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Role of Telomere in Different Hematological Diseases

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Abstract:

Background: Chromosomal integrity is essential for normal cellular function and organismal survival. Telomeres serve as protective caps that prevent chromosome ends from being recognized as DNA damage, thereby inhibiting inappropriate repair mechanisms and chromosomal fusions. Since their discovery, telomeres and telomerase have emerged as central players in aging biology and cancer research. Landmark discoveries in this field have demonstrated that telomere shortening acts as a molecular clock limiting cellular replicative capacity, while telomerase reactivation enables cellular immortality in malignant cells. Understanding telomere biology has therefore become increasingly relevant for both basic research and clinical practice.

Keywords: Telomere; Telomerase; Shelterin complex; Telomere length; Aging; Cancer; Hematological diseases

Introduction:

Telomeres are specialized nucleoprotein structures located at the ends of linear eukaryotic chromosomes, composed of repetitive DNA sequences (TTAGGG in humans) and associated binding proteins. Their primary role is to preserve chromosomal integrity by preventing chromosome ends from being recognized as DNA double-strand breaks, thereby inhibiting inappropriate DNA damage responses and chromosomal end-to-end fusions. With each round of cell division, telomeres progressively shorten due to the end-replication problem, ultimately leading to cellular senescence or apoptosis when a critical length is reached. This progressive shortening positions telomeres as key regulators of cellular aging and replicative capacity **(1).**

Telomere length homeostasis is maintained through the coordinated action of telomerase and telomere-associated protein complexes, particularly the shelterin complex. Telomerase, a ribonucleoprotein reverse transcriptase composed of telomerase reverse transcriptase (TERT) and an RNA template (TERC), counteracts telomere attrition by adding telomeric repeats to chromosome ends. While telomerase activity is tightly repressed in most somatic cells, it remains active in germline cells, stem cells, and is reactivated in the majority of human cancers, enabling unlimited cellular proliferation. Dysregulation of telomere maintenance mechanisms has been strongly implicated in aging-related disorders, malignancies, and hematological diseases, highlighting telomeres as both biomarkers and potential therapeutic targets **(2).**

Telomeres and telomerase has been a ground breaking achievement in molecular biology, shedding light on the mechanisms of aging and cancer Elizabeth Blackburn, Carol Greider, and Jack Szostak were awarded the Nobel Prize in Physiology in 2009 for their discovery of telomerase, the enzyme responsible for maintaining telomere length. Their work, published in the journal Cell in 1985 **(1),** revolutionized our understanding of cellular aging and provided insights into diseases such as cancer, where telomerase activity is often dysregulated

I. Structure:

Telomeres are non-coding nucleoprotein structures that protect the ends of eukaryotic chromosomes by discriminating natural ends from regular incidental DNA breaks Telomere length (TL) differs among diverse species.

Human telomeres are comprised of thousands of tandem repeats of the 5'-TTAGGG-3' sequence and their length typically ranges between 10 and 15 kilobase pairs (kbp). Although human telomere length has a broad distribution, there are finite upper and lower borders **(3).**

Shelterin (also called telosome) is a protein complex comprising up to six different proteins; (**Figure 1)** the composition of the complex may vary depending on the chromosomal context. Shelterin binds both the single-stranded DNA (ssDNA) and dsDNA regions of telomere **(2)**

Shelterin proteins play critical roles in telomere protection by facilitating the formation of higher-order telomere structures, inhibiting DNA damage responses, and regulating telomere length. **(2)**

It`s responsible for maintaining genetic integrity, assisting in the prevention of excessive DNA damage and regulating telomere-telomerase interaction. The complex protects telomeric DNA from unwanted degradation and chromosomal fusions. **(2)**

The key components of the shelterin complex include: **(4)**

A. Telomeric Repeat Binding Factor 1 (TRF1): Binds specifically to double-stranded telomeric DNA and regulates telomere length by inhibiting telomerase activity.
B. Telomeric Repeat Binding Factor 2 (TRF2): Protects telomeres from DNA damage responses and prevents chromosome end-to-end fusion by promoting the formation of a protective cap structure.
C. TRF1-Interacting Nuclear Factor 2 (TIN2): Serves as a bridging molecule within the shelterin complex, connecting TRF1 and TRF2 to other shelterin components.
D. Repressor/Activator Protein 1 (RAP1): Binds to telomeric DNA and regulates telomere length and stability, as well as participating in DNA repair processes at telomeres.
E. Protection of telomeres 1 (POT1) and TIN2-Interacting Protein 1: Interacts with POT1 and TIN2 to stabilize the shelterin complex and regulate telomerase access to telomeres.
F. Protection of Telomeres 1 (POT1): Binds specifically to single-stranded telomeric DNA and protects telomeres from being recognized as DNA damage sites.

