Trends in Cell Biology

CellPress
REVIEWS

Review

Classical and Nonclassical Intercellular Communication in Senescence and Ageing

Juan Antonio Fafián-Labora[1] and Ana O'Loghlen[1,*]

Intercellular communication refers to the different ways through which cells communicate with each other and transfer a variety of messages. These communication methods involve a number of different processes that occur individually or simultaneously, which change depending on the physiological or pathological context. The best characterized means of intercellular communication is the release of soluble factors that affect the function of neighboring cells. However, there are many other ways by which cells can communicate with each other. Here, we review the different means of intercellular communication including soluble factors in the context of senescence, ageing, and age-related diseases.

Highlights

Intercellular communication is a key feature in physiological and pathological conditions. We hypothesize that several means of intercellular communication occur either simultaneously or in succession.

The most studied means of intercellular communication are soluble factors. However, important alternative means are emerging.

Senescent cells are highly proactive and communicate with neighboring cells via various means of intercellular communication including but not limited to the senescence-associated secretory phenotype (SASP).

Most studies of pharmacological drugs preventing the release of soluble factors oversee the influence of these drugs on other means of communication.

Cellular Senescence: A Complex and Heterogeneous Phenotype

The discovery of a cellular phenotype termed **senescence** (see Glossary) was first identified by Moorehead and Hayflick in the 1960s. When culturing *in vitro* primary fibroblasts isolated from human donors, they observed that these cells reached a point where they lost their proliferative capacity, and termed this phenotype cellular senescence. Cleverly, they hypothesized that this phenotype could mimic ageing and be exploited as 'ageing in a Petri dish' [1,2]. The premature induction of senescence termed oncogene-induced senescence was identified *in vitro* and later confirmed to play a physiological role in preventing tumor progression *in vivo* [1,3]. However, it was not until 2011 that the van Deusen laboratory established a causative role between the activation of senescence and ageing [4]. Here, the authors established that the accumulation of $p16^{Ink4a}$ in certain organs in a prematurely aged mouse model deficient for the mitotic checkpoint protein BubR1 triggers natural features common in premature ageing. Interestingly, they showed for the first time that genetic inactivation of $p16^{Ink4a}$ ameliorated these ageing phenotypes [4]. It is now well established that the induction of cellular senescence is a hallmark of ageing [5]. Furthermore, senescence is a driver not only of ageing but also of certain age-related diseases such as cancer, osteoarthritis, atherosclerosis, Alzheimer's diseases, chronic obstructive pulmonary disease (COPD), and idiopathic pulmonary fibrosis (IPF) among others [6–11].

Although the main characteristic of senescence is a stable cell cycle arrest induced by the expression of the cell cycle inhibitors $p16^{INK4A}$ and $p21^{CIP}$, the influence that senescence has on tissue homeostasis is due to its highly proactive secretome. The **senescence-associated secretory phenotype (SASP)** could be considered the 'soul' of senescence as it is highly proactive and it changes its composition with time. It has both beneficial and detrimental effects depending on the trigger and context where senescence is induced [12,13]. However, the SASP is still not well characterized, as only a number of factors have been identified in very specific scenarios. It is important to note that senescence is a complex heterogeneous cellular phenotype that affects tissue homeostasis in many different contexts and caution should be taken when it is standardized to certain markers (Box 1). It is likely that there remain novel, unveiled characteristics of senescence that are context dependent [14].

[1]Epigenetics and Cellular Senescence Group, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK

*Correspondence:
a.ologhlen@qmul.ac.uk (A. O'Loghlen).

 https://doi.org/10.1016/j.tcb.2020.05.003

There are currently two main therapeutic strategies to deal with the presence of senescent cells in ageing and age-related diseases. One relates to the potential for selective killing of senescent cells (using pharmacological compounds termed **senolytics**). The second aims to neutralize the deleterious effects of intercellular communication, in particular the SASP, on senescent cells by using drugs denominated **senomorphics**. Here, we review the different means of intercellular communication for senescent cells and their relation to ageing and some age-related diseases, classifying them as classical, emerging, and nonclassical. We include soluble factors and extracellular matrix (ECM) remodeling proteins, but also describe additional ways by which cells communicate. We focus on how intercellular communication is regulated and its functionality in different biological and age-related pathological contexts.

SASP: Classical and Nonclassical Intercellular Communication

The SASP is a means of intercellular communication that specifically refers to senescent cells. The classical SASP is characterized by soluble factors, growth factors, and ECM remodeling enzymes [12]. However, emerging SASP and other means of intercellular communication that we have denominated nonclassical have also been described during senescence and ageing. Here, we review the current knowledge on what we call classical, emerging, and nonclassical SASP.

Classical SASP: Soluble Factors or sSASP

The sSASP can be both beneficial and detrimental for tissue homeostasis; therefore, the sSASP needs to be tightly regulated. One of the main drivers of the sSASP is a persistent DNA damage response [15]. This converges in the activation of two major regulators of the SASP: NF-κB and C/EBPβ, where NF-κB is further regulated by the transcription factor GATA4 (Figure 1A) [16]. However, the sSASP can also be induced independent of a noncanonical DNA damage response by p38MAPK [17] and by the presence of cytoplasmic chromatin fragments (CCFs). These are DNA fragments that can be released from the nucleus during senescence and activate the antiviral cyclic GMP-AMP synthase (cGAS) stimulator of interferon genes (STING) pathway [18,19]. Still, both p38MAPK and CCF activate the sSASP via NF-κB signaling.

Curiously, activation of the **inflammasome** can also control the sSASP. This is mediated by interleukin (IL)-1 signaling and IL-1α expression and is mainly involved in paracrine senescence signaling [20]. Furthermore, IL-1A is regulated by mammalian target of rapamycin (mTOR) inhibition.

