Telomere dysfunction and fusion in chronic lymphocytic leukaemia

Duncan Baird
Telomere structure

TRF1 complex - Telomere length control
- Repression of TRF1 - telomere elongation.

TRF2 complex - Telomere capping
- Dominant negative TRF2 - chromosomal fusions
- Represses DNA damage response (ATM)
- Represses NHEJ (via T-loop)

Stansel et al. EMBO 2001

The end replication problem


- Predicted losses of DNA at the terminus
- Which could ultimately result in the loss of genetic information and senescence

Various solutions to this problem

- Convert to circular DNA
- 5’ terminal protein
  - provides an OH group to prime synthesis
- 3’ hairpin → concatemers
- Replace the lost sequences
  - Terminal specific transposition
    - Drosophila
  - Unequal recombination
  - Enzymatic addition of repeats

From Molecular Biology of the gene
**Telomerase expression**

**Negative**
- Mortal primary cell strains
- Most human tissues

**Positive**
- Male and female germ line
- Most immortal cell lines
- 85% of malignancies
- Stem cells

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Kolquist et al. 1998
Normal human cells are not immortal:

- Limited replicative capacity- ‘Hayflick’ limit
- After 60-80 PD (fibroblasts)- replicative senescence

In vitro cultured fibroblast cells- TRF analysis

In the absence of telomerase, telomeres erode

Fibroblasts appear to senescence when telomere length is around 4-6kb

Telomerase expressed- Cells immortalised

Bodnar et al. 1998
Senescence and crisis

Telomeres and tumour suppression/progression?

Telomeres play a dual role in tumourigenesis

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Evidence for telomere crisis? - humans

Colorectal Carcinoma

Non reciprocal translocations

Telomere shortening

Telomerase activation

Anaphase bridging

Adenocarcinoma colon T.Ried SW837
National Cancer Institute-NCBI – SKY/M-FISH & CGH Database

Hastie et al Nature 1990

Chadeneau et al Cancer Research 1995

Rudolph et al Nat. Genet 2001
Telomere dynamics

Gradual telomere erosion
- Decrease in the mean
- Increase in the variance
Consistent with the end-replication problem

Stochastic telomeric deletion
- Severely truncated telomeres
  - <20 TTAGGG repeats
- Sporadic
- Do not accumulate
  Inconsistent with the end-replication problem

Short telomeres in senescent cells
- Shortest distribution 500bp
- Triggers DNA damage checkpoint

Allelic variation
- Long allele (6 kb)
- Short allele (1 kb)

MRC5 fibroblast clone

Our current interests....

- Mechanisms of instability
- Telomere length variation
- cis-acting determinants of telomere length
- Definition of a dysfunctional telomere
- What is the fate of a short telomere?
- Mechanistic basis of telomere fusion?
- Can telomere dynamics drive neoplastic progression?
  - Haematological malignancies
  - Solid tumours
- Does telomere length and fusion have diagnostic/prognostic value?
Definition of a dysfunctional telomere
Is telomere length important?

Are these telomeres dysfunctional and capable of fusion?
At what length do telomeres become dysfunctional?

Fusion assays now expanded to include 1/3 telomeres in the genome.
Sequence of telomere fusions

Complex fusion events

Large 1-3 kb insertions of non-telomeric genomic sequences 4/298 (1.3%) - close to fragile sites

Inverted insertion – too complicated to explain

Sister chromatid fusion

Ring chromosomes

Subtelomeric deletion

Mean deletion 2.5 kb

Deletion and micro-homology - consistent with Ku-independent end joining

TTAGGG repeats at fusion point

Mean 5.8 repeats
Telomere fusion - Key findings

1. Fusion detected in both checkpoint deficient and competent cells
2. Telomeres are short, mean of 5.8 TTAGGG repeats
3. Stochastic telomere deletion creates telomeres that are capable of fusion – accounts for fusion in normal cells with long telomeres
4. Ring chromosomes/insertions/complex insertions etc
5. Fusion events can be clonal
6. Microhomology at fusion point
7. Large deletions up to the limits of our assays (6 kb) – probably further
8. Mutational profile consistent with Ku-independent error-prone NHEJ
Can telomere erosion, dysfunction and fusion drive genomic instability during neoplastic progression?
Chronic lymphocytic leukaemia

- Most common form of leukaemia in adults
- Highly variable clinical course
- Evidence of telomere erosion
- NRTs are common chromosomal aberrations-
  - Deletion of 17p and 11q - high risk
Telomere length-CLL
Telomere length and clinical staging-CLL
Complete telomere loss-CLL
Telomere fusion-CLL
Telomere fusion-CLL

![Graph showing XpYp TTAGGG (kb) distribution across stages A, B, and C. The graph indicates a significant difference (p<0.0001) among the stages. Different markers represent XpYp Fusions and No XpYp Fusions.]
Telomere fusion-CLL

Profile indistinguishable from the in vitro data
Does telomere instability = genome instability?
What might be happening in CLL?

Ongoing cell division - with or without increases in WBC

- Fully functional checkpoints
- Telomere shortening = Apoptosis or Senescence

Telomere erosion

- Telomere dysfunction and fusion
- Anaphase-bridging, breakage and fusion = genomic instability
- Loss of checkpoint control = further telomere erosion
Telomere dynamics in CLL

• Substantial telomere erosion-
  • Prior to disease progression
  • Consistent with significant cell division
• Erosion exacerbated by ATM mutation
• Telomere length distributions are homogenous
  • Clonal growth
• Stage C patients exhibit short telomeres (mean 1.58 kb)
  • Shortest yet recorded in humans
  • Similar telomere dynamics to that observed in cells undergoing ‘crisis’ in culture
  • Telomere length as determined with STELA may a useful prognostic indicator
• Telomere fusion is detected between short telomeres
  – Frequency as high as that observed in cells in ‘crisis’ in vitro.
  – Some of which are clonal
• Telomere dysfunction is associated with genomic instability

Poor prognosis CLL patients undergo a telomeric crisis
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