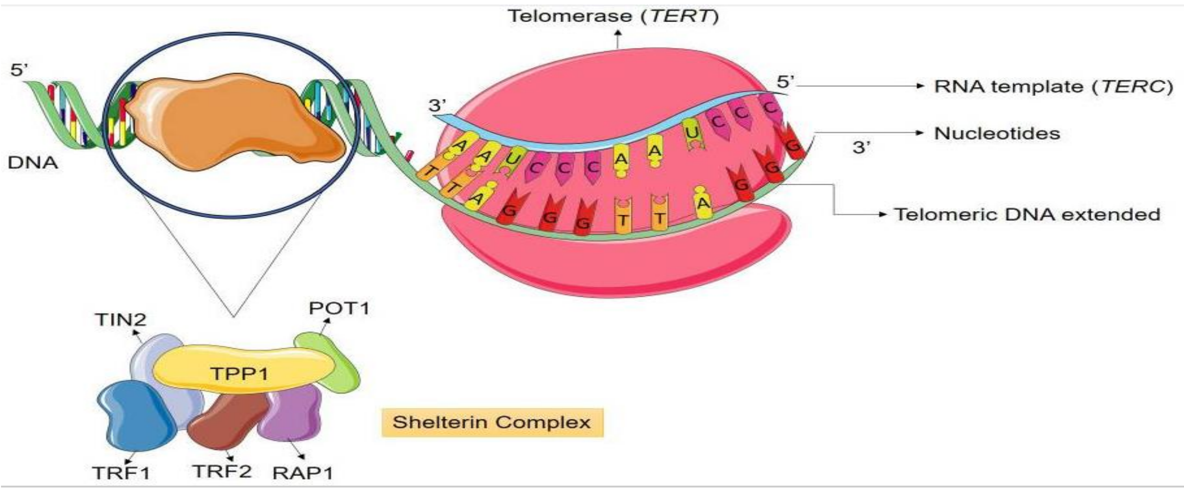

Figure (1) Sheltrin complex structure and formation

The shelterin complex protects telomeres and regulates telomere elongation. TRF1/2, RAP1, and TIN2 are associated with doublestranded DNA; POT1 and TPP1 bind to the single-stranded G-tail DNA and are responsible for recruiting telomerase to telomeres, The absence of a shelterin complex causes telomere uncapping and thereby activates damage-signaling pathways.

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Telomerase enzyme counteracts nucleotide loss during cell replication. The catalytic domain has telomerase reverse transcriptase (TERT) activity **(Figure 2)**, which utilizes the telomerase mRNA (TERC) subunit as a complementary template for the addition of nucleotides to telomeric DNA. **(5)**

The repetitive telomeric DNA sequence provides a platform for the binding of shelterin proteins and serves as a buffer against telomere shortening during DNA replication. **(6)**

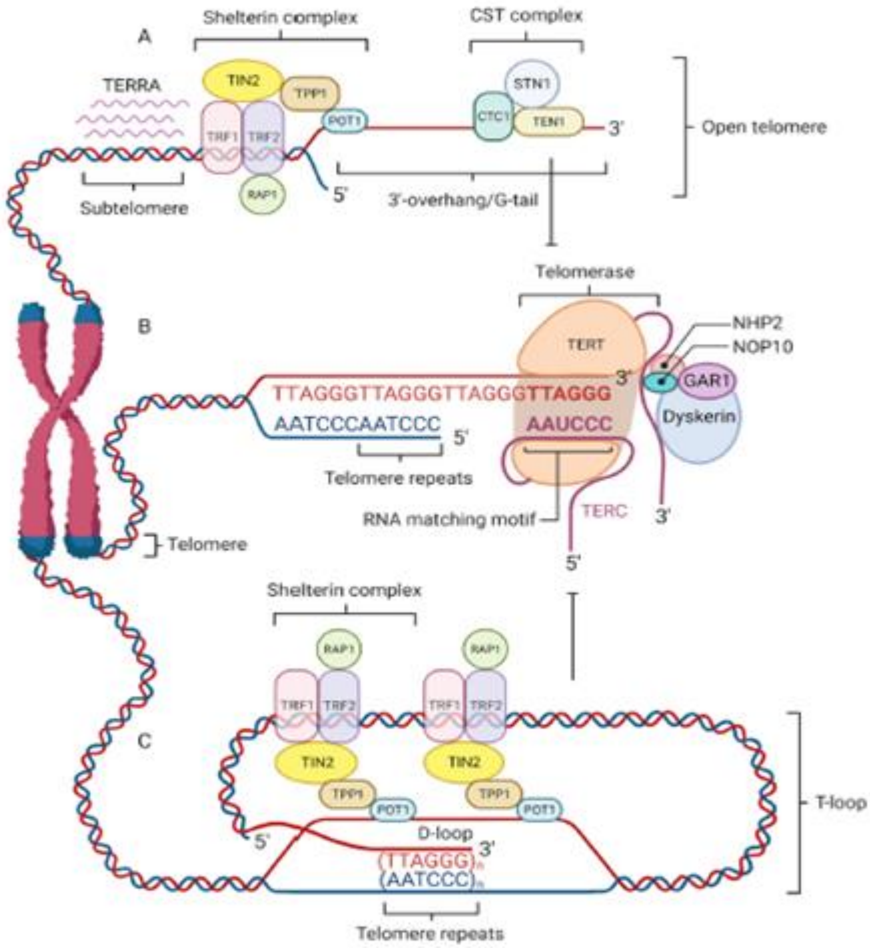

Figure (2) Telomere structure:

Four factors contribute to telomere maintenance: the shelterin complex, telomerase, telomeric

repeat-containing RNA (TERRA), and the CST complex.

Telomerase is a reverse transcriptase enzyme that carries its own RNA molecule, which is used as a template in telomere elongation. This ribonucleoprotein complex consists of TERT (telomerase reverse transcriptase), TERC (telomerase RNA component), dyskerin, NOP10, NHP2, and GAR1 (**B**). TERRA is transcribed from telomere DNA and together with the shelterin complex inhibits telomere lengthening by telomerase (**A**). The CST complex localized on the single-stranded 3′ overhang prevents telomerase from binding to the 3′-overhang and interacts with DNA Polα-primase during telomere replication (**A**).

