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Extra Views

Executing Cell Senescence

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ABSTRACT

Senescence is a permanent form of cell cycle arrest that limits the proliferation of damaged cells and may contribute to tumor suppression and aging. We recently demonstrated that some senescent cell types undergo a dramatic changes in chromatin organization that are dependent on the retinoblastoma protein and are associated with the stable repression of some E2F target genes. Here we how these changes might contribute to the stability of the senescent state.

Cellular senescence was originally defined as the cellular state that accompanies the replicative exhaustion of cultured human cells. Whereas "replicative" senescence is triggered by telomere attrition, an indistinguishable phenotype (often called premature senescence or stasis) can be induced in young cells in response to activated oncogenes, DNA damage, oxidative stress and suboptimal culture conditions.[1] Hence, both telomere loss and other stresses produce a common endpoint and, indeed, recent reports indicate that replicative senescence requires normal DNA damage responses.[2,3] Irrespective of the initiating trigger, cellular senescence involves an apparently irreversible cell cycle arrest, as well as characteristic changes in morphology and gene expression that distinguish the process from quiescence, a reversible state of cell cycle arrest. Importantly, some genes that control cellular senescence in vitro influence cancer and aging in vivo, suggesting that the process may be act in tumor suppression and organismal aging.[4]

The fact that senescence is an irreversible program that can be induced by diverse stimuli conjures up analogies to apoptosis. Apoptosis is an irrevocable cellular response to stress that acts as a potent barrier to cancer development and may also contribute to aging. Further supporting a relationship between these two programs, many of the same signals that promote apoptosis in one cell type or induce senescence in another—for example, ionizing radiation triggers apoptosis in thymocytes but senescence in fibroblasts.[5,6] The 'choice' between senescence and apoptosis is influenced by cell context and genotype, and indeed mitogenic oncogenes (e.g., Myc, E1A) that prevent senescence often promote apoptosis, whereas other genes that block apoptosis (e.g., Bcl-2) can reveal a senescence-like arrest.[7,8] Consequently, senescence parallels apoptosis in cellular stress responses.

Although diverse signal transduction pathways regulate apoptosis, a common program produces the characteristic morphological and biochemical endpoints of apoptotic cell death. This 'machinery' involves cysteine proteases known as caspases, which act to cleave cellular substrates and disassemble the cell.[9] Since proteolytic cleavage of proteins is effectively irreversible, sufficient caspase activation triggers a point of no return. Almost nothing is known about how senescence is executed, although genetic studies using viral oncoproteins implicate the p16/Rb and p53 tumor suppressor pathways in the process. Until recently, the molecular mechanisms that drive a senescent arrest to an irreversible state were unexplored.

Based on the analogy to apoptosis, we reasoned that cellular senescence should also involve a machinery that is ultimately responsible for the stability of the senescent state. In this regard, we recently reported that senescent human IMR90 fibroblasts accumulate a distinct type of heterochromatin that can be visualized microscopically by the appearance of senescence associated heterochromatic foci (SAHFs) (Fig. 1).[10] These SAHFs are enriched for histone H3 modified at lysine 9 (K9M H3) as well as its binding partner heterochromatin protein 1 (HP1), both of which are hallmarks of heterochromatin. Interestingly, SAHF formation correlates with the accumulation of K9M H3 and HP1 on some E2F target promoters, and the stable silencing of some E2F target genes. Importantly, all of these changes require Rb, and none are observed in quiescent (reversibly arrested) cells.

Might SAHFs and their associated changes reflect the action of our hypothetical senescence machinery? Several observations make this an attractive possibility. First, heterochromatin confers stable and often heritable changes in gene expression by silencing unnecessary genes.[11] Second, certain E2F responsive promoters are apparently targeted by new heterochromatin in senescent cells. Since these genes are required for S phase entry and cell cycle progression, the stable silencing of these genes would clearly prevent subsequent cell division. Finally, SAHF formation and E2F target gene silencing require the p16/Rb pathway, which is crucial for the execution of the program.

