Mechanism-based combination telomerase inhibition therapy

Inhibition of telomerase is an exciting therapeutic target, since it is required for the long-term proliferation of most cancer cells but not present in most somatic cells. However, effective telomerase inhibitors have yet to be tested in clinical trials. In this issue of *Cancer Cell*, Seimiya and coworkers (Seimiya et al., 2005) explore inhibiting tankyrase, an enzyme involved in making telomeres accessible to telomerase. Adding a partial inhibition of tankyrase to a partial inhibition of telomerase drove cancer cells into crisis and death. The combination of tankyrase and telomerase inhibitors may offer new opportunities for realizing the promise of telomerase inhibition therapy.

Progressive telomere shortening provides the mechanism by which normal human cells count divisions and eventually stop proliferating due to replicative senescence (Shay and Wright, 2004). Cancer cells require multiple mutations to become malignant, and each mutation initially occurs in a single cell and uses up many divisions before it can become widespread in a premalignant population. It is believed that the primary purpose of replicative aging is to form a barrier against the continuing proliferation of precancerous cells, since limiting the total number of available doublings would prevent cells that had divided many times during the acquisition of a few mutations from progressing to frank malignancy. The demonstration that the vast majority of cancers have upregulated telomerase in order to overcome these limits (Kim et al., 1994; Shay and Bacchetti, 1997) and that inhibiting telomerase in cultured cancer cells can drive them into crisis and apoptosis (Hahn et al., 1999; Herbert et al., 1999; Zhang et al., 1999; Shay and Wright, 2002, 2004) raises great hopes that inhibiting telomerase will provide a very effective cancer treatment. Telomerase inhibitors will have a high therapeutic ratio, since most normal human cells do not express telomerase, and telomerase inhibitors will be almost universal, since 85%–90% of all tumors express telomerase.

As our understanding of telomere biology increases, it is becoming clear that it is the shortest telomeres rather than average telomere length that cause chromosome end fusions and apoptosis in telomerase-inhibited cancer cells in crisis. This explains why most cancer cells have relatively short telomeres, since to a first approximation they only need enough telomerase to prevent the shortest telomeres from causing problems. Once a cancer cell upregulates telomerase sufficiently to maintain its shortest telomeres, there may be little additional advantage in increasing its expression in order to further elongate telomeres. It is now known that most human stem cells express telomerase, and there is some concern over the consequences to stem cells from inhibiting telomerase. However, most stem cells have telomeres much longer than most cancer cells, and most primitive stem cells may divide less frequently than cancer cells (so their rate of telomere shortening per calendar time in the absence of telomerase is predicted to be much less than cancer cells). It is hoped that the combination of longer initial telomere length and slower rates of shortening would provide a large therapeutic window between the effects of inhibiting telomerase in stem versus cancer cells.

The maintenance of telomere length in telomerase-expressing cells reflects the equal balance of telomere-shortening and lengthening activities. In addition to the amount of telomerase, a large number of factors have been found that influence the efficiency with which telomerase is actually recruited to act on telomeres. Some of the telomere-binding factors and proteins associated with telomerase are shown in Figure 1. TRF1 (telomere repeat factor 1) was the first human telomere binding protein to be identified, and it was quickly shown to be a negative regulator of telomerase: overexpressing TRF1 causes telomeres in

