Senescence and Immortalization

Lecture 18 November 3 2016
Assigned reading Chapter 10
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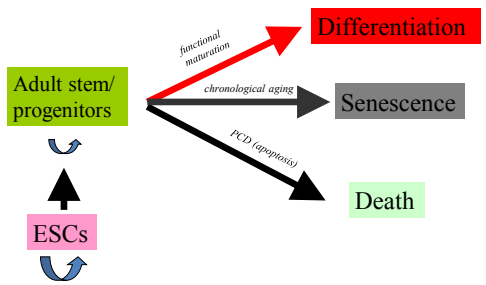
Normal Development



Slide Credit: Dr. Dean G Tang

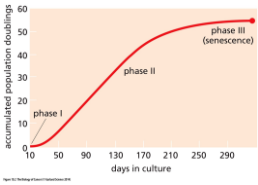
Immortalization, Senescence, Telomerase, and Cancer

1. Cell Senescence: Characteristics
2. Telomerase, Senescence, and Cancer

What is Senescence??

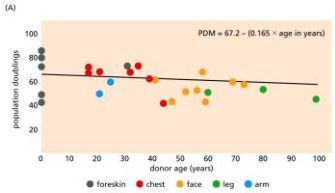
A state of cellular being characterized by:
a) metabolic activity but
b) irreversible loss of the capacity to enter active cell cycle
c) Growth factors help sustain viability but
d) Are unable to elicit usual proliferative response

Leonard Hayflick and Paul Moorhead (1961) first showed the phenomenon that cells would stop growing after a certain number of divisions.

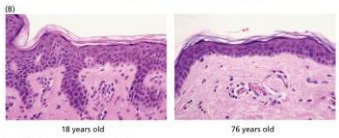


Phase I Cells divide actively to cover the culture dish
Phase II Cells proliferate robustly
Phase III Cells remain viable but do not proliferate

Normal cells have predetermined population doublings
Proliferative capacity decreases with age



The number of growth and division cycles is dictated by the species and tissue of origin and age of the donor.



Regenerative capacity declines with age.

Figure 10.4 The Biology of Cancer (© Garland Science 2014)

Characteristics of senescent cells

- Permanent growth arrest– can not be reversed by physiological stimuli
- Increased cell size– flat cells with huge cytoplasm (appearance of a fried egg)
- Increased cytoplasmic granularity
- Express senescence associated (SA) beta galactosidase
- Metabolically active

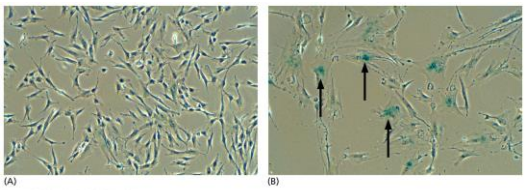
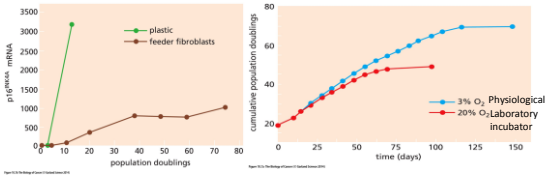


Figure 10.3 The Biology of Cancer (© Garland Science 2014)

