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Cellular senescence in vivo: From cells to tissues to pathologies

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ABSTRACT

Senescent cells accumulate during aging in a variety of tissues. Although scarce, they could influence tissue function non-cell-autonomously *via* secretion of a range of factors in their neighborhood. Recent studies support a role of senescent cells in age-related morbidity, including neurodegenerative diseases, cardiovascular pathologies, cancers, aging-associated nephrological alterations, chronic pulmonary disease and osteoarthritis, indicating that senescent cells could represent an interesting target for therapeutic exploitation across a range of pathophysiological contexts. In this article, we review data available to indicate which cell types can undergo senescence within various mammalian tissue environments and how these processes may contribute to tissue-specific pathologies associated with old age. We also consider markers used to identify senescent cells *in vitro* and *in vivo*. The data discussed may serve as an important starting point for an extended definition of molecular and functional characteristics of senescent cells in different organs and may hence promote the development and refinement of targeting strategies aimed at removing senescent cells from aging tissues.

1. Introduction

Cellular senescence is a form of stable cell cycle arrest that occurs in diploid cells when they are exposed to certain stressors, including genotoxic agents, nutrient deprivation, hypoxia, mitochondrial dysfunction and oncogene activation. Senescence was first described in primary cells by Hayflick and colleagues (Hayflick and Moorhead, 1961), when they observed that human diploid fibroblasts *in vitro* could reach only a finite number of cell divisions before their proliferation was arrested irreversibly. This biological phenomenon, referred to as the “Hayflick limit”, was later attributed to progressive telomere shortening upon consecutive rounds of cell divisions (Courtois-Cox et al., 2008). The Hayflick limit represents a physiological cellular response to counter genomic instability (Courtois-Cox et al., 2008; Hayflick and Moorhead, 1961). This phenomenon is now also referred to as replicative senescence. However, an accelerated senescence response, which is independent of telomere shortening, can also arrest growth or proliferation of diploid cells, and it is known as premature senescence (Courtois-Cox et al., 2008; d’Adda di Fagagna et al., 2003; Serrano et al., 1997). Subsequently, it was demonstrated that oncogenic stress (*e.g.*, overexpression of oncogenes and loss of tumor suppressor genes) can induce premature senescence *in vitro*, a process known as ‘oncogene-induced senescence’ (Counter et al., 1992; Serrano et al., 1997).

Many questions regarding the causes, signaling networks and mechanisms underlying the various types of cellular senescence still remain open, and current evidence is largely based on *in vitro* experiments. At the molecular level, cell cycle arrest and exit is controlled by the activation of the p53/p21^{CIP1} and the p16^{INK4a}/Rb tumor suppressor pathways (Herranz and Gil, 2018). The senescence growth arrest is often triggered by a persistent DNA damage response (DDR) caused by either intrinsic (*e.g.* oxidative damage, telomere attrition, hyperproliferation) or extrinsic insults (*e.g.* ultraviolet- or γ-irradiation, cytostatic drugs) (Herranz and Gil, 2018). In the context of the DDR signaling pathway, ATM or ATR kinases block cell-cycle progression through stabilization of p53 and transcriptional activation of the cyclin-dependent kinase (Cdk) inhibitor p21 (van Deursen, 2014). Moreover, activated oncogenes, such as oncogenic Ras, are also prominent inducers of senescence. Oncogenic Ras acts through overexpression of Cdc6 and suppression of nucleotide metabolism, resulting in aberrant DNA replication, formation of double-strand DNA breaks (DSBs) and activation of the DDR pathway (Aird et al., 2013; Di Micco et al., 2006). However, senescence caused by E2F3 activation or c-Myc inhibition is DDR-independent and involves p19^{Arf} and p16^{Ink4a} (Lazzerini Denchi et al., 2005; Nardella et al., 2011). Senescence is closely linked to profound metabolic changes (Dörr et al., 2013; Kondoh et al., 2005). Furthermore, various tumor suppressors trigger a senescent growth arrest when inactivated, including RB, PTEN, NF1 and VHL (Nardella

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et al., 2011; Shamma et al., 2009). While RB inactivation involves the DDR (Shamma et al., 2009), the other tumor suppressors mentioned are DDR-independent and induce senescence through $p19^{Arf}$ and $p16^{Ink4a}$. An interesting species-specific difference is that senescence pathways in murine cells are more dependent on $p19^{Arf}$ than those in human cells (Ben-Porath and Weinberg, 2005). Recently, the cGAS–cGAMP–STING pathway, in the context of which cGAS senses cytoplasmic DNA as a consequence of nuclear DNA damage, has been shown to connect DNA damage to inflammation, senescence and cancer *via* activation of the transcription factors IRF3 and NF-κB (Li and Chen, 2018; Yang et al., 2017). In summary, many senescence-inducing stressors or stimuli have in common that they trigger a DDR which then orchestrates downstream signaling mediated by ATM/R kinases or the cGAS–STING axis to acquire a full senescence phenotype. On the other hand, some of the stimuli induce cellular senescence through $p19^{Arf}$, $p16^{Ink4a}$ and profound metabolic changes suggesting that type, intensity and/or exposure time to senescence-inducing stimuli can play a major role in determining which senescence pathway gets triggered. However, it is important to note that current models regarding the molecular mechanisms involved in cellular senescence are largely based on *in vitro* data. To what extent each of these pathways is involved in driving cellular senescence within different tissues and cell types *in vivo* is an important issue that remains to be explored in future studies.

A primary feature of senescence cells, which distinguishes them from the state of quiescence, is irreversible proliferative withdrawal. *In vitro*, senescent cells often exhibit increased size with a flattened morphology, smooth shape, large vacuoles and possibly multiple nuclei. However, these changes in size and shape of senescent cells may not necessarily occur in the same manner in tissues *in vivo* (Rhinn et al., 2019). An additional feature of senescent cells is that they fail to respond to growth- and apoptosis-inducing stimuli (Baar et al., 2017; Yosef et al., 2016; Zeng et al., 2018). A typical senescence phenotype is characterized by a number of intrinsic and extrinsic markers, although none of them is specific or universal for senescence. At the molecular level, senescent cells are routinely characterized by upregulation of cell-cycle inhibitors such as p21 and/or p16 (Calliò et al., 2015; He et al., 2017), positive staining of senescence-associated β-galactosidase (SA-β-gal) at pH 6 (Dimri et al., 1995; He et al., 2017; Hernandez-Segura et al., 2018), formation of senescence-associated heterochromatin foci (SAHF) (Yamauchi et al., 2017), accumulation of lipofuscin, loss of lamin B1 (Shimi et al., 2011), senescence-associated distension of satellites (Swanson et al., 2013), the induction of senescence-associated DNA damage (Kim et al., 2017) and the secretion of a large number of factors, including growth factors, cytokines, chemokines, and proteases, which is commonly known as the senescence-associated secretory phenotype (SASP) (Coppe et al., 2010; Lopes-Paciencia et al., 2019). Moreover, enhanced expression of $p19^{ARF}$, p53 and PAI-1 are also observed in senescent cells and are used as senescence markers (Bernardes de Jesus and Blasco, 2012; Hernandez-Segura et al., 2018). A recent study suggested that c-Met could serve as an early marker of cellular senescence; however, the observations are based on *in vitro* investigation (Boichuck et al., 2019). Recently, Gal et al. demonstrated the use of the ImageStreamX approach as a powerful method for the detection and quantification of senescent cells in distinct tissues and cell populations (Gal et al., 2019). This novel method combines several senescence-related markers, together with the commonly used senescence-associated β-galactosidase assay and thereby offers a new solution to quantify senescent cells *in vivo*. For a summary of commonly used markers of cellular senescence, see Table 1.

