Geriatric Oncology: Cancer in Senior Adults
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Cell aging: is it an anti-cancer mechanism?

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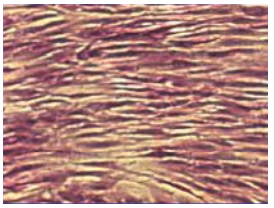
Some definitions

- 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

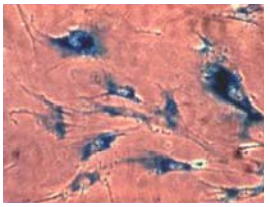
Characteristics of cell senescence

- 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



Young normal human fibroblast culture (after 20 population doublings)



Senescent normal human fibroblast culture (after 60 population doublings)

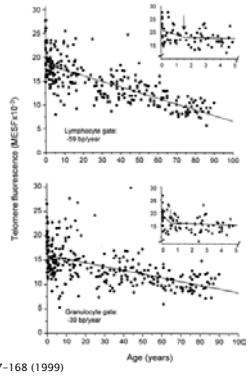
Has become an important marker for senescent cells – mostly because of a lack of other universal markers
The gene product is not different from lysosomal galactosidase
However it may become mislocalized (?) and as a result (?) can be detected at pH ~6 rather than ~4 for the lysosomal enzyme

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

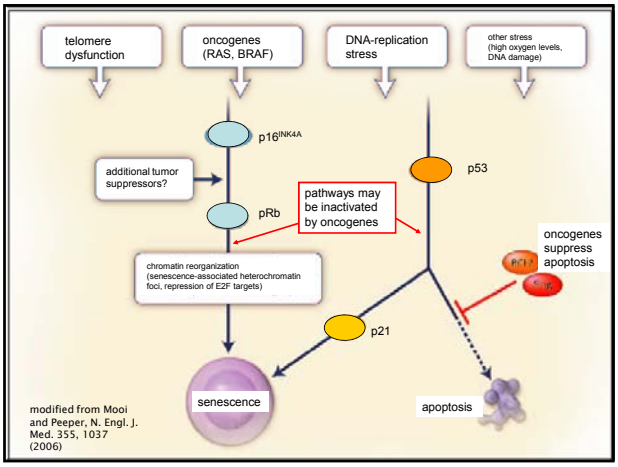
- Many cells undergo telomere shortening
  - Hematopoietic cells
  - Fibroblasts
  - Endothelial cells
  - Smooth muscle cells
- Stem cells: variable
- Germ cells: no shortening
- Results from lack of expression of TERT (telomerase reverse transcriptase) in most human cells

