Erratum to: From cell senescence to age-related diseases: differential mechanisms of action of senescence-associated secretory phenotypes

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The BMB Reports would like to correct in the reference of BMB Rep. 48(10), 549-558 titled "From cell senescence to age-related diseases: differential mechanisms of action of senescence-associated secretory phenotypes". The REFERENCE should be corrected as red highlighting in this page. This Erratum’s doi is https://doi.org/10.5483/BMBRep.2016.49.11.122.

INTRODUCTION

Cellular senescence is a process by which cells enter a state of permanent cell cycle arrest. It is commonly believed to underlie organismal aging and age-associated diseases. However, the mechanism by which cellular senescence contributes to aging and age-associated pathologies remains unclear. Recent studies showed that senescent cells exert detrimental effects on the tissue microenvironment, generating pathological facilitators or aggravators. The most significant environmental effector resulting from senescent cells is the senescence-associated secretory phenotype (SASP), which is constituted by a strikingly increased expression and secretion of diverse pro-inflammatory cytokines. Careful investigation into the components of SASPs and their mechanism of action, may improve our understanding of the pathological backgrounds of age-associated diseases. In this review, we focus on the differential expression of SASP-related genes, in addition to SASP components, during the progress of senescence. We also provide a perspective on the possible action mechanisms of SASP components, and potential contributions of SASP-expressing senescent cells, to age-associated pathologies.

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INTRODUCTION

Cellular senescence is causally implicated in biological aging; there is evidence of accumulated senescent cells in tissues of chronologically aging organisms (1-3). In addition, potential links between cellular senescence and age-related diseases, including osteoarthritis (4) and atherosclerosis (5), Parkinson’s disease (6), and cancer (7), have been reported and are supported by a recent report that removal of senescent cells can prevent or delay tissue dysfunction and extend the “healthspan” (8).

Cellular senescence refers to a permanently arrested state of cell growth that displays unresponsiveness to growth factors. It was originally described in terms of exhaustion of the replicative capacity of cultured primary fibroblasts (3) and was thought to be due in part to telomere attrition (the gradual loss of DNA at the ends of chromosomes that accompanies continuous cell division) (9, 10). It has been shown that telomere attrition generates a persistent DNA damage response, which initiates and maintains senescent growth arrest (10-12). However, cell senescence can also be induced by various stresses, including oncogenic activation, UV radiation, and chemical damage or therapeutic drug toxicity (13-15). The often unspecified causes and unclear molecular mechanisms of senescence provokes one of the fundamental questions in the biology of aging and clinical geriatrics: how does cellular senescence contribute to age-related diseases?

Despite the different causes of senescence, the senescent cells display several prominent common phenotypes, in addition to irreversible growth arrest and unresponsiveness to growth factors. Major representative phenotypes are an enlarged and flat cellular morphology with intracellular mass increase (16); increased reactive oxygen species (ROS) production and accumulation of consequent ROS-mediated damage products (17, 18); acquisition of senescence-associated β-galactosidase activity (SA-β-gal) (19); a discernible change in chromatin organization known as senescence-associated heterochromatin...
foci formation (SAHF) (20, 21); and acquisition of the senescence-associated secretory phenotype (SASP), which includes secreted inflammatory, growth-regulating, and tissue-remodeling factors (22, 23). Although these phenotypic changes are often used as indications for cellular senescence, their association to each other and their exact relevance to senescence itself, organismal aging, and age-related diseases, are not clearly understood. One established senescent feature linked to age-related pathologies is the fact that senescent cells lose their proliferation capacity and consequent normal cell turnover, thereby weakening tissue repair and regeneration, and leading to decrements in function (24). Another feature recently drawing attention is the release of SASPs which, due to their potent paracrine effects, control the functions and activities of the surrounding cells and also modify the extracellular microenvironments (22, 23). However, senescent cells express and release a variety of SASP components, with different levels of individual SASP components at different stages of senescent progress (25); thus, it is quite difficult to unravel the link between individual and combined SASP components and age-related pathologies.

