Telomerase: Key to Mortal or Immortal Road

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ABSTRACT

Gradual attrition of telomere to a critical short length elicits successive cellular response of cellular senescence and crisis. Cancer cells evade this process by maintaining functional telomeres via one of two known mechanisms of telomere maintenance. The first and most frequent mechanism involves reactivation of enzyme activity of telomerase, a ribonucleoprotein complex mainly via transcriptional up-regulation of TERT, a catalytic subunit of telomerase complex. The second mechanism utilizes telomerase-independent way termed ALT (for Alternative Lengthening of Telomere), which possibly involves recombination pathways. Thus master key for cellular immortalization is supposed to possess adequate telomere reserves. Indeed, telomerase can alone induce the immortalization under culture on feeder cell layers without generally known inactivation mechanism of tumor suppressor genes. Including this phenomena, this review will focus on telomerase and telomere-associated proteins, thereby implication of these proteins for cellular immortalization processes. **(Immune Network 2002;2(4):183-188)**

Key Words: Telomere, telomerase, telomere-binding protein, immortalization, tumorigenesis

Introduction

Normal human cells possess a limited replicative lifespan when cultured in vitro (1). In these cells, telomeres, the ends of linear chromosomes, shorten with each cell division (2,3). This observation has led to the suggestion that telomeres act as a molecular counting device that checks the number of cell divisions and limits further division, upon shortening below a threshold length (4). In contrast, the majority of cells derived from spontaneously arising human tumors proliferate indefinitely and maintain stable telomere lengths (5). This observation has led to the inference that maintenance of telomere length is a prerequisite for the acquisition of immortalized replicative capacity. In unicellular eukaryotes and in the germline of multicellular organisms, replication-associated loss of telomeric DNA is counteracted in a variety of ways. The best studied of these is the ribonucleoprotein enzyme complex, telomerase, that uses an RNA template to add repeats onto the G-rich strand, thus extending the single-stranded 3' overhang. TERT, catalytic subunit of telomerase, is important to maintain the telomere length and TERC, RNA subunit of telomerase, is essential for the enzymatic function of telomerase. Normal human somatic cells have the telomerase activity of either low or undetectable levels and progressive telomere loss occurs with each cell division. According to the telomere hypothesis of senescence, telomere erosion eventually acts as the trigger for cells to senesce, meaning that cell immortalization requires a mechanism for prevention of telomere attrition (6).

Here we summarize the regulation mechanism of telomere length, especially by telomere-binding proteins, and recent progress showing the importance of telomerase activity for telomere maintenance and their role in immortalization.

Important roles of telomere-binding proteins on protection and maintenance of chromosomal end

Telomeres are predominately double stranded. However, these end in a 30～200 nucleotide single-stranded overhang (7-9). This 3′ overhang can invade and anneal with the double-stranded region of telomeric DNA to form displacement (D)-loop in the

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same telomere (10). This overall feature is called as a telomere (T)-loop. Although T-loops can be formed on relatively short telomeres, presumably there is a minimum size below, which they cannot form.

Because only the overexpression of the catalytic subunit of telomerase (TERT) reconstitutes the telomerase activity and increases telomere length in telomerase negative cells, it seems that activation of TERT is the limiting step for the induction of telomerase activity in most cells. Therefore, much effort has been focused on the understanding of the transcriptional regulation of TERT. Besides the transcriptional regulation of TERT, there may be complex and dynamic processes regulating the actual activity of telomerase (11).

However, sufficient length of telomeric DNA does not prevent abnormalities in the integrity of 3' overhang or mutation of telomere-binding proteins from inducing telomere dysfunctions and thereby several deleterious cellular effects. Functional telomeres require a minimum length of telomere repeats, the integrity of 3' overhang and functional telomere-binding proteins (Table I). To achieve and maintain the correct telomeric structure and the integrity of 3' overhang, several telomere-binding proteins are needed. Most of them participate in some steps involved in the regulation of telomere length and/or structure. For example, the formation of T-loops is critically dependent on TRF2. It directly binds double stranded telomeric DNA and can remodel the telomeric DNA into T-loop (10). Elimination of TRF2 from telomeric DNA induces immediate deprotection of chromosomal ends and the cells show some features resembling the cellular responses to a double strand break (DSB) in the genome. In fact, some evidence indicates that components of DNA-dependent protein kinase complex are involved in signaling pathway induced by abnormal telomeres and regulate telomere integrity. For example, in cells from Ku86-deficient mice, telomeric fusions occur with sufficient length of telomeres at the fusion point (12). However, this Ku86 deficiency rescues the end-to-end chromosomal fusion induced by critically short telomeres in telomerase-deficient mice (13). Also, DNA-PKcs deficiency induces effects similar to Ku86 deficiency (14). These lines of evidence suggest that some components of DNA-PK complex have important roles in maintenance of telomere integrity and protection of telomeres from DNA repair activity.

