Cell

A New *Development* in Senescence

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Cellular senescence is implicated in several pathological responses in the adult, with important repercussions in tumor suppression, wound healing, and aging. Two studies by Muñoz-Espín et al. and Storer et al. now reveal that senescence contributes to embryonic development, suggesting a primordial role in normal physiology.

Seminal studies by Hayflick and Moorhead demonstrated that normal cells can only divide a finite number of times before they reach a state of replicative cellular senescence. Although we now believe that senescence plays wider roles in various stress responses in the adult, in this issue, Muñoz-Espín et al. (2013) and Storer et al. (2013) report the surprising result that senescence occurs under physiological conditions during mammalian embryonic development.

Cells undergoing replicative senescence downregulate cell-cycle genes and certain extracellular matrix components while upregulating genes encoding cell-cycle inhibitors, matrix degrading enzymes, particular cytokines, and immunosurveillance factors. Cellular stresses such as telomere uncapping or activation of oncogenes can trigger stable cell-cycle arrest programs with similar features, though whether different "types" of senescence exist has been debated (Shay and Roninson, 2004). The most widely used senescence marker is senescence-associated β-galactosidase activity (SAβG), which likely reflects the increased autophagy occurring in senescent cells (Young et al., 2009). Other canonical senescence markers include p53, p21, p16, and reduced RB phosphorylation, which collectively mediate the ancillary phenotypic manifestations of senescence-associated cell-cycle arrest. Affected cells often accumulate heterochromatic foci that may stabilize the senescent state, and they display altered secretory profiles that modulate immune function and/or reinforce cell-cycle arrest (Kuilman et al., 2010). A conceptual problem is that none of these markers are unique to senescent cells, and no single marker is sufficient to "diagnose" the senescent state. As a consequence, senescence has been defined by a collection of markers that are not decisive.

Senescence has been largely viewed as a stress response program. Still, hints that senescence can play some physiologic role came from studies implicating senescence in limiting certain wound-healing responses (Jun and Lau, 2010; Krizhanovsky et al., 2008). Although SAβG activity has been reported in the regressing mesonephros of birds (Nacher et al., 2006), its relevance, if any, in mammalian embryos remained unknown. The new reports imply that senescence occurs throughout mouse development. Muñoz-Espín et al. focused on the inner ear and the regressing mesonepheric tubules, whereas Storer et al. concentrated on the apical ectodermal ridge (AER) during limb formation. Both studies imply that "developmental senescence" shares some, but not all, regulatory pathways observed in the adult (Figure 1).

Both senescent states share SAβG activity and senescence-associated heterochromatin markers (HP1γ and H3K9me3), and both show reduced Ki67 staining (a proliferation marker) owing to a G1 arrest. However, developmental senescence does not appear to involve the activation of p16 or $p19^{ARF}$ and is not triggered by p53 or DNA damage. Instead, developmental senescence is mediated by p21 in a p53-independent manner but controlled instead by the TGFβ/SMAD- and PI3K/FOXO-signaling pathways. Although senescent cells in the embryo and adult each secrete factors that engage the immune system to eliminate cells and remodel tissues, the secreted cytokines and growth factors are not all the same (Figure 1).

At issue is whether these phenomena indeed represent different types of senescence or, instead, reflect fundamentally different processes. Consistent with the above observations, *p53* or *Ink4a/Arf* knockout mice do not present alterations in patterns of SAβG activity during development and do not manifest abnormalities in tissues in which senescence was observed. However, p21 null embryos revealed fewer SAβG-positive cells compared to controls and exhibited detectable developmental abnormalities in the associated tissues. Yet many of these embryonic defects are corrected in neonates. There are at least two plausible reasons why the phenotype of p21 null mice might not provide a readout of the program's potential importance. First, it is possible that p21 deletion is not sufficient to override senescence or may only delay its induction. Second, the embryo may compensate for p21 loss by engaging alternative tissue-remodeling programs.

The possibility that compensatory mechanisms may mask key roles of certain programs in development is not without precedent. Compelling evidence exists for the importance of apoptosis in embryonic development; yet, disruption of the intrinsic apoptotic program in the embryo produces only modest phenotypes. As one example, apoptosis is considered a major cell death mechanism in the developing limbs, but inactivation of proapoptotic genes in the mouse only partially prevents the removal of the interdigital tissues (Fuchs and Steller, 2011). Apparently a compensatory program exists to instruct morphogenesis

and tissue remodeling when apoptosis fails. Conversely, failure of senescence in p21 null mesonephric tubules is followed by delayed activation of apoptosis and by macrophage-mediated clearance of dying cells. It will be interesting to determine whether senescence can compensate for apoptosis deficiency during development.

So, what are the potential roles for senescence in embryonic development? That senescence and macrophage infiltration precede mesonephros involution suggests that one role of senescence is to remodel the embryonic kidney. Senescence may also have an instructive function. Indeed, Storer et al. find that the expression signature in the AER partially overlaps with that of oncogene-induced senescence, suggesting that secreted components from senescent cells influence pattern formation and proliferation of the adjacent mesenchyme. Finally, by halting the proliferation of specific cells within developing tissues, senescence may dictate the balanced outgrowth of and interplay between distinct cell populations. An example of this phenomenon may occur in the endolymphatic sac, which is not eliminated during development but instead undergoes a process of differential cellular proliferative arrest that changes the relative abundance of distinct cell populations.

