HIGHLIGHT

Bladerunners and telomerases

Mehboob A Hussain

Laboratory of Molecular Endocrinology, Howard Hughes Medical Institute, Massachusetts General Hospital, 50 Blossom Street Wellman 320, Boston, Massachusetts 02114, USA

The science fiction *Bladerunners* tells a story of bionic robots which are designed like and look indistinguishable from humans. Humans use them as slave-workers in space stations. Some of these so-called replicants realize that they have been programmed to live only a certain time, after which they cease to function – or to speak in biological terms – cease to exist, or die. A group of these robots – filled with anxiety (yes, they also have some emotions) upon their approaching death and angry at humans that they are granted only 4 years of life – set out in their anger to destroy humans and to try to persuade their designer (or rather creator?) to change their program. Alas, they have to realize that the program cannot be changed. To contain the harm to humans they are hunted down and ‘terminated’ by a so-called blade-runner (a space-age 007 with a license to kill robots).

A look into our own social behavior reveals that we have indeed realized that we have a limited lifespan and that at least some of us strive for eternal youth and longevity or an additional form of existence after the present life. A closer look at biology has given some clues to the ways the cells in our body have limited renewal capacity, thus opening the door to understanding better the processes involved in cellular senescence and perhaps a relationship between cell senescence and aging in the organism.

In 1961, Hayflick & Moorehead (1) showed that normal somatic cells have a limited replicative potential, roughly 50 cycles in young skin fibroblast cells. Prior to approaching this maximum barrier the cells slow their rate of division and manifest identifiable and predictable morphological changes characteristic of senescent cells (2). Olovnikov (3) and Watson (4) described in the 1970s that DNA polymerase primers overlay and mask a portion of terminal chromosome from DNA polymerase; a portion of the telomere is not replicated and therefore shortens with each cell division. Later, in the 1990s, molecular biology revealed that telomeres are shorter in senescent cells than in younger cells (5, 6). Since then evidence has increased that telomere shortening is the clock that results in the shift to a senescent pattern of gene expression (7, 8). This barrier to cell replication is found throughout multicellular organisms and has few exceptions. These exceptions are cancer cells (9–11), the germ cell lineage (12–14) and certain stem cell lines. These latter stem cells (hematopoietic stem cells (13), gastrointestinal crypt cells, hair follicles (15), and perhaps liver cells (16)) also demonstrate

replicative senescence, but with a much extended cellular lifespan compared with other somatic cell types. This extension correlates with transient expression of telomerase (17, 18), a reverse transcriptase comprising at least an RNA template (19) and a catalytic protein component (20), that allows them to slow the rate at which the erosion of telomere bases occurs. Telomerase expression also occurs in the germ cell line and primordial stem cells (20).

Telomerase adds DNA bases to chromosomal telomeres. In addition to its RNA template that is present in cells normally, it contains a catalytic protein component that is not found in normal, aging, somatic cells. When this catalytic protein is expressed in cells that lack it, their replicative lifespan is extended and their pattern of gene expression is typical of young cells (21). Thus, the addition of telomerase activity could ‘reset’ the clock in an *in vivo* situation. The addition of telomerase did not cause malignant transformation of the cells, thus eliminating the possibility that telomerase activity would be oncogenic rather than rejuvenating.

But is there a link between cell aging of cells and of entire organisms? Does cell aging underlie the general process of aging in the organism? New studies in mice have now advanced our understanding of the biology of telomerases. A mouse lacking the gene encoding the essential RNA component of the telomerase holoenzyme has been generated (the mTR^{−/−} mouse) (22). These mice exhibit progressive telomere shortening with each successive generation. Sixth generation (G6) mice were infertile, and had a decreased proliferative capacity of splenocytes and bone marrow cells *in vivo*, and had an increased embryonic lethality due to neural tube closure defects. Third generation (G3) mice possess shortened telomeres early in life but appear to be phenotypically normal. However, they show increased and more frequent signs of senescence as they progress through life as compared with normal mice.

The investigators found a dramatic increase in the incidence of hair graying and alopecia in aging G3 and G6 mice compared with age-matched mTR^{+/+} mice. In addition, hair graying was seen at younger ages with some of the G6 mice showing gray hair as early as at 6 months of age. The number of hair follicles in the growth phase relative to the resting phase was 2- to 3-fold lower in G3 and G6 mice compared with the wild-type animals. The histopathological findings of the aged balding mice matched those found in androgenetic

alopecia in men. In addition to the changes in hair number and color, a higher incidence of severe ulcerative skin lesions was seen in G3 and G6 mice. These skin lesions were located predominantly at anatomical sites that are exposed to chronic mechanical stress, including distal limbs, perineum, snout and throat area. Histologically these lesions appeared as ulcerations with epidermal hyperplasia, hyperkeratosis and underlying dermal fibrosis. These histological changes are reminiscent of those found after chronic superficial trauma in humans. By flow-cytometry fluorescence hybridization, the telomere length could be determined in these mice. This measurement allowed the finding of an inverse correlation between telomere length and the incidence of skin lesions, gray hair and alopecia.

Telomere shortening did not cause generalized premature aging as is found in mice. Serum chemistries of G3 and G6 animals were not different from control animals. Histology analyses did not reveal signs of arteriosclerosis and cataract. Radiographic analysis did not reveal increased osteoporosis in the null mice. Blood glucose levels were not different in the null mice as compared with control animals.