Cellular senescence is induced under physiological or pathological conditions by a variety of extrinsic or intrinsic cellular signals (Avelar et al., 2020b; He and Sharpless, 2017; Hernandez-Segura et al., 2018; Yanai and Fraifeld, 2018; Zeng et al., 2018). The persistence of senescent cells in tissues may have beneficial function as well as may alter tissue remodeling and homeostasis: transient accumulation of senescent cells in tissues is mainly associated with beneficial functions (Calcinotto et al., 2019), whereas long-term accumulation of senescent cells appears to deteriorate tissue homeostasis. Recently, transgenic mouse models have been developed to permit the elimination of senescent cells *in vivo* in mice (including the INK/ATTAC model, the p16 − 3MR model and the ARF-DTR model (Baker et al., 2011; Demaria et al., 2014; Hashimoto et al., 2016), and a series of individual studies using these mouse models have now established clear, causal contributions of senescent cells to lifespan, wound healing, tissue development and programming and to age-related functional decline in specific organ systems (Avelar et al., 2020a; Baker et al., 2016, 2011; Childs et al., 2015; Docherty et al., 2019; Farr and Khosla, 2019; Hashimoto et al., 2016; Tacutu et al., 2011). In additional studies, senolytics, drugs that specifically target senescent cells by inducing apoptosis of senescent cells, were also used for the clearance of senescent cells in mice and humans as an alternative approach to the INK/ATTAC model, and interestingly, comparable therapeutic benefits have been reported (Hickson et al., 2019; Short et al., 2019; Zhang et al., 2019). The accumulation of senescent cells with advancing age in various tissues and their role in age-related tissue alterations and pathologies, such as osteoarthritis (Price et al., 2002) and atherosclerosis (Campisi, 2005), has been documented by a number of studies (Calcinotto et al., 2019; He and Sharpless, 2017; Rhinn et al., 2019). It has also been reported that senescent cells accumulate in various tumors *in vivo*, where they are thought to influence tumor progression (Campisi, 2005; Lee and Schmitt, 2019; Zeng et al., 2018). Therefore, therapeutic approaches which can selectively clear senescent cells may be useful for the treatment or prevention of a broad range of age-related disorders.

Available evidence indicates that only specific cell types within a given tissue are susceptible to acquiring a senescent phenotype, whereas other cell types remain unaffected by senescence. To further study the cells that are being targeted or should be targeted by senolytic approaches, knowledge of their identities is required. Here, we review currently available evidence linking specific cell types in various tissues to the potential for acquiring a state of senescence *in vivo* (see main text below and Table 2). We have focused this review on tissue types where cellular senescence has been suggested to play a role in functional alterations of a given tissue *in vivo*. We have not included tissues where no clear evidence for *in vivo* senescence, based on multiple methods, is available. We also required the availability of data on cell type identity of senescent cells and demonstrated links to tissue function. The data available may serve as an important starting point for the further definition of molecular and functional characteristics of senescent cells in different organs and may hence promote the development and refinement of targeting strategies aimed at removing senescent cells from aging tissues.

2. Senescence-prone cell types and their role in physiological and pathological processes

2.1. Senescence-associated cell types in physiological processes: development, regeneration and reprogramming

2.1.1. Tissue repair and wound healing

Senescent cells have been demonstrated to play a role in wound repair and the fibrotic response in a number of tissues, including the liver (Krizhanovsky et al., 2008), skin (Jun and Lau, 2010), lung (Schafer et al., 2017) and heart (Zhu et al., 2013). In a preclinical mouse model of liver damage and fibrosis, senescent hepatic stellate cells were identified in the fibrotic lesions in liver (Krizhanovsky et al., 2008). In this study, induction of damage in mice deficient for both the p53 and $p16^{INK4A}$ genes, which show nearly complete absence of senescence, resulted in increased fibrosis, suggesting that senescence plays a role in limiting liver fibrosis. Interestingly, the contribution of senescent cells to fibrotic scar formation appears to depend on the length of time of their presence as well as their level of activation. For instance, the matricellular protein CCN1 is expressed following wound

Table 1
List of markers and methods commonly used to detect senescent cells *in vivo* and *in vitro*.

Markers	Marker type	Detection method
Senescence-associated β-galactosidase (SA-β-gal) activity	Enzymatic	Chromogenic probe (X-Gal) (Dimri et al., 1995) fluorogenic probe (FDG, C12-FDG, Spider-Gal) and ImageStreamX (Cahu and Sola, 2013; Gal et al., 2019)
Lipofuscin accumulation	Lipofuscin granules	Staining with Sudan Black B (Evangelou et al., 2017)
p53, p21, p16, p19, γH2AX, ATM, HGMB1, c-Met, lamin B1, SASP factors: IL1, IL6, IL8, MMPs, PAI1 *etc.*	Molecular biomarkers	Immunofluorescence, western blot, real-time polymerase chain reaction (Boichuck et al., 2019; Carnero, 2013; Matjusaitis et al., 2016; Wang and Dreesen, 2018)
Telomere shortening, senescence-associated heterochromatin foci (SAHF)	Chromosome-associated	Quantitative fluorescence *in situ* hybridization, immunofluorescence (Bernadotte et al., 2016; Kosar et al., 2011)

Table 2
Senescent cell types in various mammalian tissues and their contribution to physiology and/or pathological processes.