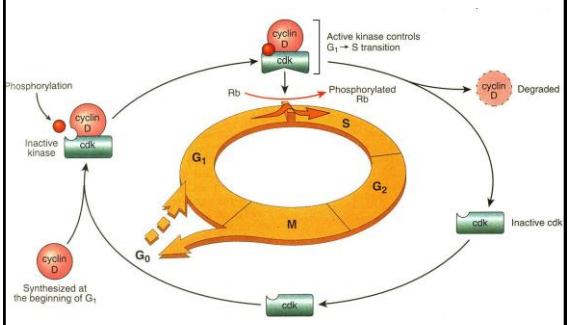
Influence of culture conditions



Higher Oxygen Levels → Reactive Oxygen Species → Oxidative damage

Hypoxia ⊣ Senescence

Senescent cells exhibit inability to enter active cell cycle



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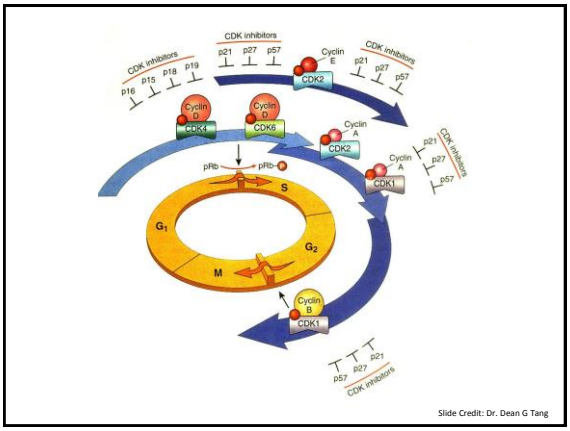

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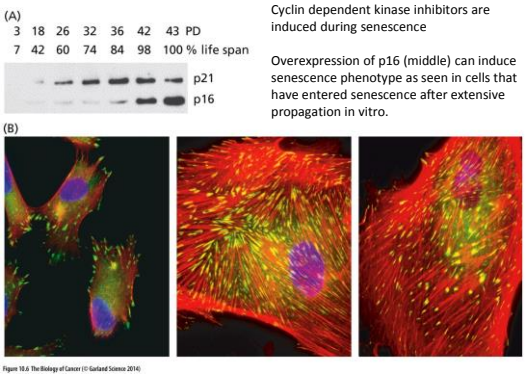
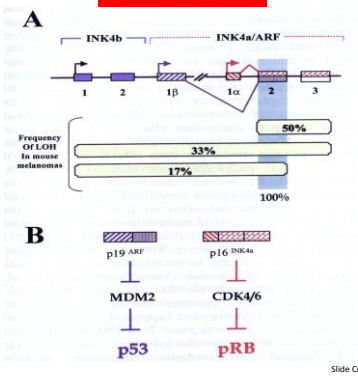


Figure 10.6 The Biology of Cancer (© Garland Science 2014)

Cyclin dependent kinase inhibitors are induced during senescence

Overexpression of p16 (middle) can induce senescence phenotype as seen in cells that have entered senescence after extensive propagation in vitro.

The INK4 locus



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Two major tumor suppressors p53 and pRb regulate senescence

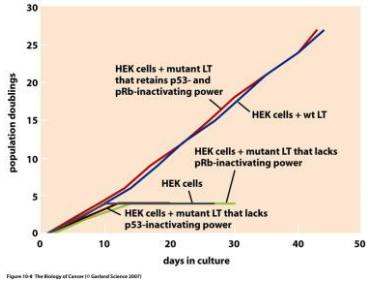
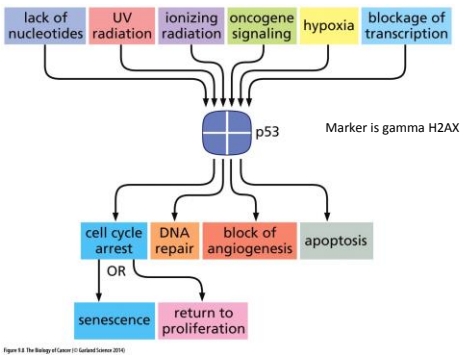


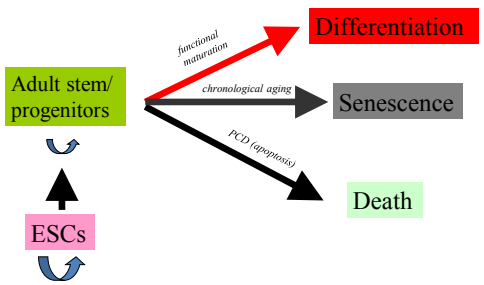
Figure 10-8 The Biology of Cancer (© Garland Science 2007)

Inactivation of both p53 and pRb is needed to escape from senescence
Both p53 and pRb are inactivated in majority of human cancers

DNA damage response can activate cell senescence



Normal Development



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Comparison between terminally differentiated and senescent cells in culture

