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The intricate nature of senescence in development and cell plasticity

Hilah Gal*, Julia Majewska* and Valery Krizhanovsky#

Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 7610001, Israel

* These authors contributed equally

Correspondence:
Valery Krizhanovsky
Department of Molecular Cell Biology,
Weizmann Institute of Science,
Rehovot 7610001,
Israel
Tel: + 972-8-934-6575
Fax: +972-8-934-4125
E-mail: valery.krizhanovsky@weizmann.ac.il

Abstract

Cellular senescence, a stable form of cell cycle arrest, accompanied by pronounced secretory activity, has functional roles in both physiological and pathological conditions. Although senescence has been linked for a long time with cancer and ageing, recent studies have revealed a functional role of senescence in development, regeneration and reprogramming. Notably, the transient presence of senescent cells may be beneficial, in contrast to the potential deleterious effects of persistent senescence in aged or chronically damaged tissues. We will discuss how senescence contributes to embryonic development, cell plasticity and tissue regeneration, as a highly coordinated and programmed cellular state.

Introduction

Cellular senescence is a stable state of cell cycle arrest. Various triggers can induce cellular senescence including DNA replication stress, telomere dysfunction, oncogene activation, oxidative stress, DNA damage, genomic instability and cell-cell fusion. These stress stimuli can lead to activation of multiple molecular pathways of senescence. Most of these pathways engage p53, and essentially all of them converge in activation of cyclin-dependent kinase (CDK) inhibitors such as, p15 (encoded by CDKN2B), p16 and p19 (encoded by CDKN2A-INK4a/ARF locus), p21 (encoded by CDKN1A), and p27 (encoded by CDKN1B) and tumor suppressor retinoblastoma (RB) protein. Inhibition of cyclin – CDK complexes by CDK inhibitors leads to a hypo-phosphorylated form of RB and results in proliferative arrest.
Senescent cells are heterogenous and exhibit a complex phenotype. Thus, they are identified by a number of senescence-related markers. The most commonly used marker, senescence-associated β-galactosidase (SA-β-gal), is based on increased lysosomal β-galactosidase activity, a typical feature of senescent cells [1-3]. However, SA-β-gal activity on its own is not an absolute marker of cellular senescence, which is rather defined by a collection of molecular identifiers, representing different characteristics of senescent cells. These include markers of cell cycle arrest machinery (e.g. p16, p53, p21), apoptosis resistance (e.g. DCR2, Bcl-xL), secretory factors (e.g. IL-6, IL-8), activation of DNA damage response (DDR) (e.g. γH2AX, p53BP1) and activation of immune surveillance genes [4-6]. Therefore, combination of senescence-related molecular markers, together with SA-β-gal activity, indicates the presence of senescent cells.
The complex nature of cellular senescence is reflected by its engagement in a number of physiological or pathological processes, with senescent cells playing either a positive or a negative role. On one hand, induction of senescence is beneficial as it limits proliferation of damaged cells and tumorigenesis, promotes wound healing and plays a role in developmental processes [7-16]. On the other hand, the long-term presence of senescent cells negatively affects restoration of tissue homeostasis and promotes pathological conditions such as tumorigenesis or tissue ageing [4, 14, 17-19]. This deleterious effect is largely mediated by the secretory profile, also known as the Senescence Associated Secretory Phenotype (SASP). It consists of pro-inflammatory cytokines, chemokines, growth factors and proteases, which promote a local inflammatory microenvironment. Activation of SASP is an essential feature of senescence cells. It can reinforce cell growth arrest of neighboring cells and modulate their microenvironment. The regulation of SASP is governed by the DNA damage response and the NF-kB, p38 and JAK-STAT signaling pathways in a coordinate manner [20-24]. It is now recognized that the secretory milieu of

senescent cells plays a critical role in tissue remodeling and regeneration. Therefore, the impact of senescence on a tissue might depend on the transient presence of senescent cells or their accumulation over time. Interestingly, recent study has shown beneficial role of long-lasting senescent vascular endothelial cells in liver sinusoids [25]. Elimination of these cells caused disruption of blood-tissue barrier with subsequent liver fibrosis. This opens new perspective on role of long-lived senescent cells in different organs.

