Telomere dysfunction in breast differentiation, aging and cancer

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Part I: The hypothesis

Consequences of telomere dysfunction in cancer: an early event?

Capped telomere → Uncapping event → Telomere fusion → Genomic instability

Breakage-fusion-bridge (BFB) cycle - McClintock, Genetics, 1941
Telomere dysfunction in cancers

- Knowledge gap: no direct evidence that telomere dysfunction occurs in human cancers

- A novel assay to detect telomere fusions to understand and monitor disease progression
Detection and analysis of fusion junction

Normal chromosomes

Dicentric chromosome

Telomere dysfunction

TAR Fusion PCR

Fusion point

Multiplex: primer sets

telomere adjacent seq.

primer

PCR product

Detection
TAR-Fusion PCR multiplex primers

**NOTE:**
TAR-PCR primers cover ~40% of ends but only ~15% of possible end-to-end combinations.
Telomere fusions present early in breast tumorigenesis

1. ~40% of tumor tissue
2. Only 15% of possible fusions covered by TAR fusion PCR
3. Stabilization at or before DCIS stage
**Summary: fusion junctions in breast tumor tissue**

<table>
<thead>
<tr>
<th>Junction Type</th>
<th>DCIS</th>
<th>Invasive</th>
</tr>
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<tbody>
<tr>
<td>Telomere to Telomere</td>
<td>7.7% (1/13)</td>
<td>6.7% (1/15)</td>
</tr>
<tr>
<td>Telomere to Subtelomere</td>
<td>92.3% (12/13)</td>
<td>80.0% (12/15)</td>
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<tr>
<td>Complex</td>
<td>0% (0/13)</td>
<td>13.3% (2/15)</td>
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*Hallmark of solid tumors: shortened telomeres and activated telomerase*
Short insertions of retrotransposon elements at invasive breast tumor tissue fusion junctions
Conclusions: fusion junction results

- Telomere fusions (dysfunction) found early in human breast tumors (DCIS).
- Potentially a highly prevalent genetic marker for tumorigenesis: breast, prostate*, ovarian*, CLL, others?

Tanaka, Abe, Huda, Tu, Beam, Grimes and Gilley, Telomere fusions in early human breast carcinoma. PNAS (2012)
Part II:

Telomere plasticity in normal human mammary repopulating cells

Note:
1) Critical to determine the biology of normal repopulating subpopulations before comparing to tumor subpopulations

2) Uncovering possible mechanisms of disease resistance and initiation
Human mammary cellular hierarchy

Cell of origin: Luminal progenitors?

Mammary stem cell (BC) → Bipotent progenitor (BC) → Luminal progenitor (LP) → Mature Luminal Cells (LC)

Mammary stem cell (BC) → Bipotent progenitor (BC) → Myoepithelial Progenitors (BC) → Mature Myoepithelial Cells (BC)

(Lim et al., 2009 Nat. Med.)
(Molyneux et al., 2010 Cell Stem Cell)
Subpopulation isolation from reduction mammoplasty

Enzymatic dissociation
trypsin/dispase/DNase

Single Cells

FACS purification
CD31- CD45-

EpCAM PE

CD49f APC

LC
LP
SC
BC
Telomere length plasticity in isolated breast subsets

20 year old

- Mammary stem cell (BC)
- Luminal progenitor (LP)
- Bipotent progenitor (BC)
- Mature Luminal Cells (LC)
- Myoepithelial Cells (BC)
- Mature Myoepithelial Progenitors (BC)

TRF

qPCR
Age-dependent telomere length shortening in the mature luminal cells.

$\text{HT1080 ladder}$

$\text{LC TRF (kb)}$

$\text{age (years)}$

$r^2 = 0.50$
Telomerase activation in luminal progenitors

<table>
<thead>
<tr>
<th>HT1080</th>
<th>HeLa</th>
<th>CHAPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>LP</td>
<td>LC</td>
</tr>
<tr>
<td>BC</td>
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</table>

TRF2  hTERT  merge

BC  LP

hTERT / TRF2

RTA (%)

Frequency of co-foci

p < 0.001
Reduced activation of telomerase in luminal progenitor cells with age

- Telomerase activity decreases with age of luminal progenitor cells

  \[ r^2 = 0.54 \]

- Telomere lengths decrease with age of differentiated luminal cells

  \[ r^2 = 0.50 \]
Telomere-associated DNA damage response in normal luminal progenitor cells

LP fraction
BP Fraction

Telomere-associated proteins

MRN complex
MRE11
Rad50
NBS1

RT-PCR confirmation
mRNA expression: Telomere and DDR genes in basal versus luminal progenitors

- MRE11
- RAD50
- ATM
- ATR
- BLM
- RAP1
- DNA-PKcs
Telomere dysfunction-induced DNA damage foci in normal human mammary LPs.

Similar results w/ 53BP1, NBS1, MRE11 and RAD50
Telomere-dysfunction DNA damage response in normal luminal progenitors
Normal breast differentiation: a double edged sword?

*Hallmark of solid tumors: shortened telomeres and activated telomerase
Disease consequences of telomere dysfunction
Cancer: Blood-borne and solid tumors (CLL, breast, prostate*, ovarian*)

Cardiovascular: telomere shortening associated with hypertension, CVD

Environmental factors & telomere dysfunction
(-via telomere shortening, oxidative damage, epigenetic remodeling, others)
Exogenous: smoking, obesity, socio-economic group, etc…
Endogenous: stress, meditation, exercise, etc….

Now include: normal cellular differentiation
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Cancer stem cell hypothesis

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