	Differentiated	Senescent
Morphology	generally big	big & flat
SA-βgal positivity	-	+
Motility	little or low	little
Saturation density	low	low
Differentiation markers	+	+/-
Differentiation functions	+	-
Cell cycle	"permanent" G_0/G_1	"permanent" G_0/G_1
Response to mitogens	low	low
Karyotypic stability	high	low

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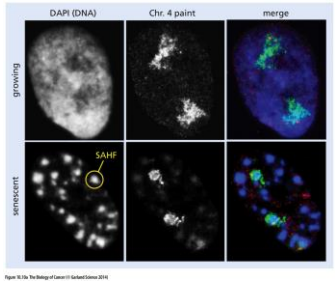
Senescence associated heterochromatic foci (SAHFs)



Markers are:
H3K9me
HP1gamma

Senescence & Senescent Cells

1) Big and flat, prominent cytoplasmic/nuclear vacuolization, less motile, decreased saturation density, and positive for SA-βgal.
2) Multiple alterations in gene expression, e.g., overexpression of collagenase and underexpression of TIMPs (tissue inhibitor of metalloproteinase).
3) Attenuated proliferative response to mitogens (EGF, PDGF, IGF-1) and unable to induce c-fos (but myc and ras induction ok).
4) "Irreversible" cell-cycle arrest at G_1/S with 2N nuclear content (but increased nuclear size); <1 PD in 2 weeks.
5) Decrease in positive regulators (cyclin D/Cdk4, cyclin E/Cdk2, etc) and increase in negative regulators (p16, p21, $p19^{ARF}$, hypo-phosphorylated RB, etc).
6) Resistance to apoptosis induction.
7) Senescent cells, to a degree, resemble terminally differentiated cells.
8) Presenescent cells often show telomere dysfunction as revealed by markers ATM activation and formation of nuclear foci containing H2AX-γ, 53BP1, MDC1,NBS1, which disappear in fully senescent cells.*
9) Fully senescent cells often possess karyotypic instability: tetraploidy, endoreduplication, aneuploidy, and other abnormal karyotypes.
10) Cellular senescence, like aging, is dominant. Therefore, immortality results from recessive changes in negative regulators (tumor suppressive genes).
11) Senescent cells accumulate senescence-associated heterochromatin foci (SAHFs), in which HMG-A proteins accumulate.
12) Senescent cells release pro-inflamatory cytokines (interleukins, IGFBPs, and TGF-beta) that act in an autocrine manner to promote senescence and in a paracrine fashion to recruit pro-inflamatory cells to promote tumorigenesis.

*Bakkenist CJ, Drissi R, Wu J, Kastan MB, Dome JS. Disappearance of the telomere dysfunction-induced stress response in fully senescent cells. Cancer Res. 2004 Jun 1;64(11):3748-52.

Slide modified from Dr. Dean G Tang

So how do we explain these effects seen in senescent cells?

Telomere

** 1978: Telomere was first found as an unusual repeated sequence motif (GGGGTT) at chromosome termini in the ciliate Tetrahymena (Blackburn & Gall, J. Mol. Biol. 120, 33-53, 1978).
**Tremendous variability: <50 bp in the hypotrichous ciliates but as long as 5100 kb in mice.
**1985-1989: Telomerase activity and telomerase uncovered (Greider CW and Blackburn EH, Cell 43, 405-413, 1985; Cell 51, 887-898, 1987; Nature 337, 331-337, 1989).

**In humans, telomeres are made up of an average of 5,000 -15,000 bp of G-rich (TTAGGG)n repeats and telomere-binding proteins.
**Each cell division loses 50-100 bp of telomeres
**When a telomere loses a critical number of base pairs, it triggers a DNA damage signal to stop cell division and initiate senescence.

