


# p53 in senescence – it's a marathon not a sprint

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## Keywords

DNA damage response; p53; SASP; senescence; transcription

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(Received 9 October 2021, revised 30 November 2021, accepted 15 December 2021)

doi:10.1111/febs.16325

The tumour suppressor p53, a stress-responsive transcription factor, plays a central role in cellular senescence. The role of p53 in senescence-associated stable proliferative arrest has been extensively studied. However, increasing evidence indicates that p53 also modulates the ability of senescent cells to produce and secrete diverse bioactive factors (collectively called the senescence-associated secretory phenotype, SASP). Senescence has been linked with both physiological and pathological conditions, the latter including ageing, cancer and other age-related disorders, in part through the SASP. Cellular functions are generally dictated by the expression profile of lineage-specific genes. Indeed, expression of SASP factors and their regulators are often biased by cell type. In addition, emerging evidence suggests that p53 contributes to deregulation of more stringent lineage-specific genes during senescence. P53 itself is also tightly regulated at the protein level. In contrast to the rapid and transient activity of p53 upon stress ('acute-p53'), during senescence and other prolonged pathological conditions, p53 activities are sustained and fine-tuned through a combination of different inputs and outputs ('chronic-p53').

## Introduction

Cellular senescence is characterised by stable exit from the cell cycle in response to various stimuli, both pathological and physiological [1]. Much like apoptosis, senescence was once viewed as an end point to the stress response, but we now know that this is far from the truth. Senescent cells are metabolically active, involving diverse sets of effector programmes depending on stressors and cell types. Although senescent cells tend to be autonomously resistant to cell death

signalling, they can be 'killed' by immune cells *in vivo*. In a physiological context, for example, fibroblasts become 'active' upon stress, followed by senescence development. During this process the secretory composition of the cells dynamically alters, from contributing towards tissue repair to eventually recruiting immune cells, which in turn eliminate the senescent cells. Throughout this process, from damage to resolution (the 'senescence life cycle'), senescence functionality is

## Abbreviations

15d-PGJ2, 15-deoxy- $\Delta$ 12,14-prostaglandin J2; ATM, ataxia-telangiectasia mutation; ATR, ataxia telangiectasia and Rad3-related protein; C/EBP $\beta$ , CCAAT/enhancer binding protein beta; CDK, cyclin-dependent kinase; CDKN, cyclin dependent kinase inhibitor; CGI, CpG-island; COX2, cyclooxygenase-2; DNA-SCARS, DNA segments with chromatin alterations reinforcing senescence; E1A, early region 1A; EDC, epidermal differentiation complex; EV, extracellular vesicle; Fboxo22, F-box protein 22; FOXO, forkhead box O; GATA4, GATA binding protein 4; Hi-C, high-throughput version of chromosome conformation capture; JAK, Janus kinase; KDM, lysine demethylase; LCE, late cornified envelope; MAPK, mitogen-activated protein kinase; MDM2, murine double minute-2; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; PAI1, plasminogen activator inhibitor-1; PML, promyelocytic leukaemia; PRMT, protein arginine methyltransferase; PTGS2, prostaglandin-endoperoxide synthase 2; PTM, posttranslational modification; RB, retinoblastoma; SASP, senescence-associated secretory phenotype; SCF, SKP1-CUL1-F-box; SERPINE1, serpin family E member 1; SOCS, suppressor of cytokine signalling; STAT, signal transducers and activators of transcription; TF, transcription factor.

crucial for tissue homeostasis [2]. However, if senescent cells persist in tissues, they can be detrimental to the tissue microenvironment, thus being involved in various pathological conditions [3].

P53, encoded by the *TP53* tumour suppressor gene, is a tetrameric transcription factor (TF), which is rapidly stabilised upon cellular stress and plays a critical role in the maintenance of cellular and genomic integrity [4]. Through a series of posttranslational modifications (PTMs) and timely proteasomal degradation due to a tight negative feedback loop, p53 activation is usually transient. If the stress is prolonged, however, p53 somehow continues to remain active and contribute to senescence and other chronic phenotypes [2]. In this review, we first overview the functional relevance of p53 in senescence focusing on the two major features, stable cell cycle arrest and the senescence-associated secretory phenotype (SASP), and then discuss the unique regulatory mechanism of how p53 and its downstream activities are fine-tuned during senescence.

