IN THE SPOTLIGHT

Turning Telomerase into a Jekyll and Hyde Case?

Raymund J. Wellinger

Summary: It may be possible to coerce telomerase to incorporate modified guanine nucleotides into telomeric repeat DNA, thereby seriously compromising the functionality of the telomeres. Thus, a guanine analogue such as 6-thio-dG could turn active telomerase into a chromosome de-protecting enzyme, the opposite of what it is normally, namely a chromosome-protecting enzyme. *Cancer Discov; 5(1); 19–21. ©2015 AACR.*

See related article by Mender et al., p. 82 (9).

Telomeres are the protecting structures at the tips of eukaryotic chromosomes. These structures are essential for genome stability, given that without them, chromosome ends are recognized as DNA double-strand breaks that require repair, the cell cycle is arrested, and these ends may be fused together to form unstable multicentric chromosomes. In most organisms, telomeric DNA is particular in that it is composed of short, head-to-tail repeated sequence elements that are rich in guanines; an example is (TTAGGG)n that is the repeat DNA for all mammals (1). However, in humans, the overall length of this repeat DNA at each telomere is neither uniform nor stable. Indeed, due to some intricacies of the basic DNA replication machinery, a DNA end will not be completely copied without some added mechanisms (2). In the absence of that, chromosome ends will lose some amount of sequences with each replication cycle, an issue known as the end-replication problem (2). Eventually, in any growing cell population, these progressive losses will lead to nonfunctional telomeres and genome instability, and the affected cells will stop dividing. This is where the enzyme telomerase provides a counteracting and telomere-stabilizing activity (1, 2). Its enzymatic function is related to reverse transcriptases, as telomerase uses a short stretch of a constitutively associated RNA molecule as a template for DNA addition onto telomeres. Specifically, telomerase will synthesize the strand containing all the guanines in the telomeric repeats (see above), whereas the other strand is thought to be filled in by conventional DNA polymerases. In human cells, telomerase is very tightly regulated in terms of cell types and developmental stage. In adults, telomerase is not detectable in most somatic tissue cells, and very little activity remains in some stem-cell niches. Given the above, telomeres shorten in dividing human somatic cells and may have important implications for cell senescence and organismal aging (3). The upside of these considerations is that because of their increased cell division rates, cancer cells would appear particularly vulnerable to impending telomere dysfunction. Unfortunately, the general deregulation of gene expression during cancer development in most cases includes reactivation of telomerase, and, therefore, a telomerase-based mechanism for telomere maintenance is reestablished in more than 80% of all cancers (4).

This almost ubiquitous requirement for telomerase in dividing cancerous cells and its virtual absence in normal somatic tissues raised significant hopes that a silver bullet target for the treatment of many cancer types had been found. The idea becomes rather straightforward: Inhibit telomerase and the eventually resulting rampant genome instability will cause cancer cell death. Non-dividing cells, and cells with adequate telomeres and dividing without telomerase, should not be affected at all if the inhibitor is sufficiently specific (4). However, for various reasons, this stratagem has not yet panned out. A number of telomerase-targeting drugs were developed, including an antisense-derived oligonucleotide against the templating region of the telomerase RNA, immunogenic peptides derived from the telomerase catalytic enzyme, or direct holoenzyme inhibitors (5). All failed in advanced pharmacologic testing or in single-agent applications in clinical trials. New compound screens have been reported, but it is too early to say whether these compounds will be more successful (6). Nevertheless, there is still hope for some of these agents, as they may be effective when applied with other compounds in combination treatments (5, 7).

An inherent problem with telomerase inhibitor–based approaches is the expected lag time between application of the agent and its effect on the cells. As mentioned above, cells with adequate telomere length will live and divide normally, even in the absence of telomerase. It is thought that this logic also applies to cancerous cells. Telomerase-inhibiting drugs may thus only affect the cells' ability to grow once a number of its telomeres become too short to function. A more direct approach to exploit the essentiality of telomeres would therefore be to develop small-molecule interference strategies that target the capping function of telomeres. Such efforts are feasible (8), but it is not clear how they will fare in the clinic. Although expected to be much more effective, this approach does have the disadvantage that the cancer specificity of the target has been lost, and any drug targeting capping may carry high risks related to toxicity.

