Review

Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP)

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ABSTRACT

The major hallmark of cellular senescence is an irreversible cell cycle arrest and thus it is a potent tumor suppressor mechanism. Genotoxic insults, e.g. oxidative stress, are important inducers of the senescent phenotype which is characterized by an accumulation of senescence-associated heterochromatic foci (SAHF) and DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). Interestingly, senescent cells secrete pro-inflammatory factors and thus the condition has been called the senescence-associated secretory phenotype (SASP). Emerging data has revealed that NF-κB signaling is the major signaling pathway which stimulates the appearance of SASP. It is known that DNA damage provokes NF-κB signaling via a variety of signaling complexes containing NEMO protein, an NF-κB essential modifier, as well as via the activation of signaling pathways of p38MAPK and RIG-1, retinoic acid inducible gene-1. Genomic instability evoked by cellular stress triggers epigenetic changes, e.g. release of HMGB1 proteins which are also potent enhancers of inflammatory responses. Moreover, environmental stress and chronic inflammation can stimulate p38MAPK and ceramide signaling and induce cellular senescence with pro-inflammatory responses. On the other hand, two cyclin-dependent kinase inhibitors, p16INK4a and p14ARF, are effective inhibitors of NF-κB signaling. We will review in detail the signaling pathways which activate NF-κB signaling and trigger SASP in senescent cells.

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Contents

1. Introduction

Fifty years ago, Hayflick and Moorhead [1] demonstrated that human diploid fibroblasts have a limited replication capacity in cell culture, i.e. exhausted cells are irreversibly growth arrested. Maximum replication in vitro, provoked by telomere attrition, characterizes only one type of cellular stress which can permanently arrest the cell cycle and induce cellular phenotype called cellular senescence. Currently, it is known that several stress-related insults, in particular those attacking DNA and inducing double strand breaks, can induce cellular senescence [2–4]. Moreover, strong mitogenic signals, e.g. mutant HRAS^{V12}, can trigger a cell cycle arrest, called oncogene-induced senescence (OIS). The senescent phenotype includes several common characteristics in addition to irreversible cell cycle arrest, e.g. (i) flat, enlarged, often multinucleated morphology, (ii) induction of senescence-associated β-galactosidase (SA-β-gal) activity, (iii) appearance of senescence-associated heterochromatic foci (SAHF) and DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), (iv) activation of tumor suppressor network, such as p16INK4a and p19ARF, and (v) secretion of pro-inflammatory mediators. Currently, there is a debate about the role of cellular senescence in organismal aging and in age-related diseases. Several studies have revealed that senescent cells will accumulate into tissues in vivo during the aging process and in many diseases and pathological conditions [5–8]. Oxidative stress and chronic inflammation can induce genotoxic injuries and trigger cellular senescence which subsequently can aggravate the pathogenesis. In cancer, OIS is a cellular host defense mechanism preventing uncontrolled proliferation of cells with genomic injuries.

Recently, a considerable amount of research has focused on the mechanisms inducing cellular senescence, particularly into the question about why/how arrested cell cycle might be associated with a secretory, generally pro-inflammatory phenotype. Genotoxic insults and the appearance of SAHF and DNA-SCARS indicate that DNA damage response is the inducer of inflammatory reaction (Section 4.1). Moreover, environmental stress involving activation of p38MAPK and ceramide signaling can induce cellular senescence and stimulate inflammatory responses (Sections 4.2 and 4.6). Genomic instability in cellular stress can trigger epigenetic changes, e.g. via release of HMGB1 proteins which could stimulate inflammatory responses (Section 4.5). Current studies have revealed that the NF-κB signaling system, the master control switch of innate immunity responses, is a crucial pathway in the induction of SASP and inflammatory responses in cellular senescence [9–11]. Vaughan and Jat [12] have recently presented a perspective on this research field. We will review here in detail the potential inducers and signaling pathways which activate NF-κB signaling and subsequently trigger SASP in cellular senescence (Fig. 1).

2. SASP: senescence-associated secretory phenotype

Judith Campisi and her group were the first to reveal that senescent cells could promote tumorigenesis in neighboring premalignant cells [13,14]. In their landmark study in 2008, they demonstrated that senescent cells, induced by genotoxic stress, secreted a myriad of factors associated with inflammation and oncogenesis [15]. They called this cellular state the senescence-associated secretory phenotype (SASP). Genotoxic stress, provoked by exhaustive replication or ionizing radiation, induced a similar type of SASP in normal human fibroblasts and epithelial cells as well as in irradiated epithelial tumor cells. Secreted factors included interleukins and chemokines, e.g. IL-1α/β, IL-6, IL-8, MCP-2 and MIP-1α, growth factors, such as bFGF, EGF and VEGF, and several matrix metalloproteinases and nitric oxide [16]. Kuilman et al. [17] demonstrated that oncogene-induced senescence (OIS) is also linked to inflammatory profile. Moreover, they revealed that IL-6 has a crucial role in the generation of OIS since its depletion abolished oncogene-induced cellular senescence, i.e. it suppressed the appearance of pro-inflammatory phenotype as well as the increase in SAHF formation and p15INK4B expression. In contrast, in SASP of normal human fibroblasts, cell surface-bound IL-1α and IL-1R were the critical signaling proteins which maintained the up-regulation of IL-6/IL-8 cytokine secretion [18]. IL-1α, but not IL-1β, was also a senescence marker of endothelial cells and vascular aging [19]. Acosta et al. [20] demonstrated that chemokine signaling via the CXCR2 receptors enhanced both replicative senescence and OIS of human fibroblasts. It seems that there are basic physiological differences between SASP and OIS. Orjalo et al. [18] observed that the secretome of normal fibroblast SASP could promote the invasive capabilities of cancer cells whereas Kuilman et al. [17] reported that secreted molecules of OIS clearly inhibited cancer cell proliferation, probably supporting the senescence of nearby cancer cells by paracrine fashion. These results imply that the secretory characteristics of SASP are dependent on cell type and cellular context, e.g. type of cell stress, its level and duration. The signaling regulation of cellular senescence needs to be clarified more thoroughly. It seems that there are several positive, cell type specific feedback loops driven by secreted inflammatory mediators which can boost autocrine senescence via the transcriptional regulation, both in SASP and OIS. For instance, Bhaumik et al. [21] observed that a robust SASP induced by IL-1α signaling stimulated a delayed expression of miR-146a/b, an inhibitor of IRAK1 mRNA, which markedly down-regulated the secretion of IL-6 and IL-8. This indicates that there are negative feedback loops against the inflammatory SASP, e.g. via expression of miRNAs.