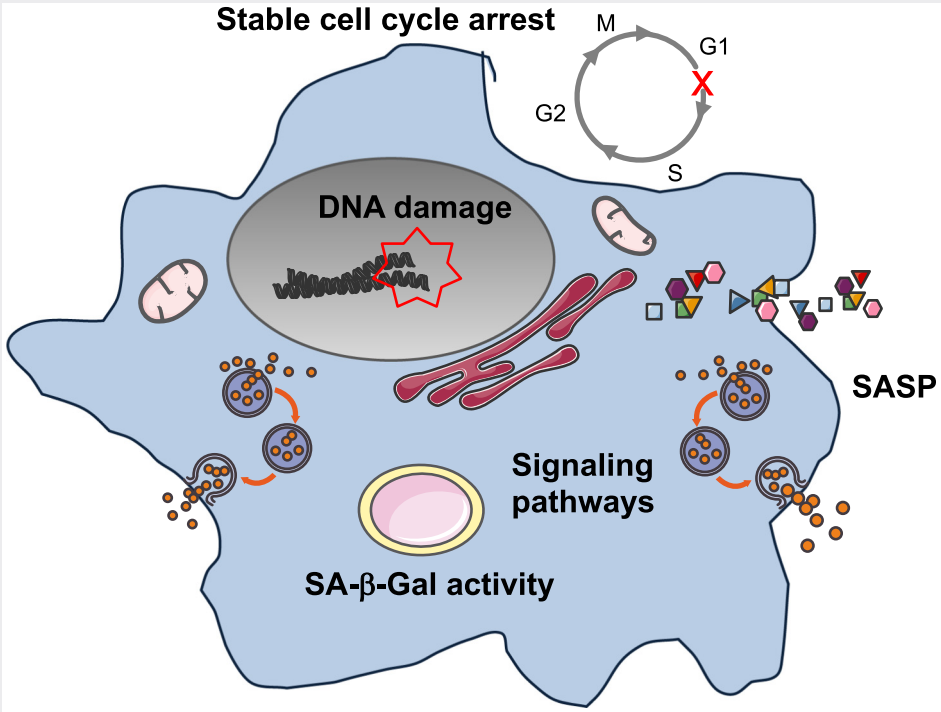
Box 1. Guidelines to Identify Senescent Cells *In Vitro*

Cellular senescence can be induced by a variety of triggers, including telomere shortening, oncogenic stress, ROS, and DNA damage. The main response of primary cells entering senescence is to induce a stable cell cycle arrest by expressing the cell cycle inhibitors *CDKN2A*, *CDKN2B*, and/or *CDKN1A* (encoding $p16^{INK4A}$, $p15^{INK4B}$, and/or $p21^{CIP}$ proteins, respectively) and showing a lack of proliferation-related markers such as Ki67, BrdU, or EdU (Figure I) [13,14]. However, as these markers are not exclusive to senescent cells but are also present in nondividing somatic cells, additional markers should be used to confirm a senescent phenotype [89]. The identification of more than three biomarkers is recommended to confirm the activation of senescence. Another marker used to identify senescence is senescence-associated beta galactosidase (SA-β-Gal) activity. An increase in SA-β-Gal is due to higher lysosomal activity in senescent cells, which could be due to an increase in the number of lysosomes in senescence [37] or an increase in lysosomal activity and can be detected by a specific stain. Finally, additional markers such as DNA damage, the release of a particular secretome that has been termed the SASP [12], or the activation of specific signalling pathways should also be verified. However, these last markers are slightly more challenging as some triggers, such as oncogene-induced senescence by $H\text{-}Ras^{G12V}$ expression, induce all of them simultaneously. Instead, other triggers, such as developmental senescence or an increase in αvβ3, do not induce a DNA damage response, which was long believed to be a key biomarker of senescence [65,73,74]. This confirms that not all biomarkers are expressed simultaneously when senescence is induced, adding intricacy to the identification of senescence.

It is important to note that senescence is an extremely complex and heterogeneous cellular phenotype that affects tissue homeostasis in many different contexts, and the generalization of standardized markers might be disadvantageous as it is likely that there are as-yet-unveiled characteristics of senescence that are context dependent [14].

Glossary

Ferroptosis: cellular death induced by accumulation of iron in the cell.
Inflammaging: chronic, low-grade inflammation characteristic of aging.
Inflammasome: congregation of immune system receptors that activate caspase-1 and induce an innate inflammatory response.
Senescence: activation of a phenotype characterized by several biomarkers including stable cell cycle arrest and a proactive secretome.
Senescence-associated secretory phenotype (SASP): soluble factors, growth factors, and matrix remodeling enzymes secreted by cells undergoing senescence.
Senolytic drugs: drugs designed to specifically kill senescent cells.
Senomorphics: chemicals or drugs that inhibit the sSASP.
Syncytiotrophoblast: outer layer of the placenta formed by the fusion of trophoblast cells.
Trans-stable-isotope labelling of amino acids in cell culture (SILAC): transfer of proteins between cells using SILAC.



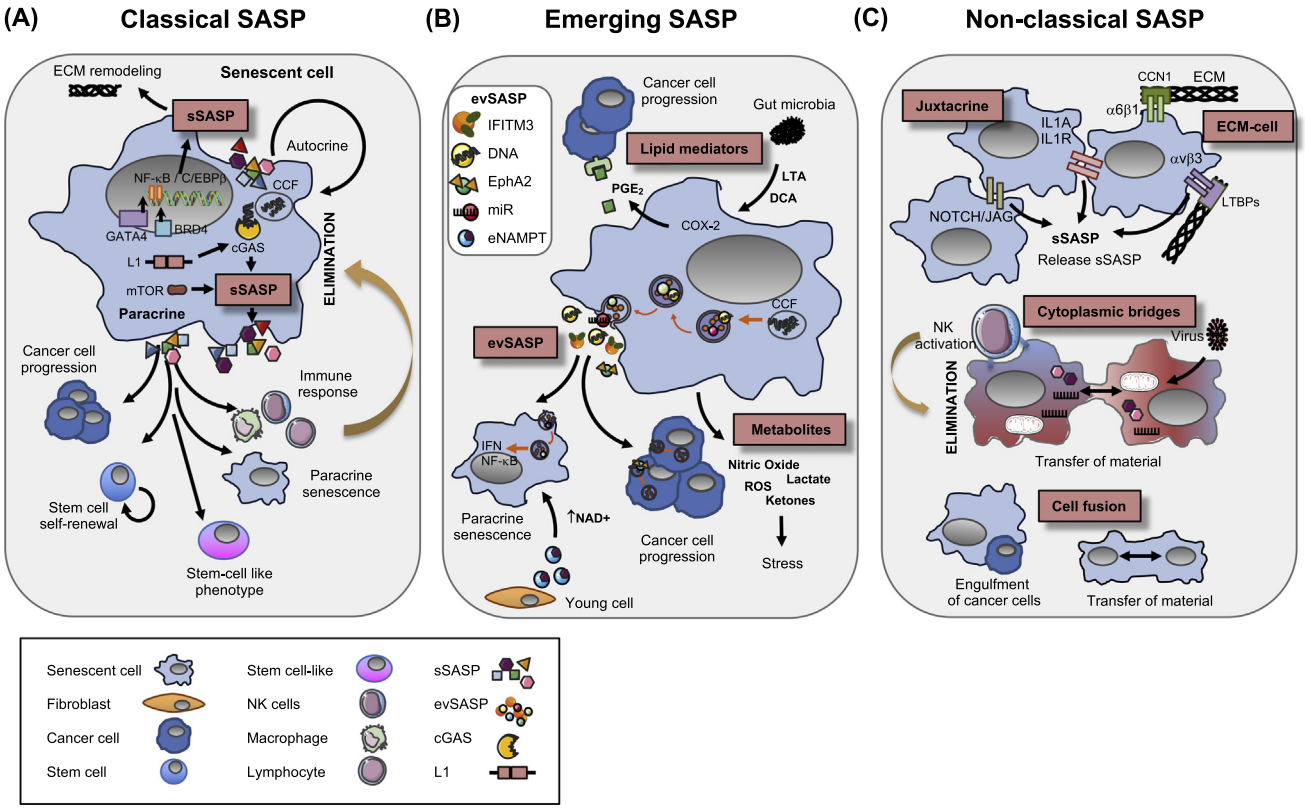
Biomarkers	Detection
Stable cell cycle arrest	• ↑ $p15^{INK4B}$, $p16^{INK4A}$ and/or $p21^{CIP1}$ • ↓ Ki67 or BrdU or EdU
DNA damage	• ↑ p-γH2AX, 53BP, ATM, ATR
Signaling pathways	• ↑ PI3K, mTOR, TGFβ, p38MAPK
SA-β-Gal activity	• ↑ β-Gal staining
SASP	• ↑ Chemokines, cytokines, growth factors, • ↑ EV

