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Developing senescence to remodel the embryo

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Cellular senescence is an irreversible form of cell cycle arrest that has been linked to several pathological conditions. In particular, senescence can function as a tumor suppressor mechanism, but is also thought to contribute to organismal aging. Paradoxically however, through the secretion of various factors, collectively termed the senescence-associated secretory phenotype (SASP), senescent cells can also have tumor-promoting and tissue-remodeling functions. In addition, senescent cells can play beneficial roles in tissue repair and wound healing, and reconciling these contradictory features from an evolutionary standpoint has been challenging. Moreover, senescent cells had not previously been documented in non-pathological conditions. Recently however, 2 studies have identified cellular senescence as a programmed mechanism that contributes to tissue patterning and remodeling during normal embryonic development. These findings have significant implications for our understanding of cellular senescence and help to clarify the paradoxes and the evolutionary origin of this process.

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Nearly half a century ago, studies by Leonard Hayflick and Paul Moorhead[1] demonstrated that normal cells can only divide a limited number of times before undergoing exhaustion at the end of their lifespan, a process called replicative senescence. Subsequently however it was demonstrated that cellular senescence could be induced prematurely by a wide variety of stimuli including oncogenic signaling, DNA-damage, oxidative stress, and chemotherapeutic drugs.[2-5] Irrespective of the stimulus, senescent cells share a number of hallmark properties. Primarily, they are non-proliferative and irreversibly arrested from the cell cycle. Driving this arrest, is the activation of potent tumor suppressor pathways mediated predominantly by the p53/p21 and p16/Rb axes.[6] These also serve as markers of the senescent state, in combination with a lack of proliferation and positive staining for senescence-associated β-galactosidase (SAβ-gal) activity. In addition however, senescent cells remain metabolically active, and it is in this way that senescent cells are able to influence their tissue microenvironment through the secretion of a myriad of proteins, collectively termed the senescence-associated secretory phenotype (SASP).[7] Indeed it is the SASP that is emerging as a key mediator of many of the biological outcomes of senescence, including the reinforced arrest and the recruitment of immune-cells to promote clearance of senescent cells.[2,8,9] However, the SASP also contributes to additional or even contradictory roles of cellular senescence, including limiting fibrosis in wounds, inducing epithelial-mesenchymal transformation (EMT) or even promoting tumor formation.[10-14] Surprisingly though, until recently, senescence had not been described in non-pathologic situations, a factor that has contributed to the controversy surrounding the process. However, recently we,[15] as well as the group of Manuel Serrano,[16] found that senescence is widespread throughout the later stages of embryonic development.

Our study demonstrated the presence of senescent cells in both mouse and chick embryos, during specific time-windows and in particular tissues. We focused on the characterization of senescence in 2 major signaling centers in the embryo, the apical ectodermal ridge (AER) of the limb and in the closing neural tube of the hindbrain, while the other study described senescence in the developing mesonephros and the endolymphatic sac of the inner ear. These cells were identified by a

combination of hallmark features including the presence of SAβ-gal activity, and an absence of BrdU incorporation. Further analysis revealed that these cells displayed enhanced expression of the cell cycle inhibitor and senescence mediator p21, and in the AER, a significant overlap with SASP proteins, including TGFβ1 and CSF1. However, in the study of Munoz et al.[16] profiling of the senescent developing mesonephros also identified similar senescence-associated genes, but found no significant overlap with the SASP of adult senescence, suggesting that within the embryo, senescence may play different roles in signaling centers compared with non-instructive tissues. Interestingly however, in both studies, developmentally senescent cells did not express other classical senescence markers found in postnatal tissues such as p16, p19, p53 or DNA damage markers. Therefore, embryonic senescence shares only some, but not all features of the senescence response observed in adult tissues. One interpretation of this is that senescence developed initially in the embryo as a basic developmental process, and a more primitive form of senescence that was subsequently adapted for its function in tumor-suppression and aging. Additionally, and not in disagreement with this, is the possibility that there are different categories of senescence, that differ in their functional role, timing, and mode of onset (**Fig. 1**). Perhaps developmental senescence represents an original remodeling and instructive process, whereas aging associated senescence is the adapted process for end-of-life arrest and protection. While oncogene-induced senescence (OIS) may represent an opportunistic amalgam of both – tumor suppressive in cells harboring mutations and damage, but instructive and detrimental if activated to excess or not removed. Indeed, if senescence in adult states is induced in response to stress and damage, factors not seen in embryonic senescence, this furthers supports that developmental senescence is a genuine programmed mechanism. More detailed comparisons of senescence in different cell types and conditions will be informative in classifying new and common markers of senescence.