The biological role of cellular senescence as well as the functions of SASP are still under debate [3,22–24]. It seems that senescent cells protect themselves from converting to cancerous growth by switching on SASP. Many of the secreted compounds are communicative and they have autocrine and paracrine effects in tissue microenvironment but also at the systemic level. Some of the pro-inflammatory cytokines and chemokines can arrest cell growth, e.g. TGF-β (Section 4.4), but in general, inflammation enhances tumorigenesis [25]. Moreover, inflammatory cytokines stimulate NF-κB signaling in senescent cells and autonomously can prevent apoptosis and thus maintain their senescent phenotype. Several studies have confirmed that apoptosis is reduced in senescent cells [26,27]. Many
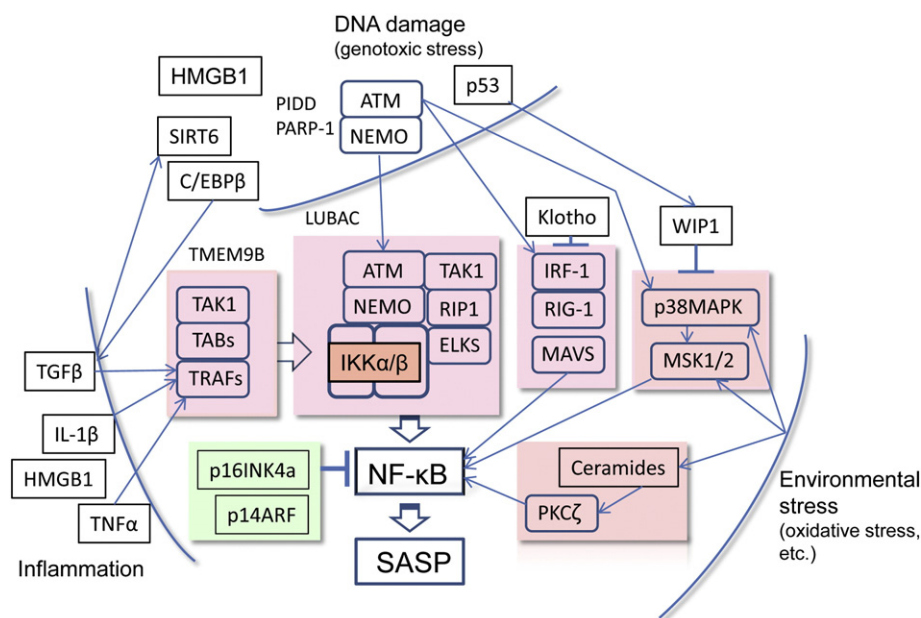
Fig. 1. A general overview on the potential signaling pathways which can control the activity of IKK and NF-κB complexes and subsequently the induction of SASP. Genotoxic stress stimulates NF-κB signaling via different pathways including the NEMO shuttle and both p38MAPK and RIG-1pathways. Environmental stress (without DNA damage) activates the p38MAPK pathway and ceramide production. Inflammatory components activate the IKK complex via signaling pathways involving complexes assembled by TRAFs and TAK1. HMGB1 can enhance inflammatory responses both in nuclei and after secretion. p16INK4a and p14ARF proteins are direct inhibitors of NF-κB signaling. *Abbreviations*: ATM, Ataxia Telangiectasia Mutated; C/EBPβ, CCAAT/enhancer binding protein β; ELKS, a protein rich in glutamic acid, leucine, lysine and serine; HMGB1, high mobility group box protein 1; IKKα/β, IκB kinase α and β; IL-1β, interleukin-1β; IRF-1, interferon regulatory factor-1; LUBAC, linear ubiquitin chain assembly complex; p38MAPK, mitogen-activated protein kinase; MAVS, mitochondrial antiviral-signaling protein; MSK1/2, mitogen- and stress-activated protein kinases 1 and 2; NEMO, NF-κB essential modifier; NF-κB, nuclear factor-κB; p14ARF, alternate reading frame product of p16INK4a; p16INK4a, cyclin-dependent kinase inhibitor 2A; PARP-1, poly(ADP-ribose) polymerase-1; PIDD, p53-induced protein with a death domain; PKCζ, protein kinase Cζ; RIG-1, retinoic acid inducible gene-1; RIP1, receptor interacting protein 1; SASP, senescence-associated secretory phenotype; SIRT6, silent information regulator 6; TAB, TAK1-binding protein; TAK1, TGFβ-activated kinase 1; TGFβ, transforming growth factor β; TMEM9B, transmembrane protein 9B; TNFα, tumor necrosis factor α; TRAFs, TNF-receptor-associated factors; WIP1, protein phosphatase 2Cδ.

chemokines can recruit immune cells into tissues and subsequently, trigger the clearance of both senescent and oncogene-damaged tumor cells. Secreted metalloproteinases support both tissue repair and tumor progression. However, chronic inflammation is detrimental in tissues and it is associated with many age-related diseases, e.g. metabolic disorders and cardiovascular and neurodegenerative diseases. The aging process itself also involves a low grade inflammation [28,29] but currently, the role of cellular senescence is still unclear in the aging process.

3. NF-κB signaling is a major inducer of SASP

There has been intensive research examining the signaling mechanisms regulating cellular senescence and SASP. It is now clear that tumor suppressor proteins p53 and pRB are involved in the appearance of cellular senescence [14,30]. Their role seems to be linked to the arrest of cell cycle rather than the induction of inflammatory SASP. Several research approaches have revealed that C/EBPβ and NF-κB transcription factors are involved in the regulation of secretory component of cellular senescence [17,18,20,31]. Both of these transcription factors have been implicated in the regulation of cellular stress and inflammatory signals. NF-κB signaling is the major immune regulator both in adaptive and innate immunity systems [32,33]. C/EBPβ is a potent transcriptional co-regulator in inflammatory responses [34,35] but also in cell cycle regulation, e.g. arresting cellular proliferation via the pRB/E2F complex [36]. There seems to be cooperation between NF-κB and C/EBPβ signaling in inflammatory regulation. For instance, Cappello et al. [37] observed that C/EBPβ stimulated the NF-κB-dependent signaling by down-regulating the expression of IκB-α, an inhibitory κB component. NF-κB and C/EBPβ have many synergistic effects, e.g. in the induction of CRP expression via the IL-1β-stimulated autocrine IL-6 loop [38,39] but in some instances, C/EBPβ can also block NF-κB signaling and inflammation [40]. It seems that C/EBPβ is a context-dependent regulator of inflammation, probably

due to its capacity to interact with several signaling molecules and transcription factors [41].

Three recently published articles using different research models have convincingly demonstrated that it is the NF-κB signaling which promotes the presence of SASP in cellular senescence [9–11]. Chien et al. [9] observed that NF-κB signaling controlled the appearance of SASP in a well-characterized H-Ras^{V12}-induced senescence model with human IMR-90 skin fibroblasts. They demonstrated that the p65 subunit of NF-κB complex was more significantly enriched into the chromatin of senescent fibroblasts as compared to that of young counterparts. Immunohistochemistry revealed that p65 was accumulating into the SAHF-positive nuclei. They also observed that p65 was clearly phosphorylated on Ser536, a transactivating modification, which correlated with increased expression and secretion of inflammatory markers. Microarray profiling showed that p53 and NF-κB controlled distinct transcriptional modules but were complementary since the down-regulation of p65 was sufficient to bypass the H-Ras^{V12}-induced senescence in human fibroblasts [9]. Rouvillain et al. [11] demonstrated that stimulation of the p53–p21Cip1 pathway predominantly evoked to cellular senescence in human fibroblasts. They also observed that senescence was associated with a prominent SASP, with IL-1α and IL-1β being the most highly up-regulated genes. Interestingly, they observed that the cell cycle arrest provoked by serum starvation induced similar inflammatory changes as oncogene-induced SASP. Rouvillain et al. [11] also revealed that the inhibition of NF-κB signaling could overcome the growth arrest induced by p53–p21Cip1 signaling which implies that the NF-κB pathway had a causative role in the induction of SASP.

