CellPress

Alternative splicing regulation of telomerase: a new paradigm?

Mandy S. Wong[1], Woodring E. Wright[1], and Jerry W. Shay[1,2]

[1] Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75390-9039, USA
[2] Center for Excellence in Genomics Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia

Alternative splicing affects approximately 95% of eukaryotic genes, greatly expanding the coding capacity of complex genomes. Although our understanding of alternative splicing has increased rapidly, current knowledge of splicing regulation has largely been derived from studies of highly expressed mRNAs. Telomerase is a key example of a protein that is alternatively spliced, but it is expressed at very low levels and although it is known that misregulation of telomerase splicing is a hallmark of nearly all cancers, the details of this process are unclear. Here we review work showing that *hTERT* expression is in part regulated by atypical alternative splicing, perhaps due to its exceptionally low expression level. We propose that these differential regulatory mechanisms may be widely applicable to other genes and may provide new opportunities for the development of cancer therapeutics.

Telomerase is an attractive yet challenging target for cancer therapeutics

Telomeres are dynamic DNA–protein structures at the end of chromosomes that prevent chromosome ends from being recognized as DNA damage [1]. Telomere repeats are bound by a shelterin protein complex comprising TRF1, TRF2, TIN2, RAP1, TPP1, and POT1 [2]. Together, the proteins of the shelterin complex recognize and bind to telomere repeats to promote the formation of a structure called a T-loop at the telomere ends by interacting with the 3′ guanine-rich termini of the telomere overhang, thereby concealing overhangs and preventing telomere degradation at DNA checkpoints [3,4]. Initially, each human chromosome is capped by 15–20 kb of telomeric TTAGGG repeats. Throughout the course of an organism's lifetime, these repeats slowly erode due to incomplete replication of the DNA lagging strand at the ends of the chromosomes (Figure 1). This process is called the end-replication problem (see Glossary) [5,6]. When a telomere becomes critically short, DNA damage signaling is induced and cell growth is arrested, resulting in replicative senescence [7,8]. The limited proliferative capacity of cells is widely accepted as an 'aging time-clock' mechanism in humans and most other large, long-lived organisms. Such cells use this counting mechanism to prevent unlimited cell growth, which could lead to the accumulation of mutations over time and potentially progression to malignancy [7,9,10].

To overcome this brake on replicative aging, cancer cells almost universally upregulate or re-express telomerase to re-elongate or maintain telomeres at lengths sufficient to avoid triggering DNA damage signaling [11]. Cancer cells have varying amounts of telomerase activity and almost all cancer cells have very short telomeres [9,10,12]. However, little is known about the regulation of this telomere maintenance program in either normal or cancer development. Telomerase is a ribonucleoprotein complex comprising a catalytic protein component with reverse transcriptase activity (*hTERT*) that uses a functional RNA component (*hTR* or *hTERC*) as a template to elongate telomeres [13]. Although telomerase is initially expressed in all cells during early fetal development, its expression is rapidly repressed to almost undetectable levels in somatic cells. Only a small subset of proliferating stem-like progenitor cells are capable of transient telomerase expression post-development [14].

Telomerase is subject to transcriptional, post-transcriptional, and epigenetic levels of control, but there is no consensus on the precise mechanisms regulating telomerase repression during development and re-expression of telomerase in cancer progression. Regardless of whether a cell has telomerase activity, almost all cells have an excess amount of *hTR* (*hTERC*), the telomerase RNA template [15]. By contrast, *hTERT* can be detected at relatively low levels in stem cells, progenitor cells, and even cancer cells. Recently it was demonstrated that both *hTR* and *hTERT* have a subpopulation in reserve that is not assembled into activate telomerase [16]. Although *hTR* is present in great excess relative to *hTERT*, only the assembled telomerase

Glossary

Alternative splicing: during pre-mRNA processing, particular exons of a gene may be included or excluded from the final mRNA. This process allows for the generation of multiple proteins with potentially multiple functions from a single gene in the genome.
End-replication problem: during DNA replication, the lagging strand cannot be completely copied because no polymerase can fill in the resulting gap after the last RNA primer is removed. As a result, each round of replication generates shorter telomeres.
Replicative senescence: also known as the Hayflick limit. After so many cellular divisions that the telomere ends are too short for the stable T-loop structure, a DNA damage signal is induced and the cell ceases to divide.
Splicing factor: proteins that affect splice site selection by directly binding to the pre-mRNA or through binding to other proteins.
Variable-number tandem repeat (VNTR): short tandem repeats of a repetitive nucleotide sequence are dispersed throughout the genome. The repeat sequence varies in length and copy number. An array of functions has been assigned to VNTRs.

Corresponding author: Shay, J.W. (jerry.shay@utsouthwestern.edu).
Keywords: RNA splicing; regulation; low-abundance transcript; telomere; cancer therapy.