(5)

II. Normal physiology:

Factors influencing telomere length (TL). Generally, TL is considered a dynamic marker reflecting genetic predisposition as well as individual environmental conditions. TL gradually decreases with age. Additional factors that have been correlated with TL decrease are stress, smoking, alcohol, obesity, chronic inflammation, viral infections as well as hormone imbalance. On the other hand, factors that have been shown to increase telomere length apart from telomerase activity are regular physical activity, low oxidative stress, antioxidants, and low stress daily life**.** **(7**)

Telomere length homeostasis is tightly regulated by a balance between telomere shortening (due to the end-replication problem) and telomere elongation (mediated by telomerase or alternative mechanisms).**(8)**

A major characteristic of telomeres is their progressive shortening that happens naturally with each division of somatic cells. The shortening occurs due to the DNA end replication problem, which consists of the inability of the cellular

machinery to effectively synthesize the chromosomes ends during replication, alongside with the lack or insufficiency of pathways that promote telomere elongation, such as telomerase activity or the alternative lengthening of telomeres (ALT **) (9)**

Telomere elongation occurs primarily by human telomerase (hTERT) activity, which is a ribo-nucleo protein enzyme specialized in neutralizing telomeric DNA attrition by synthesizing new TTAGGG repeats at chromosome ends.**(10)**

Telomerase uses its intrinsic RNA template to synthesize telomeric DNA repeats and adds ~60 nucleotides per telomere per cell cycle in a processive manner. In most cases in humans, there is a major difference in the expression of telomerase between somatic cells and germline cells, telomerase is more abundant in germline cells, which helps maintain telomere length, and is absent or less abundant in somatic cells. Not surprisingly, immortalized cells and cancer cells often have high telomerase activity. Telomerase recruitment to telomeres is mediated by the shelterin complex **(2)**

III. Function

The main functions of a telomere is to maintain chromosomal stability and prevent chromosomal degradation. Additionally, telomeres protect the ends of the chromosome from DNA end-joining to one another, damage response to DNA, and accidental DNA recombination **(11)**

The longer 3' G-rich end overhang that creates the T-loop protects the end of that chromosome from appearing as a double-stranded break in the DNA strand, thus preventing unwanted DNA repair. For these reasons, telomeres and their maintenance are essential to eukaryotic genomic stability and the longevity of cellular information. **(11)**

Telomere length homeostasis is tightly regulated and plays a critical role in cellular aging and proliferation, with short telomeres acting as a molecular clock for cellular senescence.**(12)**

Dysfunction of telomeres leads to genomic instability, cellular senescence, and is implicated in various human diseases, including cancer **(13)**

The telomere higher-order architecture must be unravelled for the telomeric tail to be rendered accessible to telomerase **(2)**

IV. Regulation:

Proper telomere regulation is essential for maintaining genomic stability, cellular proliferation, and preventing cellular senescence and apoptosis. **(14)**

A. Core Components of Telomere Regulation

1. **Telomerase Complex**:
    - Human telomerase reverse transcriptase (hTERT): The catalytic protein component that adds repetitive DNA sequences to the 3′ ends of chromosomes. Its expression is tightly regulated and is often the rate-limiting factor in telomerase activity. **Figure (**3**)**

- Human telomerase RNA (hTR): Serves as a template for telomere DNA synthesis and is ubiquitously expressed in tissues. **(14)**

2. **Telomere-Associated Proteins:**

- TRF1 and TRF2: Bind to double-stranded telomeric DNA, forming the t-loop structure that protects chromosome ends.
- POT1: Binds to single-stranded telomeric DNA, regulating telomerase access.
- PinX1: Interacts directly with hTERT, acting as a potent telomerase inhibitor **Figure** (3). **(5)**

B. Mechanisms of Telomere Regulation

1. **Transcriptional Control of hTERT:**

- The hTERT gene is located on chromosome 5p15.33. Its transcription is repressed in most somatic cells but can be activated during cellular transformation or immortalization.
- Transcriptional repressors such as Mad1, p53, pRB, and WT1 play crucial roles in downregulating hTERT expression.
- Conversely, factors like c-Myc and E2F can upregulate hTERT expression, promoting telomerase activity.

2. **Posttranslational Modifications:**

- hTERT undergoes various modifications, including phosphorylation and acetylation, which influence its stability, localization, and activity.
- These modifications can be modulated by signaling pathways responsive to growth factors, stress, and differentiation signals.

3. **Subcellular Localization:**

- The assembly of the telomerase holoenzyme and its transport to the telomeres are critical for its function.
- Regulatory proteins ensure that telomerase is active only at the telomeres, preventing inappropriate telomere elongation. Human Telomerase and Its Regulation **(5)**

Alterations in telomere length have been linked to various age-related diseases, including cancer, where telomere shortening may promote genomic instability and tumorigenesis. **(15)**

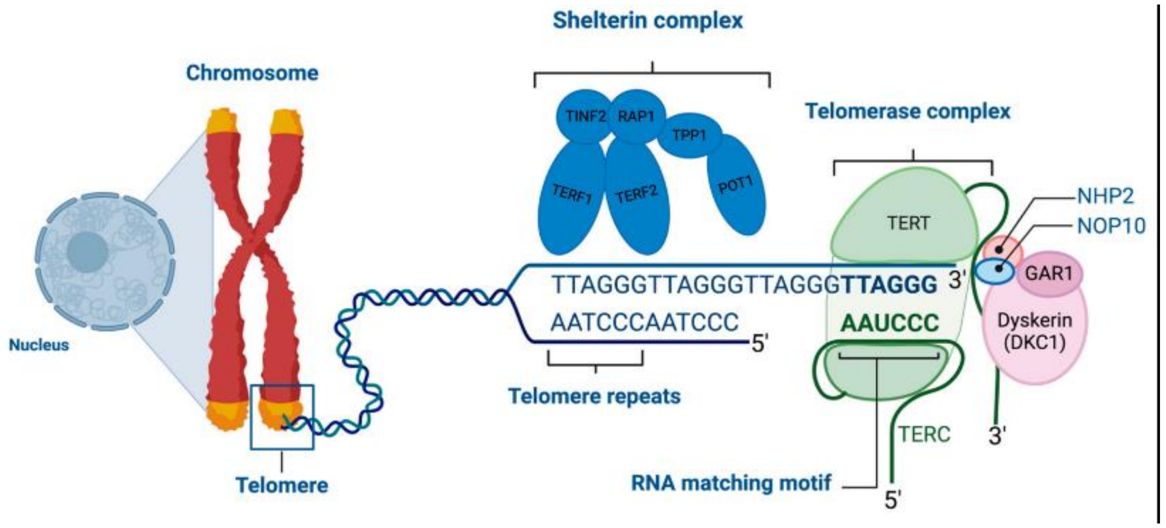

Figure (3) Telomere Regulation

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Schematic representation of telomere regulation showing the shelterin complex bound to telomeric DNA and the telomerase holoenzyme at the chromosome end. Shelterin components (TRF1, TRF2, TIN2, TPP1, POT1, RAP1) protect telomere structure and control access of telomerase, while telomerase (TERT, TERC, and associated proteins) elongates the G-rich strand to maintain telomere length and genome stability **(5)**