Telomere functions:

--- form specific complexes with telomere binding proteins
--- protect chromosome ends from exonuclease digestion
--- prevent aberrant recombination
--- prevent the chromosome ends from activating cell-cycle and DNA damage checkpoints

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Telomere and the chromosome

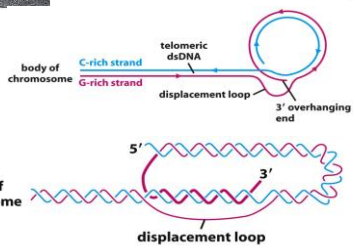
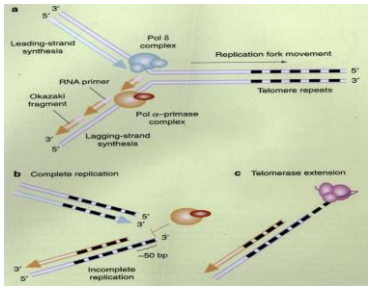


Figure 10-17: The Biology of Cancer (© Garland Science 2007)

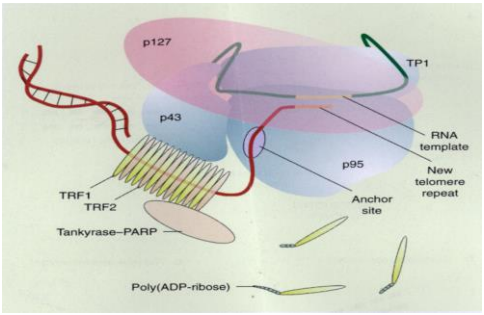
Telomere Synthesis



End replication problem:
Watson JD. Nature New Biol. 239, 197-201, 1972.

Slide Credit: Dr. Dean G Tang

Telomere and Telomerase



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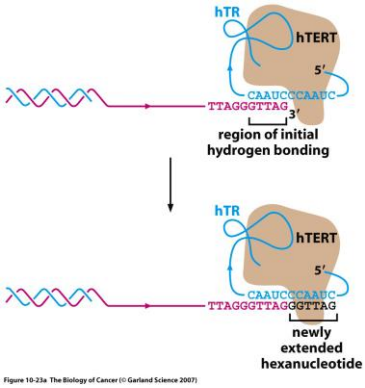


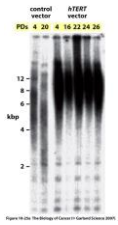
Figure 10-23a The Biology of Cancer (© Garland Science 2007)

Telomere and Telomerase Assays

**Direct measurement of telomere length

A. TRA (terminal repeat assay)
1. Digest DNA with Alu I or Hinf I.
2. Perform Southern with radiolabeled (TTAGGG)n

B. Q-FISH or flow cytometry using fluorescently-labeled probes
1. Metaphase spreads are hybridized with Cy3-labeled PNA $(CCCTAA)_3$ telomeric oligonucleotide.
2. Telomere fluorescence intensity analyzed by TFL-Telo.
3. 1 TFU = 1 kb telomere (PNAS 94, 7423-7428, 1997).
4. Flow cytometry is performed using similar procedures.



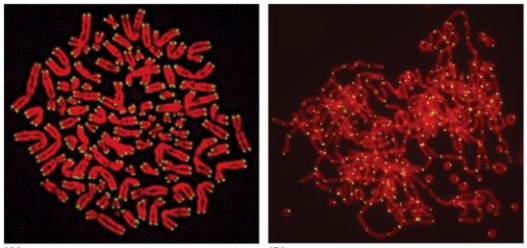
**TRAP (telomeric repeat amplification protocol)

(*Kim et al., Science 266, 2011-2015, 1994; Kim and Wu, NAR, 25, 2595-2597, 1997*)

1. Prepare cell lysates (in CHAPS buffer).
2. Add an end-labeled telomere-specific oligonucelotide substrate
(TS primer) to the lysates.
3. If telomerase is present, it adds TTAGGG repeats to the substrates.

