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Review Article

A biomarker in type 2 diabetic patients : (leukocyte telomere length)

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Abstract

At the ends of linear chromosomes are specialized nucleoprotein structures called telomeres, which guard against the activation of DNA damage response and repair processes. In order to prevent replicative senescence, genomic instability, and cell death, a variety of factors localize to telomeres to regulate their length, shape, and function.in people. Chronic inflammation and oxidative stress accelerate telomere attribution, leading to organ deterioration and replicative senescence. Telomere length can be utilized as a potential indication of biological aging and represents the total damage caused by those exposure factors. Numerous age-related illnesses, including diabetes, heart disease, and cancer, have been connected to shorter telomere length. Therefore, finding a biomarker that could offer more details about a person's cardiometabolic health in addition to (or instead of) their chronological age would be very helpful in both predicting and preventing disease. Perhaps one of these biomarkers is telomere length.

The relationship between telomere shortening and type 2 diabetes mellitus will be interpreted in this review.

Keywords : T2DM , rLTL, biomarker

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Introduction

Nucleoprotein complexes called telomeres are found at the ends of linear chromosomes. In order to prevent telomere–telomere fusion and genomic instability, they must prevent the chromosomal end from being interpreted as a free end produced by a DNA double-strand break. This would cause the DNA damage repair machinery to be activated improperly. The DNA component is made up of tandem double-stranded hexameric repeats, or TTAGGG in humans, which vary in length from 5 to 15 kb based on the kind of cell and its history of replication. It ends with a 3′ single-stranded sequence known as the 3′ G-overhang. Furthermore, a cadre of proteins that are necessary for the correct maintenance, structure, and function of telomere length bind to telomeric DNA. [1]

Telomeric DNA

At the actual ends of linear chromosomes are repeating DNA sequences called telomeres. The chromosomal ends replication and protection issues were brought about by genome linearization during evolution because the majority of prokaryotes had circular chromosomes rather than linear ones. Renewable repetitions create a unique chromatin domain with telomeres and an adjacent subtelomeric region in eukaryotic cells. Telomeres' major function is to contribute to the maintenance of genomic stability; they also have a significant impact on aging and cancer. [2] Specific tandemly-repeated sequences (TTAGGG) that contain multiple kilobases (5–15 kb) and end with 50–400 nucleotides of 3-G-rich single-strand DNA overhangs make up mammalian telomeres.

Furthermore, they can form G-quadruplexes because of the increased guanine present in the single-stranded portion of telomeres. These telomeric G-quadruplex structures participate in recombination suppression, telomerase-dependent telomere extension inhibition, and telomere protection. [3]

Telomere function

The telomeric unique structure guarantees that the end replication problem and the end protection problem, which are related to replication and protection, are addressed. [4]First, the incapacity of polymerases to completely synthesize 5' ends of DNA results in end replication problems, which cause telomeres to shrink with each DNA replication cycle. By adding telomeric repetitions to the 3' overhang, the highly specialized enzyme telomerase prolongs it and stops telomeric loss. [5]

The second issue, known as the "end protection problem," arises from the potential for the DNA damage response (DDR) machinery to identify unprotected telomeres of linear chromosomes as double strand breaks (DSBs), which can result in irreversible cell cycle arrest (replicative senescence), cellular death (apoptosis), and, in some situations, the induction of genomic instability. The end protection problem can be solved in a number of ways, including by concealing the chromosome ends in unique architectural structures (T-loops) or by using single- and double-strand telomere binding proteins (such as the six-subunit protein complex known as shelterin in mammals).Both chromosomal ends of human telomeres typically have 3' overhangs, which vary in size within the leading and lagging strands.[6]

The shelterin protein complex and particular telomeric structures, such as T-loops, are the major components of the mechanism that protects telomeres from the effects of the repair process. Although it is downregulated in the majority of somatic tissues, the telomerase enzyme, which can lengthen the telomeric tracts, is active in germline and embryonic tissues and cells, guaranteeing that offspring receive a full genome. [2]