Similar to TRF2, TRF1 binds double stranded telomeric DNA. TRF1 and its binding partner, TIN2 may facilitate or stabilize the formation and/or maintenance of T-loops (10,15). Another TRF1 binding protein, Tankyrase 1 shows poly (adenosine diphosphate-ribose) polymerase (PARP) activity and, indeed, exerts its activity on TRF1 (16). Tankyrase 1-mediated ADP-rybosylation of TRF1 decreases the telomeric DNA binding activity of TRF1 and promotes telomere elongation in a telomerase-dependent manner (17). Tankyrase 2, the homologue of Tankyrase 1, can bind to TRF1 and seems to have similar roles on telomere (18).

Whereas TRF1 and TRF2 bind double stranded telomeric DNA, Pot1 binds 3' overhang of telomeric DNA and seems to have important roles in maintaining chromosomal stability by capping the single-stranded telomeric DNA (19).

As a result, both telomere length and telomere structure are important in protection and maintenance of chromosomal end. Also, besides telomerase itself, telomere-binding proteins, as well as their associated proteins, have pivotal roles on modulation

Table I. Telomere binding proteins

Genes	Mediator for telomere association	Roles in telomeric DNA
POT1	Single stranded telomeric DNA	Telomere-length maintenance and telomere protection
TRF1	Double stranded telomeric DNA	T-loop formation
TRF2	Double stranded telomeric DNA	T-loop formation
Tankyrase 1	Via TRF1	Ribosylate TRF1
Tankyrase 2	Via TRF1	Ribosylate TRF1
TIN2	Via TRF1	Negatively regulate telomere length
RAP1	Via TRF2	Regulate telomere length
RAD50/NBS1/MRE11	Via TRF2	Maybe participate in T-loop formation
PINX1	Via TRF1	Bind TERT and inhibit its activity
Ku86	Telomere repeat?	Maybe restrict telomerase and DNA repair activity
DNA-PKcs	Ku proteins	Protect telomeres

and/or maintenance of telomere length or structure.

Telomerase and immortalization

The primary hallmark of tumor cells is loss of growth control caused by gain-of-function mutations in proto-oncogenes, and loss-of-function mutations in tumor suppressor genes. A second hallmark of tumor cells, common to almost all malignant cancers, is cellular immortality. In human tumors, cell immortalization almost always involves derepression of the gene for the catalytic subunit of telomerase, hTERT (20,21). However, it remains controversial whether the cell culture immortality of many cancer cells is a fundamental property, required for tumorigenesis, or a by-product of other genetic changes.

After finite in vitro cell division, most human somatic cells face the loss of their replicative proliferating capacity (1), and are termed cellular senescence. Among a variety of triggers for cellular senescence, the telomere shortening that occurs in each cell division as a result of the end-replication problem of DNA polymerase is recognized as "mitotic clock" to control the cellular life span, at least in human somatic cells. Given that human cells can divide only a limited number of times in culture, many researchers have wondered whether there is a "clock" that measures cell divisions. Human somatic cells were found to have shorter telomeres than sperm from the same individuals, which suggests that human somatic telomeres shorten during development (3,22). Furthermore, telomere length in somatic cells, both fibroblasts and leukocytes, from older individuals was decreased on average compared with telomere length in somatic cells from younger people (2,3). Also, telomeres were found to shorten during the passage of fibroblasts in culture (3) and in cancers compared with adjacent normal tissues (2). These results have supported the hypothesis which telomerase is shut off in human somatic cells, limiting their lifespan, whereas it is activated in cells that become immortalized in culture (6,23).