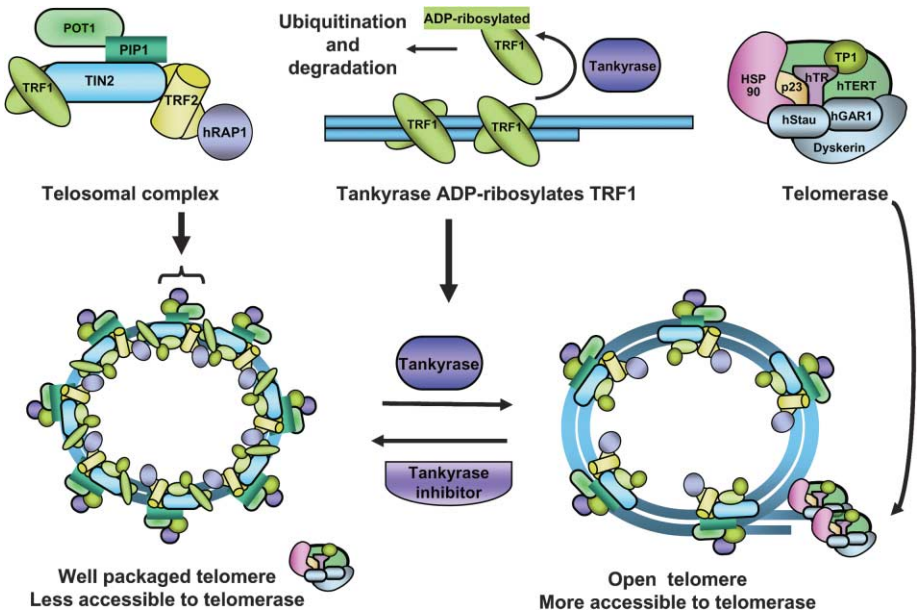

Figure 1. Tankyrase and accessibility of telomerase to telomeres

The telosomal complex is composed of the major double-stranded telomere binding proteins TRF1 and TRF2, associating through TIN2 and a POT1 interacting protein (PIP1, also known at PTOP and TINT1) with the single-stranded TTAGGG binding protein POT1. Tankyrase ADP-ribosylates TRF1, which may lead to a more open configuration allowing telomerase to have access to the single strand G-rich overhang. In the presence of a tankyrase inhibitor, the telosomal complex maintains a well-packaged telomere resulting in a configuration that is not favorable for telomerase access to the telomere. Many additional telomere/telomerase-interacting proteins are not shown.

cancer cells to shorten to a new reduced maintenance length, while dominant-negative TRF1 that removes wild-type TRF1 from telomeres causes telomere elongation (van Steensel and de Lange, 1997). TRF2 is the second major factor that binds to double-stranded telomeric DNA, and a host of factors interacting with either TRF1 or TRF2 have been identified. Recent discoveries that one of the proteins, TIN2, interacts with both TRF1 and TRF2 have led to the concept that there is a large multimolecular complex of mutually interacting factors (Ye et al., 2004; Liu et al., 2004; Houghtaling et al., 2004), so that it is now difficult to ascribe effects to the function of single factors distinguishable from their effects on the organization of the whole telomeric packaging structure.

Tankyrase was originally identified based on its ability to interact with TRF1, but it also has other nontelomeric functions. Tankyrase is a member of the PARP family that can ADP-ribosylate TRF1 and cause it to lose its ability to bind DNA (Smith et al., 1998). ADP-ribosylated TRF1 is rapidly ubiquitinated and degraded. Tankyrase is a relatively abundant cytoplasmic protein that is largely excluded from the nucleus (lacks a nuclear localization signal), and may enter the nucleus during S phase through interactions with TRF1 in order to remove telomere binding proteins from blocking progression of the replication complex. Overexpression of tankyrase with an exogenous nuclear localization signal produces telomere elongation in telomerase-expressing cells, consistent with a functional reduction in TRF1 complexes on telomeres resulting from their ADP ribosylation by tankyrase (Smith et al., 1998).

Although many cultured cancer cells can be driven into crisis by concentrations of telomerase inhibitors that only reduce telomerase activity by 50%–80%, we and others have observed tumor lines in which an 80% inhibition only resulted in telomeres shortening until a stable but reduced telomere length was achieved (our unpublished data). This reflects the fact that telomerase is recruited more efficiently to short telomeres, perhaps due to reduced binding of the multicomponent telomeric complex. As telomeres shorten, a small amount of telomerase activity can sometimes be recruited sufficiently well so that a new balance between shortening and lengthening rates is achieved. The paper by Seimiya et al. (2005) in this issue exploits the knowledge of tankyrase action by asking whether manipulating the ability to recruit telomerase to act on telomeres could prevent the establishment of a new telomere equilibrium maintenance length.

These authors used a telomerase inhibitor called MST-312, a chemical derivative of a component of green tea (Seimiya et al., 2002). Although higher concentrations of MST-312 (2 μM) inhibited telomerase efficiently and were able to drive tumor cells into crisis, Seimiya and colleagues found that lower concentrations (0.75 μM) only partially inhibited telomerase, and while telomeres shortened from 5 kb to a new stable length of almost 4 kb, the cells continued to proliferate. Seimiya et al. (2005) then picked a dose of 3-aminobenzamide (a general PARP inhibitor that inhibits tankyrase) that had minimal effects on the growth rate of the control cells. They then demonstrated that adding 3-aminobenzamide to the MST-312-exposed cells with reset telomeres at 4 kb caused them to begin shortening again until the cells entered crisis and died. This dramatically demonstrates that combinations of tankyrase and telomerase inhibitors at nontoxic doses may be an effective anticancer therapeutic approach.

In summary, changes in telomere function have been implicated in both human aging and cancer. Telomeres are essential for proper chromosomal replication, and, through proteins that bind to telomeric DNA and recruit/modify other proteins, maintain the integrity of the genome by preventing chromosomal recombination, end-end fusions, and degradation. Telomerase is a cellular reverse transcriptase enzyme complex that is not expressed in most normal human cells but is required for the long-term growth of almost all malignant human cancers. Telomerase is regulated in *cis* by telomere-associated proteins, and inhibition of telomerase results in progressive telomere shortening, leading to chromosome instability, cell cycle arrest, and apoptosis. The study by Seimiya et al. (2005) illustrates a new strategy for telomere-based molecular cancer therapeutics.

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