Slide Credit: Dr. Dean G Tang

Loss of TRF2 leads to extensive end to end fusion



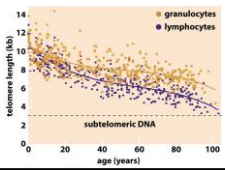
(A) WT (B) TRF2 deprived

Figure 10-11 The Biology of Cancer (© Garland Science 2007)

TRF2 is a key protein in maintaining normal telomere structure

Telomere, senescence and tumorigenesis

--- Telomeres of cultured somatic cells continuously erode until M1
--- Telomeres derived from elderly individuals tend to be shorter than those derived from young donors
---Telomeres derived from constant self-renewing tissues such as liver and GI systems tend to be shorter than most other tissues and organs.
--- Telomere length is a predicator of proliferative potential
---If M1 is overcome by transformation with viral oncogenes, telomeres continue to decrease in size until M2, a process that may be dictated by telomere length itself.
--- Whereas telomere size continuously decreases during replicative senescence, immortalized cells reach an equilibrium, albeit at shorter-than-wild-type length



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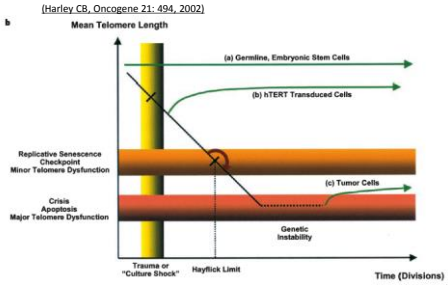
Telomere, Telomerase and the Hayflick limit

(Harley CB, Oncogene 21: 494, 2002)



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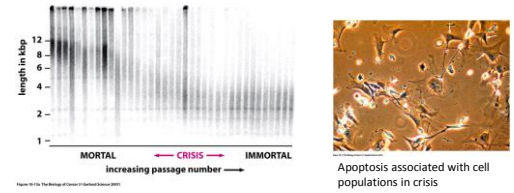
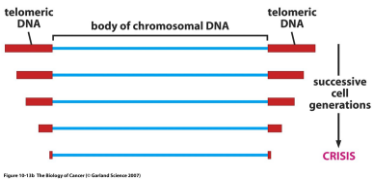
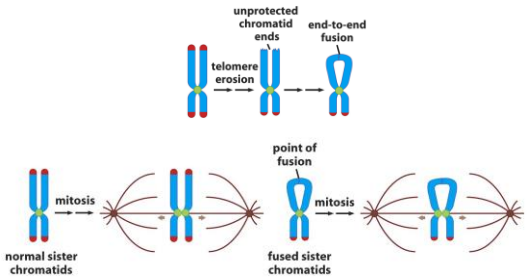


Apoptosis associated with cell populations in crisis



BFB (Breakage-Fusion-Bridge) Cycle



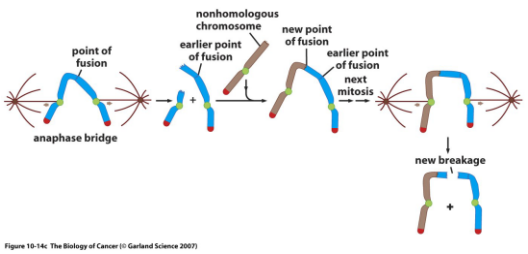
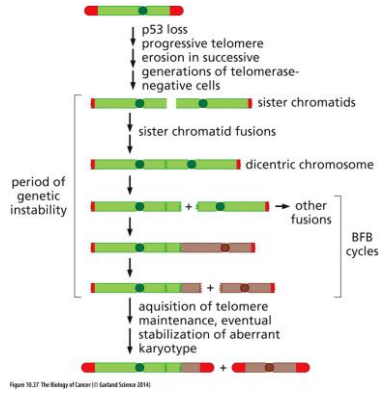
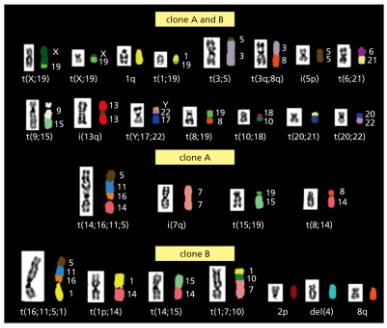


Figure 10-14c The Biology of Cancer (© Garland Science 2007)



Karyotypic chaos seen in human bladder cancer cell line



Critical Differences in Human vs Rodent Cells

	Mouse	Human
Population Doublings (PD)	10-30	60-80
Telomere length	60-100 kb	10-15 kb
Involvement of ARF-mdm2-p53	Yes	Yes
Involvement of p16-cln D-pRb	No	Yes
Telomerase activity in somatic cells	Yes	No/low
Rate of spontaneous immortalization	Very high (10^{-4}-10^{-5})	Low (10^{-7})
Conclusion	Premature senescence Induced by inappropriate culture conditions	Related to telomere shortening