V. Methods of Detection of Telomere Length

The accurate measurement of telomere length is critical for understanding telomere biology, its role in aging, and its involvement in various diseases. Multiple analytical techniques have been developed, each with distinct principles, advantages, and limitations. The major methods are outlined below.

1. Terminal Restriction Fragment (TRF) Analysis

Terminal Restriction Fragment (TRF) analysis is widely regarded as the gold standard for absolute telomere length measurement **(16)**. In this approach, genomic DNA is digested using restriction enzymes **(Figure 3)** that cleave non-telomeric DNA while preserving telomeric repeats. The resulting fragments are separated by agarose gel electrophoresis, transferred to a membrane, and hybridized with a labeled telomeric probe using the Southern blotting technique **(17)**. Signal intensity, compared to molecular size standards, is used to derive the distribution profile of telomere lengths.
TRF analysis provides absolute telomere length values and a complete distribution profile. Its long-standing use in telomere research has resulted in strong validation and reproducibility. However, the method is labor-intensive, requires large quantities of high-quality DNA, and does not resolve telomere lengths at the individual chromosome level. Its resolution is also limited for extremely short telomeres. **(18)**

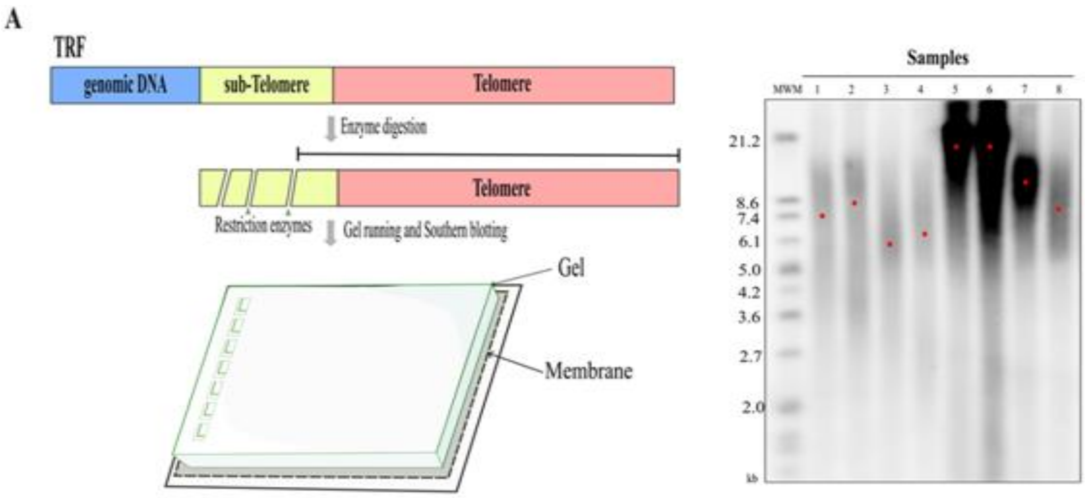

Figure (3) Terminal Restriction Fragment

DNA is digested using restriction enzymes that do not recognize telomere sequences. Gel electrophoresis is then performed, followed by blotting onto a membrane.

2. Quantitative Polymerase Chain Reaction (qPCR)

Quantitative PCR (qPCR) offers a rapid and cost-effective approach to estimate average telomere length. The method quantifies the amplification of telomeric repeats (T) relative to a single-copy reference gene (S), expressed as the T/S ratio **(Figure 4)**. This ratio correlates with average telomere length and can be standardized across samples using calibration curves.
qPCR is advantageous due to its minimal DNA requirement, high throughput capability, and suitability for large epidemiological or longitudinal studies. However, it provides only average telomere length without distribution data. Its accuracy is influenced by reference gene selection, DNA quality, and experimental variability, and it is generally less reproducible than TRF analysis. Sensitivity for very short telomeres is also reduced. **(19)**

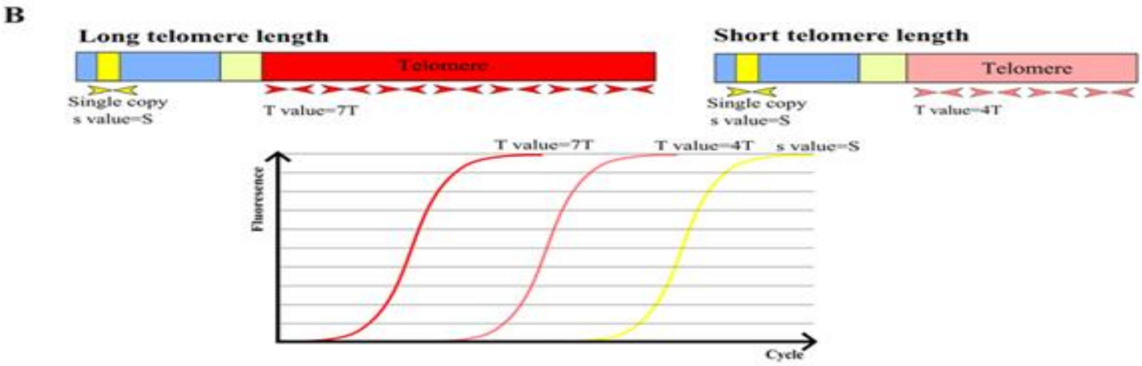

Figure (4) Quantitative PCR (qPCR)

The Q-PCR method compares the telomere length (T) to a single-copy gene (S) and represents it relative to each other, with the results expressed as a T/S ratio. We illustrate Q-PCR results from both long and short telomeres. In samples with long telomeres, the peak appears first, whereas in samples with short telomeres, the peak appears later.

