SENESCENCE

Senescence-messaging secretome: SMS-ing cellular stress

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Abstract | Oncogene-induced cellular senescence constitutes a strong anti-proliferative response, which can be set in motion following either oncogene activation or loss of tumour suppressor signalling. It serves to limit the expansion of early neoplastic cells and as such is a potent cancer-protective response to oncogenic events. Recently emerging evidence points to a crucial role in oncogene-induced cellular senescence for the 'senescence-messaging secretome' or SMS, setting the stage for cross-talk between senescent cells and their environment. How are such signals integrated into a coordinated response and what are the implications of this unexpected finding?

Cells are naturally exposed to various types of stress, including oncogenic events, putting multicellular organisms at risk. Several cell-intrinsic mechanisms, however, are in place to limit the expansion of incipient cancer cells. In addition to cell death programmes such as apoptosis and autophagy, oncogene-induced senescence (OIS) is increasingly recognized as a potent barrier against oncogenic transformation, acting to suppress the unscheduled proliferation of early neoplastic cells. OIS corresponds to a premature form of cellular senescence[1], which was first described by Hayflick as the limited replicative capacity of primary human diploid cell strains in response to prolonged passaging in culture[2]. OIS and replicative senescence share a number of characteristics, including an increase in senescence-associated β-galactosidase (SA-β-GAL) activity and the condensation of individual chromosomes into distinct heterochromatic bodies, called senescence-associated heterochromatic foci (SAHF)[3–5]. Unlike replicative senescence in human cells, however, OIS occurs independently of telomere shortening or malfunction[6]. The execution of replicative senescence as well as OIS commonly comprises the activation of bona fide tumour suppressor pathways, including the cytostatic INK4A–retinoblastoma (RB) and ARF–p53 signalling cascades. Their crucial roles in senescence are highlighted by their almost invariable inactivation in cancer[7,8]. Furthermore, genetic and epigenetic alterations in these genes may predispose to specific cancers[9]. Indeed, accumulating evidence indicates that replicative senescence and OIS act as physiologically relevant and potent barriers to tumorigenesis. In the context of benign human lesions and of several mouse models that recapitulate human disease, senescence has been detected *in vivo* and linked to inhibition of tumorigenesis (for reviews, see REFS 10,11). As has been excellently reviewed in this Series previously, cellular senescence programmes have many facets, including the engagement of DNA damage response pathways[12]. Furthermore, senescence may have important implications for ageing[13]. The roles of p53 and RB signalling pathways in senescence are undisputed, but it has become clear that other factors are also involved in this response.

The senescence-messaging secretome

It has long been recognized that cellular senescence is paralleled by significant changes in extracellular matrix (ECM)-associated factors[14]. For example, the expression of secreted factors, including insulin-like growth factor (IGF) binding protein 3 (IGFBP3)[15], IGF1 (REF. 16) and plasminogen activator inhibitor 1 (PAI1)[17], has been shown to be altered in senescence. Recent advances in detection methods and genetic manipulation have allowed us to characterize these processes more accurately. Consequently, we came to realize that, in fact, in addition to the aforementioned factors, numerous inflammatory proteins and mediators of ECM remodelling are strongly induced in senescent cells[18–20]. Several groups have reported that the implementation of cellular senescence involves, and often requires, the secretion of a plethora of factors, including key components of the Wnt, IGF1, transforming growth factor-β (TGFβ), plasmin and interleukin (IL) signalling cascades. As the currently available data suggest that one of their primary functions is to allow for communication both within and between cells, we propose to collectively name these

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At a glance

- Cellular senescence is a mechanism that blocks the proliferation of primary, and in some cases premalignant and malignant, cells. It can be activated by a plethora of stress conditions, including oncogene activation, loss of tumour suppressors or critical shortening of telomeres.
- Oncogene-induced senescence has recently been recognized as a tumour-suppressive mechanism *in vivo*, in human lesions and in several mouse tumour models.
- Recent evidence suggests that the induction of senescence requires several secreted factors, including members of Wnt, insulin, transforming growth factor-β, plasmin, interleukin and possibly also interferon signalling cascades. We term these collectively the senescence-messaging secretome (SMS).
- The SMS and its signalling cascades may converge first at the level of several plasma membrane signalling receptors.
- The use of secreted factors in senescence could provide a selective advantage to an organism, as it allows for communication between senescent cells and their microenvironment.
- Counterintuitively, senescent cells may contribute to tumorigenesis by virtue of the SMS, which can cause stromal components to senesce, thereby establishing a pro-mitogenic loop.

signalling cascades the 'senescence-messaging secretome' or SMS (after the short-message service phone text system; FIG. 1).

In this Review, we will focus on several aspects of the signalling cascades that are set in motion by these SMS factors. Emphasis will be put on the mechanisms leading to the activation (or inactivation) of SMS-associated signalling pathways. What exactly is their role in senescence? Although at first glance SMS-associated factors seem to be involved in distinct signalling pathways, it is conceivable that signals from these cascades are integrated to decide whether or not the cell engages in a senescence programme, eventually generating a binary output signal on this decision. However, from the available data it is not immediately clear how this would take place. We discuss the possibility that the first integration of SMS signalling occurs at the level of the plasma membrane. How then does the SMS regulate downstream effectors and pathways? In contrast to their tumour-suppressive role in mediating senescence, many SMS factors have been reported to also promote tumour development. What determines the switch between these two opposing functions in tumorigenesis? What could be the selective pressure for the cellular senescence response to rely on secreted factors?

SMS factors relaying senescence signalling

In this section, we describe several secreted proteins that have been shown to be involved in the induction of senescence, and explain how they complement our current knowledge on senescence signalling (TABLE 1).

IGF signalling and IGFBPs. The insulin–IGF1 pathway (FIG. 2a) is a conserved signalling route affecting several physiological and pathophysiological processes, including longevity and cancer (see below)[21,22]. A variety of papers suggest a role for IGF signalling in cellular senescence. For example, several groups have reported that IGFBPs have a causal role in the induction of senescence. IGFBPs sequester IGFs and thereby suppress IGF-dependent signalling[23]. Goldstein *et al.* reported that IGFBP3 is associated with senescence[15]. IGFBP3 and IGFBP5 levels rise in senescent human umbilical vein endothelial cells (HUVECs), in which replicative senescence is associated with an increase in SA-β-GAL activity and an induction of the cell cycle inhibitors INK4A and p53, although the associated proliferation block is not complete. Treatment of HUVECs with recombinant IGFBP3 or IGFBP5 is sufficient to slow down proliferation and provoke markers of senescence[24–26]. Whereas IGFBP3 seems to act downstream of p53 (REF. 24), both IGFBP3 and IGFBP5 regulate p53 expression and/or activity. Importantly, short hairpin RNA (shRNA)-mediated IGFBP3 or IGFBP5 depletion partially reduces SA-β-GAL positivity and concomitantly decreases population doubling time[24,25], suggesting a contribution of IGFBPs to the regulation of cellular senescence.

The importance of this protein family in senescence is further supported by a recent report that IGFBP7, overexpression of which has previously been shown to induce a senescence-like cell cycle arrest[27,28], was identified in an RNA interference screen for crucial mediators of $BRAF^{E600}$-induced senescence in human diploid fibroblasts (HDFs) and melanocytes[29]. Senescence is associated with an increased secretion of this IGFBP, and treatment with conditioned medium of senescent melanocytes or recombinant exogenous IGFBP7 is sufficient to induce senescence. IGFBP7 is not secreted by $BRAF^{E600}$-expressing melanoma cell lines, in contrast to melanoma cells not harbouring this mutation. In human melanocytic naevus cells (which often express $BRAF^{E600}$ (REFS 30, 31)), IGFBP7 levels are higher than in normal skin melanocytes, whereas its expression is lost in melanomas. This is presumably caused by the frequent methylation that the authors observed in $BRAF^{E600}$-expressing melanoma. Translating these findings to a preclinical setting, it was found that $BRAF^{E600}$-expressing melanoma xenografts are responsive to systemic IGFBP7 treatment[29].

The finding by Wajapeyee *et al.* that IGFBP7 strongly suppresses the phosphorylation of Erk suggests that it mediates its anti-proliferative effects through negative feedback signalling. Feedback inhibition of growth factor signalling has been previously proposed to have a causal role in the activation of senescence, in the context of loss of the neurofibromatosis tumour suppressor, NF1 (REF. 32). Consistent with this idea, expression of an activated mutant of ERK2 (also known as MAPK1) allows bypass of $BRAF^{E600}$-induced senescence[29]. However, a decrease in active Erk in the context of $BRAF^{E600}$-induced senescence is in apparent contrast to earlier reports, in which expression of $BRAF^{E600}$ induced activation of ERK1 (MAPK3) and ERK2 (REFS 33,34).

