REVIEW ARTICLE

Contribution of Extracellular Vesicles to Cancer Initiation through Cellular Senescence during Aging

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Abstract

Extracellular vesicles (EVs) are microvesicles secreted from cells with critical roles in cell-to-cell communication. Over the past three decades, EV biology has been characterized by many researchers. Importantly, EVs promote many steps of cancer progression, including cancer cell proliferation, metastasis, formation of pre-metastasis niches, and immunomodulation. Recently, it was shown senescent cells secrete many types of molecules, including EVs, which is termed senescence-associated secretory phenotype. The molecules secreted from cells with the senescence-associated secretory phenotype are related to inflammation, cell proliferation, and cancer initiation. It has also been shown that cellular senescence contributes to cancer progression through EVs. In this present review, the contribution of EVs to cancer biology through senescent cells and aging is summarized and discussed.

Keywords

Extracellular vesicles (EVs), exosome, cell-cell communication senescence, cancer, carcinogenesis

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Introduction

Cellular senescence and the senescence-associated secretory phenotype

Cellular senescence is a cellular state characterized by arrest of cell growth, enlarged morphology, and changes in chromatin and the secretome.[1,2] It was originally advocated by Dr. Hayflick in 1961 and is well known as the "Hayflick limit."[3,4] Cellular senescence can be categorized by external and internal changes. Damage to genomic and telomeric DNA can trigger the DNA damage response.[5–7] Senescent cells accumulate mutations in their genome[8] that trigger this DNA damage response, thus activating ataxia telangiectasia mutated (ATM) and p53, which initiate and maintain cell growth arrest.[9,10] Expression of p16INK4a, which has regulatory roles with CDK4 and p53 in cell cycle G1 progression, and Wnt family member 16 (WNT16B) is also characteristic of senescent cells[11] and contributes to the maintenance of homeostasis in organisms. These cellular states have been considered tumor suppression mechanisms.[5,12,13] However, this emerging theory appears paradoxical.[1,14] Senescent cells secrete many factors into the extracellular environment, which is known as a senescence-associated secretory phenotype (SASP).[15,16] This SASP includes secretion of many factors, including amphiregulin (AREG), granulocyte-macrophage colony-stimulating factor (GM-CSF), C-X-C chemokines (CXCLs), interleukin-6 (IL-6), interleukin-8 (IL-8), matrix metalloproteinases (MMPs), monocyte chemoattractant proteins (MCPs), plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF), and extracellular vesicles (EVs), that control cell growth, motility, differentiation, and inflammation.[17,18] In humans, there are tissue-resident senescent cells that contribute to aging and age-related diseases through the SASP.[3,19] These SASP-associated factors facilitate tumor cell invasion

and metastasis by inducing epithelial-to-mesenchymal transition.[14,15] Furthermore, it was shown in 2013 that the SASP is a key promoter of obesity-associated hepatocellular carcinoma development. Specifically, increased deoxycholic acid levels from gut microbiota in obese organisms induce the secretion of various inflammatory and tumor-promoting factors from hepatic stellate cells with the SASP. Hepatocellular carcinomas are facilitated by exposure to these factors.[20] Moreover, SASP factors promote cancer cell invasion, metastasis, and cellular epithelial-to-mesenchymal transition.[1,15,21] Therefore, SASP-associated factors influence cancer initiation and malignancy. However, this does not contradict a possible role for EVs produced by senescent cells in promoting the development of cancer.

EVs are secreted by numerous cell types

EVs were first reported in 1983 by Jonstone et al.[22] Over the past three decades, EVs, such as exosomes, macrovesicles, and ectosomes, have emerged as novel biological mediators of cell-to-cell communication.[23,24] EVs contain several types of molecules, including lipids, proteins, messenger RNAs (mRNAs), and microRNAs (miRNAs).[25] Moreover, EVs are secreted from many types of normal and pathological cells.[25] There remains confusion regarding the terminology for these secreted vesicles and distinguishing characteristics between exosomes, macrovesicles, ectosomes, etc., partially because it is difficult to distinguish between vesicle types following secretion using the currently available purification methods. In this review, to avoid such confusion, the term “extracellular vesicles” is used.[24,26]

The role of EVs in cancer biology

Recently, evidence of the importance of EVs in cancer biology has accumulated.[24] After the transfer of miRNAs and mRNAs to other cells by EVs were found in 2007,[27] it became clear that EVs affect characteristics associated with cancer malignancy, including cancer cell proliferation,[28–30] immunomodulation,[31–34] angiogenesis,[29,30,35,36] formation of pre-metastasis niches,[37] and metastasis.[38,39] Furthermore, it is possible non-invasive tests could be used to diagnose cancers based on detection of cancer-derived EVs.[40,41] EVs also have the potential to be applied in drug delivery systems[42,43] and in cancer therapy.[44,45]. As previously mentioned, the importance of EVs in cancer malignancy, progression, diagnosis, and treatment has been demonstrated. However, the role of senescent cell-derived EVs in cancer initiation remains to be elucidated.

