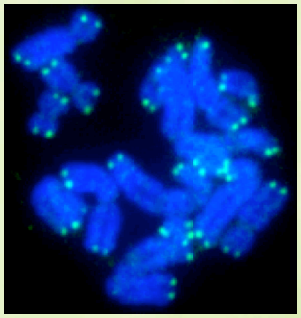
Telomeres and senescence II



A&S300-002 Jim Lund

Telomeres in mice

- Lab strains of mice have very long telomeres.
  - 30-40kb telomeres.
- *Tert* knock-out mice:
  - Normal for four generations as their telomeres shorten,
  - Premature aging phenotypes present in the 5th and generation.

Telomeres: Ageing hard or hardly ageing?
(Nature 398, 191-193, 1999)

Table 1. Aging syndromes in mouse and man

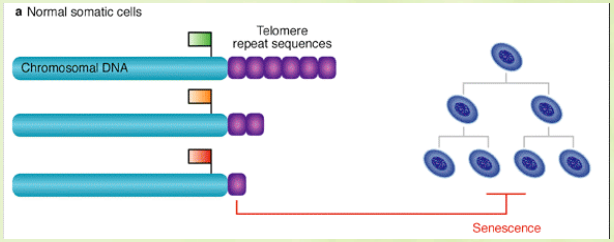
Symptoms	*mTR$^{-/-}$ mice*	*Werner's syndrome*
Shortened division capacity	+	+
Accelerated cell senescence	-	+
Premature greying	+	+
Poor wound healing	+	+
Increased cancer incidence	+	+
Gut defects	+	?
Infertility	+	+
Shortened lifespan	+	+
Decreased adipose tissue	+	+
Hair loss	+	+
Brain changes	-	-
Osteoporosis	-	+
Diabetes	-	+
Atherosclerosis	-	+
Cataract	-	+

Telomeres are one of the blocks to cancer formation

- The limited replicative potential of somatic cells blocks runaway cell division.
- Population doublings is an evolutionary trade off between cell renewal and cancer susceptibility.

Telomerase and Senescence

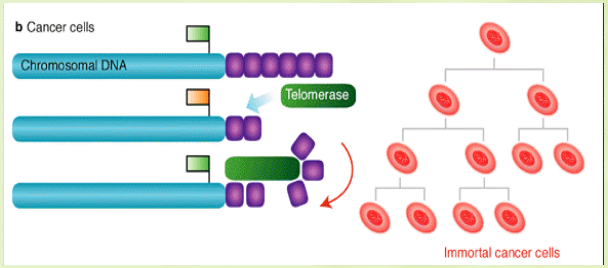
In most somatic tissues, telomerase is expressed at very low levels or not at all -- as cells divide, telomeres shorten



Short telomeres signal cells to senesce (stop dividing)

Telomerase and Cancer

The presence of telomerase in cancer cells allows them to maintain telomere length while they proliferate



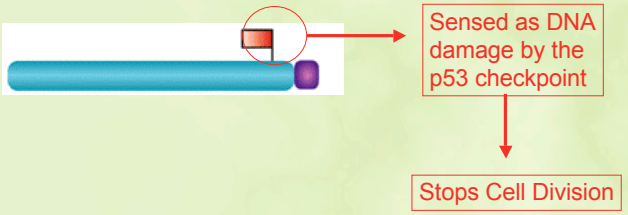
Telomerase and Cancer

Will turning off telomerase in cancer cells cause them to senesce and thereby stop progression of the disease?

Works in some cases.

Studies in an important model system, Telomerase deficient mice indicate that it will probably depend on the type of tumor.