IGFs have also been implicated in cellular senescence. For example, Chakravarthy *et al.* used skeletal muscle satellite cells from an *Igf1*-transgenic mouse to show that IGF1 delays the onset of replicative senescence, which is mediated by its cognate receptor IGF1R[35]. This is associated with decreased levels of the cyclin-dependent kinase

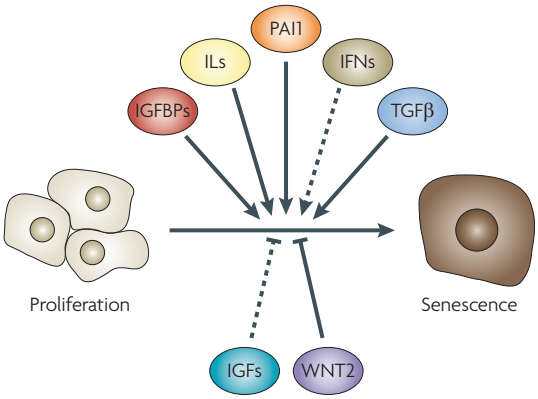

Figure 1 | **Senescence-messaging secretome (SMS) factors that contribute to the induction and maintenance of senescence.** The induction of senescence in different cell types and strains has been shown to depend on several secreted factors. These include several members of the insulin-like growth factor (IGF) binding protein (IGFBP) and interleukin (IL) families, plasminogen activator inhibitor 1 (PAI1) and transforming growth factor-β (TGFβ), which stimulate the induction of senescence, as well as WNT2, which inhibits implementation of this response. IGFs and interferons (IFNs) have also been implicated in senescence, indicated by dashed sign.

inhibitor p27 and activation of PI3K signalling. In turn, pharmacological inhibition of PI3K increases p27 levels and induces arrest of the cell cycle in G1 (REF. 35), implicating PI3K as an essential downstream target of IGF1 signalling in senescence. In line with this, the levels of the *IGF1* transcript are lower in senescent cells than in young cells[36].

Although conflicting data exist about changes in *IGF2* expression in senescence[36,37], there are indications that its protein product is involved in senescence. Androgenetic mouse embryonic fibroblasts (MEFs) — whose genomes are exclusively paternal — show a greater propensity to immortalize than normal biparental MEFs. However, parthenogenetic MEFs — whose genomes are exclusively maternal — display a complete lack of immortalization and seem to senesce prematurely[38]. In an effort to identify which imprinted genes could mediate these effects, *IGF2R*, the gene encoding the IGF2 receptor, was found as the maternally expressed gene limiting lifespan, and *IGF2* as a paternally expressed gene extending it. As IGF2R is not a typical signalling receptor, but serves to clear IGF2 from the extracellular space by lysosomal degradation (FIG. 3a), and because signalling functions for this growth factor are mainly relayed by IGF1R[39], these data support a model in which IGF2 increases lifespan in an IGF1R-dependent manner.

Thus, whereas IGF1 signalling delays replicative senescence, the situation for IGF2 signalling is less clear. Several IGFBPs are also required for the induction of cellular senescence, but their role in senescence *in vivo* is less clear, with the exception of IGFBP7. Although there is evidence that in certain tumours expression of IGFBP3 or IGFBP5 is reduced as a function of malignant progression[40–42], the role of these IGF signalling components in senescence awaits *in vivo* confirmation. Their causal role in senescence induction appears at least in part to be related to their ability to inhibit IGFs, which could open avenues for therapeutic applications. However, IGF-independent functions may also contribute (see below).

PAI1–plasmin system. Senescent cells commonly display increased levels of the ECM regulator PAI1 (REF. 17). PAI1 has long been regarded as a marker that merely correlates with, rather than contributes to, senescence[17,43]. However, Kortlever *et al.* demonstrated that it does have a fundamental role in replicative senescence[44]. This secreted factor acts in the plasmin cascade, in which the tissue-type and urokinase-type plasminogen activators (tPA and uPA, respectively) are the principal proteases for the cleavage of the zymogen plasminogen to yield plasmin (FIG. 2b). Binding of uPA to its cognate receptor, uPAR, greatly enhances this activation step. In turn, PAI1 can inhibit both plasminogen activators. Activated plasmin cleaves its targets, including several ECM and coagulation components, as well as growth factors. PAI1 levels increase in senescent cells, and overexpression of PAI1 is sufficient to drive human and murine fibroblasts into senescence, even in the absence of p53. Interestingly, in contrast to conditioned medium from wild-type or p53-depleted cells, medium from PAI1-depleted or uPA-expressing cells delays the onset of senescence in late-passage wild-type MEFs[45]. Consistent with this, interference with endogenous PAI1 in MEFs and HDFs allows escape from replicative senescence in the face of active ARF–p53 signalling. Also, shRNA-mediated depletion of glycogen synthase kinase 3β (GSK3β), as well as overexpression of PI3K, Akt or non-degradable cyclin D1, allows partial bypass of senescence[44]. This led the authors to propose a model in which PAI1 inhibits PI3K–Akt signalling, thereby activating GSK3β, resulting in the nuclear exclusion of cyclin D1 in order to cause senescence. These experiments were all performed *in vitro*. Data from the clinic reveal a more complex picture: high expression and activity of uPA, uPAR and PAI1 are all correlated with poor prognosis in several types of cancer[46]. How this relates to the role of PAI1 in senescence remains to be determined.

TGFβ. A role for TGFβ signalling in cellular senescence was shown first by Tremain *et al.*[47], who reported that introduction of a v-*ras* allele causes murine keratinocytes to hyperproliferate and display an increased lifespan. However, this is a transient phenomenon only, as these cells subsequently enter G1 cell cycle arrest, which is associated with increased expression of several senescence markers, including the cell cycle inhibitors INK4A, INK4B, ARF and p53, and also TGFβ1. Inhibition of TGFβ signalling with antibodies or by genetic means allows bypass from senescence in this cell system, with concomitant suppression of INK4A and INK4B, whereas activation of ARF and p53 remains unaffected. Conversely, treatment of keratinocytes with recombinant TGFβ1 is sufficient to raise SA-β-GAL activity.

Table 1 | **SMS factors: model systems, properties and involvement in senescence**

SMS factor(s)	Cell system	*In vitro*	Links	*In vivo*	Refs
IGF1	Murine skeletal muscle satellite	• *IGF1* transgene allows lifespan extension	PI3K activation and p27 inactivation	ND	35
IGF2 and IGF2R	Murine fibroblast (MEF)	• IGF2R KO and IGF2 transgenic parthenogenetic MEF show increased long-term proliferation • IGF2 KO biparental MEF show decreased long-term proliferation	Possible link to CDK inhibitors	ND	38
IGFBP3	Human endothelial (HUVEC)	• IGFBP3 induced in replicative senescence • IGFBP3 sufficient and required for (replicative) senescence	Bypass of replicative senescence due to $IGFBP3^{KD}$ associated with lower p53 and p21, and higher FOXO3A levels	IGFBP3 expression increases with age	24
IGFBP5	Human endothelial (HUVEC)	• IGFBP5 upregulated in replicative senescence • IGFBP5 sufficient and required for (replicative) senescence	Bypass REP due to $IGFBP5^{KD}$ associated with lower p53 and p21 and higher FOXO3A levels	ND	25
IGFBP7	Human melanocyte Human fibroblast (BJ)	• IGFBP7 upregulated in OIS ($BRAF^{E600}$) • IGFBP7 sufficient and required for ($BRAF^{E600}$) senescence • rIGFBP7 inhibits xenograft tumour growth	Bypass of OIS due to $IGFBP7^{KD}$ associated with lower INK4A and phosphoERK and higher Ac-H3K9 levels	*IGFBP7* promoter methylation in $BRAF^{E600}$-bearing melanoma Higher expression of IGFBP7 in nevi versus melanocytes and melanoma	29
PAI1	Murine fibroblast (MEF) Human fibroblast (BJ)	• PAI1 sufficient for and upregulated in senescence • PAI1 KO and KD and uPA expression and manipulation of PI3K, Akt, GSK3β and cyclin D1 allows bypass of replicative senescence	Replicative senescence-associated with lower PI3K, Akt and nuclear cyclin D1, and higher GSK3β activity and levels p53-independent	ND	44
TGFβ	Murine keratinocytes expressing v-*ras*	• TGFβ upregulated in replicative senescence • TGFβ KO, neutralizing ab or DN-TβR allows bypass of replicative senescence • TGFβ exposure increases SA-β-GAL activity	Bypass of replicative senescence due to absence of TGFβ associated with INK4B and INK4A downregulation; ARF and p53 unaffected	*Tgfb1*$^{-/-}$ v-*ras* keratinocyte xenografts often progress to malignant carcinoma	47, 51
WNT2	Human fibroblast (WI-38) Human epithelial (RPE)	• Repression of WNT2 in replicative senescence and OIS ($HRAS^{V12}$) • WNT2 KD increases amount of SAHF and HIRA foci • WNT3A exposure inhibits the formation of SAHF and HIRA foci and increases proliferation	WNT2 repression occurs independently of INK4A and p53	ND	55
IL-1α	Human endothelial (HUVEC) Human muscle (VSMC)	• IL-1α upregulated in replicative senescence (HUVEC) • 1/3 AS oligomer (HUVEC) and AS transcript (VSMC) clones show lifespan extension	ND	ND	76, 77
IL-6 and IL-8	Human fibroblast (Tig3 and IMR90)	• IL-6 and IL-8 upregulated in OIS ($BRAF^{E600}$) • IL-6, IL-6R, IL-8 and C/EBPβ KDs allow bypass of OIS	OIS bypass associated with collapse of inflammatory transcriptome and SAHF, and lower INK4B levels C/EBPβ is an essential regulator of IL-6	IL-8 expression increased in $INK4A^{+}$ non-proliferating human colon adenoma cell stretches	19
CXCR2-binding chemokines	Human fibroblast (BJ, WI-38 and IMR90)	• CXCR2 cytokines upregulated in replicative senescence and OIS ($HRAS^{V12}$/MEK) • CXCR2, IL-8 and GRO1 sufficient and required for senescence	NF-κB regulator of CXCR2 cytokines	Senescence markers and CXCR2 and its ligands increase (papillomas) Expression of CXCR2 increases (PIN)	20