4. Signaling pathways activating NF-κB system in SASP

The NF-κB system is an evolutionarily conserved signaling pathway which can be triggered not only by immune activation but also

diverse external and internal danger signals associated with senescence and the aging process, such as oxidative and genotoxic stresses. The activation of the NF-κB system is linked to several pattern recognition receptor pathways, e.g. TLRs and inflammasomes, as well as through signaling from many upstream kinase cascades via canonical and non-canonical pathways [33,42–44]. IKKα/β and NIK are the most important upstream kinases although several kinases can directly regulate the transcriptional capacity of NF-κB factors. IKKγ, generally called NEMO, is an important regulatory component of the IKK complex being linked upstream to genotoxic signals (Section 4.1) and IL-1 and TNF receptor mediated signaling [45]. NF-κB transcription factors contain both the Rel family proteins (RelA/p65, c-Rel and RelB) and NF-κB components (p50/p105 and p52/p100) which are dimerized with each other in the cytoplasm and inhibited by binding to IκB proteins (IκBα, IκBβ, IκBγ, IκBδ, IκBε, IκBζ and Bcl3). Activating kinases phosphorylate IκB proteins which are released from the complex and then degraded in proteasomes. Subsequently, the NF-κB complexes translocate into the nucleus and transactivate the expression of special sets of target genes. In addition to IκB proteins, several signaling pathways and negative feedback loops can also inhibit NF-κB signaling through different mechanisms at various levels of the signaling cascades [46–48]. We will review here the putative mechanisms which activate NF-κB signaling and trigger SASP in senescent cells (Fig. 1).

4.1. DNA damage provokes NF-κB activation via NEMO shuttle

DNA damage is the common denominator inducing cell cycle arrest and cellular senescence associated with many chromatin changes, such as the accumulation of SAHF and DNA-SCARS [4,49,50]. DNA damage is also a potent trigger for oncogene-induced senescence. Moreover, genomic stability is clearly impaired during organismal aging [51]. Several studies have demonstrated that DNA damage can stimulate a robust secretion of inflammatory mediators and is a common research model in SASP studies [9,10,50,52]. Rodier et al. [52] demonstrated that ATM kinase had a crucial role in the initiation of SASP in the DNA damage response. For example, the depletion of ATM prevented the secretion of IL-6 after irradiation in human fibroblasts. The effect of ATR was less prominent although it could also contribute to the SASP response. ATM and ATR are the key protein kinases initiating the host defense after DNA damage. Interestingly, the cytokine response was not dependent on the activation of p53 and pRB, two factors known to arrest proliferation. By profiling the secreted proteins, Rodier et al. [52] reported that ATM signaling did not regulate the entire SASP secretome although it was required for the secretion of the two major inflammatory cytokines, e.g. IL-6 and IL-8. Interestingly, ATM stimulation is known to trigger several downstream pathways, e.g. via the control of NEMO activation and p38MAPK pathways (Section 4.2) which are involved in the inflammatory responses.

NEMO protein is the regulatory subunit of the IKK complex and thus an essential modifier of NF-κB signaling [45,53]. In addition to IKK components, NEMO can interact with several other proteins associated with NF-κB signaling, e.g. ATM, ELKS, RIP1 and TAK1. Post-translational modifications, e.g. phosphorylation, sumoylation and ubiquitination, control the binding targets and the function of NEMO. It is recognized that DNA damage stimulates NF-κB signaling which consequently regulates several host defense functions related to cellular senescence, e.g. it induces resistance against apoptosis and triggers innate immunity responses. Recent studies have revealed that NEMO protein has a major role in the activation process of NF-κB signaling evoked by DNA damage. Miyamoto [54] has recently described in detail the signaling pathway driven by ATM and NEMO proteins. We have termed this cascade as the NEMO shuttle since NEMO protein is being transported between cytoplasm and nucleus [55]. Genotoxic stress, e.g. induced by oxidative stress, can trigger the RIP1-mediated assembly of the complex between NEMO, PIDD and

RIP1. PIDD is a p53-inducible protein and it is able to sensitize cells to genotoxic stress [56]. Consequently, this tripartite complex translocates into the nucleus where PIASy, a SUMO-1 ligase, sumoylates NEMO protein. Subsequently, activated ATM phosphorylates NEMO protein at Ser 85 which triggers desumoylation and facilitates its monoubiquitination at Lys277 and Lys309. This modification provokes the export of the ATM/NEMO complex into the cytoplasm where it binds to the IKKs and ELKS, a scaffold protein of IKKs. The ATM/NEMO complex activates the IKKα/β complex which triggers NF-κB signaling by phosphorylating inhibitory IκB proteins.

There are several specific modification steps which are linked to the NEMO shuttle. For instance, Stilmann et al. [57] observed that activated PARP-1, a sensor of DNA damage, could interact with NEMO and this clearly enhanced the formation of nuclear complex between ATM, NEMO and PIASy and promoted the sumoylation of NEMO. It is known that PARP-1 can activate NF-κB signaling but its functional role in the NEMO shuttle needs to be clarified. The ectopic expression of human PARP-1 in mice generated several age-related inflammatory pathologies and neoplastic lesions; this implies that PARP-1 can enhance NF-κB signaling [58]. In addition, the ATM/NEMO complex can be linearly ubiquitinated by LUBAC into the NEMO protein in cytoplasm [59]. Subsequently, that complex activates the TAK1 and IKK complex. Moreover, ATM/NEMO can enhance the ubiquitination of ELKS by XIAP which induces the binding of ELKS to TAK1 and activation of IKK complex and thus NF-κB signaling [60]. Recently, several studies have revealed that cytoplasmic ATM, activated by DNA damage, can also activate the IKK complex without NEMO, e.g. via the ATM/TRAF6/cIAP1 module which stimulates TAK1-dependent activation of IKK complex [61]. The ATM/NEMO/RIP1 complex can recruit TAK1 which activates IKKβ [62]. Interestingly, this TAK1 complex can activate also p38MAPK which stimulates cell cycle arrest. TAK1 is the TGFβ1-activated protein kinase and because TGFβ signaling has a crucial role in cellular senescence, we will discuss later the functions of TGFβ and TAK1 in Section 4.4.

Recently, Biton and Ashkenazi [63] revealed a TNF-α-dependent, autocrine feedforward signaling loop triggered by ATM/NEMO activation in persistent DNA damage. The ATM-induced ubiquitination of NEMO activates RIP1 which is a crucial step in the cytokine, in particular in the TNF-α-induced signaling pathway. In the DNA damage-induced SASP, this so-called second wave, cytokine-mediated pro-inflammatory response is augmented because the activation of RIP1 by ATM/NEMO potentiates the stimulation of JNK3 signaling through TNF-α receptor. JNK3 enhances the expression and secretion of inflammatory proteins via the AP-1 factors and enhances the secretion inflammatory components in SASP. In conclusion, the ATM-induced NEMO shuttle triggers NF-κB signaling which is controlled by its associations with PARP-1, XIAP, RIP1 and TAK1.