Although oncogenically transformed cells can extend the lifespan of human cells beyond the first growth arrest point, known as senescence, these cells eventually enter a phase known as crisis and then suffer chromosome aberration and massive cell death. Rare immortal cells are survived by telomerase activation (24-26) and this was established by studies of telomere length and telomerase activity in mortal and immortal human cultured cells. Analysis of telomere lengths in human embryonic kidney cells transformed with simian virus 40 T antigen shows a steady decline until crisis, and then telomere maintenance in the survivors. Concordantly, the telomerase enzyme is inactive in the precrisis mortal cells and is activated in the immortalized cell clones (26,27). Whereas the human telomerase RNA subunit is expressed in both telomerase-positive and telomerase-negative tissues (28,29), the hTERT mRNA is expressed in immortal, telomerase-positive cell lines but not in mortal, telomerase-negative cells (7,30,31). Furthermore, overexpression of hTERT in previously telomerase-negative cells generates an active telomerase enzyme in these cells, confirming that hTERT expression is a key step in regulating telomerase activity (32-36). Even more dramatically, ectopic expression of the telomerase catalytic subunit gene in certain telomerase-negative cells is able to extend the lifespan of these cells, confirming the model that telomerase can overcome the limits of human cell mortality, at least in some cases.

In addition, hTERT expression leads to significant lifespan extension in strains of mortal fibroblasts and retinal epithelial cells, allowing them to bypass the senescence limitation to cell growth (34,36). For example, hTERT-immortalized BJ foreskin fibroblasts have been found to proliferate for an additional 200 population doublings compared with control cell clones that can double for approximately 80 generations (37). Telomerase expression is sufficient to immortalize some cell types, such as fibroblasts, directly, but several reports have claimed that keratinocytes and other types of epithelial cells exhibit an additional mechanism of replicative aging involving the p16/pRB pathway that is independent of telomere length, and that inactivation of both mechanisms is also required for cellular immortalization (38-42).

However, in most of these reports, cells were cultured in chemically defined media and proliferated for only 15～20 doublings. It was dramatically less than the ～50 doublings described previously for the growth of keratinocytes on feeder layers (43). Furthermore, 15～20 doublings appear grossly inadequate to explain the estimated number of keratinocyte doublings occurring in vivo (44). For these reasons, several groups have developed the different model systems to examine whether the proposed p16/pRB pathway actually represented a telomere-independent second mechanism of cellular senescence or was a secondary consequence of particular culture conditions. They found that telomerase activity alone could immortalize without the inactivation of p16/pRB pathway. In other words, human keratinocytes grown on appropriate feeder layers could directly immortalized by hTERT maintenance of telomeres without any intervening inactivation of a p16/pRB pathway (45,46).

Human mammary epithelial (HME) cells have been described to exhibit the mechanism of senescence