Before discussing the action modes of SASP components, several salient features need to be mentioned. First, there are many types of SASP components, such as cytokines, chemokines, growth factors, and proteases, which are expressed and involved in senescence at different individual levels. This indicates that the overall contribution to a specific pathogenesis of the combined actions of all SASP components may be quite different from the known effects of individual SASP components. Second, the senescent cells also express and release regulatory or inhibitory factors, such as tissue inhibitors of metalloproteinases (TIMPs), plasminogen activator inhibitors (PAI), and insulin-like growth factor-binding proteins (IGFBPs). This implies that the ultimate activity of a single SASP component may not be determined only by the number of SASP components involved, but also by the combinatorial action of the SASP components along with their regulatory factors. Third, the progress of senescence alters the expression levels of SASP receptors, modifying cellular reactivity to specific SASP ligands. Fourth, the types and levels of SASP components vary among the stages of senescence progression, suggesting that senescent cells at different stages may affect the age-related pathogenesis differently. Therefore, careful consideration of the altered expression profiles of individual SASP components and their associated regulatory factors and receptors may allow us to hypothesize the possible mechanism of action by which individual SASP components modulate the surrounding tissue microenvironment, and to determine how senescent cells communicate with the surrounding cells.

In this review, we discuss various studies relating to the secretion of SASP components from senescent cells and their involvement in age-related pathologies. Here, the aforementioned features of SASP components and their associated gene products are collectively referred to as SASP-related factors. We also consider the possible action mechanisms of individual SASP components on the tissue microenvironment, and finally propose the potential contributions of senescent cells to senescence-related pathologies.

**SPECIFIC VERSUS OVERALL GENE EXPRESSION CHANGES IN SENESCENCE**

Cell size is fairly homogeneous during the early passages of human fetal fibroblasts, while cell size at the terminal stages (senescence) of the in vitro cellular life span is large and heterogeneous (26, 27). Similarly, increases in cell size (surface area) and cell mass (components) are generally seen in most cases of cellular senescence triggered by various stresses. Thus, this enlarged cell morphology and size is the most prominent senescent phenotype, which allows us to judge, by appearance, whether or not cells are progressing to senescence. However, then, do senescent cells acquire this phenotype? The enlarged cell size reflects an increase in cell mass, generally in terms of both molecular and organellar components (28-30). However, it is unclear whether the increase in mass is confined to specific cellular molecules and compartments, or whether it is caused by a random and uncontrolled increase.

The majority of senescent cells are in the G1 phase of the cell cycle (i.e., G1, S, G2, and M phase, respectively), with an overall delay seen in cell cycle progression, indicating that the G1 checkpoints are critical controls for senescence (31, 32). This stable G1 arrest is mainly executed by an interplay between the Rb and p53 tumor suppressor pathways (33, 34). Senescent cells express activated p53 transcription factor (35) and, consequently, elevated levels of p21Waf1/Cip1 (36), p15Ink4b (37, 38), and p16Ink4a (39); also, they are unable to hyperphosphorylate Rb protein in response to mitogenic stimulation (40). Activation of these cell cycle checkpoints comprises an important mechanism of cell cycle arrest in senescent cells (41). However, G1 is the phase at which the cell grows in size by synthesizing the mRNA and proteins required for cell components, as well as some specific proteins required for DNA synthesis. Once the required cell growth and mass increase has taken place, the cell enters the next phase of the cell cycle, the S phase. Senescent cells in G1 arrest, which is the result of an inability to transition from G1 to S without the cessation of synthesis of cellular molecules and components, may result in enlarged cell morphology. Ultimately, this representative senescent phenotype, i.e. progressive enlargement of cell morphology, is tightly linked with another well-known senescent feature: senescent cells remain metabolically active, which includes an overall increase in gene expression (42), despite the loss of their replicative capacity.

Enhanced protein synthesis during senescent arrest is maintained by mTOR activation and upregulated activity of phosphatidylinositol 3-kinase (PI3K), an upstream activator of mTOR (43-45). Moreover, GSK3-mediated augmentation of lipogenesis and glycogenesis has been reported to be critically linked...
with an increase in the overall mass of organelles (such as mitochondria, lysosome, Golgi, and ER) and cell granularity (46, 47). In particular, increased mass of lysosomes and mitochondria has been observed in both senescent cells and aged tissues (30, 48, 49). The combined activity of augmented lipogenesis and protein synthesis leads to the increase in organelar formation. However, the imbalance between anabolic activities, including protein synthesis and organelar biogenesis, and cell cycle progression contributes to the abnormal cell volume increase.

Alongside the overall increased synthesis of mRNAs and proteins, the senescent cells also have an extremely altered expression of specific genes, that are often referred to as senescence-associated genes. These include p53 (35, 50), p21\(^{Cip1/Waf1}\) (36), p15\(^{INK4a}\) (37, 38), p16\(^{INK4a}\) (39), vimentin (51), fibronectin (52), PAI (53), and several SASP components (54, 55).

Some of these upregulated gene expressions critically control the senescent cell itself, and contributes to the aging process and age-related diseases (36-38). Among the senescence-associated gene products, synthesis and secretion of SASP components have recently been of interest due to their potential link to various age-related diseases (24, 59).