## P53 in senescence – classical view

The senescence state is highly heterogeneous, depending on the different modes of senescence induction such as activation of certain oncogenes (oncogene-induced senescence), genotoxic reagents (DNA damage-induced senescence), replicative exhaustion and metabolic stress. These different cellular stresses often lead to persistent oxidative and genotoxic stress, either through excessive replication or inefficient DNA damage repair [5–10]. Thus, cellular senescence is considered a tumour suppressive mechanism by halting such damaged cells from further replicating. However, senescent cells also influence the tumour microenvironment due to the non-cell-autonomous aspect of senescence, represented by the SASP, and its composition and intensity are highly dependent on p53 [2].

### Cell cycle arrest

Acute activation of p53 in response to cellular stress results in the expression of genes involved in diverse effector programmes, including cell cycle, DNA repair and cell death, to mitigate cellular and tissue damage [11]. If the stress is not resolved in a timely manner, different cellular outcomes may occur such as senescence. While other downstream effectors of p53 are also involved, such as PAI1 (encoded by *SERPINE1*) and Promyelocytic leukaemia (PML) protein, p21 (encoded by *CDKN1A*), an endogenous cyclin-dependent kinase (CDK) inhibitor, plays a major role

in p53-mediated cell cycle arrest. CDKs antagonise Retinoblastoma (RB) pocket proteins through sequential phosphorylation, thus promoting cell cycle progression. In addition to the p53-p21 axis, a second barrier to cell proliferation is also involved; another CDK inhibitor, p16 (*CDKN2A*). The reliance on each pathway varies in different conditions. Classically, while in rodent cells, inhibiting either pathway appears to be sufficient for senescence bypass, blockage of both pathways is often required to achieve this in human cells. Such cooperative effects of these two major tumour suppressor pathways are also observed in senescence maintenance: p53 inactivation in established senescent human fibroblasts restores proliferation only if the p16 level is substantially low [12]. Interestingly, a more recent study has shown that timely activation of p53 is important for senescence induction [13]. Transient activation of p53 during the G2-phase leads to mitosis skip and triggers G1-tetraploid senescence. In this model, p53 is critical for senescence induction, whereas p16 is required for senescence maintenance. This model also implies that after senescence is fully established and p16 is abundant, p53 activity may be dispensable for at least some aspects of senescence effector programmes (see below) [14].

### Senescence-associated secretory phenotype

Senescent cells are not simply inert. They actively communicate with surrounding cells and tissues, affecting the local (and possibly systemic) tissue microenvironment [15]. This involves different mechanisms which either require direct cell–cell contacts (juxtacrine activities) or are mediated by secretory factors (autocrine and/or paracrine activities). The juxtacrine activities of senescent cells can be through receptor–ligand association [16], cytoplasmic bridges [15] and gap junctions [17]. The SASP components refer to ‘soluble factors’; however, senescent cells also develop the ability to produce and secrete extracellular vesicles (EVs), which carry diverse functional molecules. The functional relevance of EVs in senescence and cancer and how p53 is involved in this process are extensively discussed in recent reviews [18–20], and we focus this section on the SASP.