Trends in Cell Biology

Figure I. Biomarkers of Senescence. Abbreviations: EV, extracellular vesicle; mTOR, mammalian target of rapamycin; SA-β-Gal, senescence-associated beta galactosidase; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor β.

mTOR selectively regulates a number sSASP factors post-transcriptionally via IL-1A, but also through MK2 [mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2)] by the protein synthesis factor 4EBP1 [21,22].

Alternatively, epigenetic alterations are also known to regulate the sSASP. A recent study found that derepression of the retrotransposable element line 1 (L1) increased during senescence and ageing. This in turn activated cGAS/STING and, consequently, the sSASP [23]. Furthermore, the sSASP is regulated by global chromatin remodeling and the recruitment of BRD4 to superenhancers near sSASP genes [24]. Overall, the sSASP is tightly regulated as dysfunctional



Trends in Cell Biology

Figure 1. Intercellular Communication in Senescence and Ageing. (A) Soluble factors, growth factors, and matrix remodeling enzymes are released in the classical soluble senescence-associated secretory phenotype (sSASP) model. However, the sSASP needs to be tightly regulated. At the transcriptional level, BRD4 and GATA4 regulate the master regulators of the SASP: NF-κB and C/EBPβ. The sSASP can also be driven by LINE-1 (L1) retrotransposable elements that are derepressed during senescence and by cytoplasmic chromatin fragments (CCFs) through the activation of cyclic GMP-AMP synthase (cGAS). The sSASP is also regulated post-transcriptionally by mammalian target of rapamycin (mTOR) and downstream signalling pathways. The secreted sSASP plays many roles in the microenvironment. On the one hand, it alters the extracellular matrix (ECM) and can reinforce senescence through autocrine signalling. On the other hand, it has many functions through paracrine signalling. It can promote cancer progression, stem cell self-renewal, and stem cell-like features in damaged cells. It also has the ability to transmit senescence to neighboring cells. Importantly, the sSASP stimulates an innate and adaptive immune response favoring the elimination of senescent cells. (B) Emerging SASP factors include extracellular vesicles (EVs) (evSASP). Due to the high heterogeneity and cargo diversity of EVs released during senescence, novel functions are continually emerging. Some cargo factors, such as interferon (IFN) and ephrin-related and antioxidant proteins, are highlighted. In addition, miRNAs (miRs) and CCF DNA fragments can be loaded into evSASPs. evSASPs also exert paracrine functions to promote cancer progression and induce paracrine senescence. Furthermore, metabolites and lipid mediators that induce an oxidative microenvironment or favor tumor progression have been found. Gut microbiota release lipoteichoic acid (LTA) and deoxycholic acid (DCA), which drive a sSASP response via COX-2 and prostaglandin E2 (PGE_2) release. (C) Nonclassical SASP refers to intercellular communication in senescence not driven by released factors. Cell-to-cell communication can be mediated via receptor interaction as with interleukin-1 receptor (IL-1R) and interleukin-1 alpha (IL-1A) or via NOTCH1/JAG interaction, both of which induce sSASP. In addition, cell–ECM interaction has been described in senescence. For example, integrin α6β1 can interact with CCN1 (cysteine-rich protein 1) and induces senescence via reactive oxygen species (ROS) while αvβ3 induces sSASP. Alternatively, cytoplasmic extensions called cytoplasmic bridges also occur during senescence. Interestingly, these have been described between senescent cells and natural killer (NK) cells to activate these and promote the elimination of senescent cells. The fusion of cells can also induce senescence allowing material exchange between the fused cells. Furthermore, senescent cells can engulf cancer cells as a source of energy to maintain the high metabolic requirements of senescent cells. Abbreviations: eNAMPT, extracellular nicotinamide phosphoribosyltransferase; EphA2, ephrin A2; IFITM3, interferon-induced transmembrane protein 3; LTBP, latent TGF-binding protein.

expression and release can drive pathological conditions. This highlights the importance of understanding how the sSASP is regulated.

It was long believed that the sSASP would change in composition throughout time. Thus, it was not until recently that a comprehensive study found the sSASP to comprise two distinctive functional waves [25]. The first wave is formed by an anti-inflammatory transforming growth factor

(TGF)-β-enriched sSASP. This particular sSASP is regulated by membrane-bound NOTCH1, whose expression increases during the initial stages of the induction of senescence and mediates C/EBPβ repression. Second and through time, NOTCH1 expression decreases causing the activation of C/EBPβ, which in turn induces a proinflammatory sSASP [25].

Our advancement of knowledge of the sSASP has greatly increased, with many novel roles emerging in the past decades. On the one hand, the sSASP is important for reinforcing a stable cell cycle arrest in an autocrine manner through IL-8 and IL-6 and their corresponding receptors (Figure 1A) [26,27]. On the other hand, it acts in a paracrine fashion influencing a variety of cell types. It can induce senescence in primary fibroblasts and epithelial cells [20], while promoting tumorigenesis in cancer cells [13,14,28]. Importantly, the sSASP also acts in a paracrine fashion by mediating both an innate and an adaptive tissue response favoring the removal of senescent cells from the tissue, which is essential for the maintenance of tissue homeostasis [20,29,30]. More recently, the SASP was shown to be key for tissue regeneration via IL-6 [31,32] and cell plasticity and stemness [33,34]. Despite many recent advances in novel roles and regulation of the sSASP, there are also emerging functions as described next.

Emerging Classical SASPs

Although the SASP is classically defined as the secretome released from senescent cells, most of the current literature focuses on soluble factors, growth factors, and ECM remodeling enzymes. In this section, we aim to review emerging literature on novel SASP components such as extracellular vesicles (EVs) and noncellular metabolites and ions (Figure 1B).