**TYPES OF SENESCENCE-ASSOCIATED SECRETORY PHENOTYPE COMPONENTS**

The culture medium of senescent cells is enriched with secreted proteins (60, 61). The functional involvement of the secreted proteins in age-associated pathologies was initially recognized in a study by Campisi et al., where the secreted factors from senescent fibroblasts, especially MMP3, promoted the transformation of premalignant mammary epithelial cells (62, 63). This observation confirmed the belief that senescence might act as a tumor suppressor mechanism through the irreversible senescence arrest feature, thus emphasizing its potential to act as a double-edged sword within the tumor microenvironment. In addition, there is accumulating evidence that senescent cells secrete a variety of inflammatory cytokines, chemokines, proteases, and other immune modulators (22). As a result, it is predicted that senescent cells modify the tissue microenvironment in vivo by massive alteration of SASP expression (42). Most importantly, studies have revealed that the secretion of SASP components is conserved between human and mouse cells in vitro and in vivo (22, 23, 64-66), and it commonly occurs in the progress to senescence of various cell types, such as fibroblasts, epithelial cells, endothelial cells, and astrocytes (62, 67-69). Currently, some SASP components are used as general markers of senescence (64). These observations led us to investigate in detail the action mechanisms of SASP components in diverse age-related pathologies.

To thoroughly investigate the possible mechanisms by which SASP contributes to age-related pathologies, we have divided the SASP-related factors into three categories, depending on the mode of action that initiates the SASP activity: receptor-requiring SASP, direct-acting SASP, and SASP regulatory factors.

**Receptor-requiring SASP**

These factors include cytokines (interleukins), chemokines, and growth factors, which generally initiate their innate functions by binding to their respective receptors on the surface of target cells, and activating receptor-mediated intracellular signal transduction pathways. The most prominent SASP cytokines are interleukin (IL)-6, -8, and -1α; their mechanism of action in, and their contributions to cancer, are well understood. IL-6 secretion is markedly augmented in DNA damage and oncogene-induced senescence of mouse and human keratinocytes, melanocytes, monocytes, fibroblasts, and epithelial cells (54, 70, 71). Interestingly, IL-6 expression is upregulated by IL-1 (68, 72), which is also known to be overexpressed and secreted by senescent cells (23); this implies that the SASP component expression may be sequentially regulated in a hierarchical cascade through their autocrine activity. Among the chemokines, Groα (CXCL-1), Groβ (CXCL-2), MCP-1 (CCL-2), RANTES (CCL5), HCC-4 (CCL-16), eotaxin-3 (CCL-26), and MIP-3α (CCL-20) are reported to be secreted by senescent cells (64, 73). They exert their biological effects by interacting with their own G protein-linked transmembrane receptors (chemokine receptors). Increased expression of many growth factors such as HGF, FGF, TGFβ1 and GM-CSF, has also been reported in various types of senescent cells (54, 74). The effect of these receptor-requiring SASP components on age-related diseases, including inflammatory diseases and cancer, relies on the target cells that express their respective receptors. The target cell may be the senescent cell itself, thereby aggravating or shielding the cell from senescence progression. Alternatively, the target cells may be various types of normal cells that are nearby; in this case, the cellular environment may be gradually modified to give rise to a pathological condition, or to create an environment in which multiple pathologies may arise.

**Direct-acting SASP**

Many matrix metalloproteinases (MMPs) belong to this group. MMP family members that are consistently upregulated in human and mouse fibroblasts undergoing replicative or stress-induced senescence include stromelysin-1 and -2 (also called MMP-3 and -10, respectively) and collagenase-1 (MMP-1) (75-77). Another family of proteases that belongs to this SASP category are the serine proteases: urokinase-type or tissue-type plasminogen activators (uPA and tPA, respectively) (78, 79). These direct-acting SASP components exert their proteolytic activities on their respective substrates, mostly extracellular matrix (ECM) proteins, the extracellular portion of membrane-anchoring proteins, or some soluble molecules released from cells, thereby modifying the extracellular microenvironment. Although non-protein small molecules such as ROS, transported ions, and metabolites may also belong to this group, they will not be discussed in this review since they are not proteins.
SASP regulatory factors
Members of this group include TIMPs, PAIs, and IGFBPs. These factors do not have their own enzymatic or signal triggering activities, but modulate the activities of receptor-requiring or direct-acting SASP components by binding to them. The TIMPs comprise of a family of four protease inhibitors: TIMP1, TIMP2, TIMP3, and TIMP4. TIMPs are generally known to inhibit most of the activated MMPs, but in some cases they are known to form complexes with the latent form of MMPs, such as MMP2 and MMP9 (80). The complex of TIMP2 with latent MMP2 (pro-MMP2) serves to facilitate the activation of pro-MMP2 at the cell surface by MT1-MMP (MMP14), a membrane-anchored MMP. The role of the pro-MMP9/TIMP1 complex has yet to be elucidated (80). TIMPs are known to be highly inducible in response to many cytokines and hormones (81), suggesting that their upregulated expression in senescent cells may be the result of the autocrine activity of SASP cytokines.