Ab, antibody; Ac-H3K9, lysine 9 acetylation on histone H3; AS, antisense; C/EBPβ, CCAAT/enhancer-binding protein-β; CDK, cyclin-dependent kinase; CXCR, CXC chemokine receptor; DN, dominant-negative; FOXO, forkhead box; GSK3β, glycogen synthase kinase 3β; HUVEC, human umbilical vein endothelial cell; IGF, insulin-like growth factor; IGFBP, IGF binding protein; IL, interleukin; KD, knockdown; KO, knockout; MEF, murine embryonic fibroblast; ND, not determined; NF-κB, nuclear factor-κB; OIS, oncogene-induced senescence; PAI1, plasminogen activator inhibitor; PIN, prostatic intraepithelial neoplasia; r, recombinant; SA-β-GAL, senescence-associated β-galactosidase; SAHF, senescence-associated heterochromatic foci; SMS, senescence-messaging secretome; TβR, TGFβ receptor; TGFβ, transforming growth factor-β; uPA, urokinase-type plasminogen activator; VSMC, vascular smooth muscle cell.

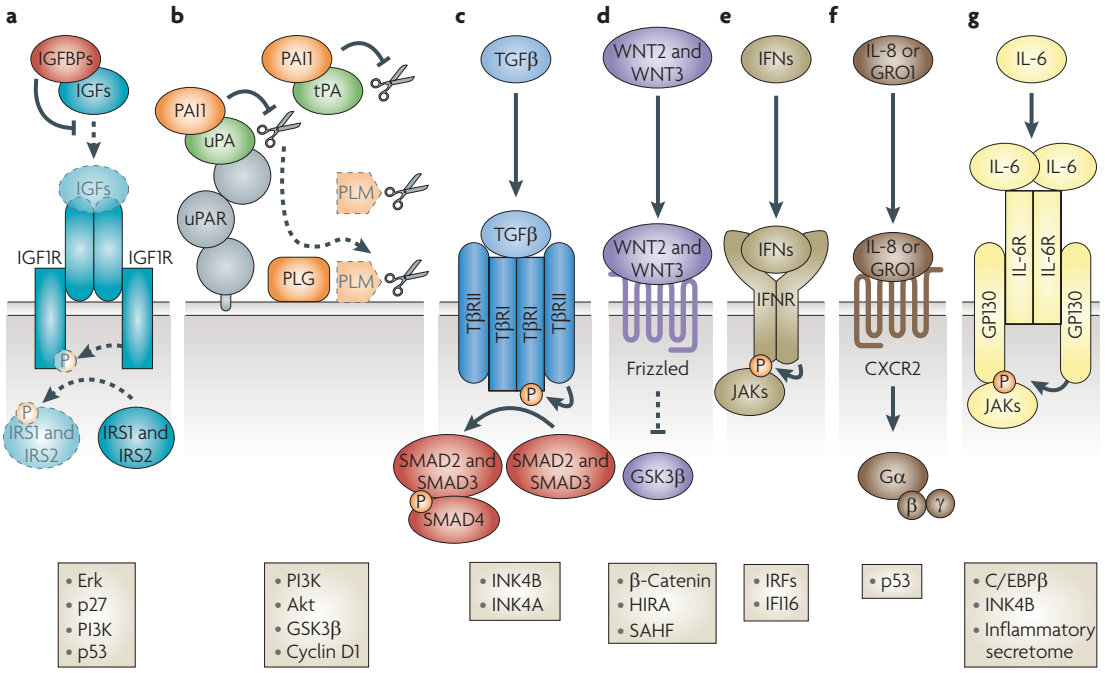

Figure 2 | **Signal transduction pathways (partly) used by the senescence-messaging secretome (SMS).** Schematic representation of signalling cascades (partly) used by SMS factors, and their targets (beige boxes) in senescence (TABLE 1). **a** | Insulin-like growth factor (IGF) binding proteins (IGFBPs) can sequester IGF1 and IGF2, thereby inhibiting their ability (shown in transparency and dashed lines) to induce phosphorylation and activation of their receptor (IGF1R) and adaptor (insulin receptor substrate; IRS) proteins. **b** | Plasminogen activator inhibitor 1 (PAI1) can inhibit both tissue-type and urokinase-type plasminogen activator (tPA and uPA, respectively), which are the principal proteases for the cleavage of the zymogen plasminogen (PLG) to yield plasmin (PLM). Binding of uPA to its receptor, uPAR, greatly enhances this activation step. Activated plasmin can cleave its targets, including several extracellular matrix and coagulation components, as well as growth factors. **c** | Active transforming growth factor-β (TGFβ) can bring TGFβ receptor I (TβRI) and TβRII together. Subsequent phosphorylation of TβRI by TβRII leads to recruitment of adaptor proteins of the SMAD family, their phosphorylation, and dimerization with SMAD4 to yield complexes that function as transcription factors. **d** | WNTs can indirectly activate their receptor, frizzled, leading to inhibition of glycogen synthase kinase 3 (GSK3β). **e** | Type I or II interferons (IFNs) can bind to their receptors, IFNAR1 and IFNAR2, or IFNGR1 and IFNGR2, respectively. Effectors of this signal transduction cascade include IFNγ inducible protein 16 (IFI16) and interferon-regulatory factors (IRFs). IRFs regulate transcription not only of interferon target genes, but also of IFNs themselves. **f** | CXCL type chemokines can bind to the CXCR2 receptor, thereby activating this G protein-coupled receptor and subsequently its adaptor G protein. **g** | Interleukin-6 (IL-6) can bind to its receptor IL-6R, leading to its complex formation with two GP130 molecules, their *trans*-autophosphorylation and recruitment and phosphorylation of adaptors of the Janus kinase (JAK) family. C/EBPβ, CCAAT/enhancer-binding protein-β; SAHF, senescence-associated heterochromatic foci.

Ligand-induced heterodimerization of type I and II TGFβ receptors (TβRI and TβRII) leads to activation of TβRI, which then recruits SMAD2 and SMAD3 proteins for phosphorylation. This provokes their heterodimerization with SMAD4, nuclear translocation of the dimers and transactivation of target genes[48] (FIG. 2c). SMADs are likely to mediate the cytostatic effect of TGFβ in senescence, although PAI1–plasmin–PI3K signalling can also contribute to the cytostatic effects of TGFβ[49]. Nuclear SMAD3 accumulates in senescent cells, and senescence as measured by SA-β-GAL positivity is reduced in *Smad3*$^{-/-}$ keratinocytes[50]. Conversely, overexpression of SMAD3, but not SMAD2 or SMAD4, in v-*ras*-expressing keratinocytes reduces proliferation and induces SA-β-GAL activity.