EVs are lipid membrane vesicles that are released by all cells and are therefore found in most biological fluids [35]. Although initially thought of as a mechanism to release unwanted components from the cell, they are now recognized as a well-established mechanism for intercellular communication. Despite this, senescent cells remove toxic cytoplasmic DNA via EVs to maintain cellular homeostasis [36]. Indeed, senescent cells release more EVs (evSASP) than proliferating cells [36–40]. Interestingly, it has been shown that these EVs induce paracrine senescence in healthy cells, highlighting their importance as intercellular communication mediators (Figure 1B) [37,39]. While the mechanisms responsible for this are unknown, several miRNAs, interferon-related proteins, and antiapoptotic proteins were found enriched in senescent-derived EVs [37,39–42]. By contrast, a protumorigenic role for EVs derived from senescent cells mediated by ephrin A2 (EphA2) has been suggested [38] adding an extra layer of complexity to the evSASP. Interestingly, several ephrin-related proteins are enriched in plasma derived from old healthy donors [43,44], although whether they are free proteins or EV associated remains to be determined.

Metabolites are small chemical byproduct molecules of metabolic activity in cells and tissues that provide functional evidence of biochemical activity [45]. Thus, several metabolites are emerging as biomarkers of senescence, ageing, and related diseases. Interestingly, metabolite regulators have also been found inside EVs conferring intrinsic metabolic activity on EVs [90]. For example, extracellular nicotinamide phosphoribosyltransferase (eNAMPT), a regulator of NAD^+, was found in EVs and decreased with ageing in mice and humans. Furthermore, EV-contained eNAMPT increased NAD^+ levels in recipient cells, delaying ageing and extending lifespan in mice [46]. Interestingly, NAMPT has been shown to regulate the SASP [47].

The extracellular metabolite profile differs between young and elderly individuals. Analysis of blood from old donors (>74 years old) showed a reduction in metabolites related to antioxidants, redox, and muscle maintenance [48]. Levels of the antioxidant molecule NAD^+ decrease in various tissues, including plasma [49], during ageing [50,51], which correlates with the reduction

in the relative NAD^+:NADH ratios found in mitochondrial dysfunction-associated senescence [52]. Together these findings are associated with the extracellular metabolic profile of senescent cells, where an increase in citrate and metabolites involved in oxidative stress were found [53]. Citrate, however, can be found associated with iron in the blood. The levels of iron are generally increased in ageing and age-related diseases as Alzheimer's and Parkinson's, leading to the generation of reactive oxygen species (ROS). ROS, lactate, ketones, glutamine, and nitric oxide (NO) are all high-energy metabolites released by senescent cells producing a toxic surrounding microenvironment [54]. Given that ions and metabolites have been poorly characterized in senescence and ageing, it will be critical to further understand their implications as emerging components of the SASP.

Lipid mediators involve a family of molecules implicated in anti- and proinflammatory mechanisms that also enhance microbial clearance [55]. A recent study found that the obesity-related gut microbiota components lipoteichoic acid (LTA) and deoxycholic acid (DCA) induce senescence in hepatic stellate cells (HSCs), promoting hepatocellular carcinoma (HCC) progression [56,57]. Interestingly, LTA induced the release of the lipid metabolite prostaglandin E2 (PGE_2) via COX-2 and suppressed the antitumor immunity. It would be interesting to determine whether other lipid mediators are implicated in tissue homeostasis maintenance as part of the emerging SASP.

Nonclassical Intercellular Communication

In this section, we review other means of intercellular communication beyond soluble and emerging factors that are also important during senescence and ageing. We call these nonclassical (or not secreted) mechanisms for intercellular communication (Figure 1C).

Juxtacrine signaling is a means of intercellular communication characterized by cells involving ligand–receptor binding. Senescent cells also communicate with their neighbor cells through cell-to-cell or juxtacrine contact. For example, IL-1A, thought to be a master regulator of soluble factor paracrine senescence [20], also regulates juxtacrine senescence [58]. Senescent cells express membrane-bound IL-1A, which interacts with the IL-1R to control the levels of IL-6 and IL-8; thus, downregulation of IL-1R or IL-1α using RNAi or blocking antibodies prevents the upregulation of IL-6 and IL-8 during senescence (Figure 1C) [58]. Furthermore, in an elegant study Hoare *et al.* showed that membrane-bound NOTCH1 expression regulates the composition of the SASP, which they found to be highly dynamic [25]. Although NOTCH1 initially drives a classical TGF-β-enriched secretome, it also contributes to senescence mediated by cell-to-cell contact through the juxtacrine NOTCH/JAG pathway in a lateral induction fashion [59]. This has been defined as secondary juxtacrine senescence, where NOTCH1 is essential for the transmission of senescence through cell-to cell signaling but not for paracrine senescence mediated by soluble factors or cell-autonomous senescence [60].

It is known that sSASP can induce ECM remodeling and stiffening, which can alter immune cell recruitment in ageing [54]. Although it is acknowledged that senescent cells secrete a variety of ECM remodeling proteins, the interaction of the senescent cell with the ECM is less well described. Senescence can be induced by the interaction between the integrin α6β1 and the matricellular protein CCN1 during wound healing, inflammation, fibrosis, heart regeneration, and cancer, activating ROS [61–64]. Furthermore, integrin αvβ3 was also shown to induce senescence by activation of the TGF-β pathway in a cell-autonomous and non-cell-autonomous fashion [65,66]. However, although ROS release was induced, there was no DNA damage associated, suggesting that the ECM–cell interaction can induce senescence via different mechanisms.

Cell-to-cell fusion is a means of intercellular communication that can be induced upon the aberrant expression of fusogenic proteins or in response to viral infection such as with measles or ERVWE1 expression [67]. It induces senescence in response to fusion and mediates an immune response not only in primary but also in cancer cells. Although the mechanisms implicated remain to be elucidated, p53 is partially needed for the induction of senescence by fusion in cancer cells [68]. One of the pathological conditions where cell fusion has been observed is cancer [67]. Interestingly, the activation of senescence by chemotherapy in cancer cells stimulates them to 'engulf' neighboring cells, which are later processed through the lysosome. This provides these cells with material and energy to sustain the required high metabolic capacity of senescent cells [69]. However, the mechanisms behind this and whether this is a form of cell fusion would need to be further addressed.

Besides cell fusion, cytoplasmic bridges also allow the exchange of biological material between cells, including RNA, proteins, and even organelles such as mitochondria and lysosomes [70,71]. Cytoplasmic bridges are membrane extensions that allow spatiotemporal interaction between nearby cells. The transfer of materials between cells has been proved in diverse cell types, such as neurons, cancer cells, and immune cells, but also senescent cells [70,71]. By performing **trans-stable-isotope labelling of amino acids in cell culture (SILAC)**, Biran *et al.* showed that senescent cells transfer protein material to natural killer (NK) cells through cytoplasmic bridges mediating their activation and increased cytotoxicity [70]. The transfer is dependent on the GTPase CDC42 and the proteins transferred are mainly involved in actin cytoskeleton reorganization and antigen presentation, although certain proteins important for the activation of NK cells such as HSPA5 and CALR were also found. Interestingly, protein transfer also happens *in vivo* [70], clear whether the transfer is mediated exclusively by cytoplasmic bridges or by additional intercellular communication mechanisms.

