholangiocytes affected the immune regulatory pathway by accelerating the migration of human monocytes and upregulating the EGF-dependent expression of TNF-α and IL-1β in THP-1 cells (Al Suraih et al., 2020).

In other studies, Alique et al. (2017) reported the function of senescent endothelial cell-derived EVs on the calcification of human aortic smooth muscle cells (HASMCs). EVs secreted from senescent human umbilical vein endothelial cells (HUVECs) contain higher amounts of the annexin proteins A2 and A6, BMP2 (bone morphogenic protein 2), and calcium than EVs secreted from young HUVECs. Therefore, Alique et al. (2017) concluded that senescent EVs promote the calcification of SMCs and suggested that these abundant proteins may be involved in the process. This EV promotion of calcification has been consistently demonstrated in experiments with EVs isolated from the plasma of elderly and young subjects (Alique et al., 2017). Galectin-3 was found to be downregulated not only in plasma EVs of elderly individuals but also in EVs secreted by senescent HUVECs. Reduction of galectin-3 expression in EVs reduced the osteogenic differentiation capacity in mesenchymal stem cells (MSCs) (Weilner et al., 2016). Recently, it was found that IFN (interferon)-induced transmembrane protein 3 (IFITM3) was secreted from cells by small EVs in senescent cells induced through overexpression of oncogenic H-RAS^{G12V}. Secreted IFITM3 was taken up by normal HFFF2 human foreskin primary fibroblasts and induced cellular senescence (Borghesan et al., 2019).

Furthermore, a recent study showed that heat shock protein 70 (HSP70), which is on the surface of EVs, may contribute to cardiac fibrosis during cellular senescence. Reduced expression of HSP70 in serum EVs in old rats, compared to that in young rats, increased fibroblast proliferation and myofibroblast differentiation (Yang et al., 2019).

miRNAs

A number of miRNAs have been identified in EVs secreted from senescent cells and have been proposed to function in recipient cells following their uptake. First, we summarize multiple miRNAs involved in the promotion of cell senescence. Notably, recent evidence has shown that miR-139-5p levels are increased in senescent osteoblast-derived EVs compared to control EVs. Additionally, senescent osteoblasts can promote the senescence and apoptosis of endothelial cells by secreting miR-139-5p in EVs. To investigate whether miR-139-5p can directly induce senescence and apoptosis of endothelial cells, experiments in which miR-139-5p was overexpressed or inhibited were performed. Overexpression of miR-139-5p induced the senescence and apoptosis of endothelial cells, whereas inhibition of miR-139-5p expression led to opposite results. These findings suggest that senescence of endothelial cells can be promoted by miR-139-5p accumulated in EVs secreted from senescent osteoblasts (Lu et al., 2021).

Another study reported the function of small EVs secreted from senescent HUVECs. Senescent HUVECs secreted a high number of small EVs containing miR-21-5p and miR-217,

which were transported and internalized by nonsenescent HUVECs. Moreover, miR-21-5p and miR-217 transported by small EVs reduced the expression levels of DNMT1 and SIRT1 in recipient cells. In addition, treatment of nonsenescent cells with small EVs derived from senescent HUVECs led to decreased proliferation and an increased senescent phenotype (Mensa et al., 2020). Using a miRNA array, Davis et al. (2017) demonstrated a difference in miRNA expression in EVs derived from the bones of old and young mice. Specifically, they found that the levels of miR-183-5p were increased in EVs secreted from the bones of aged mice. To investigate the function of EVs in aged mice, young primary bone marrow stromal cells (BMSCs) were treated with high-dose EVs. The results of this experiment confirmed that the osteogenic differentiation of the BMSCs was inhibited. In addition, overexpression of a miR-183-5p mimic promoted senescence and reduced the proliferation and differentiation of BMSCs. A significant decrease in the Hmox1 (heme oxyenase-1) protein, which is repressed by miR-183-5p, was also identified. Taken together, these results suggest that aged EVs from bone marrow with increased miR-183-5p may reduce BMSC proliferation and increase stem cell senescence (Davis et al., 2017).

Several studies have revealed the contribution of miRNAs to the repair of damaged cells by senescent cell-derived EVs. A miRNA secreted by EVs, miR-221-3p, has been reported to enhance angiogenesis and cardiac repair capacity of MSCs. Interestingly, the accumulation of miR-221-3p was significantly reduced in EVs secreted from aged MSCs compared to EVs from young MSCs.