Telomere- specific structure

A. core elements of telomerase:

The RNA subunit (TR; TERC; TER, Telomerase RNA component), which acts as a template, and the catalytic subunit (TERT, TElomerase Reverse Transcriptase) are the two main parts of this enzyme. [7]

B. proteins of shelterin:

Shelterin proteins are specialized proteins that bind telomeric DNA and shield chromosomal ends from abnormal DDR activation. [8]

Table (1). The Function of the Shelterin Complex Components in Telomere and Telomerase Regulation is listed in [9]

Components of Shelterin	Important Functions	Type of Regulator for Telomerase
TRF1	Length regulation, capping, telomere replication, DDR, mitosis, polymerization of microtubules, prevention of telomere fragility,	Negative regulator
TRF2	Length regulation, capping, DDR	Negative regulator
TIN2	Length regulation, bridging shelterin	Negative regulator
TPP1	Capping, telomere recruitment, bridging shelterin, Length regulation, prevention of telomere fragility	Connection
POT1	Protection of 3′ overhang from illegitimate recombination, DDR, regulating telomere length, catastrophic chromosome instability, and abnormal chromosome segregation.	Negative regulator
RAP1	Length regulation, DDR, subtelomeric silencing, prevention of telomere recombination and telomere fragility, transcriptional gene regulation, NF-κB pathway regulators, losses of site-specific histone, maintenance of chromosomal integrity.	Positive regulator

C. T-loops

T-loop formations occur when the G-rich 3' overhang intrudes into the double-stranded DNA (D-loop) and the telomere end folds back on itself. [8] The T-loop structure is estimated to be a few hundred nucleotides in size. The size of the T-loop and the telomericrepeat array's length are closely related. It was suggested that TRF2 (Telomere Repeat Factor 2), a shelterin protein linked to telomeres, also encourages the creation of T-loops. [10]

Telomere shortening

Replicative senescence can be brought on by progressive telomere shortening. Aging and age-related conditions like cardiovascular disease (CVD), [11] diabetes, [12] osteoarthritis, [13] glaucoma [14] and cataracts [15] are linked to replicative senescence. One short or unprotected telomere is enough to cause replicative senescence in normal cells, [16] indicating that telomeres serve as a "timer" that restricts the number of mitotic cycles. [17]

In humans, telomeric bploss varies from 30 to 200 bps each division.Cell shape, epigenetic factors, and gene expression can all change as a result of cellular senescence. Senescent cells develop the Senescence-Associated Secretory Phenotype (SASP), a phenotype that is mostly pro-inflammatory, and cause young cells to undergo senescence, which leads to tissue malfunction, the advancement of atherosclerosis, cancer, and diabetes. [18]

Additionally, it has been demonstrated that cells with short telomeres can avoid senescence and achieve immortality by either activating alternative lengthening of telomeres (ALT) or upregulating the telomerase enzyme. About 85% of all malignant tumors have active telomerase, which is different from the somatic cells, where telomerase activity is downregulated shortly after birth. [19]

Progressive telomere shortening, however, can also cause alterations in the expression of genes that are remote from telomeres, which can lead to age-related diseases like cancer. Furthermore, long before telomeres get short enough to cause DDR, telomere length can control gene expression. Gene expression can be reversibly suppressed by a phenomenon known as the telomere position effect (TPE), which is dependent on telomere length.

