Telomerase as an Anticancer Therapeutic Target

Kee-Ho Lee

Division of Radiation Cancer Research, Korea Institute of Radiological and Biological Sciences
Loss of telomere during DNA synthesis

5' 3'

5' 3'

5' 3'

5' 3'

5' 3'

5' 3'

5' 3'

5' 3'

Gap

Primer removal
Telomere length determines the physiological fate of cellular senescence and tumorigenesis.

- Germ line cells: Telomerase positive
- Stem cells
- Normal somatic cells
- Immortal tumor cells: Telomerase positive

Transforming event (p53, INK4a)

Senescence

Critical Point

Replicative Age
Clinical importance of telomerase in cancer therapy

- **Telomerase activation**
  - 85% (758 / 895) of malignant cancer
  - 14% (38 / 266) of benign and premalignant tumor
  - None of most somatic cells

- **Telomerase inhibition as an anticancer therapeutic strategy**
  - Chemosensitivity (?)
  - Radiosensitivity (?)
Telomerase Inhibitor

- **Compound targeting TERC** (telomerase RNA component)
  - Unmodified PNAs, 2-5A antisense, GRN163, GRN163L

- **Compound targeting TERT** (telomerase protein component)
  - AZT, AZT-TP, BIBR1532, BRACO-19, Telomestatin, Dibenzophenanthroline,
  - Pentacyclic acridine RHPS4, TMPyP4, 2,6 diamidoanthraquinone

- **Peptide targeting TERT** (telomerase protein component)
  - GV1001, p540-548, Vx01, TLI
Combination anticancer therapy with telomerase Inhibitor

- Telomerase Inhibitor + anticancer drug irradiation
- Anti-telomerase gene therapy
I. Chemo- and Radio-sensitizing Efficacy of Telomerase Inhibitor (?)
Generation of mice doubly null for telomerase and INK4a gene

Greenberg et al., Cell, 1999
Generation of telomerase–deficient and –rescued cells transformed by cotransfection of Myc and Ras

mTERC+/+, INK4a-/-

Myc/Ras + [ ]

mTERC Vector

G5 mTERC-/-, INK4a-/-

mTERC Vector

(mTERC; mouse Telomerase RNA Component)

Ju et al., Int J Oncol 2007; Greenberg et al., Cell 1999
### Telomere and telomerase status of cells derived from telomerase-deficient mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Telomere</th>
<th>Telomerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt mTERC+/+ INK4a-/-</td>
<td>Long</td>
<td>+</td>
</tr>
<tr>
<td>G1 mTERC-/-INK4a-/-</td>
<td>Long</td>
<td>-</td>
</tr>
<tr>
<td>G1 mTERC-/-INK4a-/- [+mTERC]</td>
<td>Long</td>
<td>+</td>
</tr>
<tr>
<td>G5 mTERC-/- INK4a-/-</td>
<td>Short</td>
<td>-</td>
</tr>
<tr>
<td>G5 mTERC-/- INK4a-/- [+mTERC]</td>
<td>Short</td>
<td>+</td>
</tr>
</tbody>
</table>

*Lee et al., Proc Natl Acad Sci U S A. 2001  
Ju et al., Int J Oncol 2007,*
Telomerase inhibition sensitize tumor cells to doxorubicin and related compounds

![Graph showing cytotoxicity vs concentration for Doxorubicin, Daunorubicin, and Actinomycin D.](image)

<table>
<thead>
<tr>
<th></th>
<th>Doxorubicin (µM)</th>
<th>Daunorubicin (µM)</th>
<th>Actinomycin D (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G5 mTERC−/− INK4a−/− [+vector]</td>
<td>IC\textsubscript{50} = 0.16 µM</td>
<td>IC\textsubscript{50} = 0.11 µM</td>
<td>IC\textsubscript{50} = 1.42 nM</td>
</tr>
<tr>
<td>G5 mTERC−/− INK4a−/− [+mTERC]</td>
<td>IC\textsubscript{50} = 1.86 µM</td>
<td>IC\textsubscript{50} = 1.17 µM</td>
<td>IC\textsubscript{50} = 23.2 nM</td>
</tr>
</tbody>
</table>