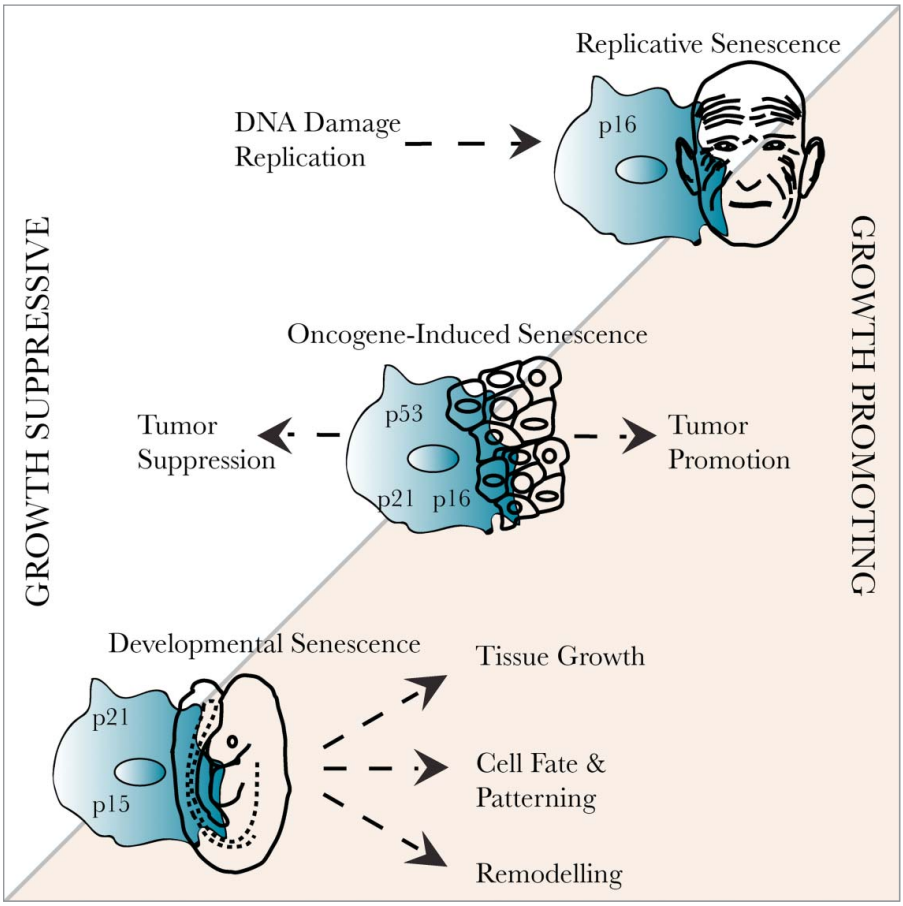

Figure 1. Schematic outline of different types of senescence, and their effect on tissue growth. Replicative senescence and tissue aging are mediated largely by p16-driven senescence, which likely contributes to growth suppression in the aged state. Developmental senescence is mediated largely by p21, likely acts in a growth-promoting manner, leading to tissue patterning, cell-fate instruction and remodeling. While oncogene-induced senescence probably combines aspects of both, having tumor/growth suppressive functions as well as growth promoting effects mediated by the SASP.

However, it is compelling that each of the tissues where we see a pronounced activation of developmental senescence, seems to share an expression profile that overlaps with OIS. For example, in addition to the AER, we identify the roof plate of the hindbrain neural tube as a primary site of senescence. As a signaling center, this structure expresses genes coding for many secreted proteins that are increased in OIS, including *BMPs, TGF*β, *WNTs, FGF*s, *Notch1*, and *VEGF*, in addition to *PAX* genes.[17] We demonstrate that this is also non-proliferative and expresses high levels of p21. The otic vesicle, another structure that stains intensely for SAβ-gal, expresses members of the class 3-Semaphorin family (*SEMA3A* and *3D*), as well as *FGFs, IGF1*, and *HOX* genes,[18] many of which are also increased in OIS. Together, this suggests that in addition to utilizing common mediators of senescence such as tumor suppressors and cell cycle inhibitors, senescent cells may actually have tissue-specific profiles that confer context-dependent functions through the SASP. Indeed, there is evidence that senescence in the adult induced by different stimuli results in significantly different profiles, or even cells with a different SASP.[19-21] Furthermore, the pronounced overlap between pre-malignant senescence profiles and embryonic patterning genes also has strong implications for the function of OIS, suggesting that during tumor initiation, senescence cells may play an instructive function in altering the local tissue environment to resemble that of an

embryonic state, supporting the notion that tumor initiation in some cases involves a reactivation of developmental pathways and helping to explain how senescence can have pro-tumorigenic functions.[21,22]

However, this still leaves the question as to why populations of cells in the embryo need to be "senescent," and here the terminology may be unclear. Perhaps the order of discovery and subsequent nomenclature of the process adds some confusion. While apoptosis was identified as a developmental process that was subsequently found in physiological and disease states, senescence was discovered the other way around, and was as such, named accordingly. However, the name should not detract from a defined cellular state with a myriad of functions that we are just beginning to discover. The hallmark feature so far is that senescent cells are irreversibly arrested from the cell cycle, which makes sense in the context of tumor suppression and damage. However, perhaps in the embryo it is a little different. Not all of the hallmark proteins that mediate irreversibility are expressed, suggesting that this feature is not fully developed or needed in the embryo. Indeed, in the embryo, as in the adult, senescent cells are removed by a process involving macrophage-mediated clearance and apoptosis, which is a senescent cells way of making sure it does not persist, programming its own removal. In adult senescence, expression of senescence-reinforcers such as p16, Il6, and IL8 were probably co-opted to maintain the arrested state. However, in each case, the senescent cells are non-proliferative, implying this must confer a property or function. Given that the establishment of signaling gradients is such a well-known mediator of developmental patterning, perhaps it is important to have a non-proliferating signaling focus that remains constant and immune to instructive signals, while the adjacent proliferating (and instructed) cells grow and extend further away from the senescent signal. To maintain their instructive window, these cells must not proliferate, to fine-tune the signaling gradient, yet remain protected from apoptosis, in an environment where apoptosis is common. Nevertheless they signal and prepare for their own removal, as an additional layer of control. While in each case, whether the SASP-like function is present or not, the programmed establishment (and subsequent removal) of senescence cells provides an additional layer of developmental remodeling.

Altogether, the identification of cellular senescence as an additional layer of developmental control in the embryo is a stimulating finding with important ramifications not just for development, but also for cancer and aging. In one way, this can be equated to the identification of apoptosis or EMT as a distinct cellular process that fine-tunes normal embryonic patterning. It will be exciting to unravel the molecular mechanisms and biological functions in the embryo, and to determine whether a deregulation of developmental senescence might play a role in the development of birth defects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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