3. Quantitative Fluorescence In Situ Hybridization (Q-FISH)

Quantitative Fluorescence In Situ Hybridization (Q-FISH) employs fluorescently labeled peptide nucleic acid (PNA) probes that bind specifically to telomeric repeats on metaphase chromosomes or interphase nuclei. High-resolution fluorescence microscopy is then used to quantify fluorescence intensity, which correlates with telomere length at individual chromosome ends. **(20)**

Q-FISH provides high-resolution, chromosome-specific telomere measurements and can identify the shortest telomeres **(figure 5 C)**. It is applicable to both metaphase spreads and interphase nuclei, including formalin-fixed paraffin-embedded (FFPE) tissue sections. However, it requires advanced fluorescence or confocal microscopy, It is sensitive to sample preparation quality, and is low throughput, making it unsuitable for large-scale studies. **(20)**

3.1 Flow-FISH

Flow-FISH is a variation of Q-FISH that combines PNA probe hybridization with flow cytometric analysis. Fluorescent probes bind to telomeres in intact cells, and fluorescence intensity is measured as cells pass through a laser beam in a flow cytometer **(figure 5 D)**. This enables quantification of average telomere length in large populations of cells and can distinguish telomere lengths across different cell subpopulations. **(21)**
Flow-FISH is clinically validated and offers higher throughput than traditional Q-FISH. Nevertheless, it is limited to cells in suspension, such as lymphocytes or cultured cells, and is unsuitable for adherent or solid tissue samples. It also requires specialized flow cytometry equipment and expertise, and it does not achieve the single-chromosome resolution of Q-FISH. **(21)**

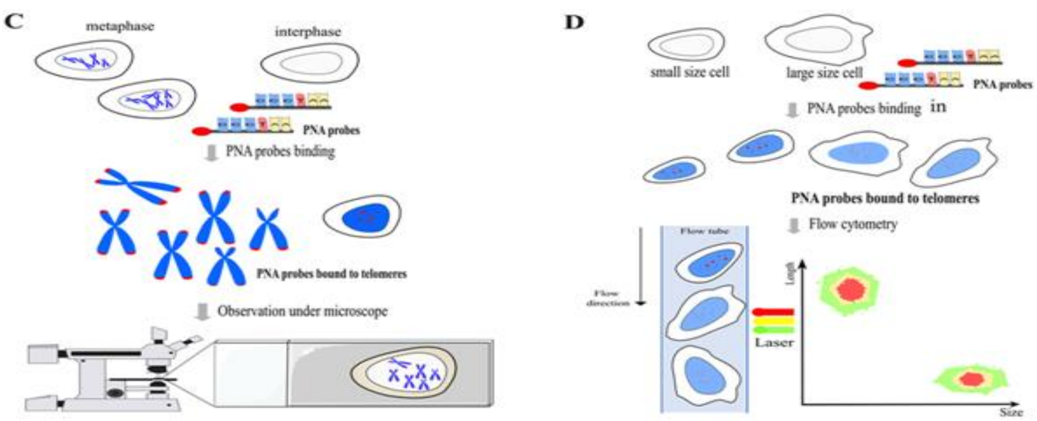

Figure (5) Quantitative Fluorescence In Situ Hybridization

C. Q-FISH: PNA probe with a fluorescent dye is used to hybridize to the telomeric DNA repeats in cells. The (*Peptide nucleic acid)* PNA bound to telomeres can be observed through fluorescence microscopy. The fluorescence intensity is proportional to the telomere length. By quantifying the fluorescence signals, researchers can determine the relative telomere length of individual chromosomes. **D. Flow-FISH:** Fluorescently labeled PNA probes specific to telomere repeats are hybridized to the telomeric DNA in cells. These cells are then passed through a flow cytometer, where the fluorescence intensity of each cell is measured. This intensity corresponds to the telomere length, allowing researchers to assess the distribution of telomere lengths in a cell population.

4. Single Telomere Length Analysis (STELA)

Single Telomere Length Analysis (STELA) is a high-resolution technique that measures individual telomeres using ligation-mediated PCR followed by Southern blotting. A specific linker is ligated to the 5′ end of the telomere, and PCR is performed with a telomere-specific primer and a primer complementary to a unique subtelomeric sequence **(Figure 6)**. The amplified products are resolved via Southern blotting to determine telomere length.
STELA excels in detecting the shortest telomeres and assessing telomere heterogeneity in small cell populations. However, it is unsuitable for chromosomes without distinct subtelomeric sequences and is ineffective when DNA is degraded or when chromosomal arm deletions occur. It is also labor-intensive and low throughput, making it impractical for large-scale studies. **(22)**.

4.1 Universal STELA (U-STELA)

Universal STELA modifies the traditional STELA method by introducing restriction enzyme digestion followed by ligation of a universal oligonucleotide to sticky DNA ends. A telorette linker is then attached to the 3′ telomeric overhang to facilitate PCR amplification **(Figure 6)**. This adaptation enables measurement of telomere length across all chromosomes. While U-STELA allows detection of critically short telomeres even from limited DNA samples, it is less accurate for long telomeres (>10 kb) and may produce artifacts, such as gel smearing, when excess template DNA is used. **(23)**.