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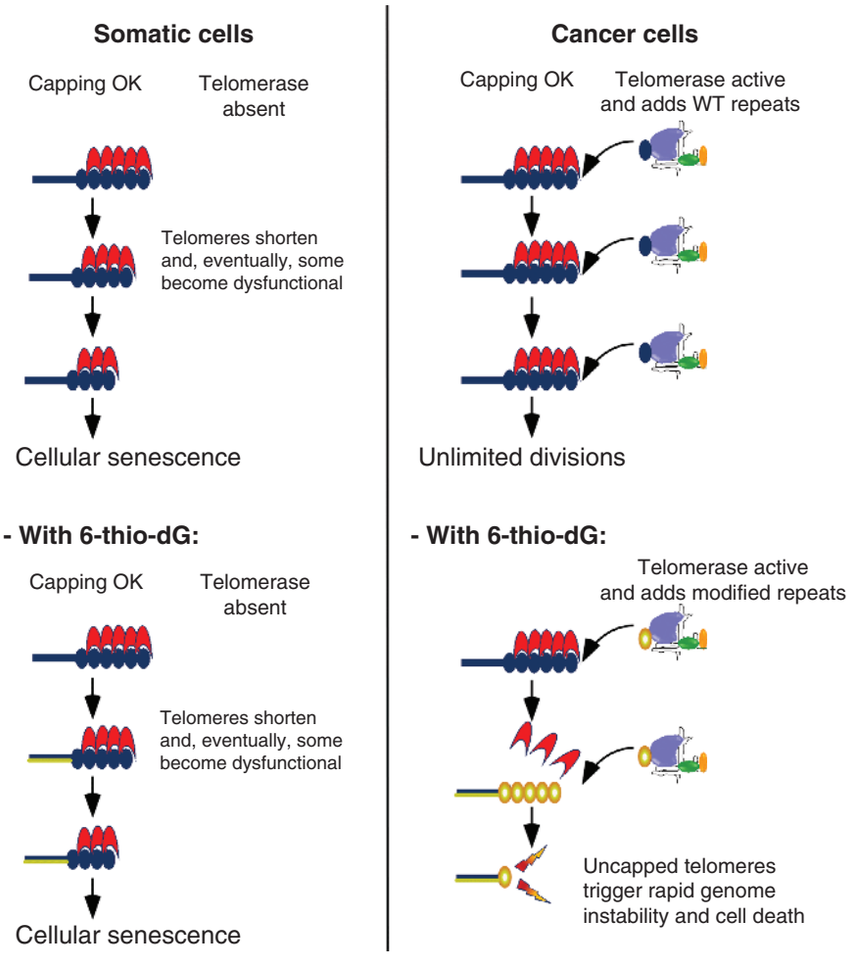

Figure 1. Coercing telomerase to change personality and induce genome instability. Top, situation in normal human somatic cells and in cancerous cells. Telomerase is virtually exclusive to cancer cells and maintains chromosome stability and capping (red caps) by replenishing telomeric repeats (blue ovals), where needed. In normal somatic cells, telomeres will slowly and progressively shorten due to the end-replication problem until some of them become dysfunctional and cause cellular senescence. Bottom, when cells are provided with pharmacologic amounts of the nucleoside analogue 6-thio-2′-deoxyguanosine (6-thio-dG), only the telomerase-containing cancerous cells will experience 6-thioguanine (6-thio-G) incorporation into telomeric repeats (yellow ovals, right). Presumably, some capping function is interfered with, causing rapid cell death. Replicative DNA polymerases may also incorporate 6-thio-G to some extent elsewhere in the genome (blue–yellow line), but the consequences of that remain unclear. WT, wild-type.