4.2. Stress responses induce p38MAPK-dependent NF-κB activation

Mammalian p38 mitogen-activated protein kinase (p38MAPK) is the signaling target of a wide variety of cellular stresses, e.g. oxidative, metabolic and endoplasmic stresses, DNA damage, heat shock and mechanical damage [64–66]. In addition, cytokines and other inflammatory mediators activate the p38MAPK signaling pathway and p38MAPKα has a crucial role in the inflammatory responses, in particular in the regulation of chemotaxis by enhancing the expression of several chemokines, e.g. MIP-1α/β, MCP-1 and CCL5 [67]. Currently, the kinases of p38MAPK pathway are potential drug discovery targets. An abundant literature indicates that p38MAPK is an important inducer of cellular senescence triggered by either DNA damage or environmental stress [68–70]. It seems that p38MAPK can activate both p53 and pRB-mediated arrest of cell proliferation, e.g. in Ras-induced premature senescence [69]. Oxidative stress activates the MKK3/6-p38 pathway and induces cellular senescence. Interestingly, Davis et al. [71] demonstrated that an inhibitor of p38MAPK, SB203580,

could revert the morphology of senescent Werner syndrome fibroblasts and increase their replication capacity to that within the normal range. Werner syndrome is a segmental progeroid syndrome where also cultured fibroblasts reveal an accelerated replicative senescence. SB203580 as well as many other p38 inhibitors is a potent suppressor of cytokine expression and secretion [72] and this implies that NF-κB signaling is involved.

Recently, Freund et al. [73] demonstrated that a constitutive p38MAPKα activity could induce SASP in human fibroblasts. The activation of p38MAPKα did not cause DNA damage or activate ATM and CHK2 indicating that p38MAPKα was able to trigger SASP without genotoxic stress. Interestingly, they observed that p38MAPKα controlled SASP via the transcriptional activation of NF-κB signaling. Depletion of RelA/p65 significantly reduced the secretion of pro-inflammatory cytokines induced by p38MAPK activity. Moreover, p53 expression repressed the p38MAPK activity which indicates that p53 can suppress the stimulation of p38MAPK pathways and provide time for DNA repair. Furthermore, it is known that p53 can inhibit NF-κB signaling via the activation of WIP1 [74]. WIP1 is a p53-inducible p38MAPK phosphatase which can also inhibit the signaling of ATM, CHK2, p53 and p38MAPK [75]. Zhang et al. [76] demonstrated that the knockdown of WIP1 induced premature senescence in human fibroblasts. Chew et al. [77] observed that WIP1 was a negative regulator of NF-κB signaling which implies that p38MAPK stimulates NF-κB signaling and subsequently induces SASP.

Many observations indicate that p38MAPK can stimulate NF-κB signaling and inflammation through diverse mechanisms [67]. It is well known that p38MAPK activates mitogen- and stress-activated protein kinases, MSK1 and MSK2, which can phosphorylate the trans-activating p65 subunit of the NF-κB complex at Ser276 and thus potentiate NF-κB signaling [78–80]. For instance, oxidative stress activates this same pathway [79]. MSK1/2 can also stimulate CREB and STAT3 transcription factors which enhance inflammatory reactions [80]. In UV radiation, CK2 kinase activates MSK2, but not MSK1, in a p38MAPK-dependent manner and subsequently, MSK2 phosphorylates p65 protein at Ser276 and triggers NF-κB signaling [81]. In 2002, Saccani et al. [82] demonstrated that p38MAPK can trigger the phosphorylation of histone H3 which induces promoter remodeling and in that way enhances the recruitment of NF-κB complexes to certain promoters. Interestingly, they observed that the phosphorylation was targeted selectively to the promoters of a subset of cytokine and chemokine genes. The p38MAPK-activated genes included IL-6, IL-8 and MCP-1 in human dendritic cells. Recently, Drobic et al. [83] described that MSK1/2 provoked nucleosomal H3 phosphorylation at Ser10 and 28 which was coupled with the binding of the multiprotein chromatin remodeler complex involving 14–3–3 protein and proteins of SWI/SNF complex. This complex was located at the promoters which were engaged by NF-κB and AP-1 binding complexes, well-known transactivators of inflammatory genes. The p38MAPK-induced phosphorylation of H3, observed by Saccani et al. [82], was probably mediated through MSK signaling. These observations indicate that the p38MAPK/MSK pathway triggers inflammatory responses targeting specifically those genes containing NF-κB factors. Saha et al. [84] observed that the activation of p38MAPK induced the acetylation of p65 protein at Lys310, probably after the H3 phosphorylation. It is known that the acetylation of p65 at Lys310 remarkably increases the transactivation capacity of the NF-κB complex and subsequently provokes inflammatory responses [85]. In addition, p38MAPK signaling can also stabilize the mRNAs of inflammatory mediators via the activation of downstream kinases, e.g. MAPKAP K2 [72]. All these observations indicate that p38MAPK activation is a potent inducer of SASP associated with cellular stress and probably with organismal aging.

4.3. Inflammasomal RIG-1 activates NF-κB signaling pathway

Inflammasomes are intracellular danger recognition complexes which consist of the receptor protein as a sensor and several associated proteins which trigger pro-inflammatory responses, including IL-1β and IL-18 secretion [86]. Pathogens and cellular stress, e.g. oxidative stress, potassium efflux and lipid accumulation, can provoke inflammasomal signaling. Recently, Liu et al. [87] demonstrated that the RIG-1 type of the inflammasome receptor was activated in cellular stress induced by irradiation or replicative senescence in human fibroblasts and endothelial cells. These treatments induced a robust, RIG-1-dependent secretion of IL-6 and IL-8. Cellular senescence also increased the expression level of RIG-1. Moreover, the expression of RIG-1 was clearly increased in various tissues taken from old mice. RIG-1, retinoic acid inducible gene-1, is a specific viral recognition system although recent studies have confirmed that it can also possess other functions e.g. in inflammation, cancer, apoptosis and senescence [88,89]. There are several upstream and downstream pathways which are linked to the activation and signaling of RIG-1. Liu et al. [87] demonstrated that genotoxic stress activated the ATM-IRF1 axis since knockdown of ATM prevented both the induction of RIG-1 and IL-6 expression. Downstream RIG-1 signaling can be linked via ASC to the caspase-1-induced IL-1β production or via mitochondrial MAVS adapter protein to NF-κB signaling [89]. Liu et al. [87] observed that silencing of MAVS, as well as RIG-1, clearly suppressed the IL-6 expression in senescent cells. MAVS protein can also be linked downstream to IRF3 activation and IFN-β production. Liu et al. [87] demonstrated that IRF3/IFN-β pathway was not activated in cellular senescence. It seems that genotoxic stress and replicative senescence activate the NF-κB system and IL-6 and IL-8 expression via the ATM/MAVS pathway. However, Liu et al. [87] did not report whether the caspase-1-mediated pathway was activated. Interestingly, knockdown of RIG-1 in senescent HUVEC cells led to the extension of their lifespan [87] which suggests that RIG-1-induced inflammatory response can aggravate cellular senescence and organismal aging.