Perhaps the most important ramification of the new work relates to its implications for the evolutionary origin of the senescence program. Most research to date has focused on senescence as a tumor-suppressive process, and it has been debated as to how evolution selects for programs that prevent a disorder that typically occurs after reproductive age (Campisi, 2003). The new work raises the possibility that senescence in the adult evolved from a primordial tissue-remodeling program that takes place in the embryo. In both settings, cells arrest in the cell cycle, partially share a common set of functional markers, have an active role in modifying the tissue microenvironment, and are ultimately recognized and cleared by the immune system (Figure 1). These features may have been adapted as part of an emergent adult stress response program that incorporated additional tumor suppressor mechanisms, such as those reliant on p53 and p16, to eliminate damaged cells and that may, in turn, contribute to organismal aging.

These studies represent another landmark in the senescence field but also raise a new range of pertinent questions. What are the developmental cues that trigger senescence in the embryo, and to what extent does this process reflect the stress-induced senescence program studied so far? What are the salient features that define a cellular senescent state? Perhaps, as our understanding of cellular senescence progresses, the hallmarks and implications of this process will broaden and evolve.

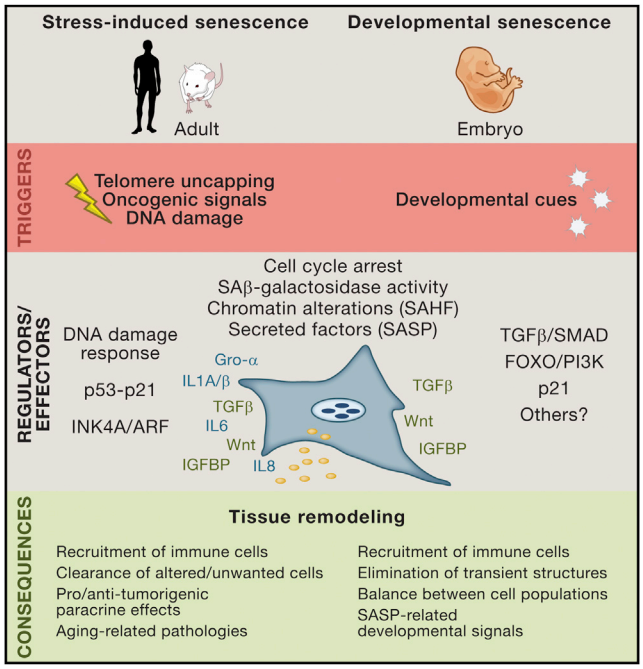

Figure 1. Main Features of Senescence in the Adult and in the Embryo

Whereas the first is induced by stress, such as telomere uncapping, oncogenic signals, or DNA damage, the second is induced by still undetermined developmental cues. Both programs share a set of features such as senescence-associated β-galactosidase activity (SAβG), senescence-associated heterochromatic foci (SAHF), and some of the members of the senescence-associated secretory phenotype (SASP) such as TGFβ, Wnt, and IGFBP family ligands. Nevertheless, differences exist. Developmental senescence does not depend on the activation of DNA damage response, p53-21, or p16 tumor suppressor pathways and does not present some of the SASP-related factors such as IL8 (Cxcl1, 2, and 5 homologs in mice) and IL6. Although additional regulators may exist, senescence in the embryo is mainly mediated by p21 and regulated by the TGFβ/SMAD- and FOXO/PI3K-signaling pathways. Tissue remodeling is a main consequence of both programs. By recruiting the immune system, senescence mediates the elimination of unwanted/transient cells or structures. Developmental senescence may additionally dictate the balance between cell populations or instruct developmental processes.

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REFERENCES

Campisi, J. (2003). Nat. Rev. Cancer *3*, 339–349.

Fuchs, Y., and Steller, H. (2011). Cell *147*, 742–758.

Jun, J.I., and Lau, L.F. (2010). Nat. Cell Biol. *12*, 676–685.

Krizhanovsky, V., Yon, M., Dickins, R.A., Hearn, S., Simon, J., Miething, C., Yee, H., Zender, L., and Lowe, S.W. (2008). Cell *134*, 657–667.

Kuilman, T., Michaloglou, C., Mooi, W.J., and Peeper, D.S. (2010). Genes Dev. *24*, 2463–2479.

Muñoz-Espín, D., Cañamero, M., Maraver, A., Gómez-López, G., Contreras, J., Murillo-Cuesta, Rodríguez-Baeza, A., Varela-Nieto, I., Rubert, J., Collado, M., et al. (2013). Cell *155*, this issue, 1104–1118.

Nacher, V., Carretero, A., Navarro, M., Armengol, C., Llombart, C., Rodríguez, A., Herrero-Fresneda, I., Ayuso, E., and Ruberte, J. (2006). J. Vasc. Res. *43*, 581–586.

Shay, J.W., and Roninson, I.B. (2004). Oncogene *23*, 2919–2933.

Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M.C., Di Giacomo, V., Yosef, R., Pilpel, N., Krizhanovsky, V., Sharpe, J., et al. (2013). Cell *155*, this issue, 1119–1130.

Young, A.R., Narita, M., Ferreira, M., Kirschner, K., Sadaie, M., Darot, J.F., Tavaré, S., Arakawa, S., Shimizu, S., Watt, F.M., and Narita, M. (2009). Genes Dev. *23*, 798–803.