Telomere and telomerase in cancer

Previous studies in mice have demonstrated antagonistic effects of telomerase loss on carcinogenesis. Telomere attrition can promote genome instability, thereby stimulating initiation of early-stage cancers, but can also inhibit tumorigenesis by promoting permanent cell growth arrest or death. Human cancers likely develop in cell lineages with low levels of telomerase, leading to telomere losses in early lesions, followed by subsequent activation of telomerase. Mouse models constitutively lacking telomerase have thus not addressed how telomere losses within telomerase-proficient cells have an impact on carcinogenesis. Using a novel transgenic mouse model, Begus-Nahrmann et al. demonstrate in this issue of the JCI that transient telomere dysfunction in telomerase-proficient animals is a potent stimulus of tumor formation.

Telomere stability and carcinogenesis: an off-again, on-again relationship

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Telomeres and telomerase in cancer

Telomeres have a mixed reputation when it comes to cancer. On the one hand, the chromosome-protective functions of telomeres (capping) can be lost with the shortening of telomeres that accompanies cell division, which in turn can limit cell proliferation. When telomeres become critically short and uncapped, they lose their ability to disguise the linear ends of chromosomes from the DNA damage and checkpoint response machinery, which -- depending on cell context -- leads to cell-cycle arrest (senescence) or cell death (1). Thus loss of telomere reserves may stymie a clone of incipient cancer cells before it can give rise to a significant tumor. On the other hand, rare cells that have sufficiently inactivated their checkpoint response machinery (e.g., via mutation) may continue to divide despite telomere losses. In the case of cultured human fibroblasts, inactivation of the p53 and p16/Rb pathways enables bypass of senescence (2). Uncapped telomeres are prone to recombination, including ligation to other uncapped telomeres, yielding dicentric chromosomes that, following a tug-of-war at mitosis, generate nondisjunction events or internal chromosome breaks. Cycles of these so-called breakage-fusion-bridge events drive gene sequence and copy number changes leading to cell dysfunction and death, which, in human fibroblasts that have bypassed senescence, is called crisis. But they also provide fertile ground from which rare variants can emerge to form tumors (3). Therefore, a question of fundamental importance is whether telomere losses play net inhibitory or stimulatory roles in carcinogenesis. A correlative question of greater practical importance is whether inhibition of the telomere-lengthening enzyme telomerase is likely to benefit cancer patients.

In humans, telomerase activity is under strict control, in part via epigenetic regulation of genes encoding its components, including the TERT catalytic protein and the TERC template RNA (4). Although telomerase can be detected in the progenitor cells of highly proliferative tissues, its activity is nonetheless insufficient for preventing age-related decreases in telomere lengths. Thus, telomeres would be expected to shorten in a runaway premalignant clone of cells. Indeed, premalignant lesions are

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characterized by extremely short telomeres, consistent with shortening limiting further cancer progression (5, 6). Accordingly, forced telomerase expression immortalizes human cultured primary fibroblasts, pointing to the strong proliferative barriers evoked by uncapped telomeres (7). Similarly, mTerc−/− mice, when crossed for several generations to allow telomeres to shorten significantly (e.g., G3), generally have fewer mature tumors, particularly when the p53-dependent checkpoint is intact (8). In contrast, genome instability driven by telomere dysfunction increases the initiation of early-stage cancer lesions. For example, later generation mTerc−/− mice carrying an ApcMin allele develop higher numbers of intestinal microadenomas than mTerc+/−ApcMin+/− or early generation mTerc−/−ApcMin−/− controls, although ultimately, the late generation mice develop fewer macroadenomas (9). These observations raise the following question: if telomerase were activated following telomere dysfunction, would the telomere dysfunction promote or inhibit carcinogenesis overall? The nearly ubiquitous presence in human cancers of telomere length–maintenance mechanisms (usually telomerase, or sometimes an alternative recombination-based mechanism called ALT) together with the capacity of telomerase inhibition to compromise tumor growth suggest that functional telomeres are critical to cancer progression (10).