The detrimental effects of accumulating senescent cells in ageing and age-associated diseases is well-established and have been extensively reviewed elsewhere [4, 19, 26, 27]. However, the role of cellular senescence as a trigger of tissue remodeling during development and upon tissue damage is becoming increasingly appreciated and will be the focus of this review. We will review the role of senescence in the developing embryo, followed by cell-fusion-induced senescence of syncytiotrophoblast, as a part of a physiological function of the placenta during pregnancy. We will then discuss the role of senescence in tissue repair and regeneration and the crosstalk between senescence and reprogramming, pointing to its potentially deleterious effect in tumor development.

Senescence in development

Developmental senescence in the embryo

Senescence plays an active role in embryonic growth and patterning [7-10]. Senescent cells are present at different stages of embryonic development, in several transitory fetal structures, including the regressing mesonephros, the endolymphatic sac of the inner ear, the apical ectodermal ridge (AER) during limb formation and several other tissues [9, 10]. As senescent cells are removed by macrophage-mediated clearance, they contribute to morphogenesis by elimination of transient structures. Activation of developmental pathways of senescence seems to differ from the senescence signaling in the adult. Embryonic senescence does not depend on the activation of the main regulators of senescence, p53 and p16, nor the activation of the DDR. Senescence in the developing embryo is mainly mediated by p21 and regulated by the TGFβ/SMAD and FOXO/PI3K signaling pathways. Remarkably, embryonic senescent cells share expression signatures with oncogene-induced senescence, especially those related to SASP [10]. Therefore, senescent cells in the embryos use SASP components to regulate temporal and spatial patterning. Interestingly, while senescence in the adult is mainly induced by stress, it is suggested that embryonic senescence is triggered by developmental cues and is aimed at tissue remodeling and organ patterning of the developing embryo [9, 10]. Therefore embryonic senescence can be

regarded as a tightly programmed instructive mechanism during mammalian embryonic development [9]. Owing that this type of senescence is not driven by DNA damage of p16/Rb pathway, future research is necessary in order to understand the differences between this and other types of senescence.

Developmental senescence in placenta

During fetus development, cell fusion at the maternal/fetal interface of the placenta results in senescent syncytiotrophoblast cells, which support fetal growth and development [7, 8]. The placenta is a transient organ, intended at fetal nourishment during pregnancy. The transfer of nutrients, respiratory gases and hormones occurs within the large multinuclear syncytiophoblast layer [28-30]. Formation of syncytiotrophoblasts is induced by a fusogen of viral origin ERVWE1, which mediates cell-cell fusion, activating the senescence response [7, 31]. Unlike the signature of programmed developmental senescence in the embryo, senescence in the placenta shares features of senescence induced by DNA damage stress stimuli and exhibits a coordinated activation of p53/p21 and p16/pRb regulatory pathways and DDR [7]. This suggests that cellular senescence during placental development, might share the same evolutionary origin with damage-induced senescence in the adults, caused by fusogens and viral infections [7, 28, 32]. Interestingly, since cellular senescence in embryonic and placental development is transient due to the immune surveillance of senescent cells [9, 10] and the transitory existence of the placenta respectively, the deleterious effects of long-term senescence are avoided.

The functional role of senescent syncytiotrophoblast can be studied noninvasively in murine models by the dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) system [33, 34], which enables assessment of the maternal circulation in vasculature of murine placenta. Mice with attenuation of the senescence program showed placental abnormalities, as observed by altered intensity of signal dynamics in the maternal circulation of placenta, accompanied by histopathological changes in placental structures. In particular, alterations were observed in *p53*$^{-/-}$, *Cdkn2a*$^{-/-}$ and *Cdkn2a*$^{-/-}$; *p53*$^{-/-}$ mice [8]. Strikingly, senescent pathways were found to be downregulated in syncytiotrophoblast of human placentas from complicated pregnancies with intrauterine growth restriction (IUGR) pathology. Therefore, cell-fusion-induced senescence of syncytiotrophoblast is an essential mechanism mediating the development and proper function of the placenta during pregnancy.