0168-9525/
 http://dx.doi.org/10.1016/j.tig.2014.07.006

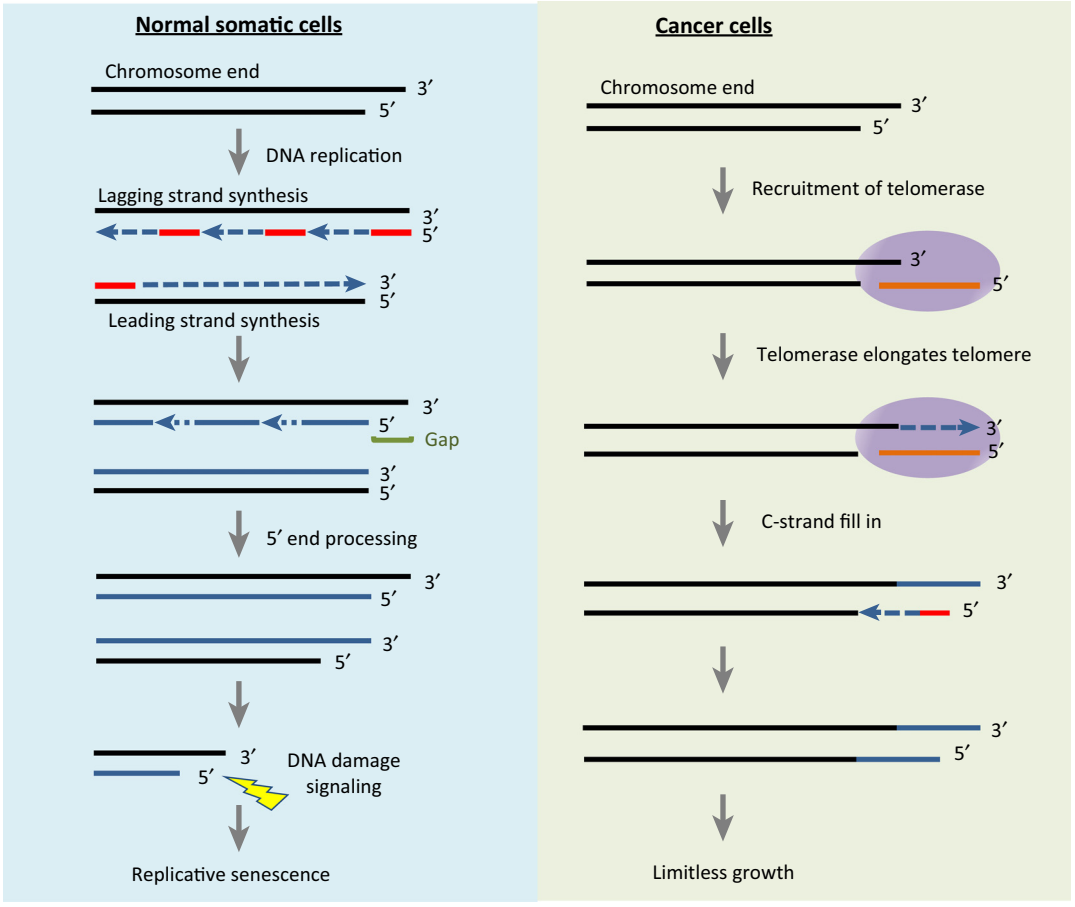

Figure 1. Telomerase expression contributes to cancer cell immortality. In normal somatic cells, telomeres shorten with every cellular division due to the end-replication problem. During DNA replication, DNA polymerase makes complementary DNA using RNA primers (in red). The RNA primers are later removed. The newly synthesized fragments are used as primers for DNA polymerase to fill in the gaps. Although replication on the leading strand is complete, replication on the lagging strand is incomplete, resulting in telomere shortening with every cell division. When a telomere reaches a critically short length, a DNA damage response is triggered and causes the cell to go into replicative senescence. In cancer cells, telomerase uses its RNA component (*hTR*, in orange) as a template for its catalytic component (*hTERT*) to elongate telomeres and evade cellular senescence. Therefore, cancer cells have unlimited proliferative capacity (e.g., become immortal), permitting additional alterations to occur in more-malignant tumors.

with both components has telomere elongation activity; therefore, both *hTR* and *hTERT* are limiting factors. The current best estimate for the number of catalytically active telomerase molecules per *in vitro* immortalized (telomerase-positive) cell or cancer cell is approximately 100–500 [17], produced from approximately 20 mRNA molecules per cell [15].

Because telomerase expression is restricted to cancer cells and some but not all proliferating stem cells, it offers a potentially highly specific target for cancer treatment. Moreover, telomeres are short in approximately 90% of primary cancers and cancer cell lines compared with the longer telomeres seen in the rarely dividing stem cells and actively dividing progenitor cells (Figure 2). Thus, inhibiting telomerase activity should result in telomere shortening, leading to apoptosis of cancer cells while having little to no effect on quiescent stem cells. Significant efforts have been expended to develop cancer therapeutics targeting telomerase, yet the development of telomerase inhibitors has been largely unsuccessful. Although many telomerase-directed therapeutic approaches demonstrate inhibitory effects in *in vitro* systems, they rarely progress beyond early-stage clinical trials due to lack of potency, low specificity, and/or increased toxicities (Box 1). A major challenge for developing an effective therapeutic agent against telomerase has been that telomerase is expressed at exceptionally low abundance even in cancer cells.

Alternative splicing is a dynamic and highly regulated process

It is surprising that almost two decades after the cloning of *hTERT* it remains unclear how telomerase is regulated. It has been suggested that during development *hTERT* is in part regulated by alternative splicing [15,18], which adds another layer of complexity to the problem of dissecting the mechanisms regulating telomerase. Mechanistically understanding *hTERT* alternative splicing offers the possibility of developing a novel anticancer agent that targets splicing to reduce telomerase activity in cancer. However, studying the alternative splicing of *hTERT* poses a new challenge because previous studies of splicing have largely been based on highly expressed genes and the splicing of *hTERT* does not appear to conform to the established norm of alternative splicing regulation.