Physiological and/or pathogenetic implications	Cell type(s)	Organ system(s)	Relevant references
Tissue repair and wound healing	Hepatic stellate cells, fibroblasts, epithelial cells, endothelial cells	Liver, skin, lung, heart	(Jun and Lau, 2010; Krizhanovsky et al., 2008; Schafer et al., 2017; Zhu et al., 2013)
Organ development, plasticity and reprogramming	Syncytiotrophoblasts, epithelial cells, fibroblasts	Mesonephros, endolymphatic sac of the inner ear, apical ectodermal ridge of developing limbs, hindbrain roofplate, neural tube, placenta, neonatal mouse hearts	(Chuprin et al., 2013; Feng et al., 2019; Munoz-Espin et al., 2013)
Chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis	Fibroblasts, epithelial cells, myofibroblasts, alveolar fibroblasts, mesenchymal stem cells, alveolar type II cells	Lung, bronchial tissue	(Chilosi et al., 2013; Hashimoto et al., 2016; Hecker et al., 2014; Kuwano et al., 1996; Lomas et al., 2012; Schafer et al., 2017)
Liver injury and damage, non-alcohol-related fatty liver disease, fibrotic changes and liver cirrhosis	Hepatic stellate cells, cholangiocytes, hepatocytes	Liver	(Aravinthan et al., 2013; Bird et al., 2018; Ferreira-Gonzalez et al., 2018; Huda et al., 2019; Kong et al., 2012; Krizhanovsky et al., 2008; Nishizawa et al., 2016)
Myocardial infarcts, myocardial fibrosis	Cardiomyocytes, myofibroblasts, cardiac progenitor cells	Heart	(Anderson et al., 2019; Lewis-McDougall et al., 2019; Torella et al., 2004; Zhu et al., 2013)
Glomerulosclerosis, diabetic nephropathy, renal fibrosis	Podocytes, endothelial cells, epithelial cells, tubulointerstitial cells	Kidney	(Chkhotua et al., 2003; Ding et al., 2001; Liu et al., 2012; Melk et al., 2004; Prattichizzo et al., 2018; Yang et al., 2018b)
Parkinson's disease, Alzheimer's disease, tauopathies, amyotrophic lateral sclerosis	Astrocytes, microglia	Brain	(Bachstetter et al., 2011; Bhat et al., 2012; Bussian et al., 2018; Chinta et al., 2018; Coppe et al., 2008; Salminen et al., 2011; Sierra et al., 2007; Trias et al., 2019)
Osteoarthritis, osteoporosis	Chondrocytes, osteoclasts, mesenchymal stem cells	Bone	(Loeser, 2009; Martin and Buckwalter, 2003; Price et al., 2002; Zhou et al., 2004)
Glaucoma, senile cataract, aging	Endothelial vascular cells, lens epithelial cells, corneal epithelial cells, corneal endothelial cells, retinal neurons ('senescence-like' phenotype based on the expression of p16 and p21)	Eye	(Fu et al., 2016; Lopez-Luppo et al., 2017; Skowronska-Krawczyk et al., 2015; Song et al., 2008; Yan et al., 2019)
Skin aging, skin cancer, wound healing	Epidermal keratinocytes, melanocytes, dermal fibroblasts	Skin	(Dimri et al., 1995; Gray-Schopfer et al., 2006; Victorelli et al., 2019; Wang et al., 2017)
Diabetes mellitus	Pancreatic β-cells	Pancreas	(Helman et al., 2016; Thompson et al., 2019)
Lipodystrophy, diabetes mellitus	Adipose progenitor cells, adipocytes, preadipocytes	Adipose tissue	(Gustafson et al., 2019; Minamino et al., 2009)
Muscle aging, muscular dystrophy and degeneration	Endothelial progenitor cells, myoblasts	Musculoskeletal system	(Baar et al., 2018; Bigot et al., 2009; Le Roux et al., 2015; Thornell et al., 2009; Yang et al., 2018a)
Aging, ovarian cancer	Leydig cells, testicular fibroblasts, spermatogenetic cells, epithelial ovarian cancer cells, theca-interstitial cells	Testes, ovary	(Bitler et al., 2011; Merz et al., 2019; Wang et al., 2018)
Atherosclerosis, degenerative vascular changes	Endothelial cells, vascular smooth muscle cells, macrophages	Arteries	(Catita et al., 2015; Childs et al., 2016; Durik et al., 2012; Lopez-Luppo et al., 2017)
Aging, autoimmune diseases and chronic infections	Thymic epithelial cells, T cells	Spleen, thymus	(Barbouti et al., 2019; Chou and Effros, 2013; Covre et al., 2018; Wang et al., 2009)
Aging, cancer, inflammation, chronic infections, Crohn's disease	Intestinal epithelial cells, fibroblasts	Gastrointestinal tract	(Cougnoux et al., 2014; Going et al., 2002b; Guo et al., 2019; Penfield et al., 2013)

induction in the skin and activates a senescence response in skin fibroblasts, including p53 and $p16^{INK4A}$ expression (Jun and Lau, 2010). A beneficial role of a SASP containing platelet-derived growth factor AA (PDGF-AA) secreted by senescent fibroblasts and endothelial cells has also been demonstrated in skin wounds (Demaria et al., 2014). Interestingly, senescent fibroblasts and endothelial cells appear very early in response to a skin wound, where they accelerate wound healing by inducing PDGF-AA-mediated myofibroblast differentiation (Demaria et al., 2014). In the p16−3MR model, which permits selective elimination of p16-positive senescent cells, premature death of transient senescent cells resulted in impaired wound healing (Demaria et al., 2014). Further, senescent cells have also been reported to accumulate and contribute to the fibrosis in mouse models of idiopathic lung fibrosis (IPF) induced by bleomycin treatment (Schafer et al., 2017). Significant upregulation of p16 and several SASP components within fibroblasts and epithelial cells, but not endothelial cells, was observed in response to bleomycin (a chemotherapeutic agent) treatment. Surprisingly, elimination of senescent cells improved bleomycin-induced lung fibrosis in the INK-ATTAC mouse model (Schafer et al., 2017). Moreover, the authors demonstrated significantly increased p16 expression in lung fibroblasts and epithelial cells within fibrotic foci in human IPF lung samples. The transient induction and accumulation of senescent cells has also been observed following limb amputation and regeneration in the salamander (Yun et al., 2015). Within the regenerating salamander limb, senescent cells were found at the level of the amputation plane, including cartilage and muscle, and within the blastema, including fibroblast, monocyte-like cells, and epidermal glands (Yun et al., 2015). Together, these lines of recent evidence suggest that cellular senescence is also required for some physiological functions.