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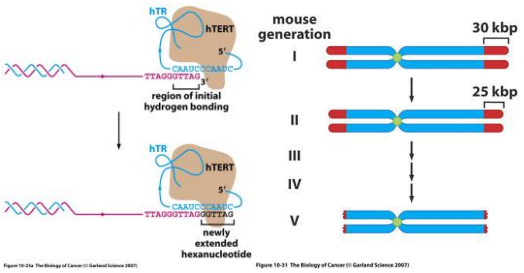

Figure 10-31 The Biology of Cancer (© Garland Science 2007)

Is telomere shortening really important: The mTR$^{-/-}$ mouse model

[1]mTR$^{-/-}$ mice lacked detectable telomerase activity yet were viable for the 6 generations analyzed. Telomerase-deficient cells could be immortalized in culture, transformed by viral oncogenes, and generated tumors in nude mice following transformation. Cells from the 4th mTR$^{-/-}$ generation onward possessed chromosome ends lacking detectable telomere repeats, aneuploidy, and chromosomal abnormalities including end-to-end fusions.

[2]Late-generation mTR$^{-/-}$ mice show defects (decreased proliferation and increased apoptosis) in high-renewable organ systems such as spermatogenesis and hematopoietic cells in bone marrow and spleen.

[3]mTR$^{-/-}$ ES cells slow down their proliferation after ~300 divisions and completely stop proliferation after 450 divisions.

[4]Late-generation mTR$^{-/-}$ mice demonstrate shortened telomere and genetic instability, shortened life span and reduced capacity to respond to stresses such as wound healing and hematopoietic ablation. There was increased incidence of spontaneous malignancies.

1. Blasco et al., Cell 91, 25-34, 1997.
2. Lee et al., Nature 392, 569-574, 1998.
3. Niida et al., Nature Genetics, 19, 203-206, 1998.
4. Rudolph et al., Cell 96, 701-712, 1999.

Slide Credit: Dr. Dean G Tang

Is telomere shortening really important: The mTR$^{-/-}$ mouse model

[1]mTR$^{-/-}$ significantly reduces tumor formation in p16^{INK4A}/p19^{ARF} null mice. Reintroduction mTR into cells restored the oncogenic potential, suggesting that telomerase activation is a cooperating event in the malignant transformation of cells containing critically short telomeres. Loss of telomere function impairs, but does not prevent tumor formation.

[2]Late-generation mTR$^{-/-}$ cells show severe telomere shortening, genomic instability, and p53 activation, leading to cell-cycle arrest and/or apoptosis. The *mTR$^{-/-}$ p53$^{-/-}$* mice showed significantly increased rate of epithelial cancer formation.

[3]mTR$^{-/-}$ mice show rapid liver cirrhosis when subjected to genetic, chemical, and surgical ablation. Telomerase gene delivery alleviated cirrhotic pathology and restored liver function.

[4]Telomere dysfunction in late-generation *mTR$^{-/-}$* mice impairs DNA repair and enhances sensitivity to ionizing radiation.

[5]Telomere dysfunction, together with p53 deficiency, promotes non-reciprocal translocations and epithelial cancers in mice.

1. Greenberg et al., Cell 97, 515-525, 1999.
2. Chin et al., Cell 97, 527-538, 1999.
3. Rudolph et al., Science 287, 1253-1258, 2000.
4. Wong et al., Nature Genetics 26, 85-88, 2000.
5. Artandi et al., Nature 406, 641-644, 2000.