Extrapolating this to an *in vivo* setting, xenografts of v-*ras*-expressing keratinocytes in immunocompromised nude mice progress more frequently from papilloma to malignant carcinoma in the absence of either TGFβ1 or SMAD3 (REFS 50,51). Although this was tested in another context, chemically induced papillomas indeed express several senescence markers[52], suggesting that TGFβ and its target SMAD3 functionally link senescence to inhibition of tumour progression in this xenograft model also. These findings are supported by *in vitro* data in human diploid fibroblasts. Inhibition of TGFβ signalling with neutralizing antibodies to TGFβ1 or TβRII reduces the number of SA-β-GAL-positive cells and the levels of the transcripts of some senescence-associated genes after treatment with either subcytotoxic levels of H_2O_2 (REF. 53) or repeated exposure to ultraviolet B[54]. In keeping with this, TGFβ has clear tumour-suppressive functions, and several of its signalling pathway components are altered in cancer[48]. The important role that TGFβ seems to have in senescence, both *in vitro* and *in vivo*, may therefore contribute to its tumour-suppressive functions.

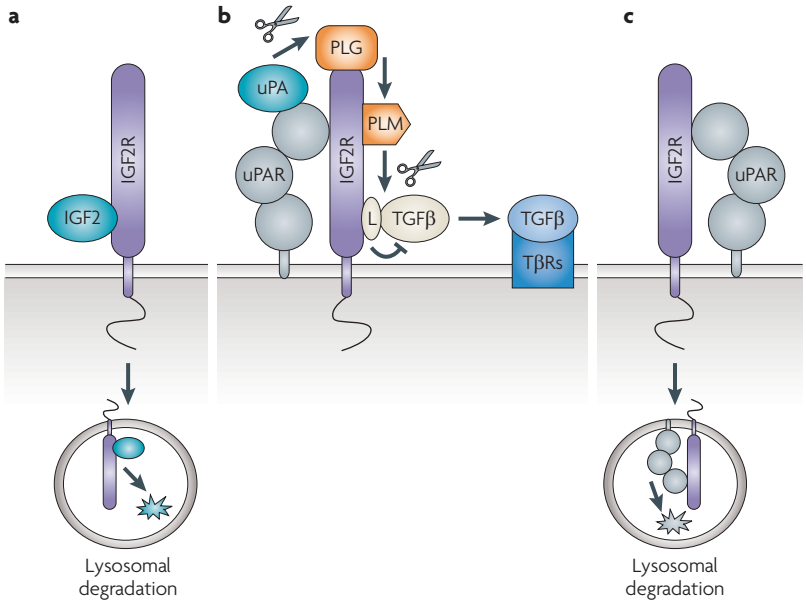

Figure 3 | **Functions of insulin-like growth factor 2 receptor (IGF2R) that may integrate senescence-messaging secretome (SMS) signalling cascades.** Several SMS signalling pathways can be integrated around IGF2R, and might thereby collectively provide a cell with instructions to undergo senescence. **a** | IGF2R is the clearance receptor for IGF2. Binding of IGF2 to the receptor leads to endocytosis and lysosomal degradation of the growth factor. **b** | IGF2R can bind to urokinase-type plasminogen (PLG) activator (uPA) receptor (uPAR), plasminogen and transforming growth factor-β (TGFβ) in its latent form (LTGFβ), and therefore functions as a scaffold to allow plasminogen activation by uPA. The activated zymogen can subsequently cleave LTGFβ to yield the mature cytokine, which can then bind to its corresponding receptors. **c** | uPAR can also be internalized and directed for lysosomal degradation by IGF2R. PLM plasmin; TβR, TGFβ receptor.

WNT2. In an effort to identify proliferation-regulatory signalling pathways connecting cellular senescence to the formation of SAHF, Peter Adams' laboratory found a novel link for the SMS factor WNT2 (REF. 55). SAHF contain several heterochromatin-associated post-translational modifications, and are thought to contribute to senescence by silencing genes that are required for cellular proliferation[4,5]. Induction of SAHF is linked to high mobility group A proteins and SUV39H1 (REFS 56,57) and requires the chromatin regulators ASF1A and HIRA[58]. Relocalization of HIRA to PML bodies is thought to initiate chromosome condensation at the onset of senescence (reviewed in REF. 5).

Ye *et al.* observed that the translocation of HIRA to PML bodies in HDF is independent of the status of p53 and RB[59]. The *WNT2* transcript is downregulated in replicative senescence and OIS, in a p53- and RB-independent manner[55]. In canonical Wnt signalling, Wnt ligands bind to Frizzled receptors, which in turn leads to activation of the cytoplasmic Dishevelled proteins. Subsequent inactivation of the kinase activity of GSK3β leads to accumulation of the multifunctional adaptor protein β-catenin, its interaction with TCF (also known as LEF) transcription factors in the nucleus, and eventually transcriptional activation of responsive genes (FIG. 2d). As a consequence of the loss of the WNT2 ligand, GSK3β is activated in senescence. This is required as well as sufficient for HIRA localization to PML bodies and SAHF formation. Overexpressed GSK3β and HIRA physically interact, and the latter is phosphorylated on serine 697 by the kinase to allow localization to PML bodies. Finally, Ye *et al.* showed that knockdown of WNT2 induces HIRA foci and SAHF, and that exogenous WNT3A allows a delay in senescence[55]. Thus, whereas Wnt signalling is sufficient to postpone senescence, the observed decrease in WNT2 levels in senescent cells might be involved in the onset of the proliferative arrest.

In line with the findings by Ye *et al.*, GSK3β depletion has been shown in two reports to bypass oncogene-induced and replicative senescence[44,60] and lead to tumour formation in nude mice in the context of oncogenic Ras^{V12} (REF. 60). By contrast, expression of an active β-catenin mutant induces senescence in MEFs[61]. Furthermore, and in contrast to the finding by Ye *et al.* that WNT3A treatment delays the onset of senescence, another report by Liu *et al.* shows the opposite in both human and murine fibroblast cells: WNT3A caused these cells to enter senescence prematurely[62]. Despite the existence of these functional connections, it is as yet unclear to what extent these *in vitro* findings, and also the dominant role of Wnt signalling in tumorigenesis[63], are related to the ability of the Wnt pathway to regulate senescence *in vivo*. An extra layer of complexity may be envisaged by the stromal secretion of Wnts, which may counteract the induction of senescence by loss of Wnt signalling in incipient neoplastic cells.

Interferons. Interferons potentially have a role in senescence. Expression of some interferon-regulated genes increases with age *in vitro*[64,65]. During cellular immortalization such genes can be downregulated, for example by epigenetic silencing[66]. Some of these intracellular targets, including interferon-regulatory factor 1 (IRF1), IRF5 and IRF7 and interferon-γ inducible protein 16 (IFI16) (FIG. 2e), have been functionally linked to senescence[67–69]. For instance, $Irf1^{-/-}$ MEFs (in spite of the fact that they do not seem to be immortal) are oncogenically transformed on expression of $HRAS^{V12}$ (REF. 67). Moreover, the onset of senescence is associated with induction of IFI16, which is sufficient as well as required for induction of this response[69]. These observations imply an involvement of interferons in the senescence response. Indeed, prolonged exposure of HDF, endothelial or biliary epithelial cells to interferon activates the senescence response[70–72]. Their cell cycle arrest depends on the activation of p53, and may be related to reduction in expression of telomerase[72–74]. It should be noted that, although several lines of evidence suggest that exposure to interferons can trigger cellular senescence and that interferon-dependent signalling pathways are involved in mediating senescence-promoting signals, it remains an unanswered question whether interferons themselves are also required for senescence. This notwithstanding, they should also be considered potential components of the SMS.

Interleukins. Exploring the secretory phenotype of senescent cells using antibody arrays, Judith Campisi's laboratory has found that the induction of senescence is accompanied by the secretion of a plethora of cytokines and other extracellular factors. This so-called senescence-associated secretory phenotype (SASP)[75] involves the emission of several inflammatory and cancer-related factors, including interleukins. An involvement of interleukins in senescence was first suggested by Maier *et al.*, who found a positive correlation between IL-1α transcript levels and the control of cellular proliferation in HUVECs[76]. Antisense oligomer-mediated depletion of IL-1α extends their lifespan (although this was observed for only one of the three antisense oligomers used). Cellular lifespan extension has been reported also for vascular smooth muscle cells transfected with antisense *IL1A*[77]. However, neither study investigated whether this correlated with altered expression of senescence markers.

