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TELOMERASE AND ITS REGULATION

วิสาตรี คงเจริญสุนทร

KONGCHAROENSUNTORN, W.

ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา

ต.แสนสุข อ.เมือง จ.ชลบุรี 20131

Department of Biology, Faculty of Science, Burapha University,

Chonburi 20131, Thailand

wisatre@bucc4.buu.ac.th

บทคัดย่อ

เทโลเมียร์คือสายนิวคลีโอไทด์สั้นๆ ที่มีองค์ประกอบของเบสกวานีนในปริมาณมากซ้ำๆ กันไม่ต่ำกว่าหนึ่งร้อยครั้ง พบบริเวณปลายของโครโมโซมของสิ่งมีชีวิตชั้นสูง เทโลเมียร์สังเคราะห์จากเอ็นไซม์เทโลเมอเรสซึ่งคือเอ็นไซม์ประเภทเดียวกับเอ็นไซม์รีเวิสทรานสคริปเทสที่พบในไวรัสเอชไอวี เอ็นไซม์เทโลเมอเรสมีหน้าที่ควบคุมกลไกการแบ่งเซลล์แบบไมโอซีสและไมโตซีส พบว่าส่วนของเทโลเมียร์บริเวณปลายโครโมโซมจะสั้นลงทุกครั้งที่เซลล์แบ่งตัวทำให้มีการขาดหายของสารพันธุกรรมทุกครั้งที่เซลล์แบ่งตัว เข้าใจว่าความสั้นยาวของส่วนเทโทเมียร์มีบทบาทในโปรแกรมควบคุมความตายของมนุษย์ (apoptosis) มีหลักฐานพบว่าการทำงานของเอ็นไซม์เทโลเมอเรสจะสูงมากและสายเทโลเมียร์จะสั้นลงในเซลล์มะเร็งหลายชนิดเช่น เซลล์มะเร็งตับ เซลล์มะเร็งลำไส้ใหญ่ ในอนาคตอาจจะมีการนำเอ็นไซม์เทโลเมอเรสและเทโลเมียร์มาใช้รักษาโรคมะเร็งหลายชนิด ถ้าสามารถจะกระตุ้นให้เอ็นไซม์เทโลเมอเรสทำงานมากขึ้นซึ่งส่งผลให้สายเทโลเมียร์ที่ปลายโครโมโซมยาวขึ้นเพื่อปกป้องการขาดหายของโครโมโซมทุกครั้งที่เซลล์แบ่งตัว

ABSTRACT

Telomere is a short repeated sequence with G-rich residues, localized at the end of chromosome. Telomeres are synthesized by telomerase which is a reverse transcriptase. Many models described how telomere length is regulated such as available of TBP, the limiting number of repeated units in each cell division and the control of both TBP and its accessory proteins. Telomere and telomerase play a pivotal role in senescence especially in the somatic cells with a high degree of cellular turnover. The increased activity of telomerase have been found in many carcinoma cell lines such as human ovarian carcinomal, hepatocellular, colorectal carcinomal cell lines. Also, cancer cells with long telomeres and a high telomerase activity can contribute in a high proliferative activity that could survive under anti-cancer drug treatment.

INTRODUCTION

Telomeres an essential component was first identified in the ciliate Tetrahymena (Counter, 1994). Telomeres are comprised of tandem repeats of DNA sequence that maintains chromosome stability. The short repeated sequences have been highly conserved among protozoa, fungi, flagellates, plants and animals. A special DNA polymerase like telomerase synthesizes telomeres at the tip of chromosomes. Telomerases dynamically control shortening and lengthening of teromeric sequence and many telomere-binding proteins (Greider, 1996). Thus, this reviews discussed on telomere structure and function, mechanisms of telomere length regulation, telomeres in cellular senescence and aging, also telomerases in cancer cells.

TELOMERE STRUCTURE AND FUNCTION

Telomeres are the short repeated sequence, rich in G residues and located at the end of the chromosome. They are essential for stabilization of linear chromosomes and the completion of chromosomal DNA replication (Yu, 1990). Telomeres found in human chromosomes and the

chromosomes of other mamals array (TTAGGG)n for 1-15 kb whereas the telomeric sequence of the ciliate *Tetrahymena* contains TTGGGG repeats (Counter, 1994). Most numbers of repeated unit in eukaryotes are different, however, they are conserved.

TELOMERASE

The G-rich strand of telomeric DNA is synthesized by telomerase, a ribonucleoprotein enzyme with an essential RNA component. The RNA component provides the template for telomerase synthesis. For example, the telomerase RNA of *Tetrahymena* contains the nine template nucleotides 5′CAACCCCAA 3′ within its 159-bp polynucleotides. The process of telomerase synthesis includes recognition of the telomerase substrates, enlongation of TTGGGG sequence, and translocation and repositionds of the terminal TTGGGGTTG sequence (Greider, 1996). Mutation at the template nucleotides causes the synthesis of the new telomerase sequence corresponding to the mutated RNA sequence (Greider, 1996). This result leads to nuclear cell division defects (Yu, 1990). In *Tetrahymena* telomerase, its telomerase protein components are composed of two polypeptides, p80 and p95. P95 binds to telomeric primers, and p80 recognizes an element of RNA secondary structure. P95 is important in the catalysis of polymerization reaction because it contains RNA dependent RNA polymerases, and DNA polymerase.

TELOMERE BINDING PROTEINS

Telomerase binding proteins (TBPS) are required to protect the ends of a chromosome. Therefore, they play an important role in telomerase length regulation. The best characterization of telomere binding proteins is represented by RAP1 (repressor/activator protein1) which is found in yeast. RAP1 favors the assembly of SIR proteins (SIR3 and SIR4) in order to form a repressive complex at the telomere (Shore, 1994). As a result, this complex acts as the limiting factor of telomere enlongation. The RAP1 truncation also results in extremely enlongated

telomeres (Shore, 1994). Rif p1 (RAP interaction factors) interacting RAP1 at C-terminal end causes silence chromatin transcription in yeast (Greider, 1996).