Next, Liu et al. [87] demonstrated that Klotho protein can inhibit the RIG-1-induced activation of NF-κB signaling and IL-6 production in cellular senescence. Klotho is a well-known anti-aging protein, i.e. its deletion accelerates the aging process whereas overexpression extends the lifespan of mice [90]. It is also known that Klotho can inhibit NF-κB signaling and prevent endothelial inflammatory responses [91]. Liu et al. [87] observed that the intracellular, but not the secreted form, clearly suppressed the RIG-1-mediated NF-κB activation and IL-6 expression. They also revealed that Klotho directly interacted with the CARD domain of RIG-1 protein and in that way could prevent the binding of downstream signaling molecules to the CARD motif. This observation suggests that the intracellular isoform of Klotho could also inhibit other inflammasomal pathways.

4.4. TGF-β–TAK1 pathway stimulates NF-κB signaling

The transforming growth factor-β (TGF-β) family of cytokines controls a diversity of homeostatic cellular functions, e.g. proliferation, differentiation, apoptosis and immune responses via a complex signaling network [92–94]. Most of the signaling connections are mediated via SMAD factors but recently many non-SMAD pathways have been revealed, e.g. the TRAF6–TAK1–p38MAPK signaling and many connections of TAK1 to the activation of IKK–NF-κB pathway [94–96]. TGF-β signaling is a potent inhibitor of proliferation e.g. in epithelial, endothelial and myeloid cells whereas it is mitogenic for many mesenchymal cells [97]. TGF-β stimulates the expression of Cdk inhibitors including p15, p19, p21 and p27 and it induces growth arrest and cellular senescence e.g. in mouse keratinocytes, human fibroblasts and hepatocellular carcinoma cells [98–100]. Several studies have demonstrated that TGF-β can trigger the expression and secretion of IL-8 in many cancer cells [101,102]. Moreover, TGF-β signaling increased with aging in the brain [103] and chronic astrocytic overproduction of TGF-β1 has promoted Alzheimer's disease-like microvascular degeneration in transgenic mice [104]. In *Caenorhabditis elegans*, the secretion of DAF-7, an orthologue of mammalian TGF-β, by chemosensory neurons induced the formation of dauer larva, a

long-lived larva stage which survives in harsh environmental conditions [105].

Oxidative stress stimulates TGF-β expression and subsequently triggers cellular senescence e.g. in human fibroblasts [106] and retinal pigment epithelial cells [107]. In both cases, treatment with neutralizing antibodies against the TGF-β isoforms and TGF-RII receptors prevented the oxidative stress-induced increase in senescence-associated biomarkers. The TGF-β1 signaling pathway was also involved in the UVB-induced premature senescence in human fibroblasts [108]. Ionizing radiation, probably via DNA damage, also stimulated the expression of TGF-β and its receptors [109–111]. The upstream mechanisms still need to be clarified to explain the increased TGF-β expression in cellular senescence. Abraham et al. [112] demonstrated that the promoter of TGF-β1 contains a functional binding site for the CCAAT/enhancer binding protein β (C/EBPβ) which enhanced the expression of TGF-β1. Interestingly, they observed that the p65 component of NF-κB could bind to C/EBPβ protein and repress TGF-β1 expression. Functional CCAAT boxes have been described in the promoters of TGF-β receptor genes, those of type I, II and III receptors [113–115]. Gomis et al. [116] observed that the cytostatic effect of TGF-β was inhibited by the expression of LIP, a C/EBPβ inhibitory isoform. These observations clearly indicate that C/EBPβ has a crucial role in the expression of TGF-β. The transcription factor C/EBPβ carries out many fundamental actions e.g. in energy metabolism, innate immunity, inflammation, cell proliferation and senescence [117,118]. Currently, it seems that C/EBPβ is strictly epigenetically regulated by arginine methyltransferase 4 (PRMT4) and interactions with remodeling complex SWI/SNF [119]. Kuilman et al. [17] and Orjalo et al. [18] demonstrated that C/EBPβ is an important regulator of IL-6 expression and inducer of SASP. Recently, Atwood and Sealy [120] demonstrated that Ras(V12)-triggered oncogene-induced senescence was mediated by the C/EBPβ1 isoform which also up-regulated the expression of IL-6 in normal fibroblasts.

The SMAD transcription factors involve the major signaling pathway downstream from the TGFβ receptors [93]. However, TGFβ receptors can also activate signaling via three non-SMAD pathways, i.e. TAK1, RhoA and PI3K-mediated signaling [94,96]. It is known that TAK1, a TGFβ-activated protein kinase 1, has a crucial role in the activation of NF-κB signaling and inflammatory responses [121–123]. Recent studies have revealed that TAK1 is not only linked to TGFβ signaling but can also regulate NF-κB activation stimulated by IL-1β and TNFα [96,124]. The key event in the activation of TAK1 is its Lys-63-dependent polyubiquitination at Lys-34 which is triggered by TRAF6 or TRAF2/5. Subsequently, TAK1 interacts with TAB1 protein and will be autoactivated by phosphorylation at Thr-178, Thr-187 and Thr-184 in the TAK1 T-loop [125,126]. Autophosphorylation of TAK1 is required for IL-1-mediated activation of IKK–NF-κB and JNK-AP-1 signaling and expression of IL-6 [125]. On the other hand, Mao et al. [127] demonstrated that in the TGFβ-induced signaling pathway, TRAF6 targets ubiquitination to TAK1 Lys-158, not Lys-34 as in IL-1β and TNF-α signaling, and subsequently TAK1 can activate IKK, p38 and JNK pathways. TAK1 interacts with the IKK complex which is a crucial step in the activation of NF-κB signaling [121]. In order to acquire specificity, the TAK1 complex is associated with TAB2, TAB3 and TAB4 adaptors which recruit other modulators, e.g. RIP1, NEMO and MKK3/6, to facilitate distinct signaling pathways [128,129]. TAK1 is also a client protein of Hsp90 which is an important stability factor for the pro-inflammatory complexes [130]. Currently, it is still unclear how the TAK1 complexes are targeted to the correct signaling pathways.

Dodeler et al. [131] identified TMEM9B protein when they studied the role of TAK1 and RIP1 in the signaling of TNFβ. TMEM9B is a glycosylated transmembrane protein localized in lysosomes, particularly in the membranes of early endosomes. The expression of TMEM9B protein is required for the activation of NF-κB by TNF-α, IL-1β and TLR ligands and subsequently for the induction of pro-inflammatory

response. They observed that TMEM9B acts in the signaling pathway downstream of RIP1, likely at the level of TAK1, but in any case upstream of IKKα/β. Dodeler et al. [131] also demonstrated that TMEM9B undergoes interactions with RIP1 and TAK1 and this leads to stimulation of p38MAPK and JNK signaling (Section 4.2). Interestingly, the TMEM9B protein is associated only with inflammatory responses, not with apoptosis. Recently, Rovillain et al. [132] undertook senescence bypass screening to reveal that the TMEM9B protein is a novel downstream effector of p53–p21Cip1 and p16INK4a–pRB pathways in human fibroblasts. Silencing of the expression of TMEM9B protein could overcome the senescence. Another protein they identified was LTB2/3, a latent TGF-binding protein 2/3, which has a major role in the activation of TGFβ signaling. The silencing of LTB2/3 also bypassed senescence confirming a crucial role of TGFβ signaling in cellular senescence. These observations clearly demonstrate that NF-κB activation and inflammatory factors have a fundamental role in the generation of SASP.