Why is senescence in the placenta essential for maintaining placenta integrity? There are multiple senescence characteristics that are necessary for placental syncytiotrophoblast formation and function [7, 8]. For instance, senescent cells are known to be resistant to

apoptosis due to the high expression levels of the anti-apoptotic proteins of the BCL-2 family [35, 36]. This mechanism of resistance can maintain senescent syncytiotrophoblast viability during pregnancy. Additionally, the flat and enlarged morphology, distinctive of senescent cells in culture and *in vivo* [3, 37], can aid in facilitating the transfer of nutrients and gases at the feto-maternal interface. The secretion of SASP components and the interaction of senescent syncytiotrophoblast with the immune system may also support feto-maternal homeostasis [26]. This can be exemplified by the CCL5 chemokine, a SASP component known to attract decidual NK cells to the vicinity of the maternal-fetal interface, supporting key placental processes of trophoblast invasion and vascular remodeling [38].

Therefore, fusion-induced senescence of syncytiotrophoblast can maintain placental integrity in a cell autonomous manner, by maintaining the non-proliferative state and supporting the viability of syncytiotrophoblast, and in a cell non-autonomous manner, by the secretion of SASP factors that are essential for placental processes, including placenta implantation, vascular remodeling and immune attraction (Figure 1).

Senescence in regeneration

Cellular senescence plays an active role in tissue repair and regeneration in a wide range of tissues, including the skin [11, 13], heart [12, 16], liver [39], skeletal muscles [40] and lungs [41, 42]. The direct contribution of cellular senescence to wound healing, as an example of tissue remodeling *in vivo,* was demonstrated for the skin wound healing process in a p16-3MR transgenic mice, which enables the selective removal of $p16^{Ink4a}$ -positive senescent cells by drug ganciclovir [11]. Wound healing was assisted by a transient burst of senescent cells, which were cleared with resolution of injury. Interestingly, premature directed elimination of senescent cells by targeting senescent cells for apoptosis, significantly delayed the rate of wound healing and caused an accumulation of excessive fibrotic scar [11]. Molecular mechanisms inducing beneficial senescence in the skin wound healing process, depend on the expression of the matricellular protein CCN1, which activates the senescence program in fibroblasts and endothelial cells, including DNA damage response, p53 and p16 [13].

Why induction of senescence program is beneficial in wound healing process? Wound repair consists of following phases: inflammation, extracellular matrix (ECM) production, tissue formation and remodeling. Activated myofibroblasts proliferate and assist in wound repair by synthesis of ECM components, which may lead to fibrosis when healing process is unregulated and activity of myofibroblasts continues unchecked. Upon damage-induced model

of fibrosis in skin and liver, senescence of ECM-producing myofibroblasts blocks their proliferation, and in addition converts them into ECM-degrading cells, exerting anti-fibrotic effect [13, 39]. In liver fibrosis model, it was shown that senescent myofibroblasts are subsequently cleared by natural killer cells accelerating resolution of fibrogenesis and wound healing process [39]. In addition, SASP consisting of inflammatory cytokines, might promote immune surveillance of senescent cells along resolution of injury [39, 43]. Therefore, in skin and liver injury-induced fibrosis, senescence of myofibroblasts is a part of normal tissue repair process limiting the extend of fibrogenesis, promoting resolution of fibrosis and wound healing.

In addition, the SASP component and growth factor PDGF-AA, is of critical importance for wound closure. Topical administration of recombinant PDGF-AA to the wounds rescued compromised wound healing potential, in the absence of senescent cells [11]. Interestingly, heart regeneration is also promoted by CCN1 and agrin, another extracellular matrix protein involved in senescence induction during cardiac remodelling [16].

Lung injury is an additional example of senescence-mediated regeneration. Acute lung injury induces cuboidal alveolar cell type 2 (AT2) to undergo differentiation into thin alveolar cell type 1 (AT1) with transitional state in route to terminal differentiation [41, 42]. Due to a dramatic change in shape, structure and mechanical properties, these transitional progenitor cells undergo extensive stretching, inducing a p53-dependent DNA damage response and senescence. Importantly, the presence of senescent progenitor cells in normal lung repair is transient, but found to be persistent in patients with Idiopathic Pulmonary Fibrosis (IPF) [41]. This suggests that senescent progenitor cells can accompany the regeneration program, but also lead to pathological conditions when persist in the tissues.

