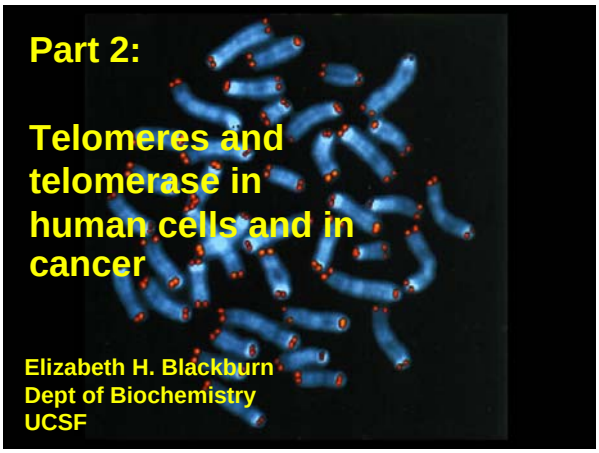
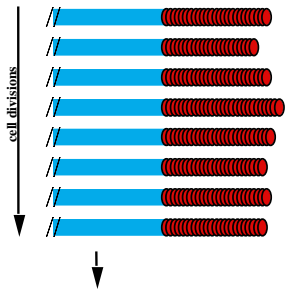


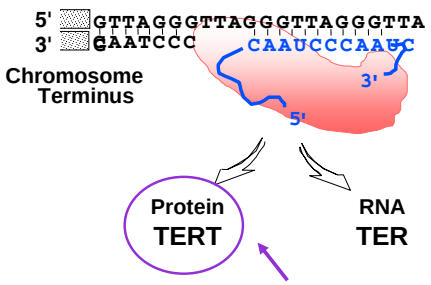
Plenty of telomerase: homeostasis balanced



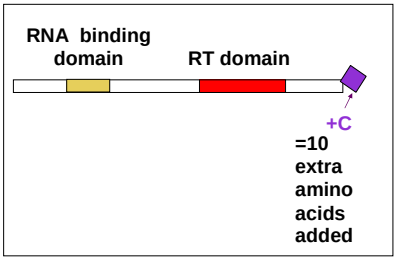
Cells keep dividing

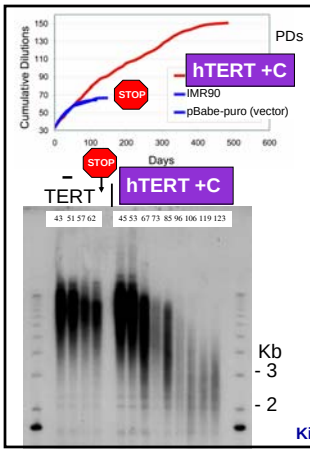
It's not just bulk telomere length…

An experiment in cultured human cells



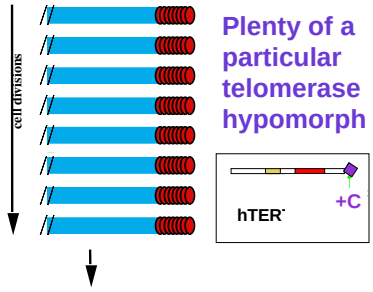
hTERT protein hypomorph +C





hTERT +C allows human primary IMR90 fibroblasts to proliferate with short telomeres

Kim, Xu and Blackburn, 2003



Despite their SHORT bulk telomere length, so it must be telomerase

Zhu et al, 2001; Kim et al, 2003

A Protective Function for Telomerase II

Telomerase stabilizes telomeres that would be too short in its absence

In humans?

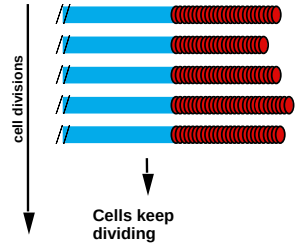
Telomerase in humans

- Telomerase is on during fetal development and remains active in various proliferative cells
  - stem cells, activated lymphocytes, hair follicles, etc

Telomerase in humans

CONTINUED…

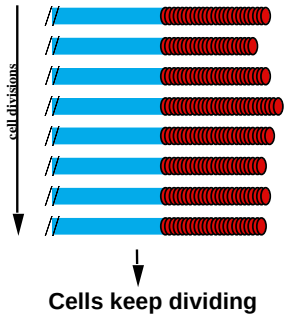
- Telomerase is down-regulated, **BUT STILL DETECTABLE**, in many other adult cells
  - epithelial
  - fibroblast
  - endothelial
- Telomerase is **HIGH** in 80-90% of invasive cancers



Telomeres replenished by telomerase

- Active: stem cells, germ cells
- Detectable: many normal adult cell types (regulated activity)
- Highly active: ~90% of human tumors

Telomerase is highly active in cancer cells

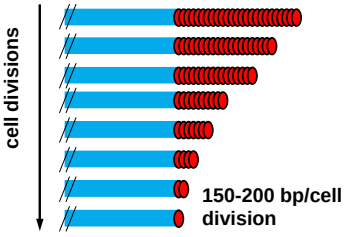


Cells keep dividing

What is expected to happen without Telomerase?