SIRT6, a member of mammalian Sirtuins, interacts with NF-κB in the chromatin and controls the expression of NF-κB target genes [133,134]. SIRT6 associates with the p65 component of NF-κB, deacetylates histone H3 Lys9 and thus inhibits the expression of NF-κB target genes. Depletion of SIRT6 induces the hyperacetylation of histones and increases the expression of NF-κB-dependent genes. The absence of SIRT6 induces a progeroid degenerative disease in mice [135]. Minagawa et al. [136] observed that SIRT6 could regulate the TGFβ-induced cellular senescence in bronchial epithelial cells. Epithelial senescence in pulmonary fibrosis increased the expression of p21Cip1 and SA-β-gal as well as the expression of SIRT6. In cell culture experiments, they observed that TGFβ increased the expression of SIRT6 in a dose-dependent manner. Overexpression of SIRT6 reduced the level of SA-β-gal staining while SIRT6 siRNA dramatically increased the presence of SA-β-gal in epithelial cells. They also observed that the increased expression of SIRT6 suppressed the secretion of IL-1β. These results suggest that SIRT6 antagonizes the cellular senescence induced by TGFβ. They also indicate that SIRT6 can repress the transcription of NF-κB dependent genes i.e. evidence that the inflammatory response is a driving force in cellular senescence.

4.5. HMGB1 potentiates NF-κB signaling and inflammatory responses

The HMGB1 protein is included into the family of high mobility group proteins which are chromatin-associated, non-histone proteins controlling e.g. transcription, replication and DNA repair. Moreover, HMGB1 is a secreted cytokine, an alerting danger signal, which reports immune system from overwhelming cellular stress and it recruits new immune cells into tissues [137–139]. Lamkanfi et al. [140] demonstrated that inflammasomes, cellular danger recognizing system, triggered the release of HMGB1 from jeopardized cells in endotoxemia. The major characteristic of HMGB1 proteins is their extremely good capacity to interact with other proteins, e.g. transcription factors, receptors, and cytokines. For instance, the binding of HMGB1 with some cytokines, e.g. IL-1β and TNF-α, clearly potentiates their inflammatory responses [141,142]. Secreted HMGB1 proteins induce inflammatory signaling by binding and activating several innate immunity receptors, e.g. RAGE, TLR2 and TLR4, which are linked to the NF-κB pathway [139]. The expression of HMGB1 is also up-regulated in several inflammatory diseases, such as atherosclerosis and ischemic injuries. Tang et al. [143] demonstrated that oxidative stress triggered the translocation of HMGB1 from the nuclei to the cytoplasm where it interacted with Beclin 1 and induced the autophagic response which improved cell survival. These studies indicate that in cellular stress, HMGB1 is released from the nuclei to facilitate cellular defense and to alert the immune system about the estimated tissue damage.

One important function of HMGB proteins is to assist transcription factors in gene expression [144]. HMGB1/2 proteins bind to histone H1 proteins on the DNA linker region between nucleosomes. It seems that

HMGB proteins enhance the local transcriptional activity impairing the inhibitory effect of H1 proteins. HMGB1 is a so-called architectural factor which enhances the DNA binding of transcriptional factors and stabilizes the protein binding to DNA. Agresti et al. [145] demonstrated that HMGB1 protein directly interacted with the p50 protein, the DNA-binding component of the NF-κB complex, and enhanced the DNA-binding of p65/p50 and p50/p50 complexes but not other NF-κB complexes. They also proved that HMGB1 protein is required for the expression of inflammatory adhesion protein VCAM-1. These observations confirmed that HMGB1 is a pro-inflammatory cytokine which can also potentiate the transcriptional activity of NF-κB factors and thus the expression of inflammatory mediators.

HMGB1 as well as HMGA2 is a chromatin protein which regulates chromatin remodeling during cellular differentiation and senescence. Narita et al. [146] observed that HMGA proteins accumulated into the chromatin of senescent fibroblasts, in particular to SAHF foci promoting their formation by p16INK4a proteins. Recently, Celona et al. [147] demonstrated that HMGB1 is a crucial protein in the nucleosome assembly, i.e. depletion of HMGB1 reduced the quantity of core, linker and variant histones and significantly decreased nucleosome assembly. This open-chromatin status increased the global gene transcription and also changed specific transcriptomes. El Gassar et al. [148] revealed that HMGB1 protein cooperated with nucleosomal H1 linker protein in the control of TNF-α transcription. They observed that the p65/RelA subunit of NF-κB was recruited to the promoter of TNF-α in endotoxin-responsive cells whereas in the tolerant cells, the presence of H1 linker protein employed HMGB1 and they formed a complex with RelB, a repressor component of NF-κB signaling. This silencing of TNF-α transcription was induced by the dimethylation of H3K9 and binding of the heterochromatin protein HP1 into the TNF-α promoter. They also described a similar silencing system in the transcription of IL-1β in the endotoxin tolerant cells. Interestingly, Funayama et al. [149] observed that SAHF-positive, senescent human fibroblasts exhibited a deficiency in their linker histone H1 proteins. Different senescence insults, such as p38MAPK stimulation, triggered the loss of chromatin-bound H1 proteins. Their results also indicated that depletion of H1 protein from chromatin was induced by posttranslational regulation. It is known that histone H1 variants have a profound effect on higher order structures in chromatin as well as in gene expression [150]. In conclusion, these observations depict a scenario where permanent changes in chromatin structure during cellular senescence evoke the transactivation of pro-inflammatory genes e.g. via p38MAPK/MSK signaling (Section 4.2) and release of pro-inflammatory HMGB1 proteins enhancing the formation of SASP. Recently, Funayama and Ishikawa [49] have reviewed in detail the structural changes observed in chromatin during cellular senescence.