Senescence is central to regeneration of complex structures such as fins or limbs in species with high regenerative potential, such as zebrafish [44] or salamanders [45]. Interestingly, in salamanders, even repeated limb amputation induces a senescence response, followed by tissue repair and regeneration, without senescent cell accumulation, due to their clearance by macrophages. The precise interplay between senescence levels and immune surveillance guarantees restructuring of damaged tissue, as depletion of macrophages in this context causes persistent senescence and impaired regeneration. Therefore, the beneficial effect of senescent cells is not limited to mammals and the transient presence of senescent cells is essential for proper tissue regeneration in multiple species.

Interplay between senescence and reprogramming

In 2006 Takahashi and Yamanaka illustrated that epigenetic memory of a somatic cell can be erased and its fate can be manipulated in the process of cellular reprogramming. Ectopic co-expression of four transcription factors; octamer-binding protein 3/4 (Oct 3/4), Sox2, Krüppel-like factor 4 (Klf4) and c-Myc, all collectively known as 'OSKM' or 'Yamanaka' factors, can reprogram somatic cells to generate induced pluripotent stem cells (iPSCs) [46]. However, the efficiency of OSKM-mediated reprogramming is low and stochastic, suggesting the existence of barriers limiting this process. Mechanistically, expression of reprogramming factors triggers a DNA damage response and cellular senescence by inducing the p53/p21 pathway, which negatively affects the transition of somatic cells into pluripotent state [47-50]. Also, senescence-associated cell cycle dependent inhibitors, namely p16, p19 and p15, serve as a barrier in iPSCs generation. Importantly, ablation of senescence effectors, by genetic inhibition of the *Ink4a/Arf* locus profoundly promoted the efficiency of iPSCs [47, 51].

The generation of mouse and human iPSCs under controlled *in vitro* conditions [46, 52-55], allowed to study plasticity of somatic cells at the cellular level, with limited ability to understand the role of the microenvironment and senescence in the process. The studies in reprogrammable mouse models were able to reveal this role and understand the effect of niches on cellular plasticity within tissues [56, 57]. Expression of four factors Oct4, Sox2, Klf4 and c-Myc in mice ('i4F mice') caused dedifferentiation and pluripotency in a variety of cell types and tissues, implying that full reprogramming can be successfully achieved *in vivo*. Interestingly, these *in vivo* generated iPSCs were highly plastic and transcriptionally similar to embryonic stem cells, apart from teratoma formation, which was reflected by embryo-like structures in i4F mice [56, 57]. Intriguingly, in i4F mice, clusters of senescent cells were found in close proximity to cells expressing the pluripotency marker Nanog [58]. Taking into account the role of senescence in limiting efficiency of iPSCs generation *in vitro*, it was tempting to verify the effect of genetic ablation of the *Ink4a/Arf* locus on cellular plasticity *in vivo*. Surprisingly, *i4F;Ink4a/Arf*-null mice with minimal level of senescent cells in tissues, were highly resistant to *in vivo* reprogramming by induction of OSKM factors. Consistently, pharmacological elimination of senescent cells by Bcl-2 family inhibitors decreased the number of Nanog positive cells, indicative of *in situ* reprogramming. The role of senescence in promoting reprograming was also shown in models of tissue-injury, in aged and progeria mice [40, 58]. Therefore, cellular plasticity can be enhanced by the presence of senescent cells in the microenvironment (Figure 2).

How is it possible to explain a suppressive role of senescence for *in vitro* reprogramming and its promoting role in reprogrammable mice? Complex genetic and epigenetic reprogramming during generation of iPSCs poses risks to genomic integrity, triggering a DNA damage response and senescence effectors [47-49]. The decision whether the cell undergoes reprogramming or senescence might depend on the extent of genomic aberrations, but also on the tissue microenvironment in the context of *in vivo* reprogramming. Recent findings indicate that expression of OSKM factors triggers cellular plasticity and damage induced senescence. These senescent cells promote reprogramming via a paracrine effect of SASP, in particular the secretion of IL-6 by senescent cells [40, 58].