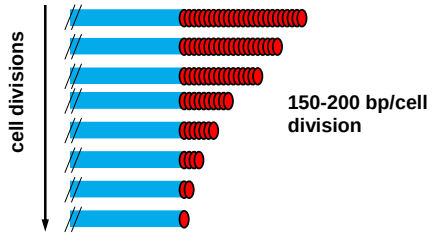
- As cells multiply, their telomeres progressively lose DNA from the ends
- After a delay, when the telomeres become too short, cells eventually cease to divide
  - senescence or cell death

OLD - Predicted, if inhibit or knock down telomerase:



- only gradual shortening of all telomeres
- DELAY before eventual senescence or apoptosis

OLD - Observed, if inhibit ~~or knock-down~~ telomerase enzyme, *but keep RNP level high*:

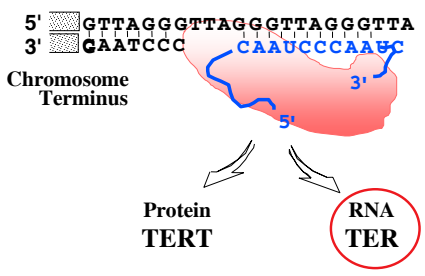


- only gradual shortening of all telomeres
- DELAY before eventual senescence or apoptosis

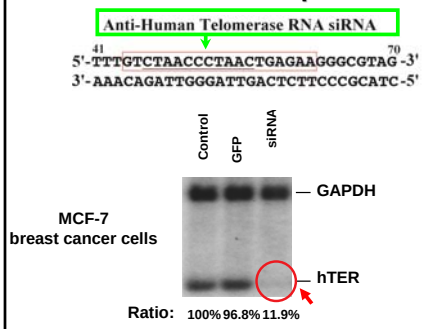
NEW - Knock-down of telomerase RNA: RAPID growth inhibition of human cancer cells

Li et al, Cancer Res 2004; Li et al, 2005

Telomerase: a telomere-synthesizing reverse transcriptase

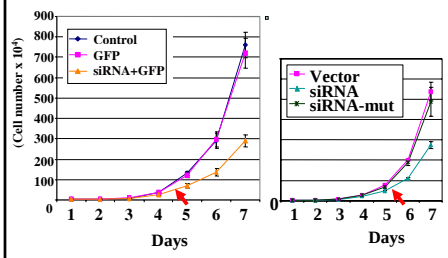


Knockdown of human telomerase RNA-hairpin siRNA



Li et al. 2004, 2005

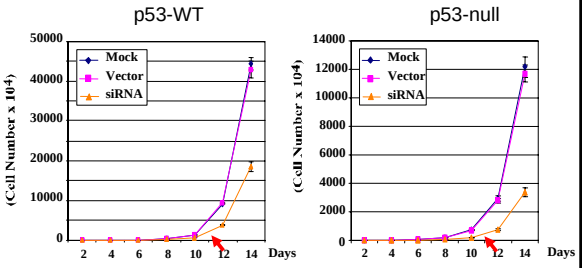
Lentiviral-expressed anti-hTER siRNA (RNAi) RAPIDLY inhibits growth of LOX melanoma cells



(bulk unselected cell populations)

Li et al 2005

Anti-hTER RNAi effects don't require p53



(bulk unselected HCT116 cell populations)

Li et al 2005

Telomerase knock-down by RNAi

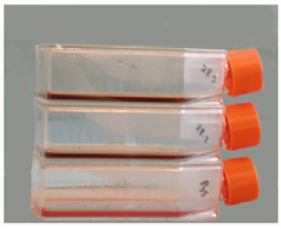
- RAPIDLY inhibits cancer cell growth and p53 is not required for this
- No telomere uncapping or DNA damage response

Does the rapid knock-down of telomerase uncap telomeres, thereby causing the growth inhibition of human cancer cells?
NO

Shang Li and E.H. Blackburn, 2004

Increased melanin production in melanoma cells expressing an anti-telomerase RNA ribozyme

Differentiation induced!



Rz Clone 5

Rz Clone 2

Pooled vector

Li et al. 2004; Bagheri et al 2006

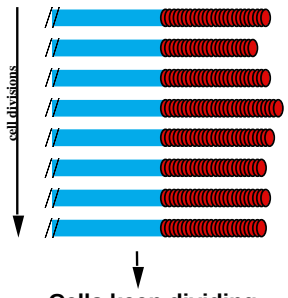
Effects of telomerase knock-down on metastasis?

YES

Knock-down of telomerase (ribozyme- or siRNA-mediated) inhibits metastasis in *in vivo* mouse melanoma models

Kashani-Sabet et al, 2004; Nosrati et al 2005; Baghari et al 2006.

Plenty of telomerase:homeostasis balanced



Cells keep dividing

Telomerase knock-down in cancer cells

RAPIDLY inhibits cancer cell growth
p53 is not required for this

NO telomere uncapping or DNA damage response

Metastasis is reduced

Li et al, Cancer Res 2004; Li et al, 2005; Bagheri et al 2006

Telomerase knock-down in cancer cells

Metastasis is reduced
HOW?

RAPIDLY downregulates cell cycle and tumor progression genes

Glucose metabolism downregulated

Cell differentiation program induced?

Li et al, Cancer Res 2004; Li et al, 2005; Bagheri et al 2006

High telomerase levels promote an undifferentiated, "stem-cell-like" phenotype?