4.6. Accumulation of ceramides triggers NF-κB activation

The senescent phenotype has a profound effect on cellular metabolism which could subsequently trigger NF-κB signaling and induce SASP. Oxidative stress is one way of provoking cellular senescence, but another, more stable type of stress is that evoked by ceramides [151,152]. Ceramides are lipid molecules composed of sphingosine and fatty acid. Ceramides are present in cell membranes but they also have important functions in cellular signaling [153,154]. Venable et al. [151] demonstrated that the ceramide level was clearly increased in senescent fibroblasts and moreover, ceramide treatment of fibroblasts induced cellular senescence involving the appearance of SA-β-gal [152]. Venable et al. [151] observed that the activity of neutral sphingomyelinase was greatly increased in senescent cells. There is clear evidence that activation of neutral sphingomyelinase 2 during aging increases the concentration of ceramides and potentiates inflammatory responses, e.g. via IL-1β receptor [155,156]. Several stress insults can trigger the accumulation of ceramides by affecting the sphingolipid metabolism [157]. For instance, different types of environmental stress such as genotoxic and oxidative stresses, many inflammatory cytokines

and cytotoxic compounds can increase the cellular ceramide level. On the other hand, ceramide and in particular, its metabolite ceramide-1-phosphate are multifunctional signaling molecules which can (i) activate several kinases, e.g. PKCζ, p38MAPK and JNK, (ii) activate protein phosphatases, such as PP1 and PP2A, (iii) stimulate phospholipase A2 [153,154,158]. Sphingolipid metabolites, i.e. ceramides, its phosphate and sphingosine 1-phosphate, can induce inflammatory responses and thus they are involved in many inflammatory diseases [159,160].

Wang et al. [161] demonstrated that ceramide can directly activate protein kinase Cζ (PKCζ). They also observed that activation is a concentration-dependent process, i.e. low concentrations activated PKCζ and JNK whereas high concentrations inhibited PKCζ activity by generating a complex between PKCζ and PAR-4, an inhibitor of PKCζ. Several studies have demonstrated that PKCζ stimulates NF-κB signaling by phosphorylating the RelA/p65 subunit [162] or IKKβ [163] and thus activates NF-κB signaling. Chang et al. [164] observed that the p62 adapter protein could form a ternary complex with PKCζ and PAR-4. They also demonstrated that binding of p62 activated the PKCζ/NF-κB signaling pathway. PKCζ is a multifunctional signaling molecule which can undergo crosstalk with many signaling pathways [165]. It is known that several cytokines, e.g. IL-1β and TNF-α, can activate NF-κB signaling via the complex between TRAF6/p62/PKCζ which then triggers IKKβ [166]. Furthermore, Bourbon et al. [167] observed that the ceramide-induced activation of PKCζ inhibited Akt signaling which subsequently arrested cell proliferation.

In addition to PKCζ signaling, sphingolipid metabolites can adjust cellular stress responses via other pathways. For instance, ceramide can stimulate ASK1/p38MAPK and JNK signaling by inducing the expression of TXNIP [158]. Ceramides can also activate C/EBP transcription factor [168] and thus stimulate TGF-β signaling (Section 4.4). Recently, Alvarez et al. [169] demonstrated that sphingosine 1-phosphate activated TRAF2 which subsequently induced Lys63-linked polyubiquitination of RIP1 and activation of IKKα/β and NF-κB signaling. On the other hand, ceramides stimulate PP2A which inhibits NF-κB signaling by dephosphorylating upstream kinases and directly RelA/p65 component and in that way it can counteract the ceramide-induced pro-inflammatory responses [170,171]. In conclusion, stress-activated ceramide signaling is a potent inducer of SASP since for instance, ceramides are tumor suppressors and p53 regulates sphingolipid metabolism [172].

5. Repression of NF-κB signaling by p16INK4a and p14ARF

There has been debate about whether SASP can enhance cellular senescence or is SASP a separate entity appearing in senescent cells. Coppe et al. [173] demonstrated convincingly that cellular senescence can appear without SASP. Ectopic expression of p16INK4a, a cyclin-dependent kinase inhibitor and a hallmark of cellular senescence, did not stimulate the secretion of pro-inflammatory cytokines or induce paracrine oncogenesis. Moreover, Coppe et al. [173] observed that the induction of SASP after DNA damage was independent of p16INK4a expression. Therefore, this study substantiated earlier observations that cell cycle arrest does not necessarily lead to SASP. Interestingly, Wolff and Naumann [174] demonstrated that p16INK4 was a potent inhibitor of NF-κB signaling, e.g. overexpression of p16INK4 repressed the transactivation of the NF-κB system. The INK4 family of proteins, including p16INK4a, p15INK4b, p18INK4c and 19INK4d, contains ankyrin repeats which are binding domains in RelA/p65 and IκB proteins [175]. Wolff and Naumann [174] observed that p16INK4 and RelA/p65 interacted, in particular after TNFα stimulation when RelA/p65 was released from IκB proteins and was translocated into the nucleus. It seems that the lack of SASP after p16INK4a overexpression [173] could be induced by the suppression of NF-κB signaling by p16INK4 proteins.

Another INK box protein, p14ARF is an effective tumor suppressor which can activate the p53/p21Cip1 pathway. Rocha et al. [176]

demonstrated that the activation of p14ARF stimulated the phosphorylation of RelA/p65 at Thr505 via the ATR/Chk1 kinase pathway. This phosphorylation site is located in the transactivation domain of RelA/p65 and its modification induced a clear, p14ARF-dependent inhibition of NF-κB signaling. The activation of p14ARF could also activate ATR/BRCA1 pathway in the nucleoli and induced the phosphorylation of p53 at Ser15 which triggered cell cycle arrest and possibly leading to apoptosis. Cheung et al. [177] have demonstrated that the collaborator of p14ARF, so-called CARF, controlled cellular fate induced by p14ARF/p53, i.e. homeostasis, senescence, cancer or apoptosis. There is also close cooperation between p14ARF and TWIST, a multifunctional basic helix–loop–helix transcription factor [178]. Kwok et al. [178] demonstrated that overexpression of TWIST down-regulated the expression level of p14ARF protein and simultaneously suppressed cellular senescence in human epithelial cells. They also observed that suppression of TWIST expression promoted cellular senescence, e.g. the appearance of phosphorylated histone H2AX. Interestingly, Sosic et al. [179] observed that transgenic mice either homozygous for twist-2 null allele or heterozygous for twist-1 and -2 alleles expressed elevated level of pro-inflammatory cytokines TNF-α, IL-1β and IL-6. They demonstrated that TWIST could bind to the promoters of these cytokines and inhibit both the basal and inducible activity. Moreover, they observed that TWIST and RelA/p65 could physically interact and this interaction repressed specifically the RelA/p65-dependent activation of cytokine genes. It seems that there is a negative feedback loop since TNF-α activated TWIST expression whereas TWIST repressed cytokine expression. It is not known whether SASP is associated with the down-regulation of TWISTs.

In addition to p16INK4a and p14ARF, there are two other families of mammalian G1 cyclin-dependent kinase inhibitors, p21Cip1 and p27Kip1 [180]. p53, activated by DNA damage, can stimulate the expression of p21Cip1 which arrests the cell cycle in the G1 phase. Instead, the expression of p27Kip1 is induced by TGF-β [181]. The activation of Ras stimulates the expression of both p21Cip1 and p27Kip1. There are differences between the accumulation and function of p16INK4a and p21Cip1 proteins [182]. It seems that p21Cip1 is involved in the cell cycle arrest whereas p16INK4a may be essential for the maintenance of the cell cycle arrest during cellular senescence. p16INK4a accumulates later than p21Cip1 and its expression correlates with the appearance of SA-β-gal and a flat morphology. Interestingly, in contrast to p16INK4a and p14ARF, it is known that p21Cip1 and p27Kip1 can increase the transcriptional activity of NF-κB [174,183]. Coppe et al. [15] demonstrated that p53 repressed the appearance of SASP. This is in line with the observations that p53 and NF-κB have antagonistic responses and p53 can inhibit, via WIP1 activation, the p38MAPK-driven NF-κB activation (Section 4.2). It seems that the p53/p21Cip1-induced activation of NF-κB is not likely to be involved in the generation of SASP. In contrast, the TGF-β/p27Kip1-enhanced transactivation of NF-κB may occur although the TAK1-dependent pathway has been studied in greater detail (Section 4.4). In conclusion, it should be emphasized that the cell cycle arrest is not automatically linked to the cellular senescence program, as discussed by Blagosklonny [184], but it requires an irreversible block of regulation which still needs to be clarified, particularly the role of NF-κB-driven appearance of SASP.