Cellular reprogramming has gained tremendous attention for its potential in regenerative medicine and aging research. *In vitro* reprogramming of cells from centenarians or patients with Hutchinson-Gilford progeria syndrome (HGPS), characterized by premature ageing, could erase several hallmarks of senescence and ageing, including oxidative stress, shortening of telomeres and epigenetic modifications [59]. Importantly, partial reprogramming *in vivo* by short term cyclic expression of OSKM factors extends lifespan in progeroid mice and improves tissue homeostasis in aged mice, without causing detectable increase in tumor development [60]. This strategy proved to be beneficial in increasing resistance to metabolic disorders, and enhancing the regenerative capacity of skeletal muscles after injury.

Senescence associated stemness in cancer development

Even though senescence and stemness are *per se* incompatible cellular states, both of them share overlapping signaling networks [50, 61]. Key components of senescence machinery, such as p16, p21, p53 and tri-methylation of lysine 9 at histone H3 (H3K9me3), play crucial role in maintenance of stem-cells by preventing their exhaustion [61]. This functional link between senescence and stemness can be seen in chemotherapy- as well as oncogene- induced senescence, which activates similar cellular signaling pathways as observed in gene signature pattern in stem cells [62, 63]. Surprisingly however, cells released from senescence by targeting senescence regulators H3K9me3 or p53 in genetically switchable model, re-entered cell cycle with strongly enhanced clonogenic growth and tumor-initiating potential compared to control tumor cells, which never experienced senescence [63] (Figure 2). This suggests that senescent cells released from cell-cycle arrest, can show cell-autonomous feature of senescence-associated-stemness, with highly aggressive growth potential and deleterious effect in tumor relapse. It may also imply that senescence is not static end point of cell-cycle but rather dynamic

state, receiving continuous signals preventing senescent cells from re-entering proliferation. Moreover, the interplay between senescence and stemness controlling signaling might be hijacked by cancer cells to promote enhanced self-renewal and tumor initiation. However, the phenomenon of spontaneous escape from senescence needs to be verified in *in vivo* settings, and further investigated.

Apart from contribution of SASP to cellular reprogramming and tissue regeneration responses, it has been shown that secretome of senescent cells can induce cancer stem cell plasticity and tumor formation in different settings [58, 64-67] (Figure 2). Interestingly, Ras-induced senescent keratinocytes show *de novo* stem cell signature in SASP-dependent manner, but with progressive decline in proliferation [15]. It opens the question about the functional role of stem cell marker expression within senescent cells. Functionally, keratinocytes transiently exposed to conditioned media of senescent cells exhibited increased stem cell properties and regenerative capacity *in vivo* demonstrating that SASP can induce cellular plasticity and stemness, promoting tissue regeneration [15]. Thus, senescent cells regulating tissue regeneration might also contribute to permissive microenvironment for tumor formation through cellular de-differentiation and expansion of stem cells, which might acquire mutations making them susceptible to cancerogenic transformation (Figure 2).

Beneficial role of senescence in cellular reprogramming and tissue regeneration might pose risk of malignant transformation. Since senescent cells are highly heterogeneous in gene expression profile [68], the contribution of senescence-associated-stemness to tumor development might be different, making it hard to predict. So far, *in vivo* models of senescence-mediated regeneration and reprogramming proved transient senescence to be beneficial while chronic senescence deleterious [15, 16, 39-42, 60, 69]. Therefore, short-term senescence-mediated regeneration followed by senescent cells clearance with senolytic might be an attractive strategy. However, signaling pathways controlling senescence and stemness cellular states in respect to tumorigenic transformation need to be thoroughly investigated.

Conclusion

Senescence plays a beneficial role in a range of physiological processes, including tissue patterning during embryogenesis, organ development, tissue repair and regeneration, and cellular reprogramming. This involvement of cellular senescence in diverse physiological processes helps to comprehend it as a highly coordinated and programmed cellular state. In this

regard, senescence might be an innate mechanism supporting normal tissue-maintenance programs and enhancing robust regeneration and repair responses in damaged tissues, which can potentially lead to tumor development when uncontrolled. Further investigations of senescence-induced cellular plasticity might have significant implications not only for regeneration, but also for a wide range of other biological phenomena.