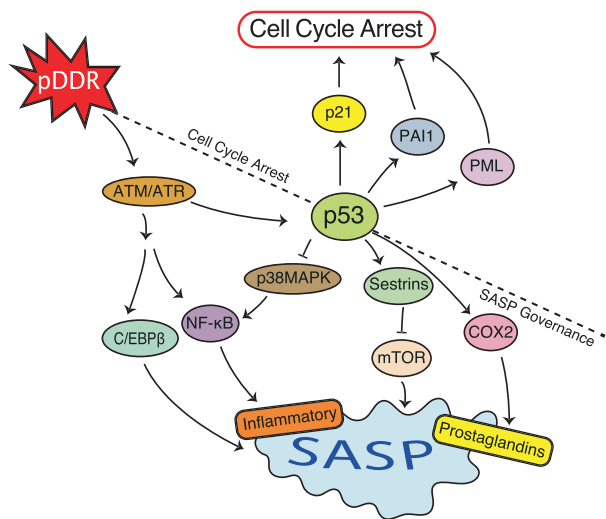
The SASP is a highly variable feature of senescence, differing in composition throughout space and time, as well as depending on the mode of senescence induction, thereby providing functional heterogeneity [2,16,21]. Broadly, the SASP is composed of inflammatory cytokines, growth factors, modulators of the extracellular matrix and angiogenic factors, among others [22]. Persistent DNA damage signalling has

been shown to be critical for the initiation and maintenance of the inflammatory SASP [23]. DNA damage responsive factors, such as ATM serine/threonine kinase (ATM), a kinase acting upstream of p53, but not p53, are required for the SASP activation. In fact, the development of both a persistent DNA damage response and the SASP, the inflammatory components in particular, were shown to increase upon p53 inactivation in human fibroblasts [24]. This inhibitory effect of p53 on the SASP can be distinguished from its role in the maintenance of cell cycle arrest (Fig. 1). As mentioned above, p53 inactivation alone is not sufficient for senescence rescue as long as p16 is active. However, p53 deficiency in senescent cells with low p16—that is, senescence can be reversed—fails to reverse the secretory phenotype, which may foster a tumorigenic microenvironment. These studies not only decouple these major senescence hallmarks (a terminal cell cycle exit and the secretory programme), but also highlight the ‘non-cell-autonomous tumour suppressive role’ of p53 [24]. Notably, p53-mediated senescent liver tumour cells exhibit a secretory phenotype which provokes an innate immune reaction, leading to the clearance of those senescent tumour cells [25]. In addition, p53 also affects the SASP composition [26,27]: for example, in mouse liver, the SASP in p53-expressing senescent hepatic stellate cells activates tumour

inhibiting macrophages whereas p53-deficient stellate cells secrete factors that activate tumorigenic macrophages, reinforcing p53-mediated non-cell-autonomous tumour suppression *in vivo* [26]. While p53 appears to negatively regulate some inflammatory subsets of SASP components in culture models, its impact on the SASP appears more complex and context-dependent *in vivo*.

How p53 modulates the SASP is not entirely clear. The inflammatory SASP is primarily regulated by two transcription factors, NF- $\kappa$ B and C/EBP $\beta$  [28]. There is a complex crosstalk between p53 and NF- $\kappa$ B, as p53 can be either a positive or negative regulator of NF- $\kappa$ B activity depending on the context [23,24]. In the case of senescence, it has been shown that NF- $\kappa$ B activation during senescence is, in part, mediated by the stress-inducible kinase p38 MAPK, which p53 appears to restrain [29]. More recently, it was shown that the DNA damage ‘sensors’, ATM and ATR serine/threonine kinase (ATR), promote the SASP through GATA Binding Protein 4 (GATA4)-mediated NF- $\kappa$ B activation [30]. The authors show that ATM/ATR inhibits p62-mediated selective autophagy degradation of GATA4. Importantly, p53, a downstream effector of ATM/ATR, is not required for GATA4 stabilisation. GATA4, a TF known to be involved in embryogenesis, appears to positively regulate NF- $\kappa$ B activation via multiple mechanisms, including through interacting with p38 MAPK [31]. Therefore, it is possible that p53 loss enhances the inflammatory SASP also through promoting a persistent DNA damage response and ATM/ATR signalling [23]. Another SASP effector that differentially modulates p53 and NF- $\kappa$ B in conditions such as aberrant activation of the JAK/STAT5 pathway is SOCS1 [28]. SOCS1 induces a unique p53-mediated SASP in part through inhibiting the NF- $\kappa$ B axis [32–34]. In addition, several studies have highlighted the role of mTOR in promoting the SASP via translational upregulation of factors involved in SASP genes [35–38]. This fits with other work demonstrating p53-dependent mTOR inhibition during senescence [39]. Finally, a new study has shown a distinct p21/RB-mediated SASP, which appears to be NF- $\kappa$ B-independent, can provoke immunosurveillance of senescent hepatocytes in mouse liver [40]. Although a specific role for p53 in the p21-dependent SASP is not examined, this study suggests that p53 may indirectly activate a non-typical SASP in certain contexts.