Intercellular Communication in Physiology and Pathology

Together, the described studies show the importance of the contribution of classical, emerging, and nonclassical intercellular communication occurring during senescence and ageing. Although we have categorized the means for intercellular communication in the different sections, the reality is that it is extremely likely that a simultaneous combination of some, if not all, is what occurs *in vivo*, which will further depend on the biological or pathological context. Here, we review which means of communication are known for senescence in a particular physiological or pathological scenario. In addition, we discuss described communication types where we can hypothesize a role for senescence in particular contexts.

Intercellular Communication Contributes to Tissue Homeostasis Maintenance

The development of the placenta is an important step during pregnancy. One of the outer layers of the placenta is the **syncytiotrophoblast** that is formed by the fusion of trophoblast cells. This fusion induces the activation of senescence *in vitro* and several markers of senescence have been observed in this structure *in vivo* [68]. Although it is unclear why cells in the syncytiotrophoblast become senescent, it is speculated that it could be a mechanism to prevent apoptosis or contribute to maternal–embryonic transport in the placenta [68]. EVs from trophoblast cells have been shown to deliver miR-enriched EVs to non-trophoblast cells to protect against viral infections by inducing autophagy [72]. Together with the ability of senescent cells to develop cytoplasmic bridges, these mechanisms seem to potentiate the exchange of materials between cells during development (Figure 2A, top panel) [70,72].

Senescence is a normal process during development, where the sSASP plays a key role in mediating the clearance of these cells by macrophages to ensure the correct formation of the embryo [73,74].

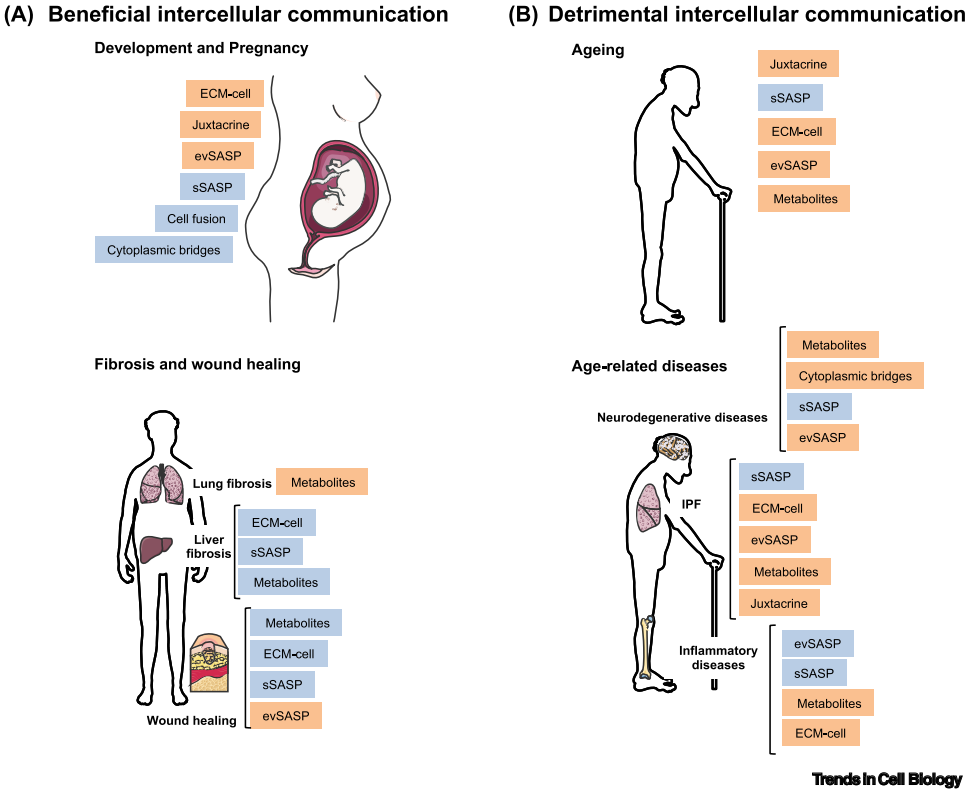


Figure 2. Intercellular Communication in Physiology and Age-Related Pathology. (A,B) Different types of intercellular communication have been described that can be (A) beneficial or (B) detrimental for tissue homeostasis and organism wellbeing. Blue boxes indicate that there is scientific evidence for a correlation between that type of intercellular communication and senescence; orange boxes show scientific evidence for the indicated particular physiological or pathological situation but not in the context of senescence. Abbreviations: ECM, extracellular matrix; evSASP, extracellular vesicle senescence-associated secretory phenotype; sSASP, soluble senescence-associated secretory phenotype.

The sSASP induces tissue remodeling and regeneration, a process in which cell fusion plays a critical role [75]. Interestingly, TGF-β as part of the sSASP seems to be key for senescence during development [73]. As integrins and NOTCH signaling regulate TGF-β in senescence [25,65] and both play key roles in development [76,77], it would be interesting to understand whether juxtacrine and ECM–cell intercellular communication occur in senescent cells at this time.

In certain scenarios such as wound healing and fibrosis, senescence is activated to prevent hyperproliferation and excessive scar formation [3,61,62]. Senescence can be mediated by contact with the ECM protein CCN1 via integrin α6β1, inducing a ROS-induced senescence response during wound healing and liver fibrosis (Figure 2A, bottom panel) [61,62]. Furthermore, the sSASP factor platelet-derived growth factor AA (PDGF-AA) released by senescent cells in the wound accelerates healing [78]. Meanwhile, the activation of senescence in liver HSCs recruits NK cells and tumor-inhibiting M1 macrophages to promote tissue resolution in fibrosis, in a p53-dependent manner [3]. Interestingly, EVs also contribute to the acceleration of wound healing closure and scar formation reduction in animal models [79], while increased NAD^+ protects against bleomycin-induced lung fibrosis [51]. Overall, during wound healing and fibrosis a combination of intercellular communication processes are described in the form of soluble and growth factors, EVs, metabolites, and interactions with the ECM, although more experimental evidence would be needed to determine the implications of their coexistence in senescence.

Intercellular Communication Can Be Detrimental in Ageing and Related Diseases

Although the incidence of cancer increases with ageing, there are already many reviews describing intercellular communication in cancer and it is therefore beyond the scope of this review [14,76,80]. Here, we review ageing and age-related neurodegenerative and inflammatory diseases.