PAI-1 is a member of the serine protease inhibitor family (serpin); also known as SERPINE1, it functions as the major inhibitor of tPA and uPA, regulating fibrinolysis (the physiological breakdown of blood clots). PAI-1 also blocks the activity of MMPs, which play a crucial role in the invasion of malignant cells across the basal lamina (82). In addition, physiological roles for PAI-1 in metabolic diseases, such as insulin resistance and cardiovascular disease, have often been reported (83, 84). PAI-2 (SERPINB2) is primarily known to be secreted by the placenta. Recently, it has also been reported to bind to several intracellular and extracellular proteins, and to play a role in the regulation of adaptive immunity (85).

IGFBPs act as carrier proteins for insulin-like growth factor (IGF) and help to maintain the half-life of circulating IGFs in many tissues, thereby modulating IGF signaling depending on the tissue context (86, 87). In humans, IGFBPs are transcribed from seven genes, IGFBP1-7, which share a 50% homology (88). Despite their similarities, the importance of IGF signaling in various pathologies, and the subtle structural differences among the IGFBPs, indicate their tremendous potential roles in modulating age-related diseases.

STAGE-SPECIFIC EXPRESSION OF SASP COMPONENTS IN THE PROGRESS OF SENESCENCE
Senescent cells express only selected SASP components at dif-
different stages of senescence progression. For example, IL-1α expression is one of the earliest events after senescent growth arrest, and its autocrine activity induces several second-wave SASP components, including IL-6 and -8, via activation of nuclear factor κB (NF-κB) and C/EBPβ (72). In cultures, our recent time-series of gene expression profiles during the progression of replicative senescence in human diploid fibroblasts (HDF) supports the idea of stage-specific SASP expression (25). These observations imply that senescent cells at different stages may have varying impacts on the progress of even the same age-related disease, such as cancer.

**POSSIBLE ACTION MECHANISMS OF SASP BY DIFFERENTIAL EXPRESSION OF SASP-RELATED FACTORS**

As described above, the overall contribution of individual SASP components to the progress of senescence itself or to age-related pathologies does not depend on the expression levels of the SASP component alone; it is also affected by the inhibitory, regulatory, or counteracting SASP molecules coexpressed by the same senescent cell. In addition, expression levels of SASP component receptors on the target cell, which may be the senescent cell itself or nearby cells, are another critical factor. Based on our previous time series of gene expression profiles for all of the SASP-related factors, including SASP components, SASP regulators, and SASP receptors (25), the possible action mechanisms of SASP components can be placed into six groups (Fig. 1).

**Expression of direct-acting SASP components alone**

Direct-acting SASP proteases (proteinases) can modulate the tissue microenvironment by regulating turnover of the ECM through processing of ECM factors, including collagen, fibronectin, and proteoglycans (Fig. 1A). By ectodomain shedding, they can also regulate the activity of certain growth factors and cytokines such as HB-EGF and TNF-α, and membrane-spanning receptors such as c-Met (89-91).

**Expression of SASP component with its receptor**

Senescent cells express a diverse array of receptor-requiring SASP factors. The effect and action mechanisms of these factors can be very different, depending on the cell types expressing their receptors. If a senescent cell expresses both a SASP component and its receptor, then the SASP component will signal to the senescent cell itself via autocrine activity (Fig. 1B). A good example is the increased expression during senescence of both IL-8 and its receptor, IL-8RB (CXCR2), and their involvement in mediating senescence (25, 64). Similar expression patterns for IL-8 and IL-8RB were also observed in our study of replicative senescence in HDF (Table 1).

**Expression of SASP component without its receptor**

If senescent cells express only a SASP component while suppressing expression of its receptor, then the SASP component will affect nearby nonsenescent cells or other target cells expressing its receptor via paracrine activity (Fig. 1C). Similar expression profiles for this group are seen for IL-17 and -20 (25), but their functional relevance to senescence or age-associated diseases has yet to be elucidated.