The complex antagonism of p53 on the typical SASP calls into question its regulation during senescence. Notably, the level of p53 is controlled through negative feedback by an E3 ubiquitin ligase complex that contains products of p53-target genes. The best-



**Fig. 1.** A simplified view of how p53 governs the cell cycle arrest and SASP independently. Persistent DNA damage response signalling (pDDR) stimulates both p53-induced cell cycle arrest and development of an inflammatory SASP. In addition to cell cycle arrest, p53 also impinges on aspects of the SASP, whilst it may also enhance others. Note, a new study showed that p21 can also provoke a specific type of SASP, which appears to be independent of NF- $\kappa$ B (see text) [40]. Dashed line indicates how cell cycle arrest and SASP regulation can be uncoupled.

known example of such E3 ubiquitin ligase is MDM2. However, Johmura et al. [41] recently showed that the SCF<sup>Fbxo22</sup>-KDM4 complex is also an E3 ubiquitin ligase for methylated p53. Since Fbxo22 is a p53-target, this SCF<sup>Fbxo22</sup>-KDM4 provides negative feedback control of the p53 level, much like the p53-MDM2 loop. Interestingly, SCF<sup>Fbxo22</sup>-KDM4 appears particularly important at the late phase of senescence for promoting the SASP, likely through p53 degradation [41]. In parallel, a positive feedback loop involving both p53 and the SASP was also reported [42]. We and others showed that COX2 (encoded by *PTGS2*), a critical enzyme for the biosynthesis of prostaglandins, contributes to the amplification of inflammatory SASP [42,43]. Interestingly, downstream products of COX2, 15d-PGJ2 and other prostaglandins, have been shown to activate RAS isoforms and related GTPases, and Wiley et al. [42] now show evidence that prostaglandins promote senescence development through activating RAS, and thereby the SASP and p53. In addition, the authors show that p53 is required for prostaglandin biosynthesis in the context of senescence; thus, COX2/prostaglandins (which activate the inflammatory SASP) and p53 reinforce each other.

Taken together, these studies suggest that the initiation of senescence perhaps requires high p53 activity to induce senescence arrest and trigger some aspects of the SASP but their dependency on p53 appears to be balanced or fine-tuned during the later stages of senescence through both positive and negative feedback regulations [41,42]. Notably, p53 activation alone is not sufficient to induce senescence [44], thus other factors such as mode of induction, duration of activation and additional signalling pathways need to be considered to understand the role of p53 during senescence.

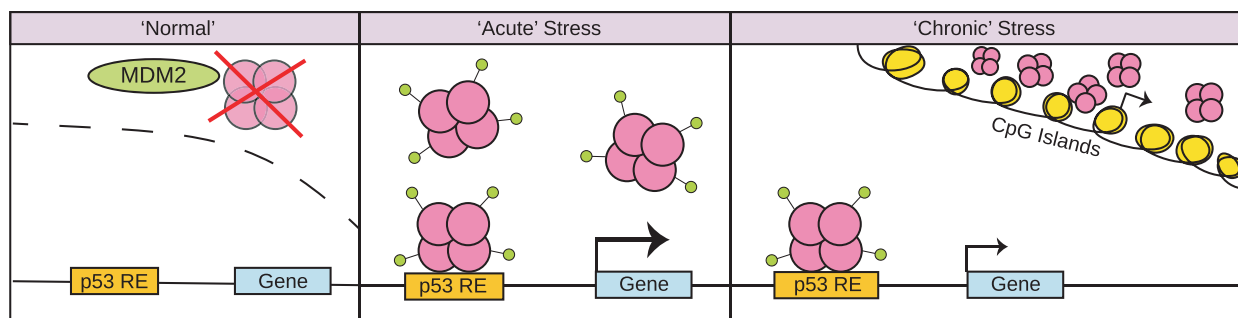
## Coordinating p53 activity during senescence