2.1.2. Development

Cells exhibiting markers and features of senescence have been documented within developing embryos in many animal models. In the context of development, cellular senescence was first described in mouse embryos between embryonic day 9.5 and 15.5 (Munoz-Espin et al., 2013; Storer et al., 2013) and was shown to be p21-dependent. Investigators performed SA-ß-gal staining of mouse embryos as a senescence marker and identified multiple tissues exhibiting the presence of senescent cells (Munoz-Espin et al., 2013; Storer et al., 2013). During mammalian embryonic development, tissues with an evident number of senescent cells included the mesonephros, the endolymphatic sac of the inner ear, the apical ectodermal ridge (AER) of the developing limb, the hindbrain roofplate, the mesonephros, and the neural tube. In the mesonephric tubules and endolymphatic sac, the detected senescence cells were identified as epithelial cells at E14.5 (Munoz-Espin et al., 2013). Furthermore, cells bearing many signatures of cellular senescence have also been identified and described in the mesonephros and endolymphatic sac of human embryos (Munoz-Espin et al., 2013), thus suggesting a role of developmentally programmed senescence in tissue remodeling and patterning. Interestingly, senescent cells have been described in the extra-embryonic tissues of the placenta in mouse and human, where cell fusion-induced senescence provides a functional interface between the mother and the developing fetus (Chuprin et al., 2013). Accordingly, placental senescent syncytiotrophoblast cells might contribute to physiological functions of the placenta.

2.1.3. Tissue plasticity and reprogramming

Forced expression of the four Yamanaka factors Oct4 (Pou5f1), Sox2, Klf4 and Myc (OSKM) induces the upregulation of senescence markers in those cells that do not undergo reprogramming. Molecularly, the lack of pluripotency occurs because of sustained upregulation of p53, $p16^{INK4a}$, and $p21^{CIP1}$ (Banito et al., 2009; Hong et al., 2009). Recent *in vivo* reprogramming studies have further elucidated our understanding of the intricate connection between cellular reprogramming and senescence (Mosteiro et al., 2016). Available evidence indicates that SASP components, such as IL6, enhance OSKM activity and facilitate reprogramming in adjacent cells. Recently, two studies have identified a role of transient senescence in heart regeneration such that elimination of senescent cells impairs proper heart regeneration (Feng et al., 2019; Sarig et al., 2019). Feng et al. identified $PDGFR^{+}$ senescent fibroblasts in neonatal mouse hearts at P3, P7 and P14, whereas no senescent cells were detected in fully-restored hearts at P21 (Feng et al., 2019). Another study uncovered a primary and beneficial role for the SASP derived from senescent cells, including epithelial cells, in promoting cell plasticity and tissue regeneration (Ritschka et al., 2017). A few additional papers are available to suggest a key role of the SASP in tissue plasticity, despite that senescent cell types were not clearly identified in these studies (Chiche et al., 2017; Mosteiro et al., 2016; Sarig et al., 2019).

2.2. Senescence-associated cell types and their pathogenic roles

2.2.1. Lung

The presence of cellular senescence is considered to be an important factor in chronic lung diseases, particularly chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). A growing body of evidence has confirmed senescence biomarkers, including p16, p21 and senescence-associated β-galactosidase activity (SA-β-gal), in both fibroblasts and epithelial cells from human IPF lung tissue (Kuwano et al., 1996; Lomas et al., 2012; Schafer et al., 2017). In this regard, human IPF cells show increased senescence propensity *ex vivo* (Yanai et al., 2015). Intratracheal injections of the chemotherapeutic agent bleomycin in mice has been shown to cause resolvable lung fibrosis that recapitulates key features of human IPF (Izbicki et al., 2002), and the age-dependent accumulation of senescent myofibroblasts in mice appears to impede fibrosis resolution following bleomycin treatment (Hecker et al., 2014). Bleomycin-induced lung injury as an IPF model in mice was used to show that, similar to human IPF, murine lung fibrosis is also characterized by accumulation of p16- and SASP-positive fibroblasts and epithelial cells (Schafer et al., 2017). Senescent cell ablation, induced by a treatment with dasatinib plus quercetin (DQ), improves pulmonary function. Using ARF-DTR mice, Hashimoto et al. provided evidence for a critical role of cellular senescence in lung aging and pathologies (Hashimoto et al., 2016), suggesting that cellular senescence represents a target for the development of therapeutics for pulmonary diseases. The ablation of $p19^{ARF}$-expressing cells using a toxin receptor-mediated cell knockout system ameliorated aging-associated lung hypofunction and reversibly restored pulmonary function in 12-month-old mice. A major senescent cell population that was identified in the lung of 12-months-old ARF-DTR mice was that of alveolar fibroblasts. Recently, another study revealed that senescent epithelial cells could contribute to the activation of Wnt/β-catenin signaling and induction of Nanog in pulmonary fibroblasts *via* increasing the expression of the SASP, ultimately resulting in pulmonary fibrosis (Chen et al., 2019). In a very recent study, serpine 1 (also known as plasminogen activator inhibitor 1 (PAI-1)) was implicated in alveolar type II cell senescence through activation of the p53-p21-Rb pathway in fibrotic lung disease (Jiang et al., 2017). Cellular senescence has also been widely implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD), presumably by impairing cell repopulation and by aberrant cytokine secretion in the context of the senescence-associated secretory phenotype. According to recently proposed pathogenic models of COPD, premature cellular senescence likely affects distinct cell types such as mesenchymal stem cells and bronchial epithelial cells (Barnes, 2017; Chilosi et al., 2013; Kuwano et al., 2016).

2.2.2. Liver

Senescence can adversely affect liver functions, cellular viability and tissue regeneration under pathological conditions. A fibrotic response is activated in the context of chronic liver damage and

consecutive reparative processes. Tissue damage to the liver can ultimately lead to cirrhosis, which is characterized by excessive tissue fibrosis that compromises liver function. Senescent hepatic stellate cells (HSCs) have been observed along the periphery of scars in the liver after the induction of liver damage and fibrotic scarring upon CCl4 administration in mice (Krizhanovsky et al., 2008). These senescent HSCs were shown to induce fibrotic resolution through diminished production of extracellular matrix (ECM) components and increased secretion of anti-fibrotic SASP factors. Upon liver damage, IL22 induces senescence in HSCs in a p53-dependent manner through STAT3, SOCS3 and IGF-1 (Kong et al., 2012; Nishizawa et al., 2016). The enterohepatic circulation of deoxycholic acid (DCA), a gut bacterial metabolite that causes DNA damage, has been shown to provoke a SASP phenotype in hepatic stellate cells (HSCs). As a result, HSCs secrete various inflammatory and tumor-promoting factors in the liver, thus facilitating hepatocellular carcinoma (HCC) development in mice (Yoshimoto et al., 2013). In a mouse model of biliary senescence, it was reported that senescent cholangiocytes induce profound alterations in the cellular microenvironment, including the recruitment of myofibroblasts and macrophages causing collagen deposition, TGFβ production and the induction of senescence in surrounding cholangiocytes and hepatocytes (Ferreira-Gonzalez et al., 2018). Of note, the inhibition of TGFβ signaling disrupted the transmission of senescence in cholangiocytes and hepatocytes, and restored liver function. Senescence in hepatocytes is indeed well documented in normal liver and different liver diseases such as chronic hepatitis C, HCC and liver cirrhosis (Frey et al., 2018; Huda et al., 2019; Kang et al., 2011; Paradis et al., 2001; Rudolph et al., 2000; Wiemann et al., 2002; Xiao et al., 2018). Teoh et al. reported the induction of p53, $p21^{Cip1}$ and $p16^{INK4A}$ in senescent hepatocytes in chronic liver diseases, although the detailed mechanism remains unclear (Teoh et al., 2010). Using a hepatocyte-specific senescence induction model (AhCre-$Mdm2^{fl/fl}$), Bird et al. demonstrated that acute liver injury could also induce senescence in hepatocyte (Bird et al., 2018).