4.2 High-Throughput STELA (HT-STELA)

High-Throughput STELA (HT-STELA) improves the scalability of STELA by employing PCR with telorette-specific primers **(Figure 6)** and analyzing fragments using automated systems such as bioanalyzers. By eliminating gel electrophoresis and Southern blotting, the workflow becomes faster and more reproducible. HT-STELA is suitable for large-scale studies, but it remains restricted to chromosomes for which primers have been designed and requires costly instrumentation. **(24)**

5. Telomere Shortest Length Assay (TeSLA)

The Telomere Shortest Length Assay (TeSLA) is designed to detect the shortest telomeres across all chromosomes. The method involves digesting and dephosphorylating genomic DNA, ligating specific TeSLA adapters, and performing PCR amplification followed by Southern blotting **(Figure 6)**. This enables detection of telomeres shorter than the threshold detectable by most other methods. TeSLA is highly sensitive, works with low DNA input, and does not require chromosome-specific subtelomeric primers. However, it cannot identify which chromosome harbors the shortest telomere, performs poorly in cases of extreme telomere heterogeneity, and is labor-intensive due to reliance on Southern blotting and long-range PCR. **(8)**

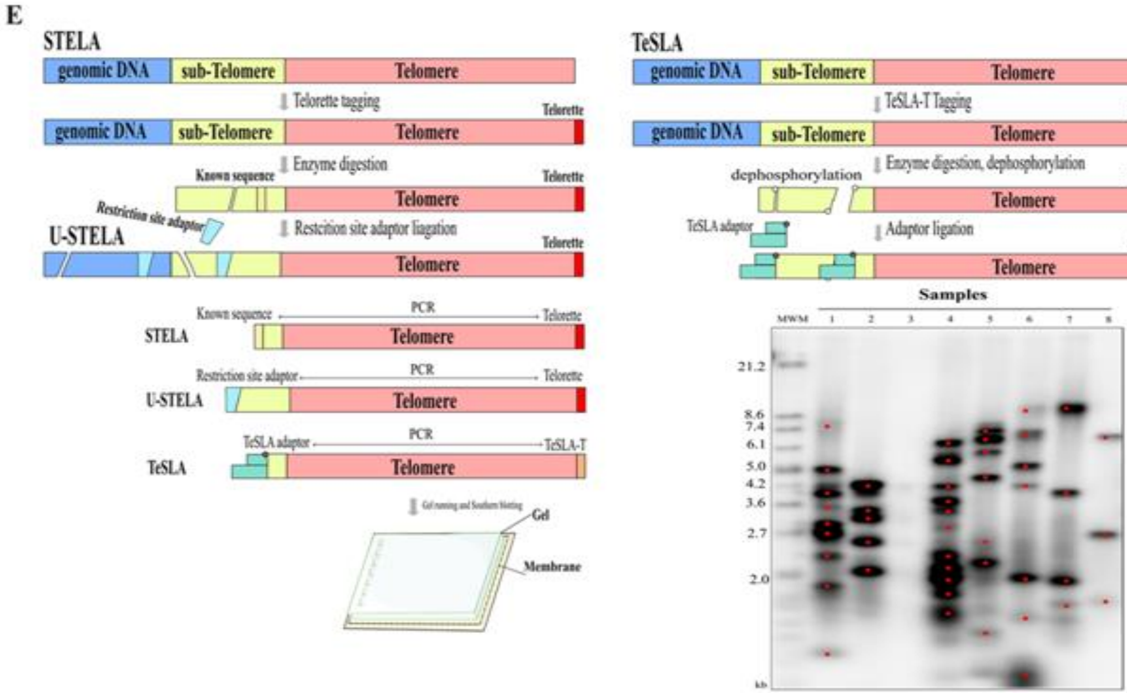

Figure (6) STELA and TeSLA

E. Southern and PCR base methods.STELA: Tagging telorette at the telomere end, and then the restriction enzyme action proceeds. PCR is performed on chromosomes known in the sub-telomere sequence to perform gel loading and Southern blotting.**U-STELA:** Tagging telorette at the telomere end, and then the restriction enzyme action proceeds. Attach binding adapters to the cleaved portion with restriction enzymes. PCR is advanced using restriction enzyme site adapter and Telorette.**TeSLA:** Tagging is performed at the end of the telomere using TeSLA-T. Enzyme digestion and dephosphorylation are performed. A TeSLA adapter that can be complementary to the dephosphorylation part as a white circle is attached. PCR was performed using TeSLA-T and TeSLA adapters.*Abbreviations: kb: kilobase pairs; MWM: Molecular weight markers*.

6. Single-Cell Telomere Length Measurement (SCT-pqPCR)

Single-Cell Telomere Length Measurement by pre-amplification quantitative PCR (SCT-pqPCR) uses whole-genome amplification of DNA from single cells, followed by qPCR to calculate the telomere-to-reference (T/R) ratio**.** This approach provides relative telomere length measurements at the single-cell level. SCT-pqPCR is valuable for detecting telomere heterogeneity within a population and is applicable to both dividing and non-dividing cells. Nevertheless, it requires careful optimization, specialized equipment, and precise normalization using multicopy reference genes. It is also sensitive to contamination and pre-PCR variability. **(25)**

7. Single Telomere Absolute-Length Rapid (STAR) Assay

The Single Telomere Absolute-Length Rapid (STAR) assay utilizes digital PCR to partition DNA into thousands of reactions, enabling precise quantification of telomeric repeats. A standard curve generated from synthetic telomere sequences is used to calculate absolute telomere lengths.

STAR is highly accurate, reproducible, and has a broad dynamic range (0.2–320 kb). It is particularly useful for studying telomere maintenance in cancer, including alternative lengthening of telomeres (ALT) mechanisms. However, it requires digital PCR instrumentation, involves higher reagent costs, and demands precise calibration with synthetic standards. **(26)**

8. Sequencing-Based Estimation Using Short-Read Next-Generation Sequencing (NGS)

Short-read whole-genome sequencing (WGS) data can be analyzed to estimate telomere length using computational tools such as Telocat, Tel-seq, and Computel. These algorithms quantify reads containing telomeric repeat sequences and normalize them to genome-wide coverage to estimate average telomere length. Sequencing-based estimation is efficient for large-scale studies and requires no additional laboratory work beyond standard WGS. However, it offers limited resolution, cannot measure telomere length at the chromosome or single-cell level, and is sensitive to sequencing biases and depth variability. It is therefore unsuitable for precise clinical diagnostics. **(27)**

Table (1) Main differences of different detection methods for Telomere Length.