EVs secreted from aged MSCs exhibited poor regenerative and cardiac repair function capacities, including proliferation, migration and inhibition of apoptosis and angiogenesis. Loss of the cardiac cell repair abilities of aged EVs was associated with decreased expression of miR-221-3p in EVs. This finding appears to have been a result of decreased miR-221-3p function, which led to activated Akt kinase by reducing the expression of PTEN (phosphatase and tensin homolog). Additionally, aged MSCs were rejuvenated and exhibited restored cardiac cell repair capacity when miR-221-3p was overexpressed in aged EVs (Sun et al., 2020).

Other regulatory factors in EVs

In addition to proteins and miRNAs, there are reports that long-chain C24:1 ceramides are secreted by EVs and serve as regulators of MSCs. C24:1 ceramide was found to be abundant in EVs of older women compared to younger women. EVs containing C24:1 were taken up by primary BMSCs and eventually induced cellular senescence (Khayrullin et al., 2019). Wong et al. (2019) identified that EVs secreted from senescent HUVECs reduced the growth kinetics and barrier integrity of young HUVECs. These functions were realized by repressing the expression levels of adherens junction proteins, including vascular endothelial-cadherin and beta-catenin. Young HUVECs treated with senescence-derived EVs eventually showed reduced potential for impairing endothelial cell migration, indicating that the accumulation of senescent endothelial cells may have led to endothelial barrier dysfunction mediated by exposure to EVs (Wong et al., 2019). Another study demonstrated a role for senescent fibroblast-derived EVs in regulating epidermal homeostasis. In contrast to EVs derived from young human dermal fibroblasts (HDFs), EVs derived from senescent HDFs inhibited keratinocyte differentiation and downregulated barrier function (Choi et al., 2020).

In another example, the generation and secretion of small EVs were found to be increased in senescent stromal cells, significantly altering gene expression patterns in recipient cancer cells and changing cellular functions, specifically increasing the aggressiveness of the cancer cells, for instance, by increasing drug resistance. Notably, a decrease in the level of SIRT1 (as seen in senescence) in senescent stromal cells induced downregulation of ATP6V1A expression, which in turn increased the production and secretion of small EVs. Overproduction and ectopic release of these EVs from senescent stromal cells led to increased drug resistance of recipient cancer cells. This enhanced drug resistance of cancer cells was blocked by inhibiting SRT2104, an agonist of SIRT1 (Han et al., 2020). In addition to the aforementioned functions of senescent cell-derived EVs, Miyazoe et al. (2020) reported that senescent cell-derived EVs contributed to the promotion of hepatocellular carcinoma (HCC) carcinogenesis. The senescence of human hepatic stellate cells (HHSteCs) was induced by etoposide treatment, as confirmed by the altered expression of various senescence markers. In addition, these authors investigated EVs secreted from HHSteCs that had not been treated with etoposide and found that the EVs secreted from senescent HHSteCs increased EGF secretion by macrophages compared to the amount of EGF carried by normal EVs. Increased EGF secreted by these macrophages eventually promoted HCC development (Miyazoe et al., 2020).

THE EFFECTS OF EV COMPONENTS AS SENESCENCE REGULATORS

Proteins

In this section, we summarize the mechanisms through which EVs secreted by nonsenescent cells affect cell senescence. Small EVs derived from fibroblasts obtained from young people have been reported to attenuate the senescence of fibroblasts in elderly individuals and patients with Hutchinson-Gilford progeria syndrome. In addition, EVs derived from young human fibroblasts showed intrinsic glutathione-S-transferase (GST) activity, as indicated by high levels of the glutathione-related protein (GSTM2) protein. GSTM2-overexpressing EVs have been shown to reduce the senescence of fibroblasts from old donors. Intraperitoneal injection of EVs derived from young sources into mice attenuated cellular senescence in the lung, liver, kidney, and brown adipose tissue (Fafián-Labora et al., 2020). Another research team reported that EVs derived from embryonic stem cells (ES) can induce rejuvenation of senescent MSCs. EVs secreted from ES contain insulin-like growth factor 1 (IGF1), which induces an antisenescence effect by activating the IGF1/PI3K/AKT pathway of senescent MSCs (Zhang et al., 2019). A recent study showed that plasma extracellular nicotinamide phosphoribosyltransferase (eNAMPT) is engulfed by EVs and that its levels are decreased in older mice and elderly individuals. EVs containing eNAMPT derived from young mice were found to increase NAD^+

synthesis and significantly extend the lifespan of aged mice (Yoshida et al., 2019).