Non-alcohol-related fatty liver disease (NAFLD) reveals features of accelerated aging, such as impaired regeneration, and an increased risk of HCC. In liver sections from patients with NAFLD, Aravinthan et al. demonstrated that hepatocytes in NAFLD lack cell cycle progression beyond G1/S phase, accumulate high-level of p21, present a larger nuclear area and γ-H2AX positive foci (Aravinthan et al., 2013; Huda et al., 2019). The presence of hepatocyte senescence positively correlated not only with the fibrotic stage of the liver but also with the diabetic state associated with NAFLD. A recent study reported a close correlation between hepatic fat accumulation and markers of hepatocyte senescence, demonstrating that the selective elimination of senescent cells using genetic approaches or senolytics reduces overall hepatic steatosis (Ogrodnik et al., 2017).

2.2.3. Cardiovascular system

A large body of evidence accumulated over the years indicates a close connection between pathways involved in cellular senescence and cardiovascular disorders (CVDs). In a study using mice, the authors observed that old animals (20–22 months) have increased left ventricular weight and cardiomyocyte volume, which was associated with reduced cardiomyocyte numbers because senescence and death of cardiac stem cells increased with age in WT mice impairing the growth and turnover of cells in the heart (Torella et al., 2004). Telomere attrition in cardiomyocytes has been implicated in several heart diseases such as hypertrophic cardiomyopathy (HCM), ischemic cardiomyopathy (ICM) and idiopathic cardiomyopathy (IDCM) (Sharifi-Sanjani et al., 2017). However, it is evident now that length-independent telomere damage can cause cardiomyocyte senescence as evidenced by increased expression of $p16^{Ink4a}$ and p21 as well as positive SA-β-gal staining (Anderson et al., 2019). In a recently published article, it was reported that endothelial cells acquire a senescent phenotype in cardiac tissues when senescence-accelerated mice were fed a high-fat high-salt diet (Gevaert et al., 2017).

Following myocardial infarcts (MI), senescence is reported to play an important role in limiting fibrosis. In a mouse model of MI, cardiac myofibroblasts were shown to develop a senescence phenotype (Meyer et al., 2016; Zhu et al., 2013). Interestingly, in the left coronary artery occlusion model, only p53 loss was sufficient to limit further fibrosis (Zhu et al., 2013), whereas dual loss of p53 and $p16^{INK4A}$ was required in a transverse aortic constriction model (Meyer et al., 2016). Therefore, it is possible that the type or extent of damage influences senescence pathways that are activated in cardiac myofibroblasts. A recent article published an extensive analysis of cardiac progenitor cells (CPCs) isolated from human subjects (aged 32–86 years) with cardiovascular diseases. In aged subjects (> 70 years old), over half of the CPCs were observed to be senescent based on senescence markers such as $p16^{INK4A}$, SA-β-gal and γH2AX (Lewis-McDougall et al., 2019). The evidence showing the role of cellular senescence in CVD and benefits of senescent cell clearance using senolytics or clearance induction in INK/ATTAC mice have been discussed in detail elsewhere (Childs et al., 2018; Shimizu and Minamino, 2019). Available data indicate that senescent cells represent a promising therapeutic target in the context of primary and secondary prevention of cardiovascular disorders.

2.2.4. Kidney

Aging is a major risk factor for kidney dysfunction and disease (Denic et al., 2016; Sturmlechner et al., 2017). Senescence markers have been observed in human and mouse renal tissues in the context of age-related kidney dysfunction and disorders (Docherty et al., 2019; Sturmlechner et al., 2017; Valentijn et al., 2018). During aging, increased renal p16 expression was observed most notably in tubular epithelial cells and to a lesser extent also in glomerular cells (including podocytes, mesangial and/or endothelial cells as well as parietal epithelium) and interstitial cells within human kidney biopsies (Chkhotua et al., 2003; Melk et al., 2004; Sis et al., 2007). Changes in p16 expression were more prominent in the cortex region compared to the medulla of the kidney. It is evident that the extent of senescent proximal tubular cells increases with advancing age in rodents, whereas no increase of senescent cells was observed in the glomeruli (Berkenkamp et al., 2014; Krishnamurthy et al., 2004). In kidney, aging-associated tubular cell senescence significantly correlated with the extent of tubular atrophy, interstitial fibrosis, glomerulosclerosis and diabetic nephropathy (Krishnamurthy et al., 2004; Liu et al., 2012; Prattichizzo et al., 2018; Verzola et al., 2008). In aging rats, kidney tubular cells developed senescence, which was detected by beta-galactosidase staining, and enhanced expression of TGF-β1 and $p21^{CIP1}$ was also observed in the tubulointerstitial cells (Ding et al., 2001). The accumulation of senescent cells (increased PAI-1 and SA-β-gal) is also seen in hyperglycemic rats and mice, where the most prominent effect is observed in the cortical and proximal tubules of the kidneys (Kitada et al., 2014; Satriano et al., 2010). Moreover, short-term sustained hyperglycemia promotes a senescence-associated secretory phenotype in endothelial cells in kidneys of a mouse model of diabetes (Prattichizzo et al., 2018). For unclear reasons, interstitial and vascular cells are extremely prone to become senescent in hypertension and glomerular disease (Westhoff et al., 2008). Other kidney diseases, such as IgA nephropathy and lupus nephritis, have also been associated with increased senescent (tubular epithelial and glomerular cells) cell burden (Liu et al., 2012; Yang et al., 2018b).