One of the mayor changes occurring in ageing is a dysfunctional inflammatory response termed '**inflammaging**', where most of the inflammatory factors described are also part of the sSASP [5]. During ageing there is an accumulation of senescent cells, which chronically release sSASP factors. This, together with a defective immune system inefficient in eliminating senescent cells, contributes to inflammaging and tissue damage in ageing [5]. However, not only the sSASP contributes to age-related dysfunction. An altered ECM due to changes in matrix stiffness impairs the access of immune cells to senescence-enriched tissues [54]. As a consequence, this impairs the interaction of senescent cells with the ECM, affecting homeostatic processes such as proliferation, apoptosis, and migration [66,76]. Interestingly, NOTCH1 expression levels decrease during ageing, which could partially explain the chronic proinflammatory SASP via C/EBPβ activation if the molecular mechanism were conserved between cancer and ageing [25].

During ageing, the number of hypothalamic stem/progenitor cell decreases inducing an ageing-related phenotype in mice. However, treatment of old mice with EVs derived from young progenitor cells ameliorates age-related functions in the hypothalamus by preventing the decrease in the

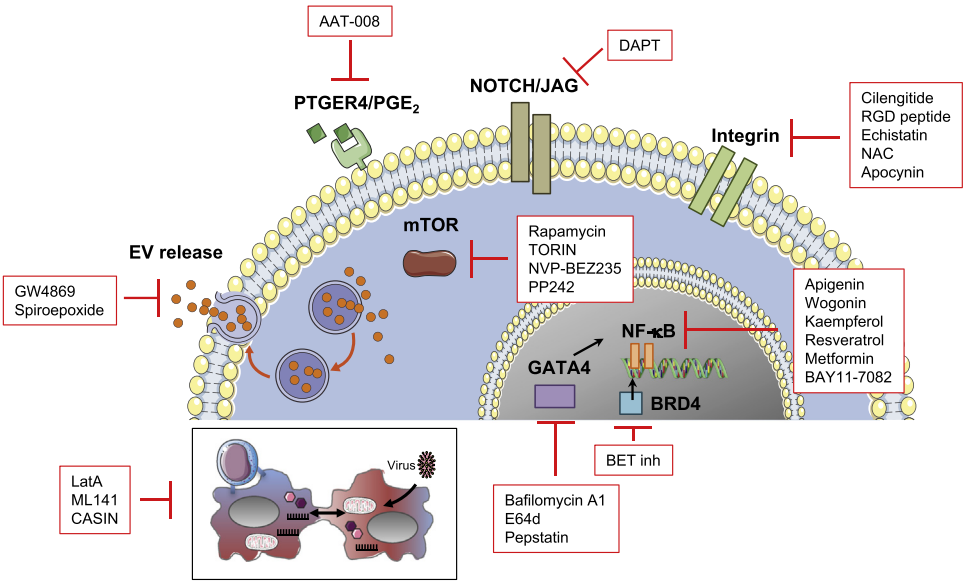


Figure 3. Pharmacological Manipulation to Prevent Intercellular Communication. Various pharmacological inhibitors have been described to prevent intercellular communication in senescence and ageing. The best characterized pharmacological drugs are those targeting the soluble senescence-associated secretory phenotype (sSASP). The sSASP can be neutralized by preventing common upstream master regulators such as GATA4, NF-κB, or BRD4. Alternatively, inhibitors can target other pathways involved in the release or processing of the sSASP, such as the mammalian target of rapamycin (mTOR) pathway. Additionally, individual components of the emerging SASP can be prevented using particular pharmacological drugs that block, for example, extracellular vesicle SASP (evSASP) release or prostaglandin E receptor 4 (PTGER4) binding to prostaglandin E2 (PGE_2). The nonclassical SASP can also be inhibited by preventing the NOTCH/JAG pathways, integrin signaling, or the formation of cytoplasmic bridges. Abbreviations: BET, bromodomain and extraterminal inhibitors; DAPT, *N*-[(3,5-difluorophenyl)acetyl]-L-alanyl-2-phenylglycine-1,1-dimethylethyl ester; LatA, latrunculin A; NAC, *N*-acetyl cysteine.

number of these cells via miR-contained EVs [41]. Similarly, old mice treated with eNAMPT-enriched vesicles – characteristic of young mice – also show delayed ageing [46], suggesting that EVs might target multiple pathways in old recipient cells [90]. Whether this is due to EV heterogeneity or to other, additional mechanisms remains to be determined. The role of EVs during ageing is still not well characterized.

Although metabolites and ions can be found in EVs, they are also present as extracellular molecules. During ageing there is an increase in the release of oxidative molecules such as NO and ROS concurrent with a decrease in those with an antioxidant capacity such as NAD^+. Together this produces an increase in oxidative stress, a driver for many hallmarks of ageing and senescence (Figure 2B, top panel) [5].

It is hypothesized that one of the consequences of ageing and related diseases is the accumulation of senescent cells and their active intercellular communication profile. For example, the use of senolytics shows amelioration of certain disease-related features in various neurodegenerative pathologies (e.g., Alzheimer's [11], Parkinson's [9,81]). As described before, many different means of intercellular communication are involved during ageing, with an inflammatory sSASP being the best characterized. Neurodegenerative diseases like Alzheimer's and Parkinson's are associated with chronic inflammation [82]. However, the release of ROS and reactive nitrogen species together with a decrease in antioxidants also greatly contributes to these pathologies [46,50,82]. Together this can increase Aβ deposition in Alzheimer's and α-synuclein truncation and aggregation in Parkinson's [82]. Interestingly, Aβ and α-synuclein can be transmitted to neighboring cells via EVs [35,83] or by cytoplasmic bridges in the case of α-synuclein [84]. Although the transfer of misfolded proteins contributes to neurodegeneration in these diseases, the release of ions such as iron also seems to contribute. Iron accumulation can be found in brain sections of multiple neurodegenerative diseases [85,86] stimulating microglia, NF-κB activation, and the release of proinflammatory cytokines [85]. Conversely, iron dysfunction leads to a form of cell death termed **ferroptosis**. Interestingly, senescent cells accumulate iron and suffer changes in the metabolism of iron [87]. As the transferrin receptor (a transporter of iron) has been found in EVs, it is tempting to speculate a role for EV in the transfer of iron in senescence, although more experimental evidence is needed (Figure 2B, bottom panel) [72].

A causative role for senescence has been found in many age-related inflammatory diseases, such as IPF, osteoarthritis, and atherosclerosis, where chronic inflammation is well characterized. This is concomitant with the release of oxidative molecules and decrease of antioxidant metabolites. It is known that NAD^+ levels are reduced in atherosclerosis, neurodegeneration, and sarcopenia [50]. Interestingly, in a recent study Jeon and colleagues found that EVs released from senescent chondrocytes transfer this phenotype to proliferating chondrocytes, inhibiting cartilage formation and contributing to an inflammatory environment in osteoarthritis [39]. The role of EVs in inflammatory diseases is highly important due to their immunoregulatory potential by either activating or inducing immunosuppression depending on the context. For example, EVs isolated from the synovial fluid of rheumatoid arthritis patients contribute to joint inflammation [72,88], while tumor-derived EVs have been shown to induce a tumor suppressive environment by inducing apoptosis in T cells via CD95 ligand (CD95L), tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRIAL), or Galectin 9 [72,80].