Based on the above reasoning, we hypothesize that Rb-mediated changes to chromatin structure underlie the stability of cellular senescence. Central to this model is the methylation of lysine 9 on histone H3. In contrast to histone acetylation, which is dynamically regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), histone methylation is extremely stable. Indeed, while many histone methyltransferases (HMTs) are known, no histone demethylases have been identified. Interestingly, Rb can associate with both HMTs and HP1, and so it is attractive to imagine that Rb acts to nucleate heterochromatin on E2F target promoters, which then could spread to other areas of the genome. Importantly, although such a mechanism is consistent with our data it remains highly speculative, and it is also possible that Rb controls heterochromatin formation in a much more indirect way. Whatever the precise mechanism, our model roughly equates lysine 9 methylation of histone H3 during senescence to the proteolytic processing of caspase substrates during apoptosis—it may act to trigger a permanent outcome.

Our model has broad implications for control of tumor suppression and senescence biology. One of the more intriguing of these is the 'gene expression profile' and growth characteristics that distinguishes quiescent and senescent cells. In principle, our study indicates that a redistribution of heterochromatin leads to silencing of growth-regulatory or unnecessary genes and, conversely, activation of senescence-associated genes. Indeed, we observed an increase in K9M H3 histone associated with the cyclin A promoter and a decrease associated with the stromelysin-1 promoter (activated during senescence)—changes that are not observed reversibly arrested quiescent cells. By analogy, similar process may involve the differentiation of some cell types.[12] Whether similar processes contribute to differentiation remains to be determined, but it is noteworthy that Rb-deficient mice show some differentiation defects.[13-15] Hence, SAHF formation may alter and maintain the overall pattern of gene expression by stabilizing a specific chromatin status.

Another intriguing element of our study pertains to Rb action. Rb is a member of a multigene family consisting of two additional members, p107 and p130. While the immortalizing viral oncoproteins target all three proteins, only Rb is mutated in human cancers. Interestingly, while Rb is often thought to repress E2F target promoters by recruiting HDACs to E2F target promoters, it has been difficult to detect on E2F target genes in growing or quiescent cells (whereas p107 and p130 are readily detected).[16] These observations suggest that Rb has functions distinct from our traditional views, and that these functions are important for its tumor suppressor activity. Interestingly, Rb loss is sufficient to prevent SAHF formation and E2F gene silencing following a senescence stimulus. Although the signals were weak, we also noticed that Rb accumulates on some E2F target promoters during senescence. Hence, Rb is activated during senescence to repress E2F target gene expression in a manner that is distinct from p107 and p130, raising the possibility that Rb directed heterochromatin formation is important for its tumor suppressor activity.

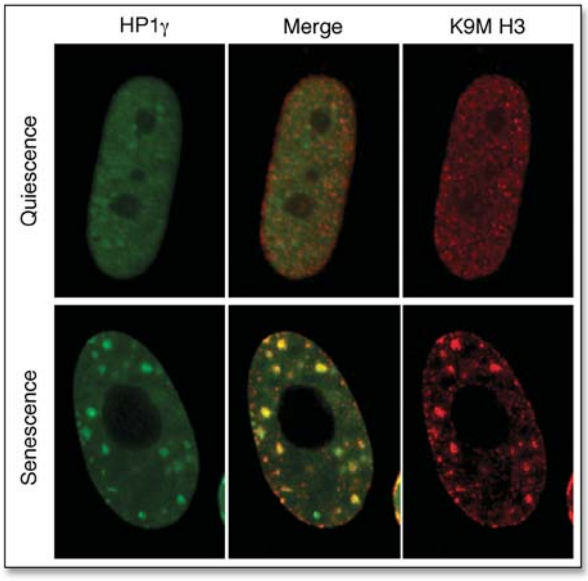

Figure 1. Senescence associated heterochromatic foci (SAHFs). Confocal images of indirect immunofluorescence of HP1γ and K9M H3 are shown in quiescent and ras-induced senescent IMR90 fibroblasts.

