International Journal of Modern Developments in Engineering and Science
Volume 1, Issue 4, April 2022
https://www.ijmdes.com | ISSN (Online): 2583-3138

Estimation of Telomeric Length as Critical Concern Towards Cellular Senescence and Age-Related Diseases

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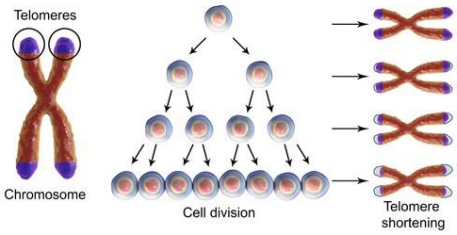
***Abstract*: Telomeres are essential genomic regions in many organisms including eukaryotes. Irregular shortening of telomeres can be life-threatening when it leads to its dysfunction and early ageing would led the particular cell to reach pre-mature cell death. Ageing organisms accumulate senescent cells that are thought to contribute to body dysfunction. Telomere shortening and damage are recognized causes of cellular senescence and ageing. The shortest telomeres that trigger DNA damage responses leading to replicative senescence in mammals. Therefore, determination of telomeric length is very important in preventing body dysfunction to take place.**



***Keywords*: Telomeres, DDR, SASP, telomere dysfunction, southern blotting, qPCR, QFISH, STELA, TeSLA, telomere syfunction assay.**

1. Introduction

Soveit theorist Alexei Olovnikov first recognized that chromosomes could not completely replicate their ends and which was designated as "end-replicative problems". [1] In the year of 1975-1977, Elizabeth Blackbum working as postdoctoral fellow at Yale University with Joseph G. Gall discovered the unusual nature of telomeres with their simple related DNA sequences composing chromosome ends. [2] Furthermore, Blackburn, Carol Greider and Jack Szostak were awarded with 2009 Nobel Prize in Physiology or Medicine for their discovery in the mechanism of how chromosomes are protected by telomeres and the enzyme telomerase. [3]

Telomeres are the genomic portions at the ends of linear chromosome. Telomeric DNA in vertebrates is made up of TTAGGG repeats bound by a set of proteins that modulate their biological functions and protect them from being recognized as DNA damage which triggers a DNA damage response (DDR). We know that standard DNA polymerase cannot fully replicate the entire genome and therefore another enzyme, telomerase which is a DNA-template independent DNA polymerase is present which prevents in formation of progressive shortened telomeres. Telomeres are most commonly found in eukaryotes. [4] They protect the terminal regions of chromosomal DNA from progressive degradation and ensuring the integrity of linear chromosomes by preventing DNA repair systems in considering the very ends of DNA strand as a double strand break.

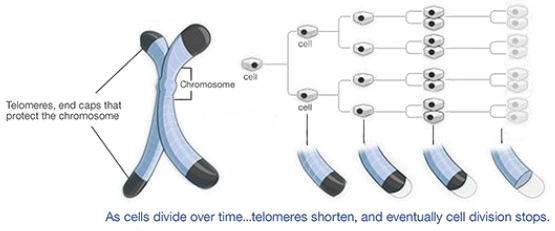


Fig. 1.

In the absence of telomerases, shortening of telomeres take place. As telomeres reach a critical length, they become unable to bind enough telomere-capping proteins and are then sensed as exposed DNA ends [5] which activates DDR pathways through the induction of cell cycle inhibitors like p21 and p16 and arrest proliferation [6]. Activation of the DDR at telomeres (termed tDDR) results in the formation of telomere associated DDR foci (TAFs) or telomere-induced DNA damage foci (TIFs), which are markers of cellular senescence in cultured cells and tissues. Telomeric DNA is hypersensitive to oxidative DNA damage, a phenomenon named TelOxidation.

Here, the main focus is to determine the length of telomeres using various sophisticated techniques and molecular approaches been discovered so far. The outcomes of such length determination have also been outlined here.

2. Materials and Methods

The methods used for determination of telomere length and dysfunction are as follows:

A. Southern Blotting

Southern blotting named after the British biologist Edwin

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Southern [7], is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. It measures mean telomere length using the length distribution of terminal restriction fragments (TRF).