Concluding Remarks and Future Directions

Recent experiments in mice have confirmed a causative, detrimental role for senescent cells in several pathologies. The selective removal of senescent cells by treatment with various senolytics has shown that their elimination improves tissue homeostasis and ameliorates disease

Outstanding Questions

Does intercellular communication in senescence play other important, unidentified physiological roles?

What other means of intercellular communication are inhibited by senomorphic drugs?

How do senomorphic drugs affect normal physiological intercellular communication?

At which time point in the disease should senomorphics be used?

symptoms. The main culprit for the pathological effect of senescent cells in these contexts is thought to be the SASP. However, as senescent cells are also important for tissue homeostasis under physiological conditions, these drugs must be used with caution. An alternative approach to prevent the damaging effect of senescent cells is by controlling or neutralizing the SASP (Figure 3). The sSASP can be blocked following various approaches, such as preventing the activation of main upstream regulators such as GATA4, NF-κB, and BRD4. Alternatively, inhibition of the secretion or processing of the sSASP can be avoided using pharmacological drugs targeting, for example, the mTOR pathway. Finally, preventing the signaling of individual sSASP factors can be considered another strategy to avoid the functions of the sSASP. However, as reviewed here, intercellular communication in senescence is much more complex than initially thought and does not exclusively rely on soluble factors. It is therefore likely that other, currently unidentified means of communication exist, highlighting the need for systematic identification and analysis of alternative means of intercellular communication in senescence and ageing (see Outstanding Questions).

Acknowledgments

We are sorry that some excellent work could not be cited due to space limitations. This work was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/P000223/1) and Barts Charity Grant (MGU0497). J.F.L. is funded by the Xunta de Galicia Fellowship (ED481B 2017/117). The figures were designed using some elements from SMART Servier Medical Art licensed under Creative Commons BY 3.0. terms.

References

1. Muñoz-Espin, D. and Serrano, M. (2014) Cellular senescence: from physiology to pathology. *Nat. Rev. Mol. Cell Biol.* 15, 482–496
2. Childs, B.G. *et al.* (2015) Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat. Med.* 21, 1424–1435
3. He, S. and Sharpless, N.E. (2017) Senescence in health and disease. *Cell* 169, 1000–1011
4. Baker, D.J. *et al.* (2011) Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236
5. Lopez-Otin, C. *et al.* (2013) The hallmarks of aging. *Cell* 153, 1194–1217
6. Childs, B.G. *et al.* (2016) Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science* 354, 472–477
7. Baker, D.J. *et al.* (2016) Naturally occurring $p16^{Ink4a}$-positive cells shorten healthy lifespan. *Nature* 530, 184–189
8. Jeon, O.H. *et al.* (2017) Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat. Med.* 23, 775–781
9. Bussian, T.J. *et al.* (2018) Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline. *Nature* 562, 578–582
10. Chilosi, M. *et al.* (2013) Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl. Res.* 162, 156–173
11. Zhang, P. *et al.* (2019) Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. *Nat. Neurosci.* 22, 719–728
12. Coppe, J.P. *et al.* (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu. Rev. Pathol.* 5, 99–118
13. Lee, S. and Schmitt, C.A. (2019) The dynamic nature of senescence in cancer. *Nat. Cell Biol.* 21, 94–101
14. Faget, D.V. *et al.* (2019) Unmasking senescence: context-dependent effects of SASP in cancer. *Nat. Rev. Cancer* 19, 439–453
15. Rodier, F. *et al.* (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat. Cell Biol.* 11, 973–979
16. Kang, C. *et al.* (2015) The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science* 349, aaa5612
17. Freund, A. *et al.* (2011) p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J.* 30, 1536–1548
18. Dou, Z. *et al.* (2017) Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature* 550, 402–406
19. Gluck, S. *et al.* (2017) Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nat. Cell Biol.* 19, 1061–1070
20. Acosta, J.C. *et al.* (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15, 978–990
21. Herranz, N. *et al.* (2015) mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat. Cell Biol.* 17, 1205–1217
22. Laberge, R.M. *et al.* (2015) mTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat. Cell Biol.* 17, 1049–1061
23. De Cecco, M. *et al.* (2019) L1 drives IFN in senescent cells and promotes age-associated inflammation. *Nature* 566, 73–78
24. Tasdemir, N. *et al.* (2016) BRD4 connects enhancer remodeling to senescence immune surveillance. *Cancer Discov.* 6, 612–629
25. Hoare, M. *et al.* (2016) NOTCH1 mediates a switch between two distinct secretomes during senescence. *Nat. Cell Biol.* 18, 979–992
26. Acosta, J.C. *et al.* (2008) Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 133, 1006–1018
27. Kuilman, T. *et al.* (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133, 1019–1031
28. Demaria, M. *et al.* (2017) Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov.* 7, 165–176
29. Kang, T.W. *et al.* (2011) Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* 479, 547–551
30. Xue, W. *et al.* (2007) Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 445, 656–660
31. Mosteiro, L. *et al.* (2016) Tissue damage and senescence provide critical signals for cellular reprogramming *in vivo*. *Science* 354, aaf4445
32. Ocampo, A. *et al.* (2016) *In vivo* amelioration of age-associated hallmarks by partial reprogramming. *Cell* 167, 1719–1733.e12
33. Ritschka, B. *et al.* (2017) The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev.* 31, 172–183