CXCR2 (also known as IL-8RB) is a promiscuous receptor that transmits signals from several CXC chemokine family members (CXCLs), including IL-8 and GRO1 (also known as GROα) (FIG. 2f). Depletion of CXCR2, or expression of the cancer cell line-derived mutant $CXCR2^{G354W}$, has recently been shown by Acosta *et al.* to allow delay of both replicative and MEK-induced senescence of HDF. During senescence, levels of CXCR2 ligands are upregulated in a CCAAT/enhancer-binding protein-β (C/EBPβ)- and nuclear factor-κB-dependent manner, and the expression levels of the receptor itself are also increased. As an shRNA against either IL-8 or GRO1 alleviates senescence, these results suggest that multiple ligands contribute to CXCR2 signalling in this context. Papillomas from mouse skin treated with DMBA and TPA, which expresses several senescence markers, display increased levels of CXCR2 and several of its ligands compared with normal skin. Similarly, human prostatic intraepithelial neoplasia (PIN) show higher expression of CXCR2 than normal prostate glands[20].

Our laboratory has also demonstrated that senescence in HDFs is associated with the activation of an inflammatory transcriptome. One of the induced cytokines secreted by senescent cells, IL-6 (FIG. 2g), is required along with its receptor (IL-6R) for cells to undergo OIS. The senescence override can be achieved by IL-6 shRNA-mediated depletion but not by antibody neutralization of IL-6. Because of the requirement for IL-6R, we concluded that IL-6 acts in a cell-autonomous mode to implement OIS. In a paracrine fashion, IL-6 was shown to promote proliferation of susceptible (cancer) cells. The transcription factor C/EBPβ was identified as an essential regulator of IL-6 and senescence, and functions together with IL-6 to amplify the oncogenic signal. The C/EBPβ–IL-6 axis is required for the formation of SAHF and contributes to the upregulation of INK4B on exposure to oncogenic stress. C/EBPβ–IL-6 signalling is required for the induction of the inflammatory transcriptome, including IL-8, in OIS. In turn, this cytokine is also required for the induction of senescence. *In vivo*, IL-8 shows a striking co-localization with arrested, INK4A-positive epithelium in human tissue sections of colon adenoma, suggesting that the interleukin-dependent network also contributes to OIS *in vivo*[19]. Thus, depending on the cellular or genetic context, SMS factors including specific interleukins elicit either mitogenic or cytostatic effects, thereby simultaneously affecting senescence and cancer.

Cytostatic effects of IL-6 have been described for several types of cancer, which would be in line with a tumour-suppressive function of this cytokine. However, a wealth of data indicates that IL-6 has pro-mitogenic or even oncogenic functions[78] (reviewed in REF 79). What determines the outcome of IL-6 signalling is as yet unclear, but it may depend on the stage of the tumour. For example, during melanoma progression, IL-6 is thought to switch from being an anti-proliferative factor to being a pro-proliferative factor[80,81]. An additional layer of complexity is that the target cell of IL-6 may also dictate the outcome of the signal. For example, whereas IL-6 contributes to the induction of senescence of non-malignant cells (in an autocrine manner)[19], its tumour-promoting function (by paracrine signalling) has been proposed to directly or indirectly target the angiogenic compartment[78]. Tumour-promoting functions have also been reported for IL-8 and GRO1. For instance, in the context of $HRAS^{V12}$-driven tumours, the HRAS-dependent increase in IL-8 expression is required for initiation of tumour-associated inflammation and neovascularization[82]. So why do tumours become refractory to the cytostatic action of the cytokine they produce? One could imagine that this is due to deficiencies in signalling pathways at or downstream of their corresponding receptors. In such a model, cells that bypass senescence could still benefit from paracrine tumour-promoting functions of the SMS. The lung adenocarcinoma $CXCR2^{G354W}$ mutant, which appears to function in a dominant-negative fashion to alleviate OIS[20], would be consistent with such a scenario.

Interdependence between the SMS, RB and p53

An important question is how we can relate the SMS to the 'classical' senescence mediators p53 and the RB pathway inhibitors INK4B and INK4A. When we consider the links between these tumour suppressors and SMS factors, a complex picture emerges . INK4B is a potential downstream target of the SMS, as its expression increases upon exposure to senescence-inducing factors and decreases when cells escape senescence in the absence of TGFβ, C/EBPβ or IL-6 (REFS 19,47). Some SMS factors, including IGFBP5, interferons and CXCR2 ligands, appear to signal through p53. This tumour suppressor is also a signalling target for IGFBP3, expression of which is in turn responsive to p53. TGFβ does not impinge on p53, as cells deficient for this cytokine escape senescence in the face of high ARF and p53 levels. This is also the case for PAI1 but, as it is an established p53 target[83], it is likely to act as an essential downstream target of p53. By contrast, the SMS factors IL-6 and WNT2 function independently of p53, as well as of INK4A. For instance, $BRAF^{E600}$-induced senescence is relayed by IL-6 irrespective of the INK4A status, and the activation of the SMS does not require INK4A[19]. Moreover, in this context p53

is not regulated at the expression level and SV40 large T antigen, which functionally inactivates RB and p53, allows only a weak bypass of $BRAF^{E600}$-induced senescence[84]. Also in the setting of replicative senescence, p53 is not necessary for the concomitant induction of IL-6 and other secreted factors[75]. Furthermore, repression of WNT2 in replicative senescence occurs even in the presence of SV40 large T antigen[55]. These findings support the view that IL-6 and WNT2 contribute to senescence independently of the INK4A and p53 pathways. On a more general note, it therefore seems that the SMS signals through, downstream of and also parallel to the INK4A–RB and p53 pathways.

Functional SMS networks

Above, we describe the role and mechanism of action (for as far as they are known) of several SMS factors. Although they all impinge on the cell cycle machinery eventually, we elaborate below on the possibility that, to control senescence, several of the components within the SMS signalling pathways physically and/or functionally interact at, or in the immediate vicinity of, the plasma membrane. Specifically, several senescence-associated signalling modules seem to be integrated by the membrane receptors IGF2R, low density lipoprotein receptor-related protein 1 (LRP1, also known as TβRV), TβRI and/or TβRII. Below, we discuss which signalling cascades these receptors feed into and how this may be integrated to allow a coordinated response to senescence-regulating signals.

Interactions of SMS signalling around IGF2R. IGF2R has an essential role in the activation of latent (L)TGFβ by plasmin[39] (FIG. 3b). LTGFβ is the inert form in which TGFβ is secreted. It consists of a disulphide-linked homodimer of TGFβ non-covalently associated with its propeptides (latency-associated peptides) and the LTGFβ binding protein (LTBP), which is disulphide-linked to latency-associated peptides[85,86]. An important mode of regulating TGFβ activity is activation by proteolysis of LTGFβ. This can be executed by several proteases, including plasmin[87]. Plasmin is cleaved and activated from its precursor plasminogen by a complex containing uPA and the membrane-linked uPAR. Interestingly, IGF2R (which is also named cation-independent mannose-6-phosphate receptor[88]) functions as a scaffold as it interacts with plasminogen[89], uPAR[90] and LTGFβ (through its mannose-6-phosphate-containing side chains)[91,92], thereby bringing LTGFβ in contact with components of the plasmin system to allow its activation (FIG. 3b). Indeed, IGF2R is required for activation of LTGFβ by plasmin[93]. At least in some settings, mini-plasmin (which is activated from its precursor mini-plasminogen, lacking four of its kringle domains compared with plasminogen) is the form of plasmin that activates LTGFβ[94]. It should be noted that the role of plasmin in LTGFβ activation has been established *in vitro*, and that null mutations of plasminogen do not lead to a TGFβ deficiency phenotype *in vivo*, but this is also true for all other known LTGFβ-activating proteases. This may be due to redundancy of these proteases, or may not have been studied in the appropriate physiological context[95].

In addition to its role in the activation of TGFβ by plasmin, IGF2R is involved in other SMS-related signalling cascades. For example, IGF2R has been implicated in IL-6 signalling. In the canonical IL-6 signalling cascade, binding of IL-6–IL-6R complexes allows cross-phosphorylation of GP130 co-receptor molecules, and subsequent recruitment of adaptor proteins transducing the signals[96] (FIG. 2f). IL-6-induced proliferation of myeloid and lymphoid cell lines is inhibited by treatment with a naturally occurring, soluble form of IGF2R or with α-IGF2 antibodies, suggesting that it relies on IGF2 signalling in a non-canonical fashion, at least in this experimental context[97]. A role for IGF2 in IL-6 signalling has been described only for pro-mitogenic signalling; whether this is also the case in the context of anti-proliferative functions of IL-6 remains to be determined. IGF2R can also direct uPAR to lysosomes (FIG. 3c). Pharmacological inhibition of lysosomal proteinases increases the concentration of uPAR in these vesicles, indicating that lysosomal targeting leads to its degradation[90].

