Cell aging: is it an anti-cancer mechanism?

Peter J. Hornsby
Professor, Dept. of Physiology
and Barshop Institute
University of Texas Health Science Center San Antonio

Some definitions

- Aging – gradual and progressive loss of normal functional characteristics at all levels – organism, organ system, tissue, cell
- Senescence – a general term for characteristics of aging, not necessarily associated with cells
- Cell aging – all and any aging process at the cell level
- Cell senescence – one form of cell aging, a state of permanent cessation of cell division into which cells may be driven by a variety of cellular events
  - originally described as a result of telomere shortening (replicative senescence)
  - now known to be an alternative (non-apoptotic) response of the cell to many forms of damage (esp. DNA damage) and to activated oncogenes

Characteristics of cell senescence

- Irreversible exit from the cell cycle
- Cells can be kept viable for 3 yrs plus in culture
- Cells cannot be stimulated to enter S phase
- Cells remain metabolically active
- Resistant to apoptosis
- Altered functions/gene expression
- For replicative senescence population doublings (cell divisions), not time in culture, determines “life span”, suggesting a counting mechanism (= progressive loss of telomere DNA leading to telomere dysfunction)

Senescence-associated β-galactosidase

Has become an important marker for senescent cells – mostly because of a lack of other universal markers
The gene product is not different from lysosomal galactosidase
However it may become mislocalized (?) and as a result (?) can be detected at pH ~6 rather than ~4 for the lysosomal enzyme

Age-related changes in tissues

- Increased number of senescence-associated β-galactosidase positive cells in tissues, as well as in pathological conditions
- Cells in many organs have shorter telomeres as a function of age
- Not clear yet that these two observations are related; e.g., β-galactosidase positive cells increase in number in tissues that do not undergo much cell division and may not undergo telomere shortening
- Alternatively, β-galactosidase positive cells may arise via DNA damage or the action of activated oncogenes

Telomere shortening in aging

- Many cells undergo telomere shortening
  - Hematopoietic cells
  - Fibroblasts
  - Endothelial cells
  - Smooth muscle cells
- Stem cells: variable
- Germ cells: no shortening
- Results from lack of expression of TERT (telomerase reverse transcriptase) in most human cells

Telomeres, telomerase and cancer

- Most human cancers are telomerase positive (80–85%)
- Most non-cancer cells have low/undetectable telomerase (exception – germ cells and stem cells) (lack expression of telomerase reverse transcriptase, TERT)
- Those cancer cells that are telomerase negative have an alternative lengthening of telomeres (ALT) pathway active (recombination based) (never active in normal cells)
- Thus >99% of cancer cells have some method for telomere maintenance

How does the lack of TERT expression exert an anti-cancer effect?

- Over long periods of time, small clones of abnormal cells appear in tissues because they have acquired multiple mutations
- By the time such a clone acquires the mutations needed for it to become a cancer cell it may also have suffered extreme telomere shortening
- Now it either has to acquire immortality (TERT expression or ALT*) or else the clone will eventually die
- Therefore, the consequence of this is that the usual lack of TERT expression and telomere shortening exerts an anti-cancer effect because it limits the growth of these abnormal clones

* = alternative lengthening of telomeres

Cooperation of Ras and SV40 large T antigen: A very simplified view

we introduced the genes for oncogenic Ras and SV40 T antigen, together with a green fluorescent protein gene, into normal cells and transplanted them in the kidney of immunodeficient mice
Experimental conversion of normal cells to cancer cells: Ras + SV40 TAg

- We introduced these oncogenes into normal human fibroblasts, normal human smooth muscle cells, and normal bovine adrenocortical cells
- Cells were transplanted under the capsule of the kidney of severely immunodeficient mice (RAG2<sup>-/-</sup>, γc<sup>-/-</sup>)
- Cells grew into malignant tumors that were visualized by green fluorescent protein (GFP); metastasis, especially to lungs, often observed
- Cells remained telomerase negative, and after some period of growth (sometimes in a secondary or tertiary serial transplant) tumors ceased growth
- Cessation of growth is caused by short telomere-based crisis and growth can be rescued by introduction of the hTERT gene (= human telomerase reverse transcriptase)

Invasion into kidney by cells expressing Ras and SV40 TAg

Progressive loss of malignant properties during serial transplantation of Ras/SV40 TAg cells

“Rescue” of Ras/SV40 TAg cells from crisis by hTERT

Cessation of growth in Ras/SV40 TAg cell tumors results from crisis
Subcutaneous tumor growth requires hTERT

bovine adrenocortical cells; Ras/SV40 Tag: above no hTERT, below + hTERT (injected subcutaneously).

The requirement for hTERT for subcutaneous growth is not linked to the need for a telomere maintenance mechanism.

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Malignancy is unrelated to telomere maintenance; but hTERT required s.c.

Table 1. Tumorigenic potential of the TERT-transduced AS17NR3R cells.

<table>
<thead>
<tr>
<th>Tumorigenic Potential</th>
<th>Genotype</th>
<th>Tumorigenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS17NR3R TERT</td>
<td>Yes</td>
<td>Y+</td>
</tr>
<tr>
<td>AS17NR3R</td>
<td>No</td>
<td>Y-</td>
</tr>
<tr>
<td>AS17NR3R + TERT</td>
<td>Yes</td>
<td>Y+</td>
</tr>
<tr>
<td>AS17NR3R + TERT</td>
<td>Yes</td>
<td>Y+</td>
</tr>
</tbody>
</table>

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Consequence of telomere shortening/dysfunction in crisis

- End-to-end fusions
  - Bridge-breakage-fusion cycles
  - Genomic instability
  - Mutations
- Mitotic catastrophe
  - Failure to separate sets of chromosomes, e.g., because of dicentrics
  - Endoreduplication
  - Giant cell formation
- Nonspecific cell death
- Loss of malignant properties

Summary

- A model combination of oncogenes, Ras and SV40 T antigen, co-operate to confer malignant properties on primary normal cells (malignancy = invasion and metastasis)
- Cells remain telomerase negative and undergo progressive telomere shortening
- Telomere dysfunction eventually results in the cessation of tumor growth due to crisis in the tumor cells
- Such tumors can be rescued from crisis by telomerase (hTERT) which restores telomere length, growth and malignant properties
- The clinical counterpart of such cells may be very small lesions that never progress – die out before acquiring telomerase expression

Cell aging as anti-cancer

- Oncogenes can directly activate the senescence program, thus aborting a potential cancer
- When senescence fails because oncogene activation overcomes senescence, a second line of defense is the lack of expression of TERT, leading to progressive telomere shortening, loss of malignant properties and a lesion that never progresses to an invasive cancer