2.2.5. Brain

Recent studies have suggested that senescent cells might also play a role in certain neurodegenerative diseases. Astrocytes in aged brains have been reported to express a characteristic SASP and higher levels of p16 than in young brains (Bhat et al., 2012; Salminen et al., 2011). In frontal cortices from Alzheimer's patients, astrocytes express higher levels of p16, γH2AX and MMP1 compared to age-matched control samples (Bhat et al., 2012; Frank-Cannon et al., 2009; Myung et al.,

2008). However, it is not clear how these cells may influence disease progression. In a mouse model of Parkinson's disease, the herbicide paraquat (PQ) was found to induce astrocytic senescence and SASP *in vitro* and *in vivo*, and senescent cell depletion in the latter protected against PQ-induced neuropathology (Chinta et al., 2018). In addition, microglia in aged mice exhibit an increased production of the proinflammatory cytokines IL-6, IL-1β and TNF-α, all of which are factors often upregulated in senescent cells (Bachstetter et al., 2011; Coppe et al., 2008; Sierra et al., 2007). Recently, higher expression of the senescent cell markers p16 and Lamin B1 in spinal cord microglia, and their role in paralysis progression was shown in a rat model of ALS (Trias et al., 2019). In a recent seminal study using a model of tauopathy, researchers found that astrocytes and microglia acquire a senescence phenotype and contribute to tau-dependent pathology and cognitive decline (Bussian et al., 2018). Notably, mouse-derived astrocytes and microglia had increased expression of senescence-associated genes, including $p16^{Ink4a}$, while a similar induction of senescence markers was not observed in oligodendrocytes. Together, these published findings indicate that senescent microglia and astrocytes play an important role in age-related brain disorders.

2.2.6. Skeletal system

The roles of senescence in mediating age-related bone loss and bone diseases have been a recent focus of investigation. Several independent studies in mice and humans provide evidence that during aging a number of cell types, such as B cells, T cells, myeloid cells and osteoblast progenitors, become senescent and develop a heterogeneous SASP in the bone microenvironment (Farr et al., 2017a, b; Jeon et al., 2018). Osteoarthritis is an age-related disease, characterized by pain, loss of cartilage, and joint inflammation, in which the overall integrity of synovial joints is compromised. Chondrocytes secrete many components of the extracellular matrix (ECM) and maintain articular cartilage, but they may enter senescence with advancing age and under pathogenic conditions (Loeser, 2009; Martin and Buckwalter, 2003; Price et al., 2002). Chondrocytes isolated from osteoarthritic joints display multiple markers of senescence including $p16^{Ink4a}$ (Zhou et al., 2004), SA-β-gal (Price et al., 2002) and also express various matrix metalloproteinases (MMPs) (Farr and Khosla, 2019). Interestingly, osteoarthritis could be prevented by eliminating senescent cells using the INK/ATTAC mouse model (Jeon et al., 2017). Osteoporosis is another disorder that develops as a result of an imbalance in bone turnover, where bone resorption by osteoclasts exceeds bone formation by osteoblasts. Aging is a major risk factor for osteoporosis as well (Farr et al., 2017b; Gnani et al., 2019) and p16 expression has been observed to be significantly upregulated in osteoblasts and osteoclasts in the bone microenvironment in old mice (Farr et al., 2016). However, osteocytes and myeloid cells were the only cell types that showed a senescence-associated SASP profile. In MSCs from the bone marrow of both young and aged donors, it was shown that aged MSCs were enlarged compared to young MSCs. Furthermore, old MSCs showed increased levels of SA-β-galactosidase and SASP (Gnani et al., 2019).

2.2.7. Eye

Senescence is an important driver of age-related cataracts (ARC) (Fu et al., 2016). A recent study indicated that the senescence-associated protein p53, as well as total laminin (LM), LMα4, and TGF-β1 are increased in the anterior lens capsules (ALCs) in the context of ARC (Yan et al., 2019). In lens epithelial cell (HLEB-3) senescence mouse models, matrix metalloproteinase-9 (MMP-9) alleviated senescence by decreasing the expression of total LM and LMα4 (Yan et al., 2019). Elevated expression of senescence-related genes $p16^{INK4a}$, $p21^{CIP1}$, $p27^{KIP1}$, and p53 has been reported in human corneal endothelial cells (Song et al., 2008).

Glaucoma is currently the leading cause of blindness worldwide and it is characterized by elevated intraocular pressure (IOP) that can lead to the progressive degeneration of the optic nerve (Skowronska-Krawczyk et al., 2015). It was recently reported that increased IOP can induce the expression of SIX6, a homeobox protein involved in eye development in mice. Interestingly, a risk variant in SIX6 was found to induce increased p16 expression and ganglion cell senescence (Skowronska-Krawczyk et al., 2015). Removal of senescent cells in a p16−3MR mouse model protects against retinal ganglion cell loss in experimental ocular hypertension (Rocha et al., 2019). Yet, it remains unclear how senescent cells can contribute to glaucoma.

Interestingly, cellular senescence has also been observed in aged human retinas (Lopez-Luppo et al., 2017). Some features of senescence have been described to occur in neurons, for example, cortical and Purkinje neurons show several senescence features like SA-β-gal activity, lipofuscin accumulation, γH2AX and macro-H2A foci, as well as IL6 expression, all in a $p21^{CIP1}$-dependent manner (Jurk et al., 2012). Interestingly, human neurons might also senesce, since there is expression of p16/Cdkn2a in pyramidal neurons in the prefrontal cortex from human brains of people over 77 years old (Kang et al., 2015), however clear evidence of *in vivo* neuronal senescence is still lacking. Neurons are post-mitotic cells and it is hence not clear to what extent these senescence-like features are indicative of true senescence states similar to those seen in replicating cell types. Retinal neurons were proposed to adopt a senescent-like state based on the expression of p21 and p16. In addition, retinal blood vessel cells (endothelial cells) displayed a senescent phenotype characterized by p16, p53 and p21 accumulation, as well as increased SA-β-gal activity and accumulation of lipofuscin (Lopez-Luppo et al., 2017). Moreover, senescent endothelial cells expressed p16 in the inner part of their cellular membrane, where p16 colocalized with the β1 integrin subunit.

2.2.8. Skin

Senescent cell numbers increase in the skin during human aging (Dimri et al., 1995; Dreesen et al., 2013; Ghosh and Capell, 2016; Ressler et al., 2006; Waaijer et al., 2012). In skin samples from human donors of different ages, there was an age-dependent increase in cellular senescence with regard to dermal fibroblasts and epidermal keratinocytes (Dimri et al., 1995). In an attempt to investigate age dependency of $p16^{INK4A}$ *in vivo*, human skin samples from the age groups 0–20, 21–70, and 71–95 years were selected, and $p16^{INK4A}$ expression was analyzed by immunohistochemistry. This study revealed that $p16^{INK4A}$ shows an age-dependent increase of expression levels in non-proliferating cells of human skin *in vivo* (Ressler et al., 2006). Furthermore, a recent study demonstrated that senescent human melanocytes accumulate in skin with advancing age, affect keratinocyte function, and drive skin aging *via* paracrine telomere dysfunction (Victorelli et al., 2019). Melanocytes have also been positively stained for multiple senescence markers, including SA-β-gal, $p16^{INK4a}$ and loss of lamin B1 within preneoplastic lesions and skin cancer (Gray-Schopfer et al., 2006; Michaloglou et al., 2005).