It thus appears that IGF2R controls several SMS signalling pathways, thereby potentially contributing to the induction of senescence (FIG. 3). This includes its function to clear IGF2 from the ECM[39,98], but also to activate TGFβ and to target uPAR for degradation. Conversely, and similarly to IL-6-dependent stimulation of proliferation, TGFβ-induced oncogenic transformation of rodent cells is dependent on IGF2 signalling[99]. In principle, these functions would put IGF2R in a good position to perform a first step of integration of cell cycle-inhibitory signals from the SMS, thereby contributing to the coordinated induction of senescence. This hypothesis predicts that IGF2R has an important role in the induction of senescence. In these contexts it is noteworthy that IGF2R is commonly deleted in hepatocellular carcinoma with the remaining allele containing one of several inactivating mutations[100]. Moreover, its functional inactivation is an early event, as deletion of IGF2R is observed in a large percentage of dysplastic lesions. IGF2R is also affected in other types of cancer including breast, lung and ovarian[39]. However, whether the functional inactivation of IGF2R in these lesions is related to the role it may have in regulating cellular senescence remains to be determined.

Interactions of SMS signalling around TβRI, TβRII and LRP1. The cytostatic activity of TGFβ is mediated by repression of proliferation-stimulating genes as well as by activation of cell cycle inhibitors, both of which occur in a TβRI and TβRII-dependent manner[48] (FIG. 4a). However, many lines of evidence indicate that other signalling cascades may contribute. Huang and colleagues have shown, for example, that LRP1 is required for the inhibition of proliferation by TGFβ, as its deficiency attenuates the response to TGFβ in MEFs, Mv1Lu (Mink fetal lung) and H1299 (human lung carcinoma) cells[101] (FIG. 4b), although this effect is context-dependent. Interestingly, IGFBP3 and, to lesser extent, IGFBP4 and IGFBP5 are also ligands for LRP1, at least

Kringle domains
Protein domains that fold into large loops stabilized by three disulphide linkages and are important for interaction of proteins with blood coagulation factors.

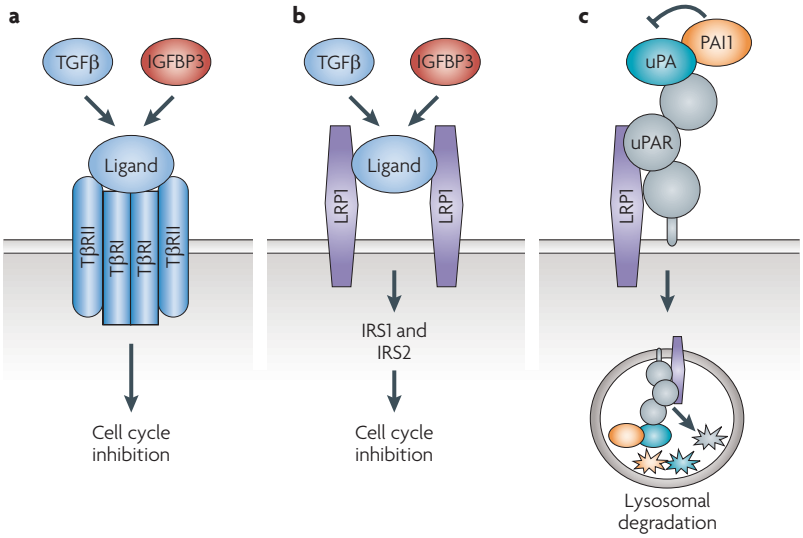

Figure 4 | **Transforming growth factor-β (TGFβ) receptor I (TβRI), TβRII and low density lipoprotein receptor-related protein 1 (LRP1) may integrate senescence-messaging secretome (SMS) signalling cascades.** TβRI, TβRII and LRP1 may act as points of convergence of SMS signalling. **a** | TβRI and TβRII can be activated not only by their canonical TGFβ ligand, but also by insulin-like growth factor receptor binding protein 3 (IGFBP3). Activation of TβRI and TβRII leads to inhibition of proliferation. **b** | TGFβ can also inhibit the cell cycle in a non-canonical manner through LRP1, in a process that requires insulin receptor substrate 1 (IRS1) and IRS2. **c** | The ability of LRP1 to target urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI1) and uPA receptor (uPAR) for lysosomal degradation constitutes an additional function for LRP1 in SMS signalling.

in Mv1Lu cells[102,103] (FIG. 4b). Hence, this constitutes a function for these IGFBPs that is independent of their ability to bind IGFs. As observed in Mv1Lu and 32D (murine myeloid) cells, the anti-proliferative effects mediated by LRP1 require insulin receptor substrate 1 (IRS1) and IRS2 (REF. 104). Those proteins are activated by, and required for, IGF-induced mitogenic signalling and activation of the insulin signalling pathway can indeed revert IGFBP3-induced inhibition of proliferation[104,105]. Another potential layer of regulation of LRP1-mediated IGFBP signalling is that IGFBP3 and IGFBP5 can be cleaved by plasmin[106,107]. Moreover, IGFBP5 can directly interact with PAI1 and this partially inhibits IGFBP5 proteolysis[108].

LRP1 has additional functions in SMS signalling. For example, it mediates the internalization and degradation of the complex of PAI1 with uPA or tPA[109–112] (FIG. 4c). On binding of this complex to uPAR, the receptor is co-internalized and targeted for lysosomal degradation[113]. Although unoccupied uPAR can be also recycled back to the cell membrane[114], the efficiency of this is probably low, and hence LRP1-mediated internalization leads to decreased surface uPAR levels[115]. Notably, IGFBP3 also inhibits cellular proliferation through TβRI and TβRII, which involves the canonical targets of the receptor, that is, SMAD proteins[116]. Full activation of TβRI and SMAD-mediated signalling by IGFBP3 requires endogenous TGFβ[117]. Conversely, small interfering RNA-mediated IGFBP3 depletion interferes with the cytostatic effect of TGFβ2 on the breast cancer cell line MDA-MB-231 (REF. 118).

Thus, LRP1, TβRI and TβRII receptors further connect TGFβ, plasmin and IGFBP signalling (FIG. 4). The functions mediated by LRP1 could all potentially contribute to the induction of senescence. Its roles in the turnover of uPA–PAI1 and tPA–PAI1 complexes, in decreasing cell membrane uPAR levels, and in mediating IGFBP3 and TGFβ signals all contribute to the inhibition of cell cycle progression. The same applies to TβRI and TβRII-dependent signalling of IGFBP3 and TGFβ. It is tempting to speculate that the shared anti-proliferative functions of LRP1, TβRI and TβRII contribute to the induction of senescence. This hypothesis would imply that these receptors and their signalling cascades are altered in cancer. The role of canonical TGFβ signalling in tumorigenesis is well established, and multiple components of its signalling pathway are known to be altered in cancer to avoid its suppressive effects[48]. There is also an indication that LRP1 has a role in cancer. In a DMBA and testosterone-induced prostate cancer model in rats, the expression of LRP1 is induced in different grades of PIN compared with normal prostatic epithelium, whereas it is lost in adenocarcinomas and undifferentiated carcinomas. The expression of uPAR is induced during the progression from PIN to tumour, and it was suggested that the loss of LRP1 underlies this phenomenon[119]. It would be interesting to determine whether the expression of LRP1 correlates with that of senescence markers.

In summary, in the context of senescence signalling, IGF2R, LRP1, TβRI and TβRII can all act cytostatically. We hypothesize that convergence of the signals they elicit occurs at the level of the membrane, where receptors serve as scaffolds to perform a first round of SMS signal integration, instructing a cell whether or not to engage in the senescence programme. Clearly, for these SMS signals, as well as those that are relayed through independent signal transduction pathways, additional integration is likely to occur intracellularly, for example at the level of signalling pathways, the transcriptome and/or even further downstream, at the level of the cell cycle machinery.