Based on the knowledge that the sequence $TTAGGG_n$ was highly conserved, the TRF analysis was developed using a $TTAGGG_n$-labelled probe and is now a widely used method for telomere measurement. Regarded as the 'gold standard' for TL measurement. While a range of telomere sizes can be imaged on Southern blots, the shortest telomeres cannot be visualized.

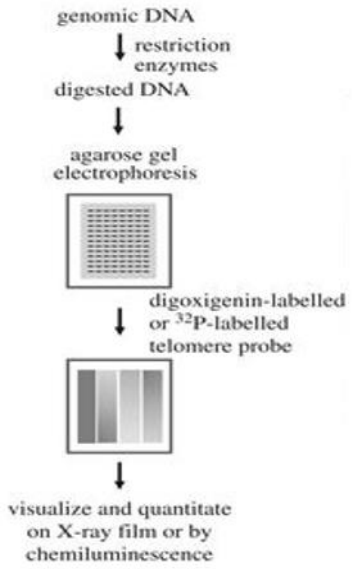

Fig. 2. Procedure to perform Southern Blotting

B. Quantitative Polymerase Chain Reaction (Q-PCR)

Q-PCR also known as real-time polymerase chain reaction is a laboratory technique of molecular biology based on polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during PCR in real time not at its end. Real-time PCR can be used quantitatively and semi-quantitatively.

Q-PCR assay is a relatively easy assay not requiring a large amount of starting DNA. This method measures telomere signal (T) to a reference single-copy gene signal (S) and thereby calculate T/S ratios. This ratio is proportional to average TL and therefore can be used to determine relative TLs. There occurs reported variability within and between samples and there may be large variations between independent laboratories because different single copy loci are used and, in some instances, the amplified control genes may not be unique in the genome affecting T/S ratio.

Moreover, Q-PCR does not provide information about the shortest telomeres. Also, Q-PCR may not be useful for cancer studies where reference single-copy gene may have been duplicated or lost due to aneuploidy. Application of Q-PCR is limited to samples that are normal diploid and karyotypically stable. [8]

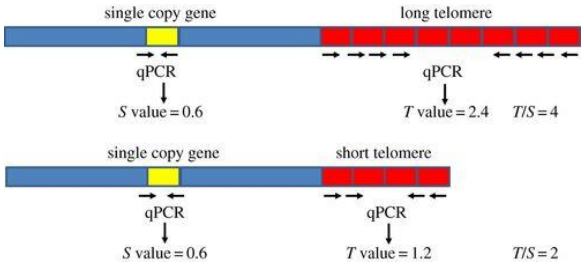

Fig. 3.

C. Quantitative Fluorescence in Situ Hybridization

Telomeric lengths can also be measured using several Q-FISH methods, which are based on similar principles but with some modifications for various applications. Interphase Q-FISH uses a microscope to determine telomere fluorescence intensity after hybridization with a fluorescent peptide nucleic acid (PNA) telomeric repeat ($CCCTAA_3$) probe. Similar experiment was also performed with metaphase spreads.

Metaphase Q-FISH can measure TL at each chromosome end with higher accuracy but simultaneously, require proliferating cells. Commercial modification of interphase Q-FISH, termed high-throughput Q-FISH (HTQ-FISH) which can use automated procedures on 384-well plates for large scale studies on fixed lymphocytes. This assay is termed Telomere Analysis Technology and is CLIA (Clinical Laboratory Improvement Amendments) certified.

Due to limits on probe hybridization, Q-FISH methods do not detect fluorescent signals at telomere at chromosome ends that have telomeric repeats below the threshold for PNA probe hybridization (so called telomere-free ends). [9]

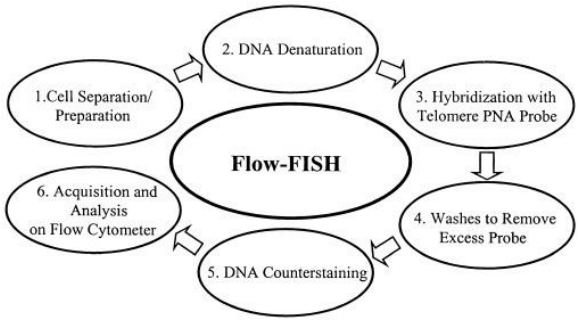

Fig. 4. Flow FISH

D. Telomere Dysfunctional Induced Foci Analysis

Telomere dysfunction assay that is useful for DNA damage studies is TIF analysis (Telomere dysfunctional Induced Foci). DDR is a set of cellular events that follows generation of DNA damage. Dysfunctional telomeres activate DDR in ageing, cancer and an increasing number of identified pathological conditions. Following DNA double strand break (DSB) generation, it becomes essential to recruit DDR proteins to sites of DNA damage. Therefore, the apical DDR kinase ataxia telangiectasia mutated (ATM) undergoes activation and phosphorylates the histone H2AX at serine 139 also ATM phosphorylated at serine 1981 (pATM) itself and p53-binding protein 1 (53BP1).