An article in this issue of *Cancer Discovery* now proposes to combine these two approaches with a single small-molecule drug (9). The concept is to supply cells with a DNA base derivative that will be converted to a true nucleotide analogue and eventually incorporated into cellular DNA during S-phase. If the modified nucleotide causes chromosomal DNA to display physicochemical anomalies, the cells will perceive it as damaged and attempt to repair it. In terms of telomeres, if telomerase uses the nucleotide analogue, telomeric repeat DNA may lose its ability to support the capping function and chromosomes become uncapped after limited passages through S-phase, ideally only one. This idea therefore combines the specificity of telomerase presence in cancerous cells with a relatively immediate chromosome uncapping caused by that telomerase activity. It is important to understand that in this case, the analogue will not be an inhibitor for the reverse-transcriptase–like enzyme, such as is the case for the nucleoside and nucleotide analogue inhibitors used to inhibit the HIV reverse transcriptase. Concerning the analogue to be used, given that telomerase synthesizes the G-rich strand on telomeres, Mender and colleagues (9) delved into the possibilities of guanine derivatives. One such compound, 6-thioguanine (6-thio-G), was prescribed in the clinic in the past, but due to toxic effects, its use has been discontinued. In the new research reported here, the authors therefore skipped ahead on the nucleotide biosynthetic pathway and argued that a compound that is closer to the actual nucleotide analogue may be more prone for incorporation into DNA and have less-toxic side effects. Thus, the investigation reported here focuses on the use of 6-thio-2′-deoxyguanosine (6-thio-dG), essentially the corresponding nucleoside for 6-thio-G (9). Remarkably, the sensitivity of cultured human cells to 6-thio-dG is quite different between telomerase-positive and telomerase-negative cells. Moreover, telltale signs of telomere uncapping and damage are very much increased in telomerase-positive cells. Encouraged by these results, the authors moved to a mouse xenograft system to evaluate human solid tumor behavior in living mice. As with the human cells in culture, preliminary toxicity assays on mice showed that 6-thio-dG is much better tolerated than 6-thio-G. Finally, paralleling the cell-culture result again, established tumors virtually stopped growing when the mice were treated with 6-thio-dG, and subsequent cellular analysis of remaining tumor tissue revealed increased telomeric DNA damage (9).

These results thus highlight the potential for telomere and telomerase-based anticancer therapies that do not involve telomerase inhibitors (see Fig. 1). Exploiting the fact that high telomerase activity is almost exclusive to cancer cells and linking that to abolishing an essential genome stability function is indeed promising. For the compound reported on in the study mentioned here, we will eventually need to

know whether it is incorporation of a 6-thio-G nucleotide into telomeric repeats that causes the reported effects. Also, further investigations could target the aspect of the telomere capping function that is interfered with. For example, do 6-thio-G–containing telomeric repeats disturb *t*-loop dynamics or simply affect the binding efficiency of some of the telomeric repeat binding proteins (TRF1 and/or TRF2)? The results in the article suggest that in the surviving tumor cells, telomeric repeat tracts shorten dramatically, despite the presence of telomerase (9). This unexpected finding is intriguing and would need some explanation. Another surprising aspect is the fact that tumor cell analyses did not reveal any other genomic DNA disturbances (9), as would be expected if the general DNA replication machinery also uses 6-thio-dGTP in places where a guanine nucleotide is needed. Therefore, 6-thio-dGTP incorporation appears to occur only to a very limited extent, and the cells are able to repair those occurrences rather efficiently. This may suggest that 6-thio-dGTP is a poor competitor for dGTP when it comes to replicative DNA polymerases, whereas it is used efficiently by telomerase, but all of these issues will need to be worked out. Time will tell whether 6-thio-dG will really be a winner and become a sought-after anticancer drug. Regardless, the general idea of using the cancer cell specificity of telomerase and coercing the enzyme to incorporate a toxic nucleotide analogue into telomeric DNA is worth exploiting aggressively. In particular, and as mentioned above, modified bases that are poorly used by replicative DNA polymerases but tolerated by telomerase could turn Dr. Jekyll into Mr. Hyde.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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