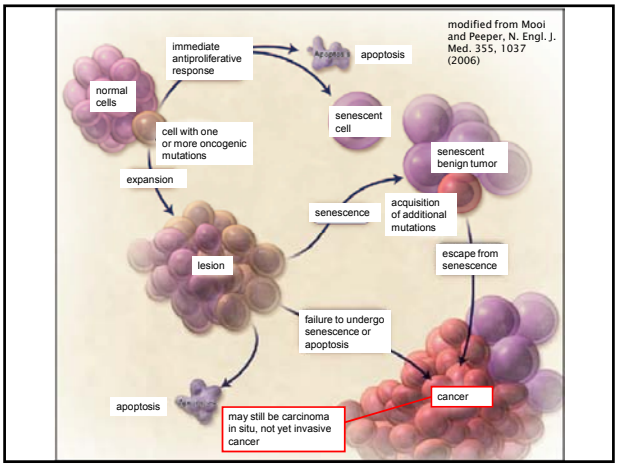


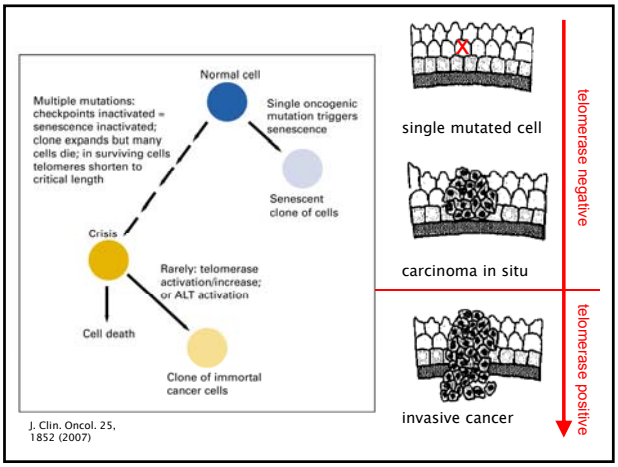
Rufer et al., J. Exp. Med., 190, 157-168 (1999)

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





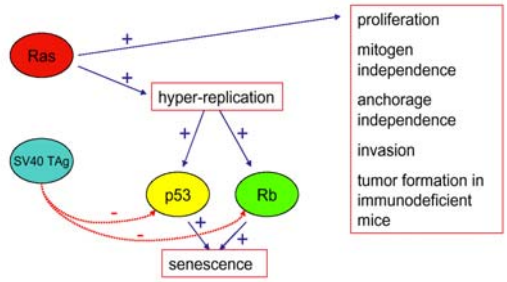


How does the lack of TERT expression exert an anti-cancer effect?

- 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



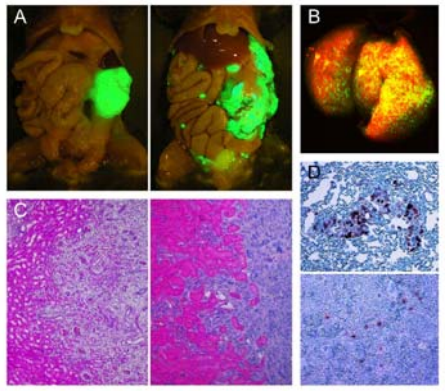
we introduced the genes for oncogenic Ras and SV40 T antigen, together with a green fluorescent protein gene, into normal cells and transplanted them in the kidney of immunodeficient mice

Experimental conversion of normal cells to cancer cells: Ras + SV40 TAg

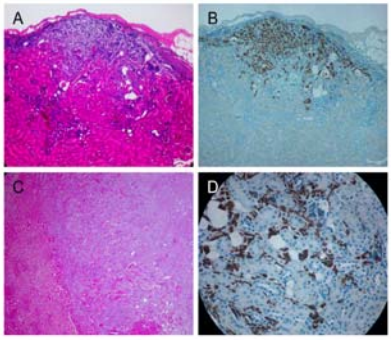
- We introduced these oncogenes into normal human fibroblasts, normal human smooth muscle cells, and normal bovine adrenocortical cells
- Cells were transplanted under the capsule of the kidney of severely immunodeficient mice (RAG2$^{-/-}$, $\gamma c^{-/-}$)
- Cells grew into malignant tumors that were 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)

Primary human fibroblasts expressing Ras, SV40 large T antigen and GFP; no hTERT

Neoplasia 7, 585 (2005)



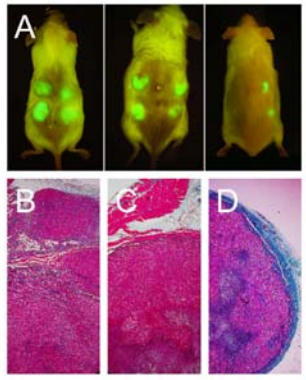
Invasion into kidney by cells expressing Ras and SV40 TAg



human smooth muscle cells expressing Ras and SV40 TAg; immunostaining for SV40 TAg

Mol. Carcinogenesis 47, 478 (2008)