Short telomeres cause growth arrest via a checkpoint



If the cells of a particular tumor still have p53, this works

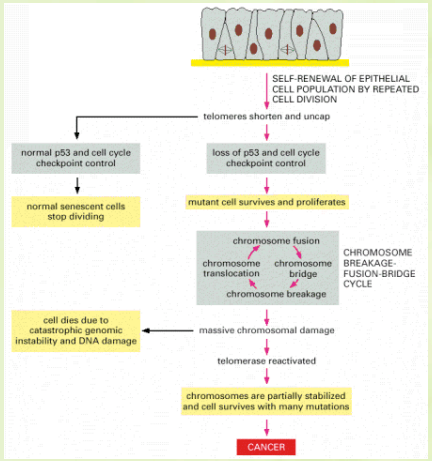
Tumor cells often lose p53

~~Sensed as DNA damage by the p53 checkpoint~~

Cell division continues without telomeres leading to chromosomal rearrangements

This genomic instability can promote tumorigenesis

Telomeres and cancer



Telomeres shorten with age

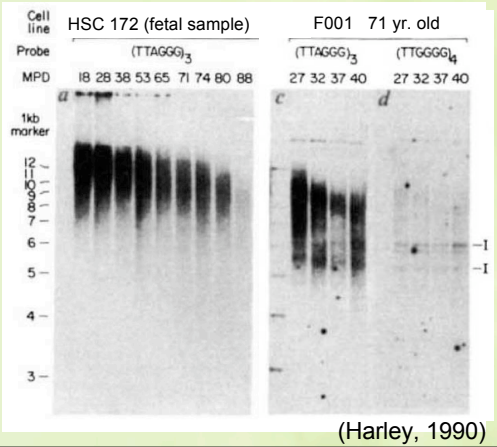
TABLE 1 Effect of donor age on telomere length in human fibroblasts

Cell strain	Age *in vivo* years	Age *in vitro* MPD (MPD max)	Mean telomere length kb ± s.d. (*n*)
HSC172	Fetal	18–28 (88)	8.6 ± 0.5 (3)
A30S	0	33 (58)	7.3 (1)
A38	24	31–33 (68)	6.9 ± 0.3 (2)
A35	70	19 (41)	6.7 (1)
F001	71	21–29 (40)	6.5 ± 0.4 (5)
F002	91	18–20 (45)	6.2 ± 0.1 (3)

Mean telomere length (the length of the terminal restriction fragment) was determined as described in Fig. 2*a* for fibroblast cell strains at the earliest available mean population doubling (MPD) in separate experiments. Strains were derived from female fetal lung (HSC172), female newborn skin (A30S), male forearm skin (A38, A35) or female abdominal skin (F001, F002). MPD at time of assay and senescence (MPD max) are indicated. The correlation between increasing donor age and decreasing telomere length is statistically significant ($P < 0.05$).

(Harley, 1990)

Telomeres shorten with age



(Harley, 1990)

Telomere variation

- Telomere lengths vary -- from cell to cell, from tissue to tissue.
  - 2X difference often observed.
- Only one telomere in a cells needs to shorten to the critical point for senescence to be triggered.
  - Hard to measure experimentally.

Telomere loss rate

- From a mechanistic basis, loss will be 8-12 bp per replication cycle.
  - 10 bp x 50 doublings = 500 bp loss.
- In vivo, mean telomere length decreases by about 15 bp per year.
  - 15 bp x 80 yr = 1200 bp loss.
  - (Harley, 1990)

Telomere loss rate

- In typical cell culture conditions, telomeres lose 50 - 90 bp per doubling.
  - 50 - 90 bp x 50 doublings = 2.5 - 4.5 kb loss.

Telomere loss rate is affected by oxidative stress:

- Under high oxygen partial pressure (double normal for cell culture), telomere loss rate is 5X higher.
  - 500 bp x 4 doublings = 2,000 bp loss
  - Cells stop dividing when telomeres shorten to 4kb after a few doublings.

(von Zglinicki et al., 1995)

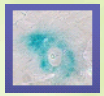
Telomere damage a source of shortening

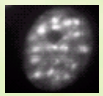
Telomere loss rate is affected by oxidative stress:

- In cell culture, telomeres shorten faster under high oxygen partial pressure, low under low oxygen partial pressure.
- In stationary phase cell culture, put the culture under oxidative stress:
  - Can detect DNA damage at telomeres, DNA nicks (single strand breaks).
  - DNA repair at telomeres seems poorer than for other DNA.