Telomeric heterochromatin spreads the subtelomeric area and silences neighboring genes to produce classic TPE. [20] Furthermore, when telomeres are long, chromosome looping brings them near to genes up to 10 Mb away from the telomere, and when telomeres are short, the same loci dissociate. Before they begin to produce signals of DNA damage, this conformation can cause broad alterations in gene regulation. For example, several genes, including DSP (Desmoplakin), exhibit variable expression based on telomere length. This phenomenon is known as the telomere position

effect over long distances (TPE-OLD), and it may be a new way whereby telomere shortening causes aging and the onset of disease. [21]

In addition to the impacts of gradually shorter telomeres described above, telomere maintenance, which is influenced by both hereditary and nongenetic factors, has been linked to mortality and aging-related disorders. Telomere-shortening processes may also be caused by nuclease activity, chemical (such as oxidative) damage, and DNA replication stress, in addition to the most well-known replication and protection issues. [22]

The association between telomere length and type 2 diabetes

It's interesting to note that telomere length may potentially be a helpful indicator of Type 2 diabetes. Even while the shortening was lessened in patients with well-controlled diabetes, it has been found that telomeres were shorter in Type 2 diabetes patients than in control subjects [23-25] .[26] Furthermore, various diabetes sequelae, including diabetic nephropathy, [27] microalbuminuria, [28] and epithelial malignancies [29] have been connected to telomere shortening. Furthermore, a fascinating discovery that has been validated by separate research is that individuals with diabetes and atherosclerotic symptoms had the shortest telomeres when compared to those with diabetes or cardiovascular disease alone. [30–32]

Measurement of leukocyte telomere length

Telomere length can be measured using a variety of techniques, each with pros and cons. The primary techniques include Southern blotting, real-time quantitative PCR (qPCR), quantitative Fluorescence In Situ Hybridization (Q-FISH), single telomere length analysis (STELA), telomere shortest length assay (TeSLA), and terminal restriction fragment analysis (also known as telomere restriction fragment analysis). [33,34] Relative telomere length assessment using quantitative PCR (qPCR) is a high throughput technique that is perfect for quantifying hundreds to thousands of samples from an epidemiological investigation since it requires little starting material, takes little time, and involves straightforward processes.

Cawthon transformed telomere biology in 2002 by creating primers that could bind to GC-rich areas [35] and enabling the use of this qPCR technique in broader population-based clinical studies.According to Cawthon's method, two distinct qPCR reactions are usually conducted, one utilizing telomere primers and the other using single-copy gene primers, in order to compare the telomere length (T) to that of a single copy gene (S). The results of qPCR provide the average relative telomere length, which is typically displayed as the T/S ratio.

This is because the telomere is a repeat sequence of (TTAGGG)n, while a single-copy gene is a distinct single sequence of that gene inside the genome. The inability to measure both the telomere length of an individual chromosome and the telomere length in absolute terms is one of the method's drawbacks. Variability in measurements and outcomes resulting from different labs' inconsistent methods, reagents, and data analysis is another disadvantage.

Additionally, the Cawthon protocol single-copy gene 36B4/RPLP0 (ribosomal protein lateral stalk subunit P0) has been utilized in various research up to this point, but it is now discovered to include multiple processed pseudogenes, which renders it inappropriate as a single copy gene. [36] A successful qPCR-based relative telomere length analysis requires a master mix, high-quality DNA, telomere and single-copy gene primers, and a well calibrated PCR instrument. Differences in data have been suggested to result from variances in the aforementioned factors. [37] Telomere PCR results have been demonstrated to be affected by a variety of commercially available master-mixes, [43] DNA extraction techniques, [38-40] DNA storage temperature and concentration, [41,42] PCR conditions, single-copy genes, and instruments.[36,44] Data analysis and the use of reference/control samples for comparison are also important considerations. Although $2^{-\Delta\Delta Ct}$ is a widely used method of determining relative telomere length, as recommended in the original Cawthon publication, there are no standard criteria for using the reference/control sample or computing the T/S ratio. The most widely used high throughput technique for measuring telomere length is qPCR; nevertheless, there have been reports of inconsistent results between labs and between association studies on clinical cohorts. As was previously said,

a number of factors influence how reliable and reproducible this method is. Even if several groups have adjusted each of the aforementioned variables to provide repeatable results, a new user may find it overwhelming to begin with such a wide collection of knowledge.

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