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Figure legends:

Figure 1. A proposed model of fusion-induced senescence of placental syncytiotrophoblast. Fusion-induced senescence of syncytiotrophoblast maintains normal placenta function in both a cell-autonomous and non-autonomous manner. In a cell autonomous manner by maintaining growth arrest of syncytiotrophoblast cells and in a cell non-autonomous manner, by the activation of central regulatory pathways of senescence and by the secretion of various SASP components, imperative for placental processes, including placenta implantation, vascular remodeling and immune cell attraction.

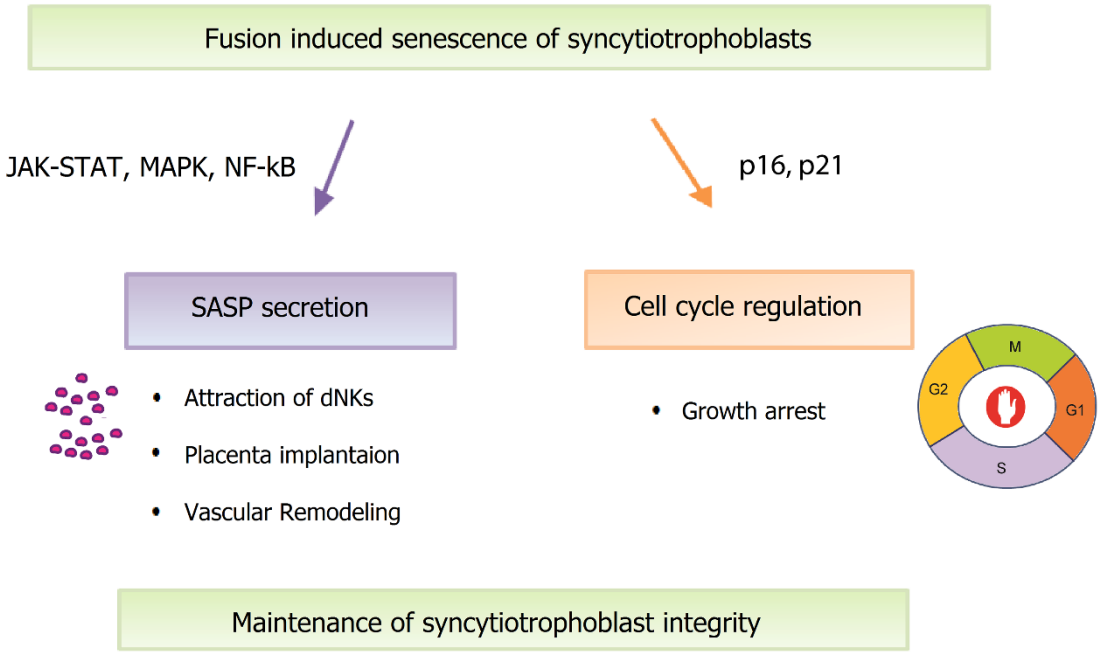

Figure 2. A proposed scheme of interplay between senescence-associated regeneration and tumor development.

DNA damage, oncogene expression and chemotherapy can induce senescence program in normal and cancer cell. Senescent cells release factors within senescence-associated secretory phenotype (SASP) that promote de-differentiation and plasticity of neighboring cells, that assists in senescence-assisted regenerative response. Some of pluripotent cells can acquire mutation making them susceptible to cancerogenic transformation. Secretome of senescent cells can also enhance tumor permissive environment resulting in tumor initiating cells. In addition, switching off senescence regulators can potentially release senescent cells from proliferative block. These cells, which re-enter cell cycle present stemness signature and high proliferative capacity, which may have profound implication in tumor initiation and its aggressiveness.

DIS – Damage Induced Senescence, OIS – Oncogene Induced Senescence, TIS – Therapy Induced Senescence, SASP – Senescence Associated Secretory Phenotype