miRNAs

Dong et al. (2019) observed that serum and EVs from systemic lupus erythematosus (SLE) patients induced the senescence of MSCs. They found that the miR-146a level was reduced in SLE patient EVs compared to healthy control EVs. Therefore, they transfected SLE MSCs with a miR-146a mimic and observed downregulated cellular senescence. On the other hand, normal MSCs transfected with miR-146a inhibitors showed higher rates of cellular senescence (Dong et al., 2019). In addition, it has been reported that miR-424-5p of EVs derived from HEK293T cells overexpressing miR-424-5p induces the senescence of granulosa cells (Yuan et al., 2021). In another study, endothelial cells secreted miR-214 through EVs, preventing the senescence of other endothelial cells and inducing endothelial cell migration and angiogenesis (van Balkom et al., 2013).

Zhang et al. (2020) found that BMSCs from old individuals (OMSCs) were rejuvenated by EVs secreted from human umbilical cord MSCs (UMSCs). The expression of senescence markers such as SA-β-gal, p53, p21, and p16 in OMSCs was decreased by treatment with EVs secreted from UMSCs. Zhang et al. (2020) also demonstrated that SIRT1 protein expression and the proliferation and migration of OMSCs were increased. By analyzing age-related miRNAs in a database, they found that UMSC EVs contained higher amounts of miR-136 than OMSCs. Moreover, miR-136 in UMSC-derived EVs attenuated the senescence of OMSCs by targeting apoptotic peptidase activating factor 1 (Apaf1) (Zhang et al., 2020).

lncRNAs

Recent evidence has shown that lncRNAs secreted by EVs can regulate the senescence of recipient cells. UMSC-derived EVs have been reported to secrete high levels of metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) lncRNA, which prevents aging-induced cardiac dysfunction by regulating NF-κB/TNF-α signaling in H9C2 cardiomyocytes. In contrast, EVs with *MALAT1* expression reduced by small interfering (si)RNA did not prevent cellular senescence (Zhu et al., 2019). Another report demonstrated that EVs derived from MSCs under hypoxia contained high levels of lncRNA *MALAT1*. *MALAT1* targeted and reduced the expression of miRNA-92a-3p, preventing miRNA-92a from targeting ATG4a, which in turn alleviated the senescence of cardiomyocytes. The authors of this study further proposed that these EVs be included in a potential therapeutic strategy for doxorubicin-induced cardiomyopathy because EVs derived from hypoxic MSCs contain abundant *MALAT1* (Xia et al., 2020).

Another research group demonstrated that the lncRNA *NEAT1* in EVs derived from macrophage migration inhibitory factor (MIF)-treated MSCs protected cardiomyocytes against doxorubicin-induced senescence by sponging miR-221-3p. This group specifically found that EVs derived from MIF-treated MSCs alleviated doxorubicin-induced senescence and cardiac injury. A microarray analysis of EVs from MIF-treated and untreated MSCs revealed that EVs derived from MIF-treated MSCs expressed *NEAT1* in higher abundance. The increase in the expression of miR-221-3p in cardiomyocytes induced by doxorubicin treatment was decreased by treatment with EVs derived from MIF-pretreated MSCs. Using a mutant form of *NEAT1* that cannot bind miR-221-3p, the authors performed reporter assays and confirmed that *NEAT1* directly repressed miR-221-3p expression and further demonstrated that miR-221-3p directly repressed SIRT2 expression. That is, *NEAT1* in EVs derived from MIF-treated MSCs targeted miR-221-3p, which in turn repressed SIRT2 expression and alleviated doxorubicin-induced cellular senescence (Zhuang et al., 2020).

circRNAs

circRNAs can be loaded into EVs and affect cellular senescence. To determine the effects of high glucose on EV components secreted from HUVECs, Wang et al. (2020) analyzed circRNA arrays and found that circRNA0077930 accumulated in EVs. When the levels of circRNA0077930 in EVs were lowered using siRNA, EVs lost their ability to induce senescence in vascular smooth muscle cells (VSMCs). circRNA0077930 induced cellular senescence by inhibiting miR-622 expression and promoting p16, p21, p53, and Kras expression. EVs secreted from HUVECs exposed to high glucose have been shown to promote VSMCs (Wang et al., 2020).