Aging-associated alterations in EVs contents

Many studies have shown EVs contents and secretion levels have an important relationship with aging.[46] EV protein,[47–50] mRNA,[51] lipid,[50] and miRNA content[52–57] typically increase in aging or senescent cells, while levels of some other molecules decrease (Table 1). In addition, many studies have shown the amount of EVs change with age.[21,47,50,53,56–59] In the case of EV-encapsulated miRNA, miRNA profiles vary with age and these miRNAs can be transferred to other cells. EV secretion from endothelial colony-forming cells was recently shown to be promoted by sirtuin-1 deficiency[60] and could possibly explain increases in EV secretion in senescent cells. By contrast, the number of EVs circulating in humans does not increase with age, which is a reflection of the inflammation status and frailty.[61] Furthermore, it has been reported EV concentrations decrease with age, where EVs in human serum are ingested by monocytes and B cells.[62] However, these do not exclude the possibility there is an alteration in EV content. As mentioned above, EV content changes with age in humans. The change in EV number remains controversial, but is probably related to other changes in the EV profile that occur with aging.

Table 1. Contents and function of EVs from senescence cells or blood samples obtained from age

Type	The alteration of component	Function	The alteration of EVs amount	Origin of EVs	References
Protein	TSN1, PODXL, IDHC, PPAP, ACBP, and ANXA5	-	increase	Urine from elderly individuals	47
	Galectin-3	inhibition of osteogenic differentiation	decrease	Plasma in elderly individuals	48
	EphA2	promotion of cancer cell proliferation	increase	senescent RPE1 pigmented epithelium cells	49
	α-Synuclein, amyloid-β (Aβ) precursor protein	-	increase	Platelet from healthy individuals	50
mRNA	IL-6, IL-12	-	increase	macrophage of elderly individuals	51
Lipid	ApoE, ApoJ	-	increase	Platelet from healthy individuals	50
miRNA	miR-31	inhibition of osteogenic differentiation of mesenchymal stem cells	increase	senescent endothelial cells	52
	miR-185-5p	cell proliferation and osteogenic differentiation inhibition	increase	aged bone marrow stromal cells (BMSCs) from elderly mice	53
	miR-133b-3p, miR-294	inhibition of TGF-β1-mediated epithelial-mesenchymal transition (EMT) in HK2 cells	decrease	bone marrow mesenchymal stem cell in older rats	54
	miR-24-3p	a novel aging biomarker	increase	healthy old individuals	55
	miR-144-3p, miR-486-5p, miR-142-5p, miR-451a, miR-25-3p, miR-145-5p, let-7f-5p	-	increase	senescent platelet	56
	miR-126-5p, miR-148a-3p, miR28-3p, miR27b-3p, miR-10a-5p, miR-215-5p	-	increase	postmenopausal no estrogenic hormone replacement therapy (HRT) group vs premenopausal group	57
	miR142-5p, miR-484, miR10b, miR-144-5p	-	decrease	postmenopausal no estrogenic hormone replacement therapy (HRT) group vs premenopausal group	57

Some studies have detected alterations in EV content by comparing healthy young and aged individuals. Levels of six proteins, TSN1, PODXL, IDHC, PPAP, ACBP, and ANXA5, in EVs isolated from urine were distinct between people aged 25-50 and 50-70 years.[47] The authors mentioned a possibility that these proteins has been implicated in cancer and cancer prognosis. In addition, the EV lipidomic profile also differed in senescent platelets.[50] An aging biomarker, miR-24-3p, was identified by assessing the miRNAs in EVs isolated from saliva from young healthy (median age, 21.0 years) and older volunteers (median age, 66.0 years).[55] Recent studies have shown levels of miR-96/-182/-183, i.e., the miR-183 cluster, increase with age in EVs from the bone marrow interstitial fluid of young (3-4 months) and aged (24-28 months) mice.[53]

Studies have shown EV components differ between diseased and healthy individuals.[63–65] Ten miRNAs from serum EVs were found to have age-related differences by comparing postmenopausal individuals not undergoing estrogenic hormone replacement therapy to premenopausal individuals.[57] In 2009, it was reported EVs may be potential markers of age-related macular degeneration pathogenesis.[66,67] Therefore, it is clear EVs contents change with age.