### Chronic versus acute phase of p53 activation

The basal p53 abundance is kept low in unstressed conditions predominantly via constant degradation by the E3 ubiquitin ligase MDM2. Control at the protein level enables rapid stabilization in response to cellular insults and the induction of target genes, including MDM2—this generates a negative feedback loop and keeps p53 under dynamic regulation in response to acute stress. If cellular stress continues or damage is too severe a cell may transition into senescence. This transition takes some time (typically ~1 week in the setting of fibroblasts in culture) during which overall p53 protein levels appear modest, but the chromatin bound p53 level is

comparable to the acute phase. The tumour suppressor p14-ARF (p19-Arf in mice), encoded by the 'Alternative Reading Frame' of *CDKN2A* (p16), interacts with and inhibits MDM2 by sequestering MDM2 in the nucleolus, leading to p53 stabilisation to promote senescence or cell death [45]. Thus, ARF might be involved in persistent expression of p53 in some contexts. As mentioned above, an additional E3 ubiquitin ligase complex, SCF<sup>Fbxo22</sup>-KDM4, also contributes to the fine-tuning of p53 levels during senescence [41]. However, protein abundance does not equate to activity and in addition to ubiquitylation, p53 receives numerous other PTMs that can influence its DNA binding and interacting partners [46]. For example, p53 is partially localised in senescence-associated nuclear bodies, such as PML bodies [47,48] and a persistent form of DNA damage foci called DNA-SCARS ('DNA segments with chromatin alterations reinforcing senescence') [49]. P53 undergoes modifications typical of the DNA damage response, such as N-terminal phosphorylation and C-terminal acetylation, in these nuclear bodies, thus reinforcing senescence. Interestingly, recent studies have shown that these nuclear bodies fuse in senescent cells to form PML-DNA-SCARS, where p53 can be sequestered through interaction with FOXO4 [49,50]. Inhibition of their interaction liberates p53 from nuclei, thus inducing cell death [49]. This exemplifies how the balance between p53-mediated effectors is shifted towards senescence, rather than cell death. Moreover, the change in signalling and metabolic states during senescence from acute to persistent DNA damage signalling has implications for p53 activity [23,27,42,51,52]. To aid with communication, we distinguish p53 in these two states as 'acute-p53' and 'chronic-p53' (Fig. 2). Differing p53 activity and the functional roles of acute- and chronic-p53 are in line with mouse models where p53 status can be reversibly switched on/off [53]: delayed activation of p53 (chronic-p53) in wild-type mice following carcinogenic whole-body irradiation suppressed irradiation-induced lymphomas. Conversely, restoration of p53 in mice at the onset of irradiation (acute-p53) did not suppress lymphomagenesis, highlighting the tumour suppressive role of chronic-p53, but not acute-p53, at least in this mouse model.

Despite such a distinction in functional relevance between acute- and chronic-p53, comprehensive knowledge about the latter in particular is still limited. To address this, we previously mapped genome-wide p53 binding sites in different cellular contexts, including acute DNA damage (acute-p53) and two oncogenic stresses (chronic-p53): oncogenic RAS<sup>G12D</sup>-induced senescence and E1A/RAS<sup>G12D</sup>-induced transformation in human diploid fibroblasts [51]. E1A is an adenoviral