There was no significant difference in the survival of G1 and G3 animals compared with wild-type animals. In contrast, a 15% decrease in spontaneous death was observed in the 3- to 12-month-old G4 and G5 animals. However, the 50% mortality mark occurred at 18 months of age for G6 mice, whereas this mark was not reached for wild-type mice until 24 months of age.

Body weight was superimposable in all groups of mice until 6 months of age. At both 10–14 and 15–18 months of age the average body weight of G6 animals was 20–25% less than early generation mTR^{-/-} or mTR^{+/-} animals. Consistent with the decrease in body weight was a diminished fat cell layer between the dermis and the skeletal muscles. In the gastrointestinal tract, villous atrophy and zonal blunting in the proximal intestine were detected in a subset of aged G6 mice.

The investigators further studied the ability of these mice to respond to defined stresses. Wound healing after application of 3 mm punch biopsies and recovery of hematological parameters after a sublethal application of 5-fluorouracil (5-FU) were used as stresses to two rapidly regenerating tissues, skin and blood respectively. Impaired wound healing and blood count reconstitution after 5-FU were found only in aged mice, whereas in young mice (up to 10–14 months of age) there was no difference from wild-type mice, although telomere length was clearly reduced in the different generations of the tested null mice.

Loss of telomere function in the mTR null mice is associated with an increase in the frequency of chromosomal fusion and aneuploidy. In animals beyond 15 months of age an increased rate of chromosomal fusions was found. In addition, the TR null mice presented with a 4- to 6-fold increase in spontaneous visible cancers. Further, the average age of

tumor formation was lower in the G4 than in the G6 mice. The main tumor types detected were from highly proliferative tissues (i.e. teratocarcinoma, lymphoma and squamous cell carcinomas). It thus appears that loss of telomere function and resulting genetic instability (i.e. chromosomal fusion) that ensues facilitates the development of certain cancers. This was undermined by the observation that chromosomal fusions and aneuploidy were increased in the tumors of mTR null mice as compared with tumors in wild-type mice.

Although some aspects of the mTR^{-/-} mouse mimic age-associated disease, many signs and symptoms classically associated with premature aging mouse models were not evident in the mTR^{-/-} mice. In particular, there was no evidence of increased cataract formation, osteoporosis, glucose intolerance or vascular disease. Thus, the telomerase null mouse does not entirely explain the process of senescence in mammals and the cues to aging and senescence – although partly uncovered – still remain elusive.

Acknowledgements

MAH is a recipient of a Juvenile Diabetes Foundation International Career Development Award. Many thanks to P Putschert for the cineastic encouragement.

References

- Hayflick L & Moorehead PS. The limited *in vitro* lifetime of human diploid strains. *Experimental Aging Research* 1961 **25** 585–621.
- Hayflick L. The biology of human aging. *Advances in Pathobiology* 1980 **7** 80–99.
- Olovnikov AM. Principle of marginality in template synthesis of polynucleotides. *Doklady Akademii Nauk SSSR* 1971 **201** 1496–1499.
- Watson JD. Origin of concatameric T7 DNA. *Nature New Biology* 1972 **239** 297–301.
- Harley CB, Futcher AB & Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990 **345** 458–460.
- Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK & Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing. *Nature* 1990 **346** 866–868.
- Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB *et al.* Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National Academy of Sciences of the USA* 1992 **89** 10114–10118.
- Allsopp RC & Harley CB. Evidence for a critical telomere length in senescent human fibroblasts. *Experimental Cell Research* 1995 **219** 130–136.
- Blasco MA, Lee HW, Hande MP, Samper E, Lansdorf PM, DePinho RA *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 1997 **91** 25–34.
- Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB *et al.* Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO Journal* 1992 **11** 1921–1929.
- Shay JW & Wright WE. Telomerase activity in human cancer. *Current Opinion in Oncology* 1996 **8** 66–71.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL *et al.* Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994 **266** 2011–2015.
- Chiu CP, Dragowska W, Kim NW, Vaziri H, Yui J, Thomas TE *et al.* Differential expression of telomerase activity in hematopoietic

- progenitors from adult human bone marrow. *Stem Cells* 1996 **14** 239–248.
- 14 Bodnar AG, Kim NW, Effros RB & Chiu CP. Mechanism of telomerase induction during T cell activation. *Experimental Cell Research* 1996 **228** 58–64.
- 15 Ramirez RD, Wright WE, Shay JW & Taylor RS. Telomerase activity concentrates in the mitotically active segments of human hair follicles. *Journal of Investigative Dermatology* 1997 **108** 113–117.
- 16 Shay JW & Bacchetti S. A survey of telomerase activity in human cancer. *European Journal of Cancer* 1997 **33** 787–791.
- 17 Counter CM, Gupta J, Harley CB, Leber B & Bacchetti S. Telomerase activity in normal leukocytes and in hematologic malignancies. *Blood* 1995 **85** 2315–2320.
- 18 Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA *et al.* Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *Journal of Immunology* 1995 **155** 3711–3715.
- 19 Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP *et al.* The RNA component of human telomerase. *Science* 1995 **269** 1236–1241.
- 20 Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J *et al.* Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997 **277** 955–959.
- 21 Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB *et al.* Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998 **279** 349–352.
- 22 Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C *et al.* Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* 1999 **96** 701–712.

Received 1 April 1999

Accepted 14 April 1999