MODELS FOR LENGTH REGULATION

There are three models that represent how proteins interact with telomeres and regulate telomere length. The first model proposes that TBPs individually limit telomere growth, so the amount of available TBP controls the number of telomere repeatd units. This model is called the limiting factor model. The telomerase activity regulation model shows that the TBPs and other accessary factors bind to telomerase, thus limiting the number of repeated units that are added at each cell division (Greider, 1996). The third model suggests that telomerase activity is not regulated by TBPs and their accessory proteins, the double-stranded telomere repeats or the G strand could be cleaved, then the telomerase enlongation process is inhibited (Greider, 1996).

TELOMERES IN CELLULAR SENESCENCE AND AGING

A lot of evidences have shown that human fibroblasts undergo a finite number of divisions in culture and senesce. The senescence of these cells may play a role in aging of the organism especially in many somatic cells that have a high degree of cellular turnover such as fibroblast, and various epithelial cells (Greider, 1996). The correlation between telomere length in different tissue and aging has shown that the more number of cell divisions, the less degree of telomere reduction. Mutation at the template site of the telomerase gene causes long telomeres and morphological changes in *Tetrahymena* cells (Yu *et al.*, 1990).

Also, the process of growth arrest and cell differentiation is associated with dramatically decreased telomerase activity (Fu *et al.*, 1999). The studies have shown that when normal human fibroblasts are transfectsed with a vector encoding telomerase catalytic subunit, theirs life span can be extended (Bodnar *et al.*, 1998). Moreover, decreased telomerase activity in pheochromocytoma cells leads to increased sensivity to apoptosis (Fu *et al.*, 1999). The latter

hypothesis gained strong support that inhibition of telomerase could be an important treatment strategy for many different kinds of cancer.

TELOMERES AND TELOMERASE IN CANCER CELLS

The characteristics of telomere shortening and telomerase activation are commonly found in tumor cells. The stabilization of telomere length in the immortalized cells is probably due to the activation of telomerase that maintain telomere length. The telomerase activity and telomere length were determined in human ovarian carcinoma cells and hepatocellular carcinoma cells. Both experiments found that telomeres are short and telomerase activity is present in both types of carcinoma cells, compared to the normal cells (Greider, 1996; Tahara *et al*, 1995; Counter *et al.*, 1994). So far, telomerase activity and telomere length contribute to the drug resistance in carcinoma cells (Kuranaga *et al.*, 2001)

The telomerase that presents in human ovarian carcinoma was tested in ascitic fluid from patients with end-stage in epithelial ovarian carcinoma, normal ovarian epithelium, and leucocytes. The assay for telomerase were performed with the extracts of these cells by adding telomere (TTAGGG)3, dGTP, and the mixtures of buffers. The yielding products were sequenced in order to detect a 6-nucleotide repeat ladder. The results were shown that telomerase activity was present in ascitic cells from patients whereas the extracts from epithelial cells and leukocytes had no detectable activity. To determine telomere length in fresh tumor cell from patients' ascitic fluid was done by southern analysis using a (CCCTAAA)3 telomeric probes. Consistantly, the results were shown that everage TRFS (Terminal Restriction Fragments) in tumorgenic cells was short (4.7$\pm$ 0.7kb in unfractionated ascites, and 4.5 $\pm$ 0.6 kb in fractionated ascitics). Therefore, this study showed that not only telomerase is specifically activated in ovarian tumor cells, but also very short telomeres have been detected (Counter *et al.*, 1994).

Telomerase activity in chronic liver disease and Hepatocellular Carcinoma (HCC) was examined in 105 frozen samples from human normal liver tissue, chronic liver disease, and

HCC. All these samples were extracted and were assays by TRAP method that is the same as telomerase assay in ovarian carcinoma, but PCR is used to detect telomere instead of telomere sequencing. The results were shown that telomerase activity was positive in 28 of 33 HCC tissue (85%) whereas it was negative in normal liver. However, very weak telomerase activity was detected in 55% (25 of 46) of chronic liver disease. This paper reported that expression of high levels of telomerase activity correlated with HCC. Also, very low activity was detected in one-half of noncacerous liver disease (Tahara *et al.*, 1995).

The role of telomerase activity and telomerase length in expression and maintainance of grug resistance among carcinoma cell lines has been investigated. The two colectal carcinoma cell lines, Lovo cells and DLD-1 cells were treated with anti-cancer drugs, *cis*-dianuninedichloroplatinuni, and 5-fluorouracil and the telomere length and telomerase activity were monitored. As the cell growth of colorectal carcinoma cell lines was accelerated during treatment of anticancer drugs, the telomere length considerately elongated, and the telomerase activity was strongly activated. This result may suggest that telomerase is essential for the continue proliferation of the colorectal carcinoma cells that acquired drug resistance (Kuranaga, 2001).

The high telomerase activity have been detected in most of immortalized cell lines and in human cancer cells. In contrast, telomerase activity is below detectable level in normal human cells. This implicated that human telomerase plays a role in cellular moralization and tumorigenesis (Li *et al.*, 1998). Also, it may be a suggestion to detect telomerase activity as a marker for malignancy, also for unlimited cell division in tumor proliferation.

CONCLUSION

As previously described, telomere stability and telomere length plays a role in cellular senescence and aging also involves in cancers. The current effects to deign specific inhibitors for telomerases may require for cancer therapies. In the next generation, the studies of telomere length regulation could help to complete the puzzles of complexities of chromosome dynamics.

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