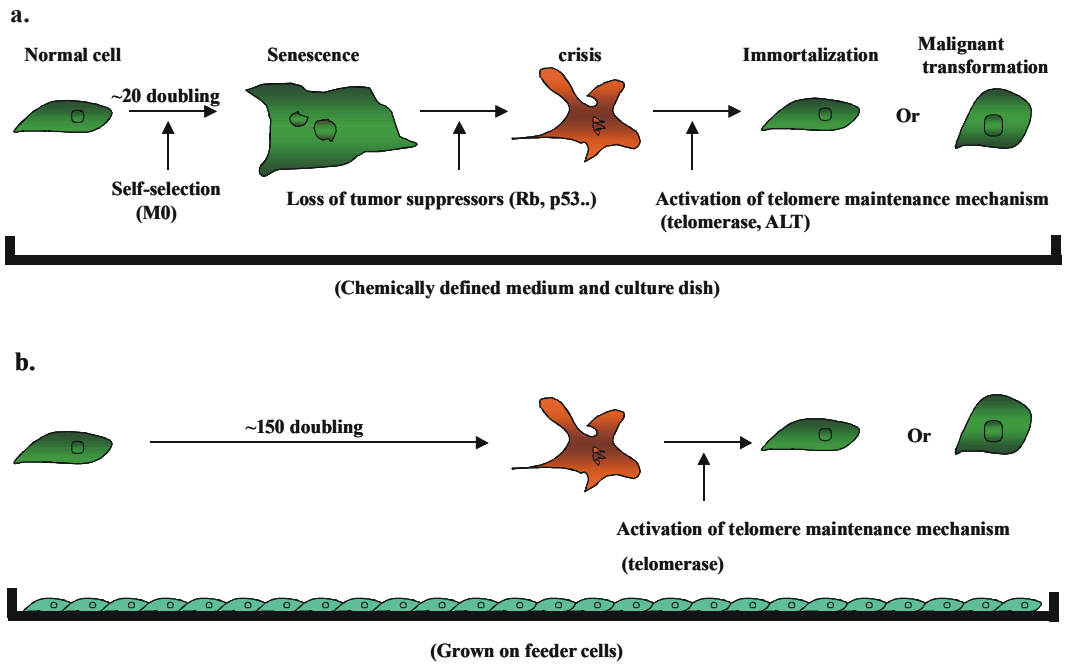

Figure 1. Schematic of telomerase hypothesis in immortalization and tumorigenesis. This diagram is based on Neumann AA et al. and modified according to recent studies.

(38,47). Generally, it has been known that HME cells appear to senesce in two stages. One is an initial growth arrest, designated mortality stage 0 (M0), occurring after about 20 doublings. This stage is apparently controlled by the Rb/p16 pathway, because M0 can either be prevented by E7 (48) or a few cells escape spontaneously with reduced p16 expression due to promoter methylation (47). The other is subsequent M1 growth arrest bypassing by E6 (49,50). In some cases, only a subpopulation of cells can emerge from mortality stage 2, which is called, as E6/E7-expressing cells will enter a period of crisis in culture. However, recently, the replicative capacity of HME cells can also be extended without inactivating p16 if these cells are cultured on feeder layers (45,47). Finally, culture of human fibroblasts under inadequate culture conditions (chemically defined medium in the presence of 0.25% serum) recapitulates the keratinocyte and HME scenarios. Such fibroblasts arrest after ~25 doublings regardless of telomere length/telomerase, but can be rescued and immortalized following transfer to adequate culture media (46). Although one cannot theoretically exclude the existence of non-telomere based mechanisms for replicative aging, these results establish that at present there is no reliable evidence for the presence of a second mechanism of replicative aging specifically in these cells.

Analysis of telomerase activity in immortal cells has led to the identification of two classes of cells. In one class, telomerase is activated. In the other class of cells, telomerase activity remains undetectable, and telomeres seem to be maintained by a telomerase-independent mechanism known as alternative lengthening of telomeres (ALT). Some of primary human tumors as well as immortalized cell lines have likewise been observed to include a population of telomerase-negative tumors, presumably surviving by ALT (51,52). Recently, it was reported that ALT could not substitute for telomerase expression and an additional function of hTERT that goes beyond its ability to elongate telomeres was required for cell transformation (53). These results clearly indicate that the ability of hTERT to elongate telomeres is not essential for facilitating function in tumorigenesis although the telomere lengthening is important to acquire the immortality.

Conclusions

Telomerase is the critical enzyme in overcoming growth limitations due to telomere dysfunction. Many scientists directly have addressed the role of telomere maintenance in control of proliferative potential and the mechanisms leading to tumorigenesis. Also, it has found that acquirement of immortality requires both telomerase activity and inactivation of oncogenes or tumor suppressors (Fig. 1a). However, from recent

results, under adequate culture conditions, telomerase is sufficient to immortalize normal cells leading to tumorigenesis. Besides, another functions of telomerase are essential for facilitating the tumorigenesis, as well as its ability to elongate telomere (Fig. 1b). Therefore, telomerase may alone extend the lifespan of mortal cells in adequate culture and contribute to experimental tumorigenesis. Among the many cellular regulatory mechanisms for immortality, the use of telomerase-based cell immortalization may offer a variety of practical applications in medical research and potentially even in improving therapies. To do this, continuing challenges for telomerase research will be required, including the elucidating the composition of telomerase complexes, the role of TERT variants, the interactions between telomerase and other components of the telomere, control of TERT transcription. To understand telomerase biology, we are currently studying the different regulation mechanisms of telomerase through interacting proteins in normal cells and immortalized cells.

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