Potential selective advantages of SMS signalling

Although the selective advantage of OIS to the organism seems obvious[8], it is less straightforward to explain why the SMS contributes to this. In fact, as discussed below, the SMS could be used by tumour cells as a strategy to acquire proliferative potential. What, then, could be the evolutionary pressure to use the SMS (FIG. 5a–c)? We believe that the most straightforward advantage of this messaging system is that it does just that: messaging and allowing for communication both within and between cells. One might hypothesize that, for example, through SMS signals, a senescent cell sends a danger signal to its microenvironment. This could be advantageous in several ways.

First, by secreting SMS factors, a senescent cell may contribute to induction of senescence in cells in its immediate vicinity (FIG. 5a). This could be beneficial in, for example, ultraviolet-damaged tissue, where increased susceptibility to undergo senescence would decrease the likelihood that a damaged cell progresses to malignancy.

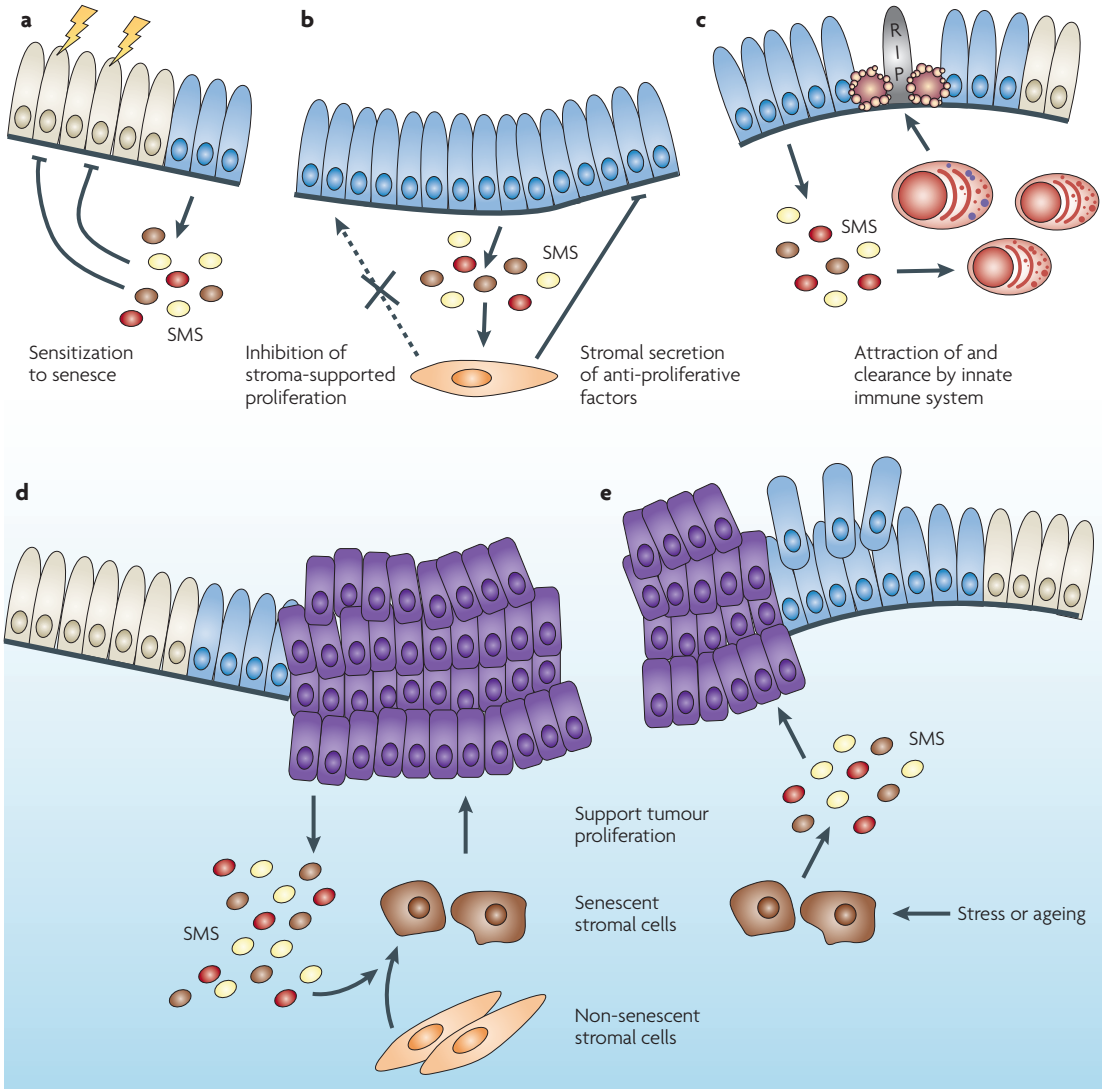

Figure 5 | **The potential benefits and threats of the association of secreted factors with senescence.** The use of senescence-messaging secretome (SMS) factors for senescence has potential positive and negative sides and constitutes an example of antagonistic pleiotropy. **a** | SMS factors secreted by senescent cells (blue) could constitute a danger signal that sensitizes normal neighbouring cells (beige) to senesce. For example, this may reduce the chance that damaged cells at risk of neoplastic transformation would fail to enter senescence. **b** | Another possible benefit is that normal stromal cells could also receive the danger signal and stop supporting growth of the incipient neoplastic cell, thereby helping to implement a full senescence response in this cell. Alternatively, they could start secreting proliferation-inhibitory (SMS) factors. **c** | A third advantage is the possible attraction of the innate immune system, which can dispose of the senescent cells. **d** | In later stages of tumorigenesis, tumour cells (purple) can misuse SMS signalling. By secreting SMS factors, cancer cells can induce senescence in stromal cells, such as fibroblasts. These in turn secrete factors that contribute to tumour growth. This model of two-way communication between tumour cells and stromal cells may explain why tumour cells commonly express several SMS factors. **e** | Stress and/or ageing can lead to an increase in the number of senescent cells, thereby potentially stimulating expansion of premalignant cells as a function of the SMS.

Along these lines, it might be advantageous to have an SMS system in place in colorectal cells, which are similarly exposed to genotoxic stress. This may serve as a fail-safe mechanism for apoptosis, which acts as a first line of defence to oncogenic events in this epithelium. Some laboratories have suggested that the SMS can induce senescence in *trans*[20,29,44]. In this way, the SMS produced by one cell can induce senescence in the next cell, possibly creating a snowball effect of senescence induction and, in turn, SMS factor production. However, it is unclear to what extent this can occur in the absence of any cooperating stress signal, and until now this has not been supported by evidence *in vivo*. For example, immunohistochemical staining of human colon adenoma resections reveal stretches of IL-8-producing, arrested cells alternating with proliferating cell groups

that are immuno-negative for this cytokine[19]. Thus, at least in this setting, senescence effects in *trans* of SMS factors seem to be spatially restricted. This may be explained by the existence of a senescence threshold for cells residing in the immediate environment of a senescent cell. In primary human fibroblasts, senescence is induced by IL-6 only in the context of additional stress or cytostatic signalling[19]. Conceivably, the cell integrates several stress and proliferation-regulating signals to come to a final binary output, instructing it whether or not to enter senescence. It is likely that multiple factors, both intracellular and extracellular, contribute to the susceptibility to SMS.

A second advantage of the SMS could be that neighbouring cells may contribute to restriction of the proliferative capacity of an SMS-producing cell (FIG. 5b). For example, by the production of SMS factors, a senescent cell may alert cells in its vicinity, which in turn could stop secreting growth factors that normally support proliferation of the neighbouring pre-neoplastic cell. By altering mitogenic support from neighbouring cells, senescence could be reinforced. As a variation on this, stromal cells may secrete proliferation-inhibitory (SMS) factors, thereby creating a gradient of cytostatic factors. They potentially interfere with processes that are being executed at the plasma membrane, particularly growth factor signalling. Indeed, one of the remarkable properties of senescent cells is that they fail to respond to growth factors[14]. Could this be caused by direct interference through the SMS? In support of this hypothesis, it has been shown that TGFβ inhibits tyrosine phosphorylation and activation of IRS1, which is an essential mediator of insulin and IGF signalling in mouse 3T3-L1 fibroblasts and rat FRTL-5 thyroid epithelial cells[120,121]. Similarly, whereas IRS1 can be recruited to the IL-6 receptor in response to IL-6 (REF. 122), the latter inhibits phosphorylation of IRS1 in primary murine hepatocytes and hepatocellular carcinoma[123–125]. Moreover, IGFBP3 inhibits IRS function, possibly by recruitment to LRP1 (REF. 104). Thus, SMS factors can block growth factor signalling directly, by inhibition at the receptor level, thereby potentially contributing to the induction of senescence. This would be compatible with the aforementioned model of negative feedback signalling that is required for the induction of senescence that is induced by loss of a tumour suppressor[32]. The activation of negative feedback signalling could collaborate with a block in growth factor signalling at the level of membrane receptors, thereby collaborating to effectively shut down mitogenic signalling. It should be noted that it is at present unclear whether negative feedback signalling equally effectively contributes to growth arrest in the context of OIS, as expression of an activated oncogene is likely to interfere with shutdown of specific growth factor signalling cascades, thereby counteracting the negative feedback loop.