Other EV molecules

In addition to proteins and RNAs, other components can be loaded into EVs and delivered to surrounding cells or target cells and can affect the senescence of the recipient cells. Zhao et al. (2021) injected chitosan hydrogel into EVs derived from MSCs and exposed them to senescent dermal fibroblasts (DFLs), which caused rejuvenation of the DFLs. Another study showed that EVs derived from human induced pluripotent stem cells (iPSCs) attenuated the senescence rate of HDFs (Oh et al., 2018), and EVs derived from gingival-derived MSCs attenuated the senescence of HUVECs that had been caused by oxidative stress (Shi et al., 2021). Additionally, Dorronsoro's team showed that EVs derived from young BMSCs reduced cellular senescence. Specifically, they found that EVs derived from human ES-derived MSCs (hESC-MSCs) reduced the senescence of MEFs (murine embryonic fibroblasts) and human IMR-90 fibroblasts. Additionally, they confirmed that EVs derived from young MSCs extended the lifespan of mice (Dorronsoro et al., 2021). Tofiño-Vian's team reported that EVs secreted from adipose-derived MSCs could reduce the cellular senescence of osteoarthritic osteoblasts induced by IL-1β treatment (Tofiño-Vian et al., 2017).

CONCLUSION

In this review, we summarized the effects of EV components derived from senescent cells as well as the effects of EV components secreted from other cells on senescent cells (Fig. 1). According to most studies, the senescence of surrounding cells is accelerated by EVs secreted from senescent cells, and a representative phenotype of senescent cells is acquired. The accumulation of senescent cells can be prevented by targeting and regulating these senescent EVs. In contrast, we have referred to various studies showing that EVs secreted from other cells show potential as senolytic drugs for the rejuve-

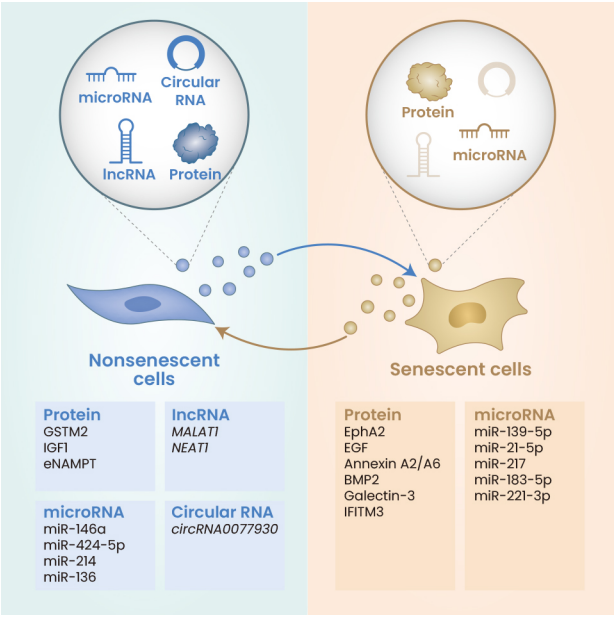

Fig. 1. Senescent cells and nonsenescent cells influence each other through secreted EVs. EVs are secreted by both senescent and nonsenescent cells and can be taken up by other cells. EVs contain various substances, such as DNA, proteins, miRNAs, lncRNAs, circRNAs, and lipids (see enlarged picture of the EVs). Components included in the EVs secreted from senescent cells (bottom, right) and from nonsenescent cells (bottom, left) are listed by type. See the text for details. Created with BioRender.com.

nation of senescent cells. As promising biological materials for diagnosis and therapy, interest in EVs for the treatment of various diseases has increased. Through studies on various mechanisms of EVs, application potential is expected to be found for regulating cellular senescence and improving aging outcomes using EVs (Reiner et al., 2017).

As people age, the incidence of chronic and other diseases increases. Therefore, interest in delaying aging and aging-related diseases by removing senescent cells with senolytic drugs has increased (Childs et al., 2015; Ogrodnik et al., 2019). For example, dasatinib, a tyrosine kinase inhibitor, was the first senolytic to be discovered and can be used to eliminate senescent cells (Zhu et al., 2015). High levels of HSP90 in senescent cells stabilize antiapoptotic signals. Indeed, one research group reported that treatment with the HSP90 inhibitor 17-DMAG successfully eliminated senescent cells via apoptosis (Fuhrmann-Stroissnigg et al., 2017).

Considerable research is still needed before EVs can be used as senolytic drugs for the treatment of diseases. Since EVs are naturally very heterogeneous, quality control is challenging, as is the establishment of effective EV generation and delivery methods. In addition, it is essential to analyze the safety and toxicity of EVs intended for use as therapeutic agents (Wiklander et al., 2019).

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CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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