UVA and UVB rays are known to contribute to skin aging and cancer development. UVA is a weak mutagen; however, it penetrates into the dermis and contributes to oxidative stress and tissue inflammation. UVB is more mutagenic as it directly interacts with DNA to generates dipyrimidine photoproducts, resulting in DNA damage (Wang and Dreesen, 2018). A recent study provided evidence that a chronic low dose exposure to UVB results in the accumulation of DNA damage and senescent cells featuring low expression levels of lamin B1 within the mouse epidermis, but not within the dermis (Wang et al., 2017).

2.2.9. Pancreas

Aging has been associated compromised β-cell functions both in mice and humans (Arda et al., 2016; Helman et al., 2016). Forced p16 expression in β-cells was sufficient to induce senescence and was associated with increased insulin secretion (Helman et al., 2016). Immunostaining and gene expression analyses of human islets revealed that p16 expression was higher in β-cells of aged subjects compared to young individuals (Helman et al., 2016). It has also been shown that a

subset of β-cells acquires a SASP during the natural history of type 1 diabetes (T1D) in humans as well as in the non-obese diabetic (NOD) mouse model (Thompson et al., 2019), raising the possibility that senescent β-cells may play a role in the pathogenesis of T1D.

2.2.10. Adipose tissue

Available evidence indicates that the accumulation of senescent preadipocytes and adipocytes in both subcutaneous and visceral adipose tissue is an important contributor to adipose tissue dysfunction and inflammation in aging (Ghosh et al., 2019; Mancuso and Bouchard, 2019; Stout et al., 2014; Tchkonia et al., 2010). Adipose progenitor cells derived from hypertrophic obese individuals are characterized by an increased frequency of senescence and are associated with an impaired adipogenic potential (Gustafson et al., 2019). Excessive calorie intake in mice promotes senescence in adipose tissue *via* ROS-mediated activation of p53 and p21 (Minamino et al., 2009). Diabetes is also associated with cellular senescence in adipose tissue (Gustafson et al., 2019; Minamino et al., 2009). Adipose tissues from diabetic humans feature increased SA-βgal activity, as well as elevated levels of p53, TNF-α, and MCP-1 expression compared to nondiabetic individuals (Minamino et al., 2009). Senescent adipocytes upregulate proinflammatory factors, including CCL2 and TNF-α, while they downregulate anti-inflammatory factors, such as adiponectin, and promote the development of impaired insulin sensitivity and glucose tolerance (Minamino et al., 2009).

2.2.11. Skeletal muscle

Recent evidence shows senescent cells can chronically interfere with stem cell function and drive aging of the musculoskeletal system (Baar et al., 2018). Multiple studies demonstrated that senescence of muscle stem cells occurs in mouse models of muscle dystrophies, such as Duchenne muscular dystrophy or Steinert's disease (Bigot et al., 2009; Le Roux et al., 2015; Thornell et al., 2009; Zhang et al., 2016). In myotonic dystrophy type 1 (DM1 or Steinert's disease), senescence of human satellite cell-derived myoblasts correlates with a lower proliferative rate than seen in age-matched controls and has been implicated in the progressive atrophy and degeneration of DM1 muscles (Baar et al., 2018; Bigot et al., 2009; Thornell et al., 2009). Moreover, muscle biopsies from the M. vastus lateralis in young (18–25 years) and old (65–86 years) men and women were employed to examine and quantify senescence markers in human skeletal muscle, using p16 and γH2AX as markers of cellular senescence in young and old human skeletal muscle (Dungan et al., 2017). In an interesting and recent study, longitudinal changes of $p16^{INK4a+}$ senescent cells in skeletal muscle were examined in young men (aged 22.5 ± 1.7 years) before and after resistance exercise (0 h and 48 h) with multiple biopsies. The authors reported that the senescent cells in muscle were decreased by roughly 50 % after 48 h of exercise, and immunohistochemical analyses of muscle sections using anti-$p16^{INK4a}$ and anti-CD34 antibodies, respectively, confirmed that the detected senescent cells were endothelial progenitor cells (Yang et al., 2018a).

2.2.12. Reproductive system

There is evidence for an age-related decline in male reproductive functions (Harris et al., 2011; Honore, 1978; Kuhnert and Nieschlag, 2004; Meacham and Murray, 1994; Merz et al., 2019). Recently published data indicate that senescent cells may accumulate with age in mammalian testes (Merz et al., 2019; Wang et al., 2018). A statistically significant age-dependent increase in the percentage of p21-expressing cells was observed for testicular fibroblasts (4-fold) and Leydig cells (8-fold) in old dog testes (Merz et al., 2019). The age-dependent morphological changes also included an increased mean number of Leydig cells per intertubular triangle (2.95-fold) as well as a decreased spermatogenesis score. Results of a recent study suggested that ginsenoside Rg1 (an extract of *Panax ginseng* used in traditional Chinese medicine) ameliorates testicular senescence levels in D-GAL-treated mice *via* anti-inflammatory and anti-oxidative mechanisms (Wang et al., 2018). In addition, the percentage of SA-βgal-positive cells and IL-6 levels in testicular tissues were significantly decreased, and the expression of p19, p53 and p21 was downregulated after the treatment with Rg1.

Concerning the female reproductive tract, in a recent study it was observed that significant cellular senescence occurs within the ovaries in theca-interstitial cells. These cells secrete SASP chemokine (C-C motif) ligand 5 (CCL5) during aging (Shen et al., 2019), thus senescent theca-interstitial cells may impair follicle development and maturation during ovarian aging. In a study on murine ovarian cancer, Bitler et al. demonstrated that Wnt5a antagonizes canonical Wnt/β-catenin signaling and induces cellular senescence in epithelial ovarian cancer cells by activating the histone repressor A/promyelocytic leukemia senescence pathway, suggesting that this pathway could be exploited for the development of anti-cancer therapeutics (Bitler et al., 2011). Increased cellular senescence (as evidenced by the number of SA-βgal-positive cells) has also been found in the uterus of older women (over 45 years old) relative to younger women (less than 45 years old) (Laser et al., 2010). Uterine tissue from the older women demonstrated higher level of senescence in small fibroids that are primarily composed of smooth muscle cells, although the cell type was not specified.