### Novel mouse models addressing roles for telomeres and telomerase in carcinogenesis

To address the capacity of telomerase to support carcinogenesis following telomere dysfunction, in this issue of the JCI, Begus-Nahrmann et al. report on their creation of a mouse carrying a liver-specific doxycycline-inducible (DOX-inducible) transgene encoding a dominant-negative form of TRF2 (11). TRF2 is a component of a protein complex called Shelterin and plays critical roles in telomere capping, in part by preventing the ATM checkpoint kinase from recognizing the telomere as broken DNA (12). A clever feature of this system is that, since transient telomere dysfunction (TTD, i.e., uncapping) can be induced at any time in animals possessing functional telomerase, TTD effects can be addressed at different stages of cancer progression.

When the transgenic mice were treated at 15 days of age with a hepatocellular carcinoma–inducing (HCC-inducing) agent diethylnitrosamine (DEN), followed by treatment with DOX at 2 to 3 months of age to induce TTD prior to the development of tumors, the numbers of microscopic dystrophic foci and fully developed tumors appearing at 6 to 12 months of age were increased compared with those in mice in which telomere capping was maintained. TTD induction also elevated rates of chromosome aberrations, suggesting that higher rates of oncogenic mutations enhanced tumor genesis. In contrast, DEN-treated G3 mTerc−/− mutants developed less numerous and smaller tumors than even the non–DOX induced TTD strain, despite increased numbers of chromosome aberrations and dystrophic foci (Table 1). Therefore, TTD enhances the initiation of HCC cancers, but persistent telomere dysfunction is deleterious to cancer cell survival, and thus telomerase facilitates the development into mature tumors of early lesions that have experienced telomere dysfunction. Furthermore, by inducing TTD in mice with established HCC at 11 to 13 months of age and following tumor growth using MRI, the authors observed increased tumor size in the DOX-treated mice relative to the controls, indicating that TTD can also aid in cancer progression (11).

Curiously, telomere lengths in TTD-induced tumors were shorter than those in tumors from mice in which telomere dysfunction was not induced. The authors suggest that TTD specifically enhances tumor formation in cells with short telomeres. How this short telomere phenotype is maintained in the presence of telomerase is unclear, but it is interesting that modest telomere lengths are often found in telomere-positive cancers and that there are correlations between chromosome aberrations and short telomeres in human tumors (13), suggesting that short telomeres may convey some advantage to cancer cells.

Findings complementary to those of Begus-Nahrmann et al. have just been published by the DePinho group, which engineered systems for restoring telomerase activity within an mTert−/− background (14, 15). p53+/−p53−/− mutant mice (naturally possessing telomerase) displayed early prostate cancer lesions by nine weeks of age and developed large and invasive adenocarcinomas by 24 weeks. Although G3/G4 mTert−/−Pten−/−p53−/− mice also showed cancer initiation by nine weeks, few tumors progressed further, and those that did remained small and were accompanied by high levels of apoptosis and DNA damage checkpoint activation compared with telomerase-positive counterparts. Thus, although critical telomere shortening due to telomerase deficiency may aid cancer initiation, progression is hampered by subsequent apoptosis and DNA-damage responses. Importantly, telomerase-deficient G3/G4 mice in which telomerase was restored at the point of cancer initiation developed invasive carcinomas after 24 weeks, similarly to naturally telomerase-proficient mice. Moreover, 25% of these mice also displayed skeletal metastases, again suggesting that periods of TDD-induced genome instability, followed by telomerase-dependent stabilization, can promote cancer progression (14). Similar results were obtained using mTert- and Atm-deficient mice in which induction of transgenic mTert stimulated T cell lymphomas. Of note, subsequent inactivation of telomerase in the tumors selected for telomere lengthening by ALT, again point-

### Table 1

Characteristics of HCC tumors

<table>
<thead>
<tr>
<th>Strain</th>
<th>Critically short telomeres</th>
<th>Chromosome aberrations</th>
<th>γ-H2AX foci</th>
<th>Microscopic tumor foci</th>
<th>Macroscopic tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTerc−/− TTD−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>mTerc−/− TTD+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>G3 mTerc−/−</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Comparison of telomerase-proficient mice (mTerc−/+ ) subjected to transient telomere dysfunction during tumorigenesis (TTD−). TTD− controls, and late generation telomerase-deficient mice (G3 mTerc−/−). The number of plus symbols indicates relative frequencies. Microscopic foci reflect presumptive early lesions. Note that TTD− and G3 mTerc−/− mice each have increased chromosome aberrations and microscopic tumor foci, but ongoing telomere dysfunction and genome-wide DNA breaks in the later strain (indicated by elevated numbers of γ-H2AX foci) are associated with fewer (and smaller) macroscopic tumors.
ing to the importance of telomere main-
tenance in mature tumors (15). Together,
the findings from the two research groups
indicate that transient telomere dysfunc-
tion prior to, concomitant with, or fol-
lowing the initiation of cancer can drive
tumorigenesis, provided it is supported by
subsequent telomere stabilization.