**Regulation of counteracting SASP components**

Senescent cells also regulate counteracting SASP components (SASPC) that block specific SASP-triggered signaling events (Fig. 1D). Anti-inflammatory cytokines such as IL-4, -10, -13, and -35 may act as SASPC. IL-10 and -13 expression was found to be downregulated during replicative senescence of HDF (25), implying that proinflammatory signaling can be activated in senescence.

**Expression of SASP regulatory factors**

Senescent cells express regulatory SASP components (SASPc) that prolong or promote certain SASP-mediated signaling events by binding to their respective receptors or to the SASP component itself (Fig. 1E). Representative examples are the IGFBPs, which are IGF signaling regulators. Insulin/IGF-mediated signaling is known to be a significant contributor to biological aging in many organisms (92, 93). Therefore, it is not surprising that the IGFBPs would have a role in senescence and aging. Increased expression of IGFBPs such as IGFBP2, IGFBP5, and IGFBP7, and their involvement in cell senescence, organismal aging, and age-associated diseases have been reported recently.

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**Table 1. Combined activity of interleukins and expression of their respective receptors**

| Names | Expression changes during senescence | Differential action mechanisms |
|-------|------------------------------------|-------------------------------|
|       | Interleukins | Receptors | Young cell | Senescent cell |
| IL1A, IL1B, IL12, IL17, IL20 | Up | Down | High sensitivity | Paracrine action only |
| IL6, IL8, IL21 | Up | Up | Suppressed overall action | Para- & autocrine action |
| IL13, IL15, IL18 | Down | Up | Paracrine action only | High sensitivity |
| IL3, IL10, IL17 | Down | Down | Para- & autocrine action | Loss of overall activity |

*This table was created by reanalyzing previously reported results (90).
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Expression changes during senescence

Possible action mechanisms

| Expression changes during senescence | Young cell | Senescent cell |
|-------------------------------------|------------|----------------|
| MMP1, MMP3, MMP12                 | Up         | Combined activity of MMPs (MMP2, MMP11, MMP20, MMP27) and TIMP4 |
| MMP2, MMP11, MMP20, MMP27          | Down       | Combined activity of MMPs (MMP1, MMP3, MMP12) and TIMPs (TIMP1, TIMP2, TIMP3) |
| TIMP1, TIMP2, TIMP3                | Up         |                |
| TIMP4                              | Down       |                |

*This table was created by reanalyzing previously reported results (90).
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Fig. 2. Potential roles of senescent cells in age-associated pathologies.

ports which show that IL-6 and -8 released from senescent cells promote the epithelial-mesenchymal transition and invasiveness of premalignant epithelial cells in culture (54). Representative SASP components in this class may include IL-6, -8, -21, and -32 (Table 1 and Fig. 2).

Class III: senescent cells act as target cells expressing SASP receptors only
In some cases, senescent cells express high levels of certain SASP receptors alone, without expressing their respective SASP components, whereas nearby young cells express the SASP components. In this scenario, the senescent cell becomes susceptible to these SASP components, probably making itself a target cell by a one-sided paracrine communication from the proliferating young cell to the senescent cell. This class may include IL-7, -13, -15, -18, -19, -23, -25, and -34 (Table 1 and Fig. 2). However, it is unclear whether these SASP components act to promote senescence or to destine the senescent cell to become a source of pathogenesis.

Class IV: senescent cells lose cell-to-cell communication by suppressing expression of SASP components and their respective receptors
If senescent cells suppress the expression of specific SASP components and their receptors, they lose their autocrine and paracrine communication. IL-3, -10, -17a, -17b, and -17c are in this class (Table 1 and Fig. 2). Interestingly, IL-10 is known to act as an anti-inflammatory cytokine and has a protective role against atherosclerosis (99). Therefore, losing IL-10-mediated communication may accelerate other inflammatory responses and result in pathogenic progression.

We have proposed four possible mechanistic contributions of senescent cells to age-associated pathologies by referring only to the differential expression of interleukins and their receptors. However, all four of these scenarios may occur at the same time in the same tissue, suggesting that their orchestrated actions are involved in promoting senescence-associated pathogenic tissue environments and remodeling. Moreover, considering all of the contributions made by the other SASP-related factors, it is challenging to explain the roles of senescence in pathogenesis. Nevertheless, elucidating SASP-mediated extracellular microenvironmental remodeling, in addition to senescence-related intracellular signaling, is crucial in establishing...
a correct and complete understanding of senescence and its related pathologies. More detailed studies of the integrity of the SASP-related factors, in terms of the components and their functional interconnections, are needed to develop and exploit appropriate pro senescent or anti senescent therapeutic strategies.

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