EV functions in aging individuals and senescent cells

Studies on the SASP and EVs have raised questions concerning the contribution of EVs to cancer initiation due to alterations in EV profiles during aging. In recent years, it has been found cell senescence mechanisms promote carcinogenesis, which appears to be a "double-edged sword."[20,46] As mentioned above, one aspect of the SASP is its anti-cancer function. For example, EV secretion maintains homeostasis by discarding accumulated DNA.[58] An inhibition of EV secretion will be induced the innate immune response, senescence-like cell-

cycle arrest, and/or apoptosis in normal human cells. However, other aspects of the SASP play a role in cancer initiation and disease promotion. It was reported senescent platelets secrete EVs containing miRNAs associated with atherosclerosis, inflammation, and neurodegeneration.[56] The authors investigated EVs from concentrated human donor platelets, which were stored for 0 to 5 days. Upregulation of miR-144-3p, miR-486-5p, miR-142-5p, miR-451a, miR-25-3p, miR-145-5p, and let-7f-5p, which contribute to certain age-related diseases, was observed in EVs from senescent platelets. Recent studies have shown EV-derived miR-183-5p from aged bone marrow reduced stem cell proliferation and inhibited osteogenic differentiation, contributing to stem cell senescence *in vitro*.[53]

In addition, EVs in fetal bovine and human serum contribute to cell growth and promote survival.[68] Many cell lines, including human U87 glioblastoma, human embryonic kidney 293T, HeLa cervical cancer, human SH-SY5Y neuroblastoma, and mouse N2a neuroblastoma cells, grow in EV-depleted serum. However, this growth is enhanced upon the addition of EVs to the serum. The authors discussed the possibility circulating EVs support cell growth and survival *in vivo*. Endothelial cell EV-derived miR-214 also promote migration and angiogenesis of neighbor cells by reducing cell senescence through repression of ATM by miR-214 expression *in vitro* and *in vivo*.[69] The authors suggested that the senescent cells with reduced miR-214 levels rescue from entering senescence by incorporating miR-214 from neighboring miR-214–producing cells via the exosomal shuttle. As mentioned previously, it is possibility that these entering senescent cell have been accumulated the mutation in the genome. Importantly, the EPH receptor A2 (EphA2), which is found in EVs, promotes cancer cell proliferation[49] and EVs from senescent cells are enriched in EphA2. These EphA2-containing EVs bind to ephrin-A1 on the surface of cancer cells and, thus, promote cancer cell proliferation through EphA2/ephrin-A1 reverse signaling. The authors suggested EVs from senescent cells have pro-tumorigenic functions. Furthermore, EVs from senescent cells contain certain disease-related components, such as those associated with atherosclerosis, inflammation, and neurodegeneration. Moreover, EV secretion is upregulated with aging. Inflammation has a well-known relationship with cancer initiation.[17] Importantly, the inflammation-associated EV content increases in the bloodstream with aging.[49,51,70] One possible explanation is EVs promote cancer initiation through such changes in their profile with age, such as EV-associated induction of inflammation and the uptake of EVs containing disease-related components. However, it is a juvenile field and there is not sufficient evidence for cancer initiation by EVs secreted from senescent cells.

Prospect

Through the extensive efforts of many researchers, different aspects of cancer malignancy have been associated with cell-derived EVs. Recently, it was revealed senescent cells secrete EVs with a different profile than EVs from non-senescent cells. Furthermore, EVs from senescent cells are associated with age-related diseases, such as inflammation, atherosclerosis, and neurodegeneration, and promotion of cancer cell proliferation. Although it is not clear the contribution of EVs to the cancer initiation, but the traditional hypothesis is inflammation and immune responses contribute to cancer initiation.[71–74] Moreover, EVs from senescent cells promote cancer progression by activating cell signaling. We can speculate EV secretion from senescent cells contributes to cancer initiation through aging. However, this area of research has yet to be fully explored. Further studies are needed to determine the effect of age-related alterations in circulating EVs on cancer initiation.

Author Disclosure

The author has no conflicts of interest.

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