In addition to addressing roles for TTD
and telomerase in carcinogenesis, both
sets of findings have revealed additional
insights. Of particular note, HCCs in mice
with TTD had changes in gene expression
and chromosome aberrations similar to
those observed in human HCCs, including
gains in chromosome 15, which carries the
c-Myc locus linked to human liver carci-
genesis (11). Furthermore, prostate tumors
emerging from mTert+/Pten+/Smad4+/– mice
in which telomerase activity was restored
revealed losses in Smad4, encoding a TGF–B
family member. Remarkably, Pten+/+;p53+/–;
Smad4+/– mice were particularly prone to
prostate cancer, including metastases to
bone (14). Thus, despite differences in
human and mouse telomere biology (see
below), the mouse models have proven
themselves valuable guides on the path to
understanding human cancer.

Implications of the new findings
The new findings suggest that TTD in cells
possessing active telomerase or in whose
progeny telomerase can become activated
can contribute to cancer progression. Telom-
erase inhibitors are being actively tested in
clinical trials for cancer, and the new find-
rings raise the possibility that short-term
telomerase inhibition in mature tumors will
do more harm than good, i.e., TTD may
stimulate the appearance of new mutant
clones, some of which could promote tumor
progression. By the same token, the new
findings are consistent with evidence that
long-term inhibition of telomerase may be
of therapeutic benefit. Also of note, inhibi-
tion of telomerase may favor the appearance
of tumor subclones that use ALT to main-
tain telomeres, although as described in the
next section, ALT probably emerges at lower
frequencies in human than in murine pre-
malignant cells. Thus, studies of telomerase
inhibitors as potential therapies for human
cancer certainly remain important avenues
of investigation.

Caveats based on differences
between mice and humans

It is important to note that the new findings
might overestimate the importance of TDD
in promoting carcinogenesis in humans
because of several key differences between
mouse and human telomere biology. Tele-
more lengths of inbred mouse lines are
approximately five times those of humans.
Secondly, telomerase activity is less restrict-
ed in mice (16), and thus cells that have
incurred a period of TDD are more likely
to be rescued by telomerase in mice than
in humans. Finally, although human and
murine cells share p53-dependent check-
point responses to telomere dysfunction,
human cells possess additional responses,
including a p16/INK4a-dependent check-
point (17, 18). This may help prevent
human cells from bypassing checkpoints to
adopt telomerase or ALT-based mechanisms
of telomere maintenance (which occur at
higher frequencies in mice). Considering
these factors, it appears that humans may
have evolved a system designed to use telo-
merase shortening as a guard against cancer,
whereas mice, which generally maintain
telomeres in a capped state, respond less
robustly when capping is lost. These con-
siderations may in part explain the approxi-
mately 10,000-fold higher rates of cancer,
likely that in human cells, the robust
checkpoint responses to telomere dysfunc-
tion coupled with controls on telomerase enable
telomeres to subserve an anticancer function.

Nonetheless, in settings where telomeres are
pathologically short, e.g., due to high muco-
sal cell turnover caused by immune-mediat-
ed damage in inflammatory bowel disease or
due to telomerase deficiency in dyskeratosis
congenita patients (8), the protumorigenic
effects of TTD may be magnified. In these
conditions, perhaps the large numbers of
cells with telomere dysfunction compared
with the small number of premalignant cells
with short telomeres in normal individuals
provide greater opportunity for emergence of
tumorigenic cells overall. Additional
investigations, including detailed studies of
telomere dynamics at different stages of car-
icogenesis in human tissues, are needed to
evaluate these ideas further.