Method	Principle	Advantages	Limitations
Terminal Restriction Fragment (TRF)	Restriction enzyme digestion of genomic DNA, Southern blot hybridization with telomeric probe.	Absolute length measurement; full length distribution; historically validated.	Labor-intensive; high DNA requirement; low resolution for very short telomeres; no chromosome-specific data.
Quantitative PCR (qPCR)	Compares amplification of telomeric repeats (T) to single-copy gene (S); T/S ratio correlates with length.	Fast; cost-effective; low DNA input; high throughput.	Provides average length only; influenced by reference gene and DNA quality; less reproducible than TRF.
Q-FISH	Fluorescent PNA probes hybridize to telomeres on chromosomes/nuclei; signal measured by microscopy.	High resolution; chromosome-specific; detects shortest telomeres; applicable to FFPE samples.	Requires advanced microscopy; low throughput; sensitive to preparation quality.
Flow-FISH	Combines PNA probe hybridization with flow cytometry to measure telomere fluorescence in cell populations.	Higher throughput than Q-FISH; distinguishes cell subsets; clinically validated.	Limited to cells in suspension; requires flow cytometer; lacks single-chromosome resolution.
STELA	Ligation-mediated PCR targeting unique subtelomeric sequences; Southern blot detection.	Measures individual telomeres; detects shortest telomeres; assesses heterogeneity.	Chromosome-specific; requires intact DNA; labor-intensive; low throughput.
U-STELA	Adds restriction digestion and universal oligonucleotide ligation for pan-chromosomal measurement.	Detects short telomeres from limited DNA; broader applicability.	Inaccurate for long telomeres; PCR artifacts possible.
HT-STELA	STELA variant using automated fragment analysis (e.g., bioanalyzer) instead of gels/blotting.	Faster; scalable; reproducible.	Requires expensive instruments; limited to designed primer targets.
TeSLA	Adapter ligation and PCR/Southern blot to detect shortest telomeres across all chromosomes.	Highly sensitive; low DNA input; detects very short telomeres.	Cannot identify specific chromosomes; labor-intensive; limited in highly heterogeneous samples.
SCT-pqPCR	Whole-genome amplification of single cells followed by qPCR T/R	Single-cell resolution; detects intra-population	Sensitive to contamination; requires careful normalization; specialized

Method	Principle	Advantages	Limitations
	ratio analysis.	variation.	equipment needed.
STAR Assay	Digital PCR quantification of telomeric repeats; absolute length from synthetic standards.	Highly accurate; broad range (0.2–320 kb); reproducible.	Requires digital PCR systems; high cost; precise calibration needed.
Short-Read NGS Estimation	Bioinformatic analysis of WGS data for telomeric repeat content.	No extra lab work if WGS available; scalable; suitable for population studies.	Limited resolution; sensitive to sequencing bias; not suitable for clinical diagnostics.

V. Telomere and disease

Telomeropathies are characterized by premature telomere shortening caused by mutations in genes involved in telomere maintenance or the DNA damage response (DDR) system. Based on the age of disease onset, severity, and type of organ systems affected **(5)**

Telomeropathies can be divided into three groups: (I) telomeropathies with very early onset and severe course (e.g., Revesz syndrome, Hoyeraal–Hreidarsson syndrome, and Coats plus disease); (II) dyskeratosis congenita (DKC); and (III) cryptic telomeropathy. **(28)**

DNA stressors include numerous endogenous and exogenous factors such as mitochondrial dysfunction, cigarette smoking, alcohol consumption, inflammation, a high-fat diet, and other lifestyle and environmental actors. **(28)**

Iatrogenically, inducers of cell senescence include chemotherapy and radiation. Most importantly, the relationship between these inducers and cellular senescence is the production of reactive oxygen species. Researchers believe that G-rich telomeres are especially susceptible to oxidative stress. **(29)** Additionally, telomeres have a repressed DNA damage response, leading to inefficient DNA repair if exposed to oxidative damage. **(30)**

It has also been reported that telomere length is inversely correlated with patients who suffer from psychosocial stress and major depressive disorder due to increased oxidative stress and inflammatory factors. Notably, it has been reported that those who participate in increased physical activity levels have longer telomeres**. (31)**

VI. Telomere and cancer :

In cancerous cells, high telomerase activity overcomes the limitation of replication

thus avoiding activation of the DNA damage signaling pathway **(32)**. Several studies have shown a correlation between telomere length and the risk of different cancers (**33)**.

The risk for cancer development aggravates in relation to the decrease in telomere length and after the disease is consolidated the telomeres tend to elongate once again. This observation shows that critically shortened and dysfunctional telomeres contribute to genetic instability and oncogenesis **(34)**

While the synthesis of telomeres by the reverse transcriptase, telomerase, is absent in most human somatic cells, it is found in greater than 90% of tumorigenic cells and in-vitro immortalized cells**. (35)**

TERTp mutations are likely to be the most important mechanism participating in telomerase reactivation and TERT expression adjustment. It represents the most common non-coding mutations in cancer **(36)**

In cancerous cells, they are generally associated with higher levels of TERT expression. The two most frequent TERTp mutations are C228T and C250T transitions found at -124 bp and -146 bp, respectively from the site of the transcription start **(37**)

Telomere and Telomerase in Human Cancer Cells with malignant potential can adapt and escape replicative senescence, by suppressing DNA damage response

mechanisms or by over-activating telomere maintenance pathways, such as telomerase activity, and thus acquiring immortality and initiating the tumorigenesis process **(38).**

