PML is required for telomere stability in non-neoplastic human cells

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Acute Promyelocytic Leukemia (APL)  
15;17 translocation, PML/RARα fusion protein

APL cells have short telomeres!
Telomere maintenance in Mammalian Cells

- **Replicating Somatic cells**: Gradually lose telomeric sequences
- **Germ and Stem cells**: Maintain telomeres length through Telomerase activity
- **Cancer cells**: 85-90% Telomerase-positive cells maintain telomeres through Telomerase reactivation, 10-15% ALT cells maintain telomeres through Alternative Lengthening of telomeres involving PML
Hallmark of the ALT cells: ALT associated PML bodies (APBs)

PML colocalize with telomere DNA

Is this the enhancement of a physiologic function of PML?
PML localizes at telomeres in normal and telomerase positive cells

Immuno-FISH w/telomere probe and α-PML Ab
Low frequency of PML/Telomere colocalization in Normal and Telomerase+ cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>n° cells scored</th>
<th>n° PML spots</th>
<th>n° PML/Tel coloc</th>
<th>PML bodies/cell (mean)</th>
<th>PML/Tel coloc/cell (mean)</th>
<th>PML/Tel coloc/cell (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI38</td>
<td>50</td>
<td>372</td>
<td>52</td>
<td>7,44</td>
<td>1,04</td>
<td>14</td>
</tr>
<tr>
<td>MRC-5</td>
<td>50</td>
<td>406</td>
<td>25</td>
<td>8,12</td>
<td>0,5</td>
<td>6</td>
</tr>
<tr>
<td>MEFs</td>
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<td>451</td>
<td>18</td>
<td>9,02</td>
<td>0,36</td>
<td>4</td>
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<tr>
<td>U937</td>
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<td>856</td>
<td>293</td>
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<td>5,86</td>
<td>34,2</td>
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<td>HeLa</td>
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<td>200</td>
<td>44</td>
<td>4</td>
<td>0,88</td>
<td>22</td>
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<tr>
<td>A549</td>
<td>50</td>
<td>77</td>
<td>20</td>
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<td>0,4</td>
<td>26</td>
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<tr>
<td>U2OS</td>
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<td>220</td>
<td>5,52</td>
<td>4,4</td>
<td>80</td>
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<tr>
<td>SK-LU-1</td>
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<td>1578</td>
<td>436</td>
<td>31,43</td>
<td>9,3</td>
<td>29,6</td>
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<tr>
<td>SA-OS-2</td>
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<td>312</td>
<td>181</td>
<td>6,26</td>
<td>3,62</td>
<td>58</td>
</tr>
</tbody>
</table>
Automated Confocal Analysis on High Cell Number

Labelling...
- DNA
- EdU (active S phase)
- pH2AX
- Telomeres
- PML

X: PML center
Y: Tel center
Adjacency Condition: dXY < rPML

N cells: 4137
N Tel/PML: 2690

Events/cell

Tot. PML NBs

PML/Tel
The telomeric localization of PML is not Cell Cycle–dependent
Damaged telomeres are labelled by $\gamma$-H2AX producing TIFs

$\gamma$-H2AX Signal  Telomere Signal

Damage marks at telomeres generate damaged Telomeres Induced Foci = TIFs
PML-Telomere association increases with fibroblasts senescence

- **Proliferating**
  - γH2AX
  - Tel
  - PML

- **Near Senescent**

**Graphs:**
- **TIFs**
  - P: 40
  - NS: 10
  - p < 0.0001
- **PML NBs**
  - P: 80
  - NS: 20
  - p < 0.0001
- **%PML/Tel**
  - P: 100
  - NS: 50
  - p < 0.0001
- **%PML/TIFs**
  - P: 80
  - NS: 20
  - p < 0.001
Automated Confocal Analysis on Proliferating or Near Senescent Fibroblasts

n early = 12397
n late = 15621
Role of PML in naturally dysfunctional telomeres of non-tumor cells

TERT/TERC mutations cause accelerated telomere shortening and organ failure in rare human diseases:

- aplastic anemia/myelodysplasia
- idiopathic pulmonary fibrosis (IPF)
- liver cirrosis
- dyskeratosis congenita

**CLINICAL CASE:**
52 Y/O woman with TERT mutation associated with idiopathic pulmonary fibrosis, liver disease, mild bone marrow failure
PML Telomere Association Increases in TERT-deficient T-cells

Age-matched control T-cells

TERT-mut T-cells

TIFs

\[ \text{Events/cell} \]

\[ \text{CTRL} \quad \text{Pt} \quad \text{CTRL} \quad \text{Pt} \]

\[ \text{Day7} \quad \text{Day21} \quad \text{Day7} \quad \text{Day21} \]

\[ P < 0.01 \]

PML NBs

\[ \text{Events/cell} \]

\[ \text{CTRL} \quad \text{Pt} \quad \text{CTRL} \quad \text{Pt} \]

\[ \text{Day7} \quad \text{Day21} \quad \text{Day7} \quad \text{Day21} \]

\[ P < 0.1 \]

\[ P < 0.1 \]

%PML/Tel

\[ \text{CTRL} \quad \text{Pt} \quad \text{CTRL} \quad \text{Pt} \]

\[ \text{Day7} \quad \text{Day21} \quad \text{Day7} \quad \text{Day21} \]

\[ P < 0.001 \]

\[ P < 0.0001 \]
RHPS4 Drug-induced specific telomere damage increases PML/Telomere association.
PML/Tel colocalization increase after TRF2 depletion

Telomeric damage leads to PML/TIF colocalization
PML knockdown by RNAi in normal human fibroblasts

CTRL  PML KD1  PML KD2

Day 0  Day 6  Day 10

CTRL  PML KD1  PML KD2  CTRL  PML KD1  PML KD2  CTRL  PML KD1  PML KD2

αPML

αPML

αActin
PML depletion induces a senescence phenotype in normal human fibroblasts.
PML depletion induces Telomere dysfunction (TIFs) in normal human fibroblasts

Human cells need the PML protein for the integrity of the telomeric structures
PML depleted fibroblasts showing TIFs activate p53

CTRL

PML KD1

TIF negative
p53 negative
p53 positive

γH2AX
p53
Tel

TIF negative
1 TIF/nucleus
2,3 TIFs/nucleus
>3 TIFs/nucleus

CTRL
PML KD1

% cells

% cells
PML depletion induces binucleation and nuclear abnormalities

CTRL

PML KD1

Binucleated Cells

% 25 20 15 10 5 0

WT CTRL PML KD1

Abnormal Cells (%)

0% 20% 40% 60% 80% 100%

WT CTRL PML KD1

Normal MN Buds NPBs
PML depletion induces chromosomal abnormalities in normal human fibroblasts.
PML/RARα impairs PML/Telomere association in normal human hematopoietic progenitors
PML/RARα expression induces telomeres shortening in pre-leukemic mice bone marrow.
CONCLUSIONS

1) The Promyelocytic Leukemia Protein (PML) can be found at telomeres in telomerase positive cancer and normal human cells.

2) Telomere damage increases PML/Telomere association

3) PML association with telomeres increases upon telomerase loss of function

4) PML is required for telomere stability, preventing telomere dysfunctions and chromosomal aberrations

5) PML/RARα alters the telomeric function of PML, possibly causing telomeres shortening
PML depletion impairs growth in IL-2 stimulated T cells

This effect is stronger in TERT-mutated T cells