Slide Credit: Dr. Dean G Tang

Human vs Mouse Tumors

1. The majority of mouse tumors are sarcomas and leukemias whereas 80% of the human tumors are carcinomas - cancer of epithelia where rapid cell turnover occurs.
2. Most of the experimental therapeutics that work in mouse fail in human, why???
3. The answer may partly lie in the behavior of telomeres, and the relationship between telomere shortening, replicative cell senescence, and genetic instability.
4. In human, telomerase is suppressed or shut down and telomere shortening leads to replicative cell senescence. In mice, cells have long telomeres and retain telomerase activity, thus no telomere-dependent replicative senescence. However, in the 5-6th generation of $TERC^{-/-}$ cells, the mice begin to show various abnormalities, including increased incidence of cancer, raising the possibility that natural telomere shortening helps

Slide Credit: Dr. Dean G Tang

Telomerase-independent telomere maintenance

**A significant number of immortal or tumor cell lines have no detectable telomerase activity and also no defect in proliferation and growth.
**These cells have unusually long telomeres (up to 50 kb; ~30 kb longer than that observed in the longest telomerase-positive cell lines).
**The ALT (alternative lengthening of telomeres) pathway of telomere maintenance (EMBO J., 14, 4240-4248, 1995; Nature Genetics, 26, 447-450, 2000). ALT occurs by means of homologous recombination and copying switching (i.e., DNA sequences are copied from telomere to telomere).

Slide Credit: Dr. Dean G Tang

Telomere-independent functions of telomerase

1. Telomerase is anti-apoptotic (Cao et al., *Oncogene* 21, 3130-3138, 2002).
2. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism (Stewart et al., *PNAS* 99, 12606, 2002; Chang and DePinho, *PNAS* 99, 12520-12522, 2002).
3. Telomerase enhances DNA repair and genomic stability (*Oncogene* 22, 131-146, 2003).
4. TERT promotes cellular and organismal survival independently of telomerase activity. Lee J, Sung YH, Cheong C, Choi YS, Jeon HK, Sun W, Hahn WC, Ishikawa F, Lee HW. Oncogene. 2008 Jun 12;27(26):3754-60.

Slide Credit: Dr. Dean G Tang

Dysregulation of Telomerase during Tumorigenesis

1) Telomerase activity and hTERT (telomerase reverse transcriptase) expression are low or absent in most somatic cells and primary tissues, due to
   a) transcriptional repression by WT1 and Mad,
   b) transcriptional repression by histone deacetylation.

2) In immortalized or cancer cells, hTERT activity is 'reactivated' due to
   a) transcriptional upregulation by myc, E2F1 etc,
   b) gene amplification,
   c) various signaling pathways such as c-Abl, bFGF, 14-3-3, Hsp90, Akt, PKC, etc,
   d) epigenetic chromatin remodeling.

3) Telomerase activity is normally associated with proliferation: cycling cells have high while differentiating cells have low telomerase activity. Due to this correlation, normal cells have relatively longer telomeres than tumor cells because the latter have undergone more cell divisions.

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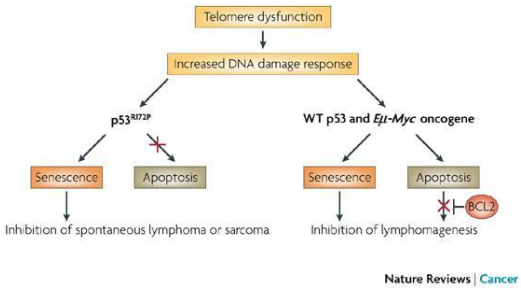
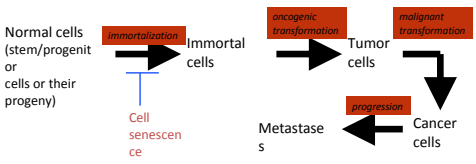
Evidence of senescence as a tumor suppression mechanism



Tumor Development



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Senescence as a Therapeutic Alternative

1. DNA damage is able to induce senescence in p53-wt tumor cells in vitro and in vivo. p53 and p21 appear to play a critical role in the onset of senescence while p16 is involved in maintenance of senescence (te Poele et al., *Cancer Res.* 62, 1876-1883, 2002).
2. Senescence induction appears to contribute significantly to the efficacy of anti-neoplastic drugs (Schmitt et al., *Cell* 109, 335-346, 2002; *Cancer Cell* 1, 289-296, 2002; *JCI*, 113, 169-174, 2004).

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