2.2.13. Spleen, thymus and peripheral blood

Aging is associated with a range of immunological alterations that increase the susceptibility to infection, autoimmune disease, cancer and reduced responsiveness to vaccination (Sidler et al., 2013). Transcriptome analyses showed an increased expression of senescence-related genes in mature and old spleens (24-month-old) as well as in thymus when compared to young tissues (one-month-old), consistent with an accumulation of senescent cells in old animals in these tissues (Aw et al., 2009; Effros, 2004; Sidler et al., 2013). Cellular senescence affects thymic epithelial cells (TECs) during human thymic involution, suggesting a role for senescence in this process (Barbouti et al., 2019). An increase in senescent lymphocytes in the aging spleen has also been suggested based on an increased number of γ-H2AX foci-positive cells and a modest increase in p16 expression in spleen from 42-month old mice (Wang et al., 2009). T cell replicative senescence is defined by a terminal state characterized by dysregulated immune function, loss of the CD28 costimulatory molecule, shortened telomeres and elevated production of proinflammatory cytokines (Chou and Effros, 2013; Xu and Larbi, 2017). Senescent $CD8^+$ T cells, which accumulate in the elderly, have been shown to frequently bear antigen specificity against cytomegalovirus (CMV), suggesting that this common and persistent infection may drive immune senescence and result in functional and phenotypic changes to the T cell repertoire (Chou and Effros, 2013). Moreover, senescent T cells have also been identified in patients with certain cancers, autoimmune diseases and chronic infections, such as HIV (Deeks, 2011; Effros, 2007). The accumulation of circulating T cells with multiple features of telomere dependent-senescence, including elevated expression of CD57, KLRG-1, γ-H2AX, short telomeres and low hTERT expression, has been reported to occur during human cutaneous *L.braziliensis* infection (Covre et al., 2018). This expanded population of T cells was found within the $CD45RA^+CD27^-$ subset, and produced high levels of inflammatory cytokines, analogous to the SASP in senescent non-lymphoid cells.

2.2.14. Gastrointestinal tract

Cellular senescence may play an important role in the pathogenesis of several diseases affecting the gastrointestinal tract. A few studies are available to suggest a role of cellular senescence in esophageal cancers, including esophageal adenocarcinoma, Barrett's esophagus, and esophageal squamous cell carcinoma (Going et al., 2002a; Penfield et al., 2013). Going et al. showed the presence of cellular senescence in normal and dysplastic epithelium from the upper GI tract by using an intensity-weighted scoring system for SA-β-gal (Going et al., 2002a). In this study, histological specimens from 28 patients with Barrett's esophagus and 15 patients with gastric adenocarcinoma were assessed for

senescence. Later, Penfield et al. evaluated the presence of senescence in a series of 11 patients who had undergone endoscopic mucosal resection for Barrett's esophagus (Penfield et al., 2013). Cellular senescence was identified using two methods: SA-β-gal staining on Barrett's epithelium and reduced expression of multiple DNA cell cycle associated genes from disrupted Barrett's tissue (Penfield et al., 2013).

A link between the induction of senescence in intestinal epithelial cells and the subsequent secretion of cancer-promoting growth factors has been provided by a study with colibactin-producing *Escherichia coli* (Cougnoux et al., 2014). Notably, colibactin-dependent induction of Myc in colonic epithelial cells increased intracellular microRNA levels of miR-20a-5p, which then prevented p53 degradation, ultimately resulting in a senescent state with secretion of tumor -promoting growth factors. Similarly, *Helicobacter pylori*, a microaerophile, has been shown to cause cellular senescence *via* certain cytotoxic proteins. *H. pylori* cytotoxin CagA expression induces cellular senescence of human gastric non-polarized epithelial cells, and it is assumed that it may play an important in the pathogenesis of gastric ulcers (Lewinska and Wnuk, 2017; Saito et al., 2010). However, *in vivo* evidence of a direct role of *H. pylori*cytotoxin CagA in gastric senescence is not yet available.

Using primary human colon tissue, Guo et al. found a significant accumulation of senescent fibroblasts in normal tissues from individuals with advanced adenomas or carcinomas in comparison with individuals with no polyps or CRC (Guo et al., 2019). This observation suggests that an increased accumulation of senescent stromal cells may mediate polyp initiation and/or the adenoma–CRC transition. Interestingly, the authors demonstrated further that senescent colon fibroblasts exhibit pro-oncogenic effects on colon epithelial cells (Guo et al., 2019). Crohn's disease (CD) and ulcerative colitis are the principal types of inflammatory bowel disease, feature a bimodal age distribution, and ~10–15 % of the affected population is older than 60 years (Saygili et al., 2016). The inflammatory lesions of Crohn's disease occur most commonly in the terminal ileum and colon (Wang et al., 2019). Upon examination of tissues from patients with early Crohn's disease, p16+ cells were observed in the stem cell niches of the intestine. The expression of a second senescence marker, p21, was also observed in the transient amplifying (TA) cell niches and the columnar cells of the intestine (Wang et al., 2019).

3. Concluding remarks

There is plenty of evidence suggesting that a variety of cellular stressors and various insults are key contributors to *in vivo* cellular senescence, which in turn is thought to contribute to age-related tissue dysfunctions and pathologies. Even a relatively small percentage of senescent cells in organs may impair tissue homeostasis and regeneration, decrease organ function, and contribute to aging phenotypes, as demonstrated by those studies where senescent cells were either genetically or pharmacologically ablated (Baker et al., 2016, 2011; Short et al., 2019; Zhang et al., 2019). Though our knowledge about senescence and senescent cell types *in vivo* as well as their roles in various diseases and aging has increased considerably over the last years, the quantification of senescent cells in human tissues and a comprehensive analysis of their roles in aging and pathologies lag behind. In addition, considering that functions have been ascribed to senescent cells in a variety of diverging biological processes such as repair, wound healing, development, diseases and aging, we still need to better characterize the nature and origin of senescent cells, their phenotypes and functions. As described in this review, a large number of studies have identified specific cell types in various organs, which can acquire a senescent phenotype during aging or under specific pathological conditions. Nevertheless, our understanding of these cells in terms of their transcriptional and protein expression profiles, morphological features and functional characteristics, including differences in the extent to which and/or mode by which they contribute to disease and aging in different tissues *in vivo* still remains limited. Further, the development of optimized methods for the isolation of specific senescent cell types from various organs for an in-depth analysis of their genome, epigenome, transcriptome, proteome and metabolome can speed up efforts to identify novel combinations of markers for senescence states, and can help to shed light on possible functional differences that may exist between senescent cells in different tissues. Moreover, a detailed and comparative analysis of these tissue-specific senescent cells *in vivo* may also help to better understand cell type-specific susceptibility for senescence. Therefore, despite recent progress in our understanding of the physiological and pathological roles of cellular senescence, many questions remain regarding tissue- and cell-type-specific contributions of senescent cells and these questions will drive research in the field of senescence for the years to come.

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