Cellular Senescence in Aging Primates

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The aging of organisms is characterized by a gradual functional decline of all organ systems. Mammalian somatic cells in culture display a limited proliferative life span, at the end of which they undergo an irreversible cell cycle arrest known as replicative senescence. Whether cellular senescence contributes to organismal aging has been controversial. To reinvestigate this question, we assayed the skin of aging baboons for telomere dysfunction, a recently discovered biomarker of cellular senescence (*1*).

Like humans, baboons have a relatively long life span and show age-dependent telomere shortening (*2*). Baboon skin fibroblasts undergo replicative senescence upon serial passage in culture, with characteristics identical to those of human fibroblasts. Senescent baboon fibroblasts display DNA damage foci that contain phosphorylated histone H2AX (γ-H2AX), activated ataxia-telangiectasia mutated (ATM) kinase ATM(Ser^{1981}), and p53 binding protein (53BP1), and they express activated p53(Ser^{15}) and elevated levels of p21^{CIP1}. More than 80% of senescent baboon fibroblasts in vitro display telomere dysfunction–induced foci (TIFs), as determined by the colocalization of γ-H2AX with telomeres.

Full-thickness skin biopsies were obtained from 30 baboons (15 male and 15 female), ranging in age from 5 to 30 years, that were born and raised at the Southwest Regional Primate Center under controlled conditions. The tissue was harvested from the medial aspect of the forearm, a surface that is relatively protected from radiation and injury. The number of dermal fibroblast nuclei containing foci of 53BP1, a marker of DNA double-strand breakage (DSB), increased exponentially with age and reached a value of 30 to 35% in very old (25 to 30 years old) animals (Fig. 1A and fig. S1A). As was found in cultured fibroblasts, 100% of 53BP1 foci colocalized with foci formed by γ-H2AX, another marker of DSB. The majority of 53BP1 foci (62 ± 7.7%) colocalized with telomeric DNA, thus classifying them as TIFs (fig. S1B) (*1*). The frequency of TIF-positive nuclei increased exponentially with age, reaching a value of 15 to 20% in very old animals (Fig. 1B). Dysfunctional telomeres in dermal fibroblasts activate the ATM kinase, as evidenced by the colocalization of γ-H2AX and ATM(Ser^{1981}) (fig. S1C). Ninety-five percent of DNA damage–positive cells displayed staining for four distinct markers of heterochromatin: HP1-β, HIRA (fig. S1, D and E), and histone H3 dimethylated or trimethylated on Lys^{9}. These data indicate that telomere dysfunction in dermal fibroblasts of baboons activates the ATM signaling pathway and leads to extensive formation of heterochromatin, a hallmark of cellular senescence (*3*). Finally, we observed a high correlation between the presence of TIFs and up-regulation of p16^{INK4A} (fig. S1, F and G). Preliminary analyses indicate that these age-dependent changes are not confined to dermal fibroblasts.

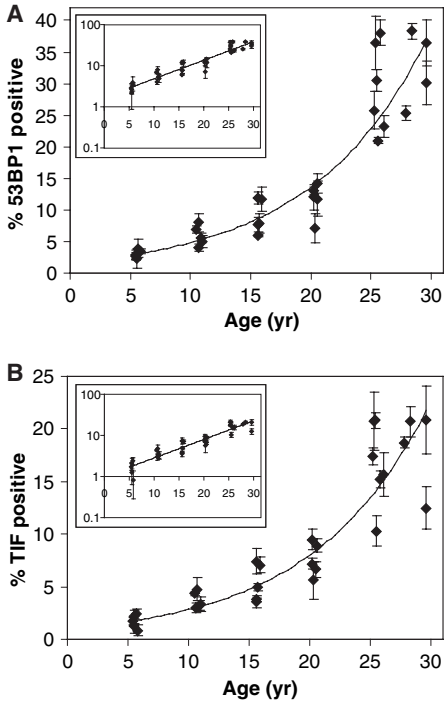

Fig. 1. Telomere dysfunction in skin fibroblasts of living baboons increases with donor age. Skin biopsies were obtained, processed, and imaged, and dermal fibroblasts were scored for the presence of (**A**) 53BP1 foci and (**B**) TIFs as described in (*7*). Thirty individuals in six age groups (five animals per group) were analyzed. Each point represents one animal; there is considerable overlap between the data points, especially at younger ages. 200 to 600 fibroblasts were scored for each animal (error bars show SD). Exponential regressions gave the best fit when applied to the data points [exponential: (A) $R^2 = 0.9136$; (B) $R^2 = 0.8849$; linear: (A) $R^2 = 0.7856$; (B) $R^2 = 0.7884$]. The insets (upper left in each panel) show the same data points plotted on a semi-log scale to further illustrate the exponential accumulation of 53BP1 foci and TIFs with age.

Although replicative senescence of cultured cells has been known for some time, its physiological relevance and contribution to organismal aging have been questioned. This skepticism is largely because of the sparse staining of aged tissues with the senescence-associated β-galactosidase biomarker (*4*). Confidence in this marker has been eroded by findings that its expression can be induced in immortalized cells and even reversed under some conditions (*4*). By using three recently discovered biomarkers (telomere dysfunction, activation of the ATM DNA-damage response, and heterochromatinization of the nuclear genome), we have provided evidence that senescent cells exist in vivo and can account for >15% of the cell population in aged animals. Heterochromatinization is triggered by both replicative and oncogene-induced senescence, is believed to be irreversible, and is associated with profound changes in gene expression (*3*). Thus, the presence of senescent cells in intact tissues at such high frequencies may have profound physiological consequences.

Although we have found a clear in vivo association between telomere dysfunction and senescence, the telomeric DNA damage may not be exclusively due to replicative exhaustion. Oxidative stress increases the rate of telomere attrition, and telomere dysfunction may be triggered by effects other than overt telomere shortening (*5*, *6*). Given the known age-dependent accumulation of oxidative damage in many tissues, it may turn out that telomeres can function in vivo both as replicative and chronological clocks.

References and Notes

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Supporting Online Material

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Materials and Methods
Fig. S1
References

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