While our studies were conducted in IMR90 human diploid fibroblasts (as well as WI-38 fibroblasts), we do not see SAHFs in all 'senescent' cells. In particular, mouse embryo fibroblasts (MEFs)—which normally have marked heterochromatin foci—and BJ fibroblasts do not display pronounced SAHF formation upon the induction of senescence. Although this may indicate that the formation of SAHFs per se are not important for senescence, they may ultimately reflect the differential contribution of p16 to senescence in different cell types. In this regard, studies by Campisi and colleagues have recently shown that senescent WI-38 cells express higher p16 levels than senescent BJ cells, and that p53 inactivation is sufficient to restore proliferation in senescent BJ cells but not in senescent WI-38 cells.[17] Depletion of p16 by stable RNAi allows senescent WI-38 cells to proliferate in response to p53 inactivation. In addition, p16 is dispensable for senescence in MEFs, which can readily reenter the cycle following inactivation of Rb.[14] In principle the failure of p16 to trigger Rb mediated heterochromatin may explain why MEFs are more readily immortalized than human cells and why disruption of Rb in senescent MEFs allows cell cycle reentry and proliferation. Confirming these ideas will require a more detailed understanding of Rb action in different cell types.

Although our study provides some of the first insights into the effector mechanisms of senescence, many unanswered questions remain. First, are SAHFs a cause or consequence of senescence? Although all of our data indicate a close correlation, there is as yet no direct evidence for a causal role. By identifying additional players involved in SAHF formation it will be possible to determine whether their disruption increases the probability that cells will evade or escape senescence. Second, how general is the appearance of SAHFs and their underlying biochemical changes? Clearly, it will be important to determine whether the underlying chromatin changes occurring in SAHF-positive cells also occur in senescent cells lacking microscopically visible SAHFs (e.g., BJ fibroblasts). Third, can

understanding SAHFs or their underlying biology ultimately be used to identify new markers of senescence? Indeed, it is unclear whether current senescence markers actually participate in its biology (in contrast to the 'TUNEL' assay or caspase activation in apoptosis). The potential involvement of distinct chromatin changes linked to senescence may ultimately produce a desired tool for characterizing the process in vivo, perhaps providing proof the process contributes to human aging or pathology.

Further studies of SAHFs may also provide insights into chromatin dynamics. Presumably, SAHF formation involves the action of an HMT that may also have tumor suppressor functions. Although SUV39H1 is an obvious candidate, our preliminary analysis suggests that another HMT may be more important (unpublished observations). Also, the regulation of SAHF formation and maintenance may provide new insights into gene silencing. Although we have yet to observe circumstances in which SAHF-positive IMR90 cells will proliferate, studies suggest that some E2F genes can be induced in association with unscheduled DNA synthesis. Understanding whether and how SAHFs might be reversed may produce insights into the mechanism of maintenance of heterochromatin. Thus, we anticipate that the study of SAHFs and their related chromatin changes provides a new experimental system to study the relationship between cell cycle and chromatin structure in normal human cells.

References

1. Serrano M, Blasco MA. Putting the stress on senescence. Curr Opin Cell Biol 2001; 13:748-53.
2. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. Curr Biol 2003; 13:1549-56.
3. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. DNA damage checkpoint response in telomereinitiated senescence. Nature 2003; 426:194-8.
4. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, et al. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002; 415:45-53.
5. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, et al. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 1993; 362:849-52.
6. Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. Genes Dev 1994; 8:2540-51.
7. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993; 74:957-67.
8. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, et al. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002; 109:335-46.
9. Thornberry NA, Lazebnik Y. Caspases: Enemies within. Science 1998; 281:1312-6.
10. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003; 113:703-16.
11. Francastel C, Schubeler D, Martin DI, Groudine M. Nuclear compartmentalization and gene activity. Nat Rev Mol Cell Biol 2000; 1:137-43.
12. Lunyak VV, Burgess R, Prefontaine GG, Nelson C, Sze SH, Chenoweth J, et al. Corepressor-dependent silencing of chromosomal regions encoding neuronal genes. Science 2002; 298:1747-52.
13. Clarke AR, Maandag ER, van Roon M, van der Lugt NM, van der Valk M, Hooper ML, et al. Requirement for a functional Rb-1 gene in murine development. Nature 1992; 359:328-30.
14. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. Nature 1992; 359:295-300.
15. Lee EY, Chang CY, Hu N, Wang YC, Lai CC, Herrup K, et al. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 1992; 359:288-94.
16. Takahashi Y, Rayman JB, Dynlacht BD. Analysis of promoter binding by the E2F and pRB families in vivo: Distinct E2F proteins mediate activation and repression. Genes Dev 2000; 14:804-16.
17. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, et al. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. Embo J 2003; 22:4212-22.