**Fig. 2.** 'Acute' versus 'Chronic' p53 activity. Under 'normal' conditions p53 is degraded due to its interaction with the E3 ubiquitin ligase, MDM2. Under 'acute' stress, p53 is rapidly stabilised and activates gene expression by binding to its p53 response element (p53 RE). Under 'chronic' stress, such as in senescence, p53 has been shown to not only bind to the p53 RE, but also to a large number of CpG island-type promoters.

oncprotein, which not only completely blocks RAS-induced senescence but can also transform cells when expressed together with oncogenic RAS. While senescent cells are typically resistant to cell death, E1A stabilises p53, thus sensitising cells to p53-dependent apoptosis [51]. Therefore, RAS-induced senescence (non-proliferative, not apoptotic) and E1A/RAS-transformed (proliferative, apoptotic) cells exhibit highly distinct phenotypes. In this model, we showed that the binding landscape of p53 differs depending on whether p53 is acutely or chronically activated: in contrast to acute-p53, which mostly showed sharp peaks at non-CpG-island (CGI) promoters, chronic-p53 peaks tended to be wider, reflecting its preferential association with 'open' CGI promoters (Fig. 2). Together with p53-dependent transcriptomic data, we generated 'p53 knowledge-based pathway models' in the chronic conditions (<http://australian-systemsbiology.org/tp53/>) [51]. Interestingly, the non-CGI p53 peaks are associated with cell cycle, DNA damage and apoptotic genes, matching the acute binding response. In contrast, CGI peaks are associated with, among others, RNA metabolism and processing, and fatty acid biogenesis—the latter are repressive targets of p53. We also observed RAS-induced senescence and E1A/RAS-transformed cells display unique phenotypes yet overlap in the p53 binding landscape. These data reinforce the critical role of cellular context, including p53 PTMs, the epigenetic landscape and higher-order chromatin topology, and the proteomic network in regulating p53 activity.

### P53-mediated tissue-inappropriate gene regulation during senescence

One striking example of context-dependent chronic-p53-targets can be seen at the epidermal differentiation complex (EDC), a ~1.5-Mb locus containing a series

of genes whose products are involved in the final stage of keratinocyte differentiation, namely cornification, a unique form of programmed cell death, which contributes to the skin barrier function [54]. Thus, these genes are regulated during epidermal differentiation in a spatiotemporal manner through a timely reduction of facultative heterochromatin activity (marked by histone H3K27me3) [55]. These genes have such a specialised function that they are tightly silenced in other cell types, such as fibroblasts, where the EDC is enriched for histone H3K9me3 (a marker of constitutive heterochromatin) and heavily compacted at the nuclear periphery, exemplifying a stable silencing mechanism of 'lineage-inappropriate genes' [56]. However, our recent study revealed that, in fibroblasts, genes within this locus, most notably *Late cornified envelope 2* (*LCE2*) genes, are ectopically de-silenced during senescence but not upon acute DNA damage treatment nor E1A/RAS-transformation, in a p53-dependent manner [56]. Surprisingly, the *LCE2* genes are induced without apparent loss of the H3K9me3 mark, but rather through physical decompaction of the locus. During keratinocyte terminal differentiation in culture, the induction of those genes is accompanied by reduction of the H3K27me3 mark but no physical decompaction. Notably, in contrast to fibroblasts, in keratinocytes, the EDC locus is localised at the nuclear interior, which is transcriptionally permissive. When comparing Hi-C maps, we observed a similar increase in specific chromatin contacts within the EDC locus both during senescence in fibroblasts and terminally differentiation in keratinocytes. These data suggest that during fibroblast senescence, the EDC locus loses globally condensed structure, but gains specific keratinocyte-like chromatin loop formation. This is reminiscent of our recent study showing that the enhancer–promoter network is rewired at a number of the SASP components, leading to 3D configurations of the regulatory



elements similarly to macrophages, professional cells which produce those cytokines [57].