Open questions
Several questions are raised by the new sets
of findings: might transient inhibition of
telomerase in cancer patients be potentially
harmful, and will sustained inhibition be
required for therapeutic benefit? How sig-
nificant is the possibility that telomerase
inhibition will select for ALT-dependent
tumor subclones? Furthermore, at what
stages of tumorigenesis does function-
ally important telomere uncapping occur?
Assays designed to address telomere capping
(rather than telomere length) will be useful
in addressing this question (1, 19, 20). Finally,
do the broad age-related declines in telomere
lengths in multiple tissues serve to promote
carcinogenesis in the elderly? Although telomere
shortening in rare cells that are dividing out of
control within a young individual may serve
to inhibit cancer progression, if most cells
within an elderly individual naturally have
shortened telomeres, the net effect may be
to promote cancer. Answers to these ques-
tions will aid in tailoring telomere-related
cancer therapies for young and old alike.

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Therapeutic potential of a peptide targeting BCL-2 cell guardians in cancer

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A promising approach to cancer therapy is to elicit apoptosis with “BH3 mimetic” drugs, which target proteins of the BCL-2 family. As of yet, however, such drugs can target only certain BCL-2 family proteins. Hence, in this issue of the *JCI*, LaBelle et al. assess instead the therapeutic potential of a “stapled” BH3 peptide from the BIM protein, which inactivates all its prosurvival relatives. The peptide killed cultured hematologic tumor cells and abated growth of a leukemia xenograft, without perturbing the hematopoietic compartment. Hence, such peptides might eventually provide a new way to treat refractory leukemias.

It is increasingly accepted that most, if not all, conventional cytotoxic cancer therapies rely upon eliciting programmed cell death (apoptosis) in the tumor cells, a process regulated principally by the BCL-2 protein family (1). Interactions among the members of this family serve as a switch determining whether the cell will live or die. In response to intracellular damage, the distant cousins of BCL-2, called BH3-only proteins because they bear only the third of the four homology domains that characterize this family, are activated and convey the cell death warrant. They use their BH3 domain to engage and neutralize their prosurvival relatives. The peptide killed cultured hematologic tumor cells and abated growth of a leukemia xenograft, without perturbing the hematopoietic compartment. Hence, such peptides might eventually provide a new way to treat refractory leukemias.

The prospects of BH3 mimetics

Oncologists have long dreamed of drugs that would directly flip the apoptotic switch in cancer cells. That dream inspired the development of the first BH3 mimetic drugs (reviewed in refs. 1, 2), the best studied of which are ABT-737 (3) and its orally bioavailable derivative ABT-263 (4). These drugs are specific for certain prosurvival BCL-2 family members; for example, they bind BCL-2 and BCL-XL with high affinity but not MCL-1 (Figure 1A). Consequently, as single agents they kill cells whose survival depends primarily on BCL-2 and/or BCL-XL but not those containing sufficient MCL-1 to restrain BAX, unless another agent inactivates or eliminates MCL-1 (5). The many promising preclinical findings with ABT-737 and ABT-263 have led to clinical trials of the latter. Notably, with chronic lymphocytic leukemia, which is sustained by high levels of BCL-2, ABT-263 has shown substantial efficacy as a single agent in one-third of patients, even in cases refractory to all conventional therapies and with poor prognostic markers (6).

In vitro and in vivo, ABT-263 and ABT-737 can greatly augment the action of diverse conventional chemotherapeutics (3, 4), most likely because those agents diminish active MCL-1 levels, but which drug combinations will be tolerable in the clinic remains unclear. For example, the ability of ABT-737 and ABT-263 to engage BCL-XL, which is the principal guardian of platelet survival (7), provokes a transient thrombocytopenia, and that has proven to be the dose-limiting toxicity for ABT-263 (6). Consequently, a novel BH3-mimetic highly specific for BCL-2 (ABT-199), which has recently entered clinical trial, should permit higher doses and have even greater promise for chronic lymphocytic leukemia and other diseases sustained by BCL-2 (1).

Eventually, BH3 mimetics that effectively target other family members (e.g., MCL-1) are likely to be developed, but the path to such agents is arduous, because as yet no defined organic skeleton adequately mimics the α-helix assumed by the 16- to 26-residue BH3 domain upon binding to the hydrophobic groove on the surface of its prosurvival relatives. Hence, the development of a new BH3 mimetic typically commences with screens of some 10⁶ chemical building blocks for binding to the BCL-2 family protein target; the resulting weak “hits” must be modified iteratively over...