34. Milanovic, M. *et al.* (2018) Senescence-associated reprogramming promotes cancer stemness. *Nature* 553, 96–100
35. van Niel, G. *et al.* (2018) Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* 19, 213–228
36. Takahashi, A. *et al.* (2017) Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nat. Commun.* 8, 15287
37. Borghesan, M. *et al.* (2019) Small extracellular vesicles are key regulators of non-cell autonomous intercellular communication in senescence via the interferon protein IFITM3. *Cell Rep.* 27, 3956–3971.e6
38. Takasugi, M. *et al.* (2017) Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. *Nat. Commun.* 8, 15729
39. Jeon, O.H. *et al.* (2019) Senescence cell-associated extracellular vesicles serve as osteoarthritis disease and therapeutic markers. *JCI Insight* 4, 125019
40. Terlecki-Zaniewicz, L. *et al.* (2018) Small extracellular vesicles and their miRNA cargo are anti-apoptotic members of the senescence-associated secretory phenotype. *Aging (Albany NY)* 10, 1103–1132
41. Zhang, Y. *et al.* (2017) Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. *Nature* 548, 52–57
42. Basisty, N. *et al.* (2020) A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol.* 18, e3000599
43. Tanaka, T. *et al.* (2018) Plasma proteomic signature of age in healthy humans. *Aging Cell* 17, e12799
44. Lehallier, B. *et al.* (2019) Undulating changes in human plasma proteome profiles across the lifespan. *Nat. Med.* 25, 1843–1850
45. Patti, G.J. *et al.* (2012) Innovation: metabolomics: the apogee of the omics trilogy. *Nat. Rev. Mol. Cell Biol.* 13, 263–269
46. Yoshida, M. *et al.* (2019) Extracellular vesicle-contained eNAMPT delays aging and extends lifespan in mice. *Cell Metab.* 30, 329–342.e5
47. Nacarelli, T. *et al.* (2019) NAD^+ metabolism governs the proinflammatory senescence-associated secretome. *Nat. Cell Biol.* 21, 397–407
48. Chaleckis, R. *et al.* (2016) Individual variability in human blood metabolites identifies age-related differences. *Proc. Natl. Acad. Sci. U. S. A.* 113, 4252–4259
49. Clement, J. *et al.* (2019) The plasma NAD^+ metabolome is dysregulated in "normal" aging. *Rejuvenation Res.* 22, 121–130
50. Fang, E.F. *et al.* (2017) NAD^+ in aging: molecular mechanisms and translational implications. *Trends Mol. Med.* 23, 899–916
51. Ralto, K.M. *et al.* (2020) NAD^+ homeostasis in renal health and disease. *Nat. Rev. Nephrol.* 16, 99–111
52. Wiley, C.D. *et al.* (2016) Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab.* 23, 303–314
53. James, E.L. *et al.* (2015) Senescent human fibroblasts show increased glycolysis and redox homeostasis with extracellular metabolomes that overlap with those of irreparable DNA damage, aging, and disease. *J. Proteome Res.* 14, 1854–1871
54. Fane, M. and Weeraratna, A.T. (2019) How the ageing microenvironment influences tumour progression. *Nat. Rev. Cancer* 20, 89–106
55. Serhan, C.N. (2014) Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510, 92–101
56. Loo, T.M. *et al.* (2017) Gut microbiota promotes obesity-associated liver cancer through PGE_2-mediated suppression of antitumor immunity. *Cancer Discov.* 7, 522–538
57. Yoshimoto, S. *et al.* (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499, 97–101
58. Orjalo, A.V. *et al.* (2009) Cell surface-bound IL-1α is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17031–17036
59. Parry, A.J. *et al.* (2018) NOTCH-mediated non-cell autonomous regulation of chromatin structure during senescence. *Nat. Commun.* 9, 1840
60. Teo, Y.V. *et al.* (2019) Notch signaling mediates secondary senescence. *Cell Rep.* 27, 997–1007.e5
61. Kim, K.H. *et al.* (2013) Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. *Mol. Cell. Biol.* 33, 2078–2090
62. Jun, J.I. and Lau, L.F. (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat. Cell Biol.* 12, 676–685
63. Feng, T. *et al.* (2019) CCN1-induced cellular senescence promotes heart regeneration. *Circulation* 139, 2495–2498
64. Jun, J.I. *et al.* (2015) The matricellular protein CCN1 mediates neutrophil efferocytosis in cutaneous wound healing. *Nat. Commun.* 6, 7386
65. Rapisarda, V. *et al.* (2017) Integrin beta 3 regulates cellular senescence by activating the TGF-β pathway. *Cell Rep.* 18, 2480–2493
66. Borghesan, M. and O'Loghlen, A. (2017) Integrins in senescence and aging. *Cell Cycle* 16, 909–910
67. Duelli, D. and Lazebnik, Y. (2007) Cell-to-cell fusion as a link between viruses and cancer. *Nat. Rev. Cancer* 7, 968–976
68. Chuprin, A. *et al.* (2013) Cell fusion induced by ERVWE1 or measles virus causes cellular senescence. *Genes Dev.* 27, 2356–2366
69. Tonnessen-Murray, C.A. *et al.* (2019) Chemotherapy-induced senescent cancer cells engulf other cells to enhance their survival. *J. Cell Biol.* 218, 3827–3844
70. Biran, A. *et al.* (2015) Senescent cells communicate via intercellular protein transfer. *Genes Dev.* 29, 791–802
71. Davis, D.M. and Sowinski, S. (2008) Membrane nanotubes: dynamic long-distance connections between animal cells. *Nat. Rev. Mol. Cell Biol.* 9, 431–436
72. Kalluri, R. and LeBleu, V.S. (2020) The biology, function, and biomedical applications of exosomes. *Science* 367, eaau6977
73. Muñoz-Espin, D. *et al.* (2013) Programmed cell senescence during mammalian embryonic development. *Cell* 155, 1104–1118
74. Storer, M. *et al.* (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* 155, 1119–1130
75. Ogle, B.M. *et al.* (2005) Biological implications of cell fusion. *Nat. Rev. Mol. Cell Biol.* 6, 567–575
76. Seguin, L. *et al.* (2015) Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. *Trends Cell Biol.* 25, 234–240
77. Kopan, R. and Ilagan, M.X. (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* 137, 216–233
78. Demaria, M. *et al.* (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell* 31, 722–733
79. Cabral, J. *et al.* (2018) Extracellular vesicles as modulators of wound healing. *Adv. Drug Deliv. Rev.* 129, 394–406
80. O'Loghlen, A. (2018) Role for extracellular vesicles in the tumour microenvironment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373, 20160488
81. Chinta, S.J. *et al.* (2018) Cellular senescence is induced by the environmental neurotoxin paraquat and contributes to neuropathology linked to Parkinson's disease. *Cell Rep.* 22, 930–940
82. Hou, Y. *et al.* (2019) Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 15, 565–581
83. Thompson, A.G. *et al.* (2016) Extracellular vesicles in neurodegenerative disease – pathogenesis to biomarkers. *Nat. Rev. Neurol.* 12, 346–357
84. Abounit, S. *et al.* (2016) Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes. *EMBO J.* 35, 2120–2138
85. Ashraf, A. *et al.* (2018) The aging of iron man. *Front. Aging Neurosci.* 10, 65
86. Crielaard, B.J. *et al.* (2017) Targeting iron metabolism in drug discovery and delivery. *Nat. Rev. Drug Discov.* 16, 400–423
87. Masaldan, S. *et al.* (2018) Iron accumulation in senescent cells is coupled with impaired ferritinophagy and inhibition of ferroptosis. *Redox Biol.* 14, 100–115
88. Boilard, E. *et al.* (2010) Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 327, 580–583
89. Sharpless, N.E. and Sherr, C.J. (2015) Forging a signature of *in vivo* senescence. *Nat. Rev. Cancer* 15, 397–408
90. Fafián-Labora, J. *et al.* (2020) Small extracellular vesicles have GST activity and ameliorate senescence-related tissue damage. *Cell Metab.* In press. https://doi.org/10.1016/j.cmet.2020.06.004