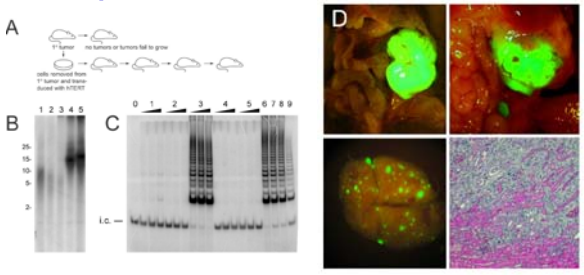
Progressive loss of malignant properties during serial transplantation of Ras/SV40 TAg cells



bovine adrenocortical cells expressing Ras/SV40 TAg (A) serial subcutaneous transplantation of fragments of tumor from kidney; (B, C, D) serial transplants – trichrome stain

Cancer Res. 64, 6144 (2004)

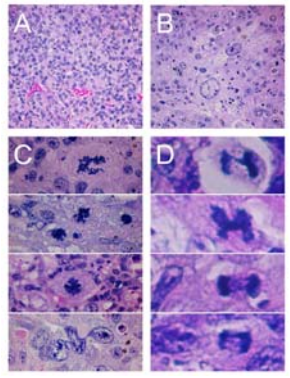
"Rescue" of Ras/SV40 TAg cells from crisis by hTERT



human fibroblasts expressing Ras/SV40 TAg
(A) serial transplantation and rescue with hTERT; (B) telomere length; (C) telomerase activity; (D) restoration of malignant properties

Neoplasia 7, 585 (2005)

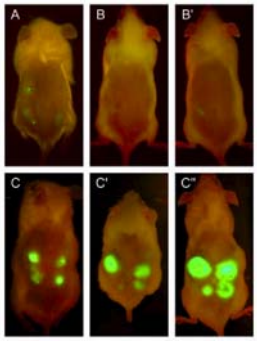
Cessation of growth in Ras/SV40 TAg cell tumors results from crisis



bovine adrenocortical cells expressing Ras/SV40 TAg (A, B) serial transplantation of cells in kidney: A = primary tumor, B = tertiary tumor; (C) abnormal chromosomes; (D) anaphase bridges

Cancer Res. 64, 6144 (2004)

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

Cancer Res. 64, 6144 (2004)

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

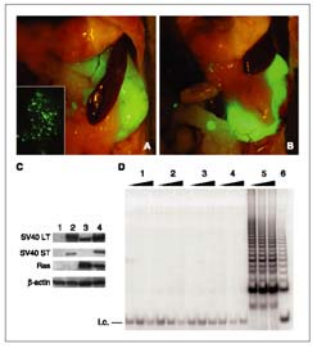


Table 1. Tumorigenic potential of Ras^{V12G}-transduced ALT+ fibroblast cell lines

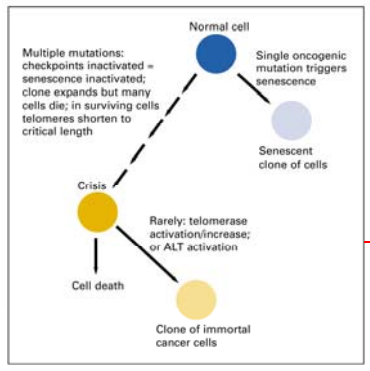
Fibroblast strain	Genetic modification	Tumor incidence	
		S.c.	Subrenal capsule
GM847	Ras	0/7	7/7
GM847	Ras + hTERT	5/7	7/7
GM639	Ras	0/3	3/3
VA13	Ras	0/3	3/3

NOTE: Three SV40-immortalized ALT+ cell lines were transduced with Ras^{V12G}; the incidence of tumors following s.c. injection was compared with that in the subrenal capsule assay. The formation of tumors by Ras/hTERT-transduced GM847 cells is also shown. Mice were sacrificed at 35 days following cell transplantation.

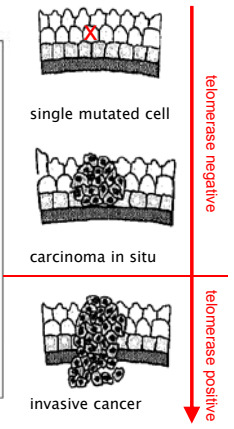
Cancer Res. 65, 6512 (2005)

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



J. Clin. Oncol. 25, 1852 (2007)



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