Dysfunctions affecting the telomere complex might expose the DNA to enzymatic degradation leading to breaks, deletions, fusions and accumulation of DNA mutations. The consequence of this genomic instability are cancer progression and worse prognosis for the afflicted patients **(39).**

VII. Telomeres and Hematopoietic Diseases

The lifespan of mature hematopoietic cells is very limited, demanding their constant production and replacement by the bone marrow. It is expected that hematopoietic cells replicate 50 to 200 times before proceeding to replicative Senescence before they cease dividing **(40)**

The telomere attrition in stem cell is accelerated during an individual's early years of life, when one stem cell will replicate from 15 to 30 times. During the adult life, between 50 and 60 years, telomere shortening progresses more slowly, however, after this period it accelerates once again. In T lymphocytes 33 base pairs of telomeric sequence are usually lost every year, while B lymphocytes only lose around 15 base pairs a year**(40)**

Although the standard model of oncogenesis for the majority of cancers predicts the presence of telomerase over expression and consequent telomere elongation, some cancer subtypes are able to maintain an indefinite proliferation by expressing telomerase but not necessarily elongating the telomeres, as is the case of hematological neoplasms **(41)**

Most hematological neoplasms possess an uncertain etiology, however, there are some predisposing factors. Malignant transformations initiate due to complex

interactions between genetic factors such as chromosomal rearrangements, aneuploidies, punctual mutations, deletions, insertions, duplications and amplifications. Among them, chromosomal translocations and aneuploidies are the main biomarkers for genetic instability and development of hematological malignancies **(42)**

Hematological disease can be divided into:

A. Inherited bone marrow failure syndromes (IBMF)

It comprise a set of diverse diseases, in which the production of one or more blood cell lines fails, Clinical manifestations vary depending on the type and number of blood cell lines involved and may include various combinations of anemia, leukopenia, and/or thrombocytopenia **(43)**. Despite having heterogeneous phenotypes and various underlying pathogenetic mechanisms, all IBMFs have a higher risk of MDS and/or AML development. Among all patients with IBMF, those with DC and Fanconi anemia presented with the highest risk. **(43)**

The dysfunctional shortening of telomeres may be present in congenital anemias, as in Fanconi anemia or Shwachman Diamond syndrome, but disease progression and telomere shortening do not have a strong association. However, telomere shortening alongside chromosomal mutations and telomerase reactivation may be predisposing factors for the development of hematological neoplasms such as myelodysplastic syndrome and leukemias **(44)**

Hematological disorders usually present similar clinical signs amongst each other, making an early differential diagnosis necessary. The occurrence of telomere shortening in hematological malignancies is associated with major genomic rearrangements and DNA damage that is not detected in cells with longer telomeres. This observation leads to the understanding that the study and analyses of TL is of utmost importance for a better prognosis of patients in the clinical practice**(45).**

B. Aplastic anemia

Short telomeres in patients with acquired severe aplastic anemia were initially thought to result from hematopoietic stress. Currently, it is believed that the destruction of HSPCs by the patient's immune system results in increased mitotic demand on a limited pool of stem cells, and subsequently a probable reduction in TL **(46)**

Although telomere shortening in marrow failure is caused by loss-of-function mutations in the telomerase complex (*TERC* and *TERT*) genes, it was later found that these mutations were also observed in patients without DC. A minority of patients with acquired aplastic anemia harbor telomerase mutations and show short telomeres **(47)**

C. Myelodysplastic syndrome

MDS patients express a progressive shortening of the telomeres which leads to

persistent physiological damage to the DNA and other classical MDS characteristics, demonstrating a correlation between TL, MDS pathophysiology and progression to malignancy as acute myeloid leukemia **(48)**.

The most common neoplasms related to shorter telomeres and age are MDS and AML Patients with short telomeres present a greater risk for developing MDS, even though only a minor percentage actually does**(49)**

VIII. Telomeres in Acute leukemia

Amongst acute leukemias, Acute lymphoblastic leukemia (ALL) patients express shorter telomeres than AML patients, however, as the diseases progress, AML telomeres tend to shorten in an increased pace compared to ALL telomeres. ALL expressing a malignant phenotype for B lymphocytes present shorter telomeres than those from AML**(50).**

The TL of patients afflicted by AML and those with a highrisk for MDS were analyzed simultaneously; in both cases TL was similar (**49)**

Telomeres in (Acute promyelocyic leukemia) APL patients are shorter in comparison to healthy controls and their length is comparable to that of patients afflicted by regular AML. Patients in complete remission from APL demonstrate considerable telomere elongation, suggesting that TL is a predictive marker for patient prognosis **(10)**

In a study by Warny et al., TL was analyzed in three stages, the first was at disease diagnosis, the second was during treatment, where telomere elongation was already observed, and the third at patient relapse when it was estimated that TL was shorter than at the time of diagnosis. **(51)**

Patients with complex cytogenetics tend to express shorter telomeres in comparison to those with a normal karyotype. The TL also changes in comparison to treatment outset and complete remission, showing an elongation pattern in the bone marrow cells from patients at treatment completion, when compared

to normal cells. **(52)**

IX. Telomerase-targeted Cancer Immunotherapy

Because upregulated telomerase activity is significant in tumor cells, hTERT makes an attractive tumor antigen for telomerase-targeted cancer immunotherapy. Several approaches exist, including oligonucleotide inhibitors, immunotherapeutic approaches, and telomerase-directed gene therapy**. (11)**

Oligonucleotide inhibitors are modified nucleic acids that inhibit telomerase, inducing telomere shortening and forcing cellular senescence and apoptosis. Immunotherapeutic approaches use high-avidity T lymphocytes that are reactive against the catalytic enzyme. Finally, telomerase-directed gene therapy involves the selective killing of tumor cells by targeting telomerase promoters. **(53)**

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