6. IKK-dependent but NF-κB-independent regulation

One key question in the regulation of SASP generation is whether all roads lead to NF-κB activation. The secretory component of SASP involves cytokines, chemokines, growth factors and metalloproteinases, i.e. all of these are mostly inflammatory elements. NF-κB signaling is the master regulator of inflammatory responses (Section 3) and thus it seems that the NF-κB system is the principal mechanism in the induction of SASP. The NF-κB system is an ancient signaling pathway which issues alerts in response to a wide variety of insults

and cellular stress to facilitate innate immunity responses and to establish cellular defense in order to maintain homeostasis. The NF-κB system integrates a complex signaling network in which secreted, mostly communicative, inflammatory factors are only a part of the repertoire of NF-κB responses [33,183]. Moreover, it should be noted that most of the signaling pathways target the IKK complex (Fig. 1), which means that IKKα and IKKβ can activate NF-κB-independent targets, e.g. β-catenin, histone H3, TSC1, FOXO3a and several nuclear co-activators. IKKα has a crucial role in epidermal morphogenesis during development [185] and in the maintenance of skin homeostasis [186]. Perkins [183] and Cariot [187] have recently reviewed in detail the targets of IKK proteins and their functions. In addition, the NF-κB system participates in crosstalk with several transcription factors which join together different signaling pathways, e.g. JNK, p53 and Wnt. For instance, cellular stress stimulates both the NF-κB and p53 signaling pathways but actually, they trigger many antagonistic functions [74,188]. Currently, it is known that different signaling pathways oscillate within a wide timescale, most likely also in the generation of SASP and cellular senescence. Recently, Mengel et al. [189] have reviewed the oscillatory control in NF-κB, p53 and Wnt signaling.

Cellular senescence involves several morphological and energy metabolic changes during the generation of the senescent phenotype. It is known that NF-κB signaling can regulate energy homeostasis [190,191] and the major housekeeping system, i.e. the autophagic cleansing system [192,193]. Currently, there is a debate about the role of autophagy in the generation of cellular senescence. Some studies have indicated that autophagy is an inducer of senescent phenotype [194–196] and others have reported that impairment of autophagy triggers premature senescence [197]. Cellular stress induces autophagocytosis, a self-eating process, which has many crucial functions in cellular survival. Moreover, the autophagic degradation capacity declines with aging in many tissues [198]. Recent studies have demonstrated that p53 and p14ARF are crucial inducers of autophagy e.g. in genotoxic stress [196]. These factors act mutually as inhibitors of NF-κB signaling (Section 5), but in fact, it is the IKK complex, not the NF-κB system, which is a potent inducer of autophagy [199,200] probably enhancing autophagocytosis if there is a genotoxic stress and during the appearance of senescent phenotype. Narita et al. [201] described a cellular compartment called TASCC, a TOR-autophagy spatial coupling compartment, where mTOR became accumulated into autolysosomes during RAS-induced senescence. TASCC combines two basic cellular activities, protein synthesis governed by mTOR and degradation driven by autophagy. Interestingly, they demonstrated that secretory IL-6 and IL-8 were enriched in TASCC in secretory senescent cells and could be involved in the appearance of SASP. Moreover, IKKβ is a potent activator of mTOR by inhibiting TSC1 [202] and thus the signaling pathways activating IKKβ after genotoxic stress, e.g. ATM-stimulated complexes, could trigger SASP via TASCC activation. It is possible that in acute cellular stress, the role of IKK-dependent, NF-κB-independent signaling is augmented since stress triggers the expression of two heat shock proteins, Hsp70 and Hsp90, which dissociate the IKK complexes and prevent the activation of the NF-κB system but not that of IKKs. We have recently reviewed this topic [203]. In conclusion, it seems that the IKK complex is an important player in the regulation of cellular senescence, in particular in the induction of SASP.

7. Conclusions

Emerging studies indicate that the NF-κB signaling pathway controls the generation of cellular phenotype called SASP, i.e. the secretion of inflammatory factors, e.g. cytokines and chemokines, increases in senescent cells. Cellular senescence is generally induced by genotoxic injuries which arrest the cell cycle of damaged cells and thus prevent their conversion into cancerous cells. For instance,

oxidative stress associated with inflammation can jeopardize DNA integrity, in particular with aging and diseases, and activate the senescence program in proliferating cells. Several studies have revealed that the number of senescent cells increases in the tissues of old animals as well as in many diseases. In cancer, this state has been called OIS, oncogene-induced senescence. Recent studies have clearly indicated that SASP is not required for the generation of cellular senescence and it seems that secretory component is only a common characteristic of senescent phenotype. Most of secreted compounds are linked to inflammation and they can have both beneficial and detrimental effects [204]. Many of the pro-inflammatory factors are alarm-issuing molecules which recruit monocytes and macrophages into tissues to assure local homeostasis. In addition, secreted molecules can arrest the proliferation of neighboring cells and thus function as growth suppressors, e.g. in tumors, but autonomously, also to maintain their own arrested, secretory phenotype. Activation of the innate immunity system and the presence of a low-grade inflammation are general characteristics of organismal aging [29]. The aging process is also associated with a clear activation of the NF-κB system in many tissues [205,206]. However, it needs to be clarified whether cellular senescence is involved in this activation or whether genotoxic injuries appearing in conjunction with aging stimulate a secretory phenotype also in non-senescent cells.

Studies on cellular senescence have revealed that genotoxic stress, particularly DNA damage, and the activation of ATM, a DNA damage-inducible kinase, can trigger the activation of the NF-κB system via several signaling pathways, i.e. NEMO shuttle as well as p38MAPK and RIG-1 pathways (Fig. 1). The age-related increase in ceramides is also a potent inducer of NF-κB signaling. It seems that epigenetic changes, both in cellular senescence and organismal aging, can enhance the transcription of NF-κB-dependent inflammatory genes, e.g. via changes in HMGB1 and SIRT6 function. On the other hand, the NF-κB system is strictly regulated, e.g. via negative feedback loops, and its efficiency oscillates with other transcription factors. The NF-κB system is an ancient defense mechanism which has a crucial role in morphogenesis and the survival of the organism but later in life, an inappropriate immune defense could have detrimental effects. This phenomenon refers to antagonistic pleiotropy, a well-known hypothesis to account for the aging process.

Acknowledgments

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