While exactly how p53 regulates the EDC locus remains unclear, in our chronic-p53 datasets in senescence fibroblasts described above, p53 binding and a concomitant increase in histone H3K27ac (an active enhancer mark) deposition were detected in this locus, suggesting a direct involvement of p53 in inducing *LCE2* genes. It is possible that the physical decompaction of this heterochromatic locus allows p53 access to the locus. Consistent with this idea, p53 depletion does not affect decompaction of this locus. In addition, enforced expression of p16 is sufficient to induce senescence (p16-induced senescence), which is a unique type of senescence, lacking both a persistent DNA damage response (thus p53 activation is minimal) and the inflammatory SASP [23]. In p16-induced senescent fibroblasts, we found substantial decompaction of the EDC locus but no *LCE2* induction. Therefore, we reasoned that these two senescence effectors, the p53 pathway and the SASP, might cooperatively contribute to *LCE2* deregulation. Indeed, p53 and a SASP-driving TF, C/EBP $\beta$ , are both required for most *LCE2* genes during senescence, where double knockdown of these TFs results in a more robust inhibition of *LCE2* induction than single knockdown. Moreover, C/EBP $\beta$  ChIP-seq uncovered its binding at multiple *LCE2* promoters [56]. This potentially highlights an additional layer of crosstalk between p53 and the SASP. Interestingly, in a transcriptomic dataset derived from a mouse arthritis model [58], synovial fibroblasts in the chronic phase appear to express higher levels of senescence markers (including *Cdkn1a*, encoding p21), SASP-related cytokines, and some EDC genes compared to in an acute phase [56]. Although the direct involvement of p53 and the epigenetic conditions in those synovial fibroblasts derived from arthritis tissues need to be validated, the data support a link between p53-mediated lineage inappropriate gene expression and senescence *in vivo*.

Functional relevance of p53-mediated lineage inappropriate gene expression remains to be elucidated. The involvement of cell type-specific 3D chromatin architecture implies that a similar deregulation of other lineage-specific genes might occur during senescence in different cell types. In addition to the altered accessibility of p53 (and/or other TFs), parallel signalling pathways would also modulate p53's activities, as we saw in senescent fibroblasts, where p53 and inflammatory signalling work together to activate *LCE2* transcription [56]. Moreover, human fibroblasts with enforced expression of *LCE2A* exhibit a limited replicative lifespan, thus the p53-*LCE2* axis might play a

role in senescence and other pathological conditions [56]. Interestingly, it was previously suggested that *LCE1* genes, which also reside at the EDC locus, are p53-targets in glioblastoma, lung, and colon cancer cell lines [59]. The authors showed that *LCE1* interacts with and inhibits the activity of the arginine methyltransferase, PRMT5 [59], which is widely expressed in cancer and has anti-senescence activity in human osteosarcoma cell lines [60].

## Conclusions

Here we have highlighted the distinct nature of acute and chronic-p53 in cellular senescence, not only for p53 *per se* but also for its target genes. While the former mostly represents the 'classic' view of p53 activities, we propose that the latter in part involves higher-order chromatin structural alterations. Thus, these two types of p53 can control genes in fundamentally different ways. Despite the notion that a delayed activation of p53 is more tumour suppressive than acute p53 activation in mice [53], our comprehensive understanding of chronic-p53 targets is limited. It is also not entirely clear how the 'quantity' of p53 is balanced with diverse inputs, both positive and negative, in prolonged stress conditions such as cellular senescence. 'Qualitative' identification of chronic-p53(-complexes) would also need further exploration. Finally, wider analysis of cell type-specific chronic-p53-targets in diverse settings may provide additional insights into the unique functionality of p53 in senescence and senescence-associated disorders, such as cancer, aging, and chronic inflammatory conditions.

## Acknowledgements

This work was supported by Cancer Research UK Cambridge Institute Core Grant (C9545/A29580 to MN). MN was also supported by the Biotechnology and Biological Science Research Council (BB/S013466/1, BB/T013486/1) and Diabetes UK via BIRAX and the British Council (65BX18MNIB). We thank Drs Andrew Young and Ioana Olan for their critical reading of the manuscript.

## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

ES and MN conceived the review and wrote the manuscript.

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