As the SMS contains several inflammatory factors, a third possible advantage of the SMS is the ability to attract other cells, such as immune cells (FIG. 5c). For instance, in a mouse model of liver carcinoma driven by inducible shRNA-mediated depletion of p53 and $HRAS^{V12}$ expression, Scott Lowe's laboratory showed that reactivation of p53 in the tumour results in a robust senescence response[126]. Soon after, the senescent tumour cells are cleared by innate immune cells. In a model of liver fibrosis, activated hepatic stellate cells were shown to be able to undergo senescence *in vivo*[127]. Clearance of these hepatic stellate cells by natural killer cells is required to limit their proliferation and terminate fibrosis. Thus, secreted factors might contribute to the eventual clearance of pre-neoplastic, senescent cells. Consistent with this view, melanocytic naevi (which display several senescence markers[84]) often gradually regress, although this can take several decades.

Misuse of SMS signalling by cancer cells

As discussed above, several observations point to an essential tumour-suppressive role for the SMS in the regulation of OIS. However, a potential drawback of the SMS is that tumour cells may misuse it to support their own proliferation. For example, in addition to its potential tumour-suppressive function in the induction of OIS, IL-6 has a dark side, in that it can contribute to tumour growth (FIG. 5d,e). Indeed, besides its function as an autocrine growth factor in some types of cancer, IL-6 contributes to a plethora of other pro-tumorigenic processes in a paracrine manner[78,79]. For example, IL-6 secretion by $HRAS^{V12}$-driven cancer cells stimulates tumour growth in a cell-non-autonomous manner. Pro-tumorigenic functions have also been described for other SMS factors, including TGFβ[48], IL-1 (REF. 128), IL-8 (REFS 82,129) and GRO1 (REF. 130). These seemingly opposite functions of IL-6 are in fact not mutually exclusive because, as discussed above, the differing outcomes might relate to the identity (cell type and tumour stage) of the target cell, or to the mode of IL-6 signalling (autocrine versus paracrine). Even endocrine signalling could perhaps have a role. This could become relevant in the case of large benign, senescent tumours, or in the case of ageing, in which senescent cells accumulate[131,132]. In this context, it is noteworthy that IL-6 antibody therapy has been proposed to treat chronic inflammatory diseases[133] as well as tumours carrying an activated $HRAS^{V12}$ allele[134]. The finding that IL-6 is required in a cell-autonomous manner for OIS[19] suggests that this therapy would not affect the pool of IL-6 required for the senescence response, and thus would not interfere with the feasibility of that strategy. Only if the paracrine pool of IL-6 also contributes to the senescence response, as described above, could this therapy have adverse effects.

An interesting scenario for how SMS factors produced by cancer cells can contribute to tumour growth comes from a study by Yang *et al.*[130] (FIG. 5d). They used immortalized human ovarian surface epithelial (HOSE) cells, which are transformed by expression of $HRAS^{V12}$ and, as a result, express increased levels of several cytokines, including GRO1. Expression of GRO1 is both required and sufficient for oncogenic transformation of these cells. Besides its potential function in apoptosis resistance, GRO1 also communicates with the tumour stroma. Exogenous GRO1 is able to induce senescence in primary ovarian stromal fibroblasts, and co-injection

Antagonistic pleiotropy
The hypothesis that genes with beneficial effects early in life are favoured by selection even if they have detrimental effects at later ages.

of GRO1-treated or GRO1-expressing fibroblasts causes non-tumorigenic HOSE cells to become tumorigenic. A model was proposed in which stromal fibroblasts are the target cells for the tumorigenic action of the SMS, including GRO1, which is secreted by the tumour cells. In turn, these fibroblasts secrete several factors that contribute to tumour growth[130].

Consistent with this, previous results by Campisi and co-workers illustrated that senescent cells can create a microenvironment that stimulates the proliferation of preneoplastic cells *in vitro* and *in vivo*[8,135] (FIG. 5e). In fact, the secretory profile of senescent cells resembles that of cancer-associated fibroblasts[8]. In keeping with this, stromal cells of hepatocellular carcinoma[136] and ovarian cancer-associated fibroblasts[130] display several markers of senescence. These observations elegantly illustrate the dual nature that is associated with the SMS, which constitutes an example of antagonistic pleiotropy[137,138]. Moreover, as (some of) these factors are important contributors to late-stage cancer progression, it is conceivable that a selective pressure exists preventing irreversible loss-of-function genetic and epigenetic alterations, providing an explanation for why such factors do not seem to be commonly inactivated by mutation or loss of expression in cancer cells.

SMS factors and ageing

The identification of cellular senescence as a cause of long-term cell cycle arrest *in vivo* raises the possibility that it is linked to ageing. Senescent cells are associated with tissue damage as well as malignant transformation, both of which are considered ageing phenotypes. There is increasing evidence that replicative senescence *in vitro* reflects, at least to some extent, the loss of regenerative capacity in the elderly. For instance, the pool of senescent cells increases in ageing renewable tissue[131,132]. Several mechanisms, including telomere shortening, have been proposed to mechanistically explain this[18,132,139,140]. However, somewhat counterintuitively, mice carrying an extra copy of either *Arf* or *Trp53* (both of which are implicated in senescence) display an increased lifespan[141].

Several SMS factors are also linked with ageing. In particular, expression of IL-6 (REF. 79), GRO1 (REF. 142), PAI1 (REF. 17), and TGFβ[143] increases with age, mimicking their behaviour in cellular senescence. Conversely, IGF1 protein levels decrease both in the elderly[144] and in cellular senescence. This raises the possibility that the SMS also contributes to the ageing process in an autocrine, paracrine and perhaps even endocrine fashion, thereby mechanistically linking senescence and ageing. In this regard it is worthwhile to note that IGF signalling has an established role in ageing, and that inhibition of this pathway increases the lifespan in mice and several other organisms[22].

Concluding remarks

Traditionally, cellular senescence has been regarded as a strictly intracellular response, with the entire signalling circuitry taking place within the boundaries of the cell membrane. Recent findings have taught us that this might be only part of the picture. Several SMS factors have now been demonstrated to be associated with, and contribute to, this proliferative arrest, which conceivably operates hand in hand with death programmes such as autophagy and apoptosis in preventing tumour progression. The SMS allows for signal modulation by communication, thereby implementing and fine-tuning the senescence response. In this way, it can warn the microenvironment of the cell(s) under stress, a phenomenon that may well allow for two-way communication, affecting the senescent cell(s) in turn. Such intercellular interactions may explain why secreted factors have evolved to contribute to the senescence programme. The signal transduction cascades elicited by the SMS are likely to interact to allow for an integration of several proliferation-regulating and stress signals. This might be initiated at an unanticipated level, namely by, and in the vicinity of, cell membrane receptors. However, despite increasing *in vitro* evidence, the role of many of these interactions in cellular senescence awaits *in vivo* confirmation. Elucidating their contribution and mechanism of actions is likely to increase our understanding of the fundamental processes meditating cellular senescence. Furthermore, the SMS is likely to contain new senescence biomarkers, which are urgently needed to improve the characterization and our understanding of the contribution to, and mechanism of, senescence in tumour suppression.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
IGF2R
UniProtKB: http://www.uniprot.org
β-catenin | β-galactosidase | ARF | ASF1A | BRAF | C/EBPβ | CXCR2 | ERK2 | HIRA | HRAS | IFI16 | IGF1R | IGFBP3 | IGFBP4 | IGFBP5 | IGFBP7 | IL-1α | IL-6 | IL-6R | IL-8 | INK4A | INK4B | IRF1 | IRF5 | IRF7 | IRS1 | IRS2 | LRP1 | NF1 | p27 | p53 | PAI1 | RB | SMAD2 | SMAD3 | SMAD4 | SUV39H1 | TβRI | TβRII | TGFβ1 | TGFβ2 | tPA | uPA | uPAR | WNT2 | WNT3A

FURTHER INFORMATION
D. S. Peeper's homepage: http://research.nki.nl/peeperlab/index.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF