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Emerging role of extracellular vesicles as a senescence-associated secretory phenotype: Insights into the pathophysiology of lung diseases

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ABSTRACT

Aging is a major risk factor for the development of chronic lung diseases such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and lung cancer. A main aspect of aging is the impaired function of maintaining homeostasis in the organs and body, which is associated with cellular senescence. Cellular senescence is recognized as the state of irreversible cell cycle arrest in response to a variety of cellular stresses. Senescent cells are not simply cell cycle-arrested cells; they also affect bystander cells through the secretion of bioactive molecules, termed the senescence-associated secretory phenotype (SASP). Many studies strongly indicate that senescent cells in the lungs are associated with the pathogenesis of age-related lung diseases by releasing SASP factors. Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are released from almost all cell types and are recognized as important mediators of intercellular communication. They have been shown to carry and transfer a wide variety of molecules, such as microRNAs, messenger RNAs, DNA, and proteins, which can contribute to physiological functions and the pathology of various diseases. Increasing evidence suggests that EVs secreted from senescent cells have unique characteristics and contribute to modulating the phenotype of recipient cells similar to SASP factors. Thus, the EVs secreted from senescent cells, namely, senescence-associated EVs, appear to be a novel SASP factor. In this review, we summarize the current knowledge linking senescence-associated EVs to the SASP factor and discuss the roles of these EVs in age-related lung diseases.

1. Introduction

Aging is a major risk factor for the development of chronic lung diseases such as chronic obstructive lung disease (COPD), idiopathic pulmonary fibrosis (IPF), and lung cancer (Kuwano et al., 2015, 2016; Thannickal et al., 2015). Increased cellular senescence is one of the major hallmarks of aging (López-Otín et al., 2013). The respiratory tract plays a role in gas exchange, thus is an organ of first contact for most environmental exposures and various cell types in the lung are exposed to different types of cellular stresses such as reactive oxygen species (ROS), radiation, drugs, and infections, which induce cellular senescence. A large body of evidence indicates that cellular senescence is involved in disease pathogenesis in terms of not only impaired regeneration but also the secretion of bioactive molecules, termed the senescence-associated secretory phenotype (SASP) (Kumar et al., 2014; Selman and Pardo, 2014). In addition, recent studies have shown that the removal of senescent cells can be an efficacious therapeutic strategy for lung diseases (Hashimoto et al., 2016; Schafer et al., 2017). Therefore, it is critical to understand the pathophysiological mechanisms that link cellular senescence to age-related lung diseases to develop more effective prevention strategies and to provide optimal therapies.

Extracellular vesicles (EVs) include a wide variety of small membranous vesicles, ranging from approximately 50 nm to a few micrometers in size, which are released into the extracellular environment by almost all cell types. EVs are often categorized as

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exosomes, microvesicles, and apoptotic bodies, bases on their size, biogenesis, and secretion mechanisms (Raposo and Stoorvogel, 2013; Yáñez-Mó et al., 2015). Exosomes are generated by the inward and reverse budding of an endosomal membrane and are released into the extracellular fluid by the fusion of the multivesicular bodies (MVBs) with the plasma membrane (Colombo et al., 2014; Robbins and Morelli, 2014). Microvesicles, which are larger than exosomes, are generated from the plasma membrane via shedding or budding in normal circumstances or in response to stimuli. Apoptotic bodies are a few micrometers in diameter and are released from the plasma membrane during cell apoptosis via indiscriminate blebbing. Although the origins of these vesicles have been defined, current methodologies cannot distinguish among those different types of EVs. Thus, in this review, we use the term EV as a general term for all types of vesicles in the extracellular fluid (Gould and Raposo, 2013), whereas we refer to each vesicle type specifically when the origin of isolated vesicles is known.

A growing number of studies have already confirmed that EVs are important mediators of intercellular communication through the transfer of their contents such as proteins, messenger RNAs (mRNAs), microRNAs (miRNAs) and DNA (Kosaka et al., 2010b; Raposo and Stoorvogel, 2013). Recently, increasing evidence suggests that EVs secreted from senescent cells have unique characteristics and contribute to modulating the phenotype of recipient cells such as accelerated senescence, resulting in inflammation, stem cell dysfunction, and cancer progression, similar to SASP factors (Abbas et al., 2017; Davis et al., 2017; Takasugi et al., 2017; Weilner et al., 2016b). Thus, the EVs secreted from senescent cells, namely, senescence-associated EVs, appear to be a novel SASP factor.

In this review, we summarize the current knowledge linking senescence-associated EVs to the SASP factor. Furthermore, we discuss the pathological roles of these EVs in age-related lung diseases.