Lee et al., Proc Natl Acad Sci U S A. 2001
Telomere length rather than telomerase is a principle determinant for enhancing chemosensitivity.
Telomerase-rescue renders telomere dysfunctional cells more resistance to radiation.
Telomere length rather than telomerase is a principle determinant for enhancing radiosensitivity.
Myc/Ras-transformed cells

Untransformed normal cells

\[ \gamma - \text{Irradiation (Gy)} \]

\[ \text{Survival (%)} \]

- G5 mTERC-/- INK4a-/- [+Vector+myc/ras]
- G5 mTERC-/- INK4a-/- [+mTERC+myc/ras]
- G6 mTERC-/-
- G2 mTERC-/-
- mTERC+/+
Combination radiotherapy with telomerase inhibition is selectively cytotoxic to tumor cells.

G6 mTERC−/− vs G6 mTERC−/-p53−/- [+Myc/Ras] labeled with GFP
Telomere dysfunctional cells are sensitive to DSB inducing agents including irradiation.

Both critically short telomere length and telomerase inhibition is required for the enhancement of chemo- and radio-sensitivity.
Chromosomal End-to-End Fusion in Telomere Dysfunctional Cells

G5 mTERC−/− INK4a−/−

G5 mTERC−/− INK4a−/− [+mTERC]
Correlation between chemosensitivity and chromosomal fusion of telomere dysfunctional cells

Lee et al., Proc Natl Acad Sci U S A. 2001; Ju et al Int J Oncology 2007; Park et al BBRC 2011
Telomere dysfunctional cells exhibit chromosomal instability with chromosomal end-to-end fusion.

Telomerase inhibitor can be applicable for human cancers with critically short telomere.

Sufficient division is required for the sensitization after the treatment of telomerase inhibitor.
II. Combination Anticancer Therapy with Telomerase Inhibition

Applicable for p53 Mutant Tumor?
p53 plays a crucial role in the physiological response during telomere dysfunction

Telomere shortening $\rightarrow$ p53 $\rightarrow$ Senescence

Telomere binding protein $\rightarrow$ p53 $\rightarrow$ Apoptosis

TRF2 dysfunction

Critically short telomere $\rightarrow$ p53 $\rightarrow$ Sterility of reproductive organ

Critically short telomere $\rightarrow$ p53 $\rightarrow$ Enhanced sensitization to anticancer agents
p53 plays a crucial role in the physiological response during telomere dysfunction

- **Shortened telomere**: p53 → Senescence → Proliferation
- **TRF2 dysfunction**: p53 → Apoptosis
- **Critically short telomere**: p53 → Sterility of reproductive organ
- **Impairment of sensitization (?)**: Enhanced sensitization to anticancer agents
- **Attenuation of sterility**: p53
Telomerase Inhibition can sensitize telomerase positive tumor cells to radiation, irrespective of p53 Status.
Telomere dysfunctional cells are susceptible to paclitaxel

Park et al., Biochem Biophys Res Commun 2011
Telomere dysfunctional cells are susceptible to microtubule disrupting drugs

Table 1 Comparison of chemosensitivity of p53-/-mTERC-/- MEF cultures to other microtubule inhibiting agents

Values are IC50 determined as the mean ± S.D. of three separate experiments for each agent.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Drug (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vinblastine</td>
</tr>
<tr>
<td>G6 mTERC-/-p53-/- [+vector]</td>
<td>1.77</td>
</tr>
<tr>
<td>G6 mTERC-/-p53-/- [+mTERC]</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* The IC50 of each agent was determined using the MTS assay. Values shown are means ± SE of three to five independent experiments.

**H.F.:** Hypersensitivity factors were calculated as the ratio of the IC50 of WT fibroblasts to the IC50 of KO, DKO, and TKO fibroblasts.

* The IC50 of WT fibroblasts was compared with the IC50 of KO fibroblasts.