This method is conducted on interphase cells in-vitro or in tissue sections and which generally involves using two

antibodies, one to a shelterin protein such as TRF2 and other to an antibody that recognizes DNA double-stranded breaks, such as gamma H2SX or 53BP1. Another approach is to use a PNA telomeric repeat probe to co-localize with gamma H2AX or 53BP1. However, this assay does not provide information about TL, only those that are so short or uncapped appear as damaged DNA. In combination with Telomere restriction fragment analysis (TRF) or with Q-FISH, TIF analysis could provide information about the average TL when telomeres are sufficiently short to initiate onset of disease.[10]

E. Single Telomere Length Analysis

Single TElomere Length Analysis (STELA) was designed to measure telomeres on individual chromosomes. STELA measures the abundance of the shortest telomeres using a combination of ligation, PCR-based methods and Southern blot analysis. However, since not all chromosome ends have unique sequences for the design of primers, and this restricts the use of STELA. This problem was resolved upon using Universal STELA (U-STELA) method which is able to detect telomeres from every chromosome end and thereby making it possible to monitor changes in the shortest telomere in cells. [11]

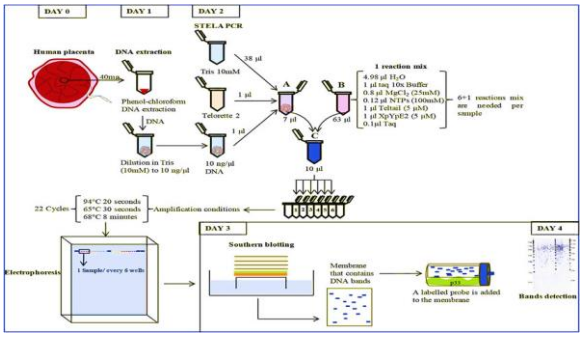

Fig. 5.

F. Telomere Shortest Length Assay (TeSLA)

TeSLA requires small amounts (less than one microgram) of starting DNA. This method employs an improved ligation and digestion strategy, the classic Southern blot analysis with hyper-sensitive digoxigenin-labelled probe and user-friendly image-processing software to automatically measure the distribution of telomeres at different lengths. Additionally, TeSLA does not amplify ITSs that are very large in some animals. [12]

3. Discussion and Conclusion

Positive outcomes after determining length of telomeres:

- As shortening of telomere can be life-threatening, therefore modern methods to determine the length of telomere would facilitate in early detection of any disease that would be associated for the same.
- For cancer cells, unregulated increase of length of telomeres allows infinite cell divisions which ultimately brings to the fate of tumor, therefore upon detecting certain abnormality if any of described methods performed by extracting the genome portion from the blood of patient, then the abnormality can be ascertained at earlier and further destructive behavior can be prevented.
- Determination of telomere length would facilitate us to understand the size of telomere and if length found below critical size, certain methods can be performed to prevent the cells facing SASP.

Even after posing such tremendous benefits with identification of telomeric length, there still remains few unanswered questions which needs to be resolved. They are:

- How to detect if a person needs to undergo his/her telomeric length determination?
- Will there be any specific phenotypic change in behavior in the person before SASP begin to take over so that early detection would be possible?
- Even if any person has been detected priory, what effective method could be executed to relief the person from undergoing pre-mature ageing or facing untimely death?

4. Future Prospects Need to be Taken Care Off

- Awareness must be raised among people to take step towards regular body check to get chance to detect the telomeric length as a routine checkup.
- A unique device needs to be identified which would help in identifying the phenotypically behavioral changes if found associated with telomeric shortening before SASP.
- STELA can prove to be very costly, so identification of new modification in southern blotting or QPCR need to be figured out which could easily determine the shortest telomeric repeats.

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