2. Cellular senescence and the SASP

A main aspect of aging is the impaired function of maintaining homeostasis in the organs and body, which is associated with cellular senescence (López-Otín et al., 2013). Cellular senescence is recognized as the state of irreversible cell cycle arrest in response to a variety of cellular stresses. In the 1960s, Hayflick et al. first used the term “replicative senescence” to describe the phenomenon of the limited proliferative capacity of normal human fibroblasts after serial passaging *in vitro* (Hayflick, 1965; Hayflick and Moorhead, 1961). It is now clear that this replicative senescence is genetically caused by telomere shortening (Harley et al., 1990). In the 1990s, several studies revealed that senescence can also be induced more rapidly even without telomere loss or dysfunction when normal cells are exposed to various exogenous stresses such as oxidative stress, DNA damage, and the activation of certain oncogenes (Campisi and d'Adda di Fagagna, 2007; Serrano and Blasco, 2001). These cellular stresses induce stable cell cycle arrest, which is accompanied by morphological changes of the cell that are typically associated with cellular senescence. This type of senescence has been termed premature senescence. Therefore, the concept of cellular senescence has now been expanded to include premature senescence in addition to replicative senescence.

Emerging evidence suggests that senescent cells are not simply cell cycle-arrested cells but also affect bystander cells through the secretion of bioactive molecules such as growth factors, cytokines, chemokines, and matrix proteases that comprise the SASP (Coppé et al., 2008; Rodier et al., 2009). Recent studies indicate that a persistent DNA damage response (DDR) signal plays a key role in the regulation of the SASP (Campisi and d'Adda di Fagagna, 2007). Rodier et al. discovered that severe DNA damage causes a persistent DDR signal and initiation of the SASP, whereas minimal DNA damage does not result in cytokine secretions (Rodier et al., 2009). By contrast, cells that are induced to senesce by p16INK4a expression, without DNA damage, fail to induce the SASP. In addition, the knockdown of the kinases Checkpoint kinase 2 or Ataxia–telangiectasia mutated, or the DDR component Nibrin, suppresses the expression of many SASP factors such as IL-6 and IL-8, which are major cytokines of the senescent secretome (Rodier et al., 2009). Thus, the SASP is activated by DNA damage and is performed through stress-response signaling. Moreover, accumulating evidence suggests that nuclear factor (NF)-κB is required for the induction of SASP factors (Acosta et al., 2008). Although the mechanism of NF-κB activation in senescent cells is not yet completely understood, p38 mitogen-activated protein kinase (MAPK), the mammalian target of rapamycin, and autophagy are proposed to regulate this activation (Freund et al., 2011; Kang et al., 2015; Laberge et al., 2015).

SASP factors reportedly act in an autocrine manner to reinforce the senescence cell cycle arrest and stimulate the migration of phagocytic cells that can remove senescent cells and damaged cells, which are in turn involved in a variety of homeostatic and pathological roles such as tumor suppression, organismal homoeostasis, organ and tissue development, wound healing, and the antifibrogenic response to acute damage (Acosta et al., 2008; Jun and Lau, 2010; Krizhanovsky et al., 2008; Kuilman et al., 2008; Storer et al., 2013; van Deursen, 2014; Wajapeyee et al., 2008) (Fig. 1A). However, many SASP factors are known to promote local and potentially systemic inflammation, disrupt tissue architecture, and enhance malignant transformation (Ancrile et al., 2007; Park et al., 2010; Sparmann and Bar-Sagi, 2004). In addition, senescent cells are known to remain viable for a long time and accumulate with aging in the tissues and organs (Fig. 1A). As a result, SASP factors are reportedly involved in fibrosis and tumorigenesis (Storer et al., 2013; van Deursen, 2014). Taken together, the SASP appears to have both beneficial and deleterious effects depending on the context and cell type.

3. EVs in intracellular communication

It has recently come to light that EVs provide a novel means of intercellular communication through the transfer of their contents. In 1996, Raposo and colleagues reported the first major breakthrough of this function in demonstrating that B-lymphocytes released exosomes containing major histocompatibility class (MHC) II and induced antigen-specific MHC class II-restricted T cell responses (Raposo et al., 1996). The second breakthrough was described by the group of Jan Lotvall in 2007, who showed that variable RNAs such as miRNAs, long noncoding RNAs (lncRNAs), and mRNAs in exosomes can be transported between cells (Valadi et al., 2007). In 2010, three research groups showed that these miRNAs can be transferred to bystander cells and are able to function within them (Kosaka et al., 2010a; Pegtel et al., 2010; Zhang et al., 2010). Since then, a growing number of studies have focused on intracellular communication via EVs not only in the context of homeostasis but also with respect to the pathophysiology of various diseases.

Currently, EVs are known to be secreted from almost all cell types and have been identified in all body fluids evaluated to date, such as plasma (Caby, 2005; Krizhanovsky et al., 2008), bronchoalveolar lavage (Admyre et al., 2003), and sputum (Porro et al., 2010). EVs contain various functional molecular constituents of their cell of origin, such as proteins, nucleic acids (e.g., miRNA, mRNA and DNA), lipids, and metabolites. Vesiclepedia, a database of EV contents, reports that 92,897 proteins, 27,642 mRNAs, 4934

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