**H.F.:** Hypersensitivity factors were calculated as the ratio of the IC50 of WT fibroblasts to the IC50 of KO, DKO, and TKO fibroblasts.

* p < 0.05 (Student's t-test), the IC50 of KO, DKO, or TKO fibroblasts compared with the IC50 of WT fibroblasts.

* p < 0.001 (Student's t-test), the IC50 of KO, DKO, or TKO fibroblasts compared with the IC50 of WT fibroblasts.

* n.d.: not determined.
Paclitaxel induces chromosomal fusion in telomere dysfunctional cells
Paclitaxel induced enlargement and multinucleation in telomere dysfunctional cells
Telomerase inhibition sensitizes telomerase positive tumor cells to anticancer agents, irrespective of p53 status.

Telomere dysfunctional cells are susceptible to microtubule disrupting drugs.
III. Side Effect of Combination Anticancer Therapy with Telomerase Inhibitor
Increased Incidence of skin lesions, alopecia, and hair graying in aged telomerase-deficient mice

Rudolph et al., Cell
Delayed wound healing in aged telomerase-deficient mice

mTERC+/+ 18 month

G6mTERC-/- 18 month

Day 4

Day 6

mTERC+/+ 18 month

G6mTERC-/- 18 month
## Summary of possible side effects observed in aged telomerase-deficient mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>mTERC+/+</th>
<th>G6mTERC-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Artherosclerosis</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Peripheral RBC &amp; WBC counts</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Blood chemistry</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Cataract</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>Male fecundity</td>
<td>12-15 months</td>
<td>Infertile</td>
</tr>
<tr>
<td>Hair graying/ alopecia</td>
<td>25%</td>
<td>60%</td>
</tr>
<tr>
<td>Skin histology</td>
<td>Normal</td>
<td>Decrease of hair follicles</td>
</tr>
<tr>
<td>Ulcerative skin legions</td>
<td>10%</td>
<td>37%</td>
</tr>
<tr>
<td>Wound healing</td>
<td>Normal</td>
<td>Delayed re epithelilization</td>
</tr>
<tr>
<td>Cancer Incidence</td>
<td>3.3%</td>
<td>19%</td>
</tr>
<tr>
<td>Life span</td>
<td>24 months</td>
<td>18 months</td>
</tr>
</tbody>
</table>
IV. What kind of human cancers can be applicable?
Human cancers applicable for combination anticancer-therapy with telomerase Inhibitor

- Cancers with telomerase positive
- Cancers with short telomere length
- Cancers with minimal telomere for the end capping of chromosome
- Recurrent cancers after surgery or radiotherapy
- Somatic cancer including HCC, colon, pancreatic cancer etc.
Possible effect of combination anticancer therapy: telomerase inhibitor and existing anticancer agents in HCC

- **HCC**
  - Telomerase activity
  - Short telomere

- **Cirrhosis**
  - No telomerase activity
  - Short telomere
  - **Senescent** hepatocytes

- **Normal liver**
  - No telomerase activity
  - Long telomere
  - Regenerating hepatocytes

**Anticancer drugs with telomerase inhibitor**

- Further telomere shortening
- Increased CIN
- Sensitization to drug

- Short telomere
- No telomerase activity
- **Senescent** hepatocytes

- Long telomere
- No telomerase activity
  - Regenerating hepatocytes
Senescent death is a major mechanism of combined anticancer therapy with telomerase inhibitor. 

Park et al., Biochem Biophys Res Commun 2011
Yun et al., Mol Cancer Res 2009
Conclusion

- Critically short telomeres as well as telomerase inhibition is key factors to sensitize tumor cells to anticancer drugs including irradiation.

- The anticancer therapy combined with telomerase inhibition is useful to both p53-deficient and p53-proficient cells.

- Abundant mitotic or senescent death might be a mechanistic basis for enhanced chemo- and radio-sensitivity.

- The present results suggest that telomerase inhibition might be a promising anti-oncologic regimen for enhancing anticancer therapeutic efficacy.
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