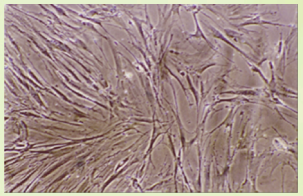
Senescence morphology

- Senescent cells become flattened, enlarged and have increased β-galactosidase activity
- Increased size of nucleus and nucleoli
- Increased number of multinucleated cells
- Increased number of lysosomes, Golgi and cytoplasmic microfilaments

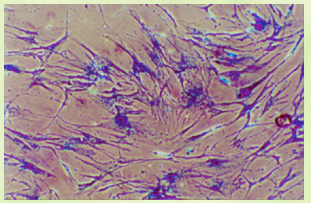




Senescent human fibroblasts

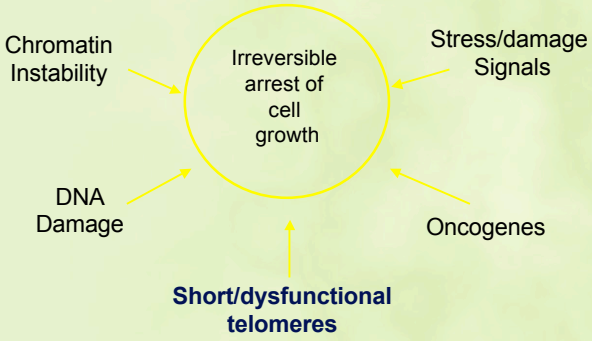


'Young'
Presenescent

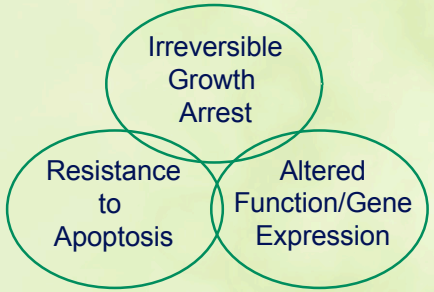


'Aged'
Senescent

Cellular Senescence: Arrests Cell Growth In response to Potential Cancer-Causing Events



Senescent/'aged' cells: Many characteristics change



Senescence is a progressive process

Table 12.2 **Progressive Differentiation of HH8 Fibroblasts and Alterations in Their Mitotic and Synthetic Capabilities**

		Distribution of cell types (%)							Macromolecular synthetic activity		
Mitotic?	Culture	I	II	III	IV	V	VI	VII	RNA	Protein	Collagen
Yes	PD17	18	70	10	1	0.5	0.5	0	1.00	1.00	1.00
Yes	PD30	3	78	16	2	0.5	0.5	0	1.33	5.6	1.4
Yes	PD55	0	1	79	14	3	3	0	3.36	7.9	4.3
No	PM 1 week	0	0	30	18	40	12	0	6.51	9.5	5.7
No	PM 3 week	0	0	3	31	43	23	0	9.93	11.2	6.2
No	PM 7 week	0	0	0	16	32	51	1	11.5	12.6	6.3
No	PM 11 week	0	0	0	4	5	87	4	9.5	12.4	9.6
No	PM 24 week	0	0	0	0	2	89	9	10.8	12.9	9.7
No	PM 40 week	0	0	0	0	0	88	12	11.6	11.8	10.2

Source: data from table 1 of Bayreuther et al. (1992).

Note: PD, population doubling generation number; PM, postmitotic weeks in culture; cell types I–VII are described in the text.

Fibroblasts grown in culture for 55 population doublings, about 300 days. Then they were maintained in culture an additional 300 days.

•Cell types:
- •I-III are mitotic.
- •IV-VI are post-mitotic and differ in size.
- •VII apoptotic or transformed.

Bayreuther et al., 1992a,b

Senescence is a progressive process

Table 12.3 **Age-dependent Distribution of Fibroblast Cell Types in the Skin of Human Donors**

	Fibroblast cell type (%)							
Age (years)	MFI	MFII	MFIII	Sum MF	PMFIV	PMFV	PMFVI	SUM PMF
10	15	59	13	87	3	1	9	13
30	7	54	12	73	11	3	13	27
50	0	38	30	68	6	7	19	32
70	0	6	39	45	14	5	36	55
90	0	2	12	14	21	8	57	86

Source: data from table 1 of Bayreuther et al. (1992).

Note: MF, mitotic fibroblast stage I, II, III; PMF, post-mitotic fibroblast stage IV, V, VI.

Fibroblasts samples from people of different ages.

•Cell types:
- •I-III are mitotic.
- •IV-VI are post-mitotic.

Bayreuther et al., 1992a,b

Telomere shortening: not universal

Some animals do not undergo telomere shortening but still age:

- Rabbits and hares (Forsyth et al., 2005).
- Some invertebrates:
  - *Drosophila melanogaster* (don't have telomeres).
  - *Podospora* (a filamentous fungus)

- Senescence can happen in the absence of telomere shortening, for example in Hutchinson-Gilford progeria.
- Senescence also occurs in non-dividing cells (ie., neurons).