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Telomerase: A link between cancer and biological immortality

Prathibha S S [1,*], Rhythima K [2], Rohan Alag [3], Shalini Singh [4] and Sneha Alwal [5]

[1] *Department of Biotechnology and Research, K.V.M. College of Science and Technology, Kokkothamangalam P.O., Cherthala, India- 688527.*
[2] *Department of Biosciences, Vellore Institute of Technology, Vellore, India- 632014.*
[3] *Department of Biotechnology, School of Biotechnology, Jawaharlal Nehru University, New Delhi, India- 110067.*
[4] *Department of Biotechnology, Noida Institute of Engineering and Technology, Greater Noida, India- 201310.*
[5] *Department of Oncology, Osmania Medical College, Hyderabad, Telangana, India- 500095.*

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Abstract

Cancer is a leading cause of death worldwide, with approximately 18.1 million new cases and 9.6 million fatalities annually. Many studies have shown that telomeres and telomerase play crucial roles in the progression of almost all types of cancer. The length of telomeres is reduced with each cell division, and when they become too short, the cells can no longer divide and die. Telomerase is responsible for maintaining the length of the telomere during replication. In healthy cells, telomerase is only active during embryonic development and gamete formation. Telomerase is inactive in most somatic cells and highly active in germ cells, stem cells, and cancer cells. This activity ensures that somatic cells can only divide a finite number of times (Hayflick limit) before they reach senescence and enter apoptosis. However, cancer cells express high levels of telomerase and can divide indefinitely. This makes them immortal, eating away from the host's resources, and acquiring properties, such as loss of cell adhesion, metastasis, and loss of contact inhibition, which creates tissue lumps wherever the tumor develops. Many recent advances in science and technology have shown the significant role of telomerase in cancer and aging, which also opens a wide door for cancer treatment, hoping for patients back to life. In this review, we discuss the significance of telomerase and its effect on cancer progression and various research and therapeutic aspects of telomerase.

Keywords: Telomerase; Cancer; Tumor suppressor genes; Proto-oncogene; Oncogene; Metastases; DNA repair; Contact inhibition; Cell adhesion; Apoptosis; Hayflick Limit; Aging; Biological immortality

1. Introduction

Human curiosity and indigeneity lead to the discovery of the cell -The basic structural and functional unit of life by Robert Hooke in 1665, which paved a path to many of today's scientific advancements. German scientists Theodor Schwann and Matthias Jakob Schleiden initially presented the cell theory in 1838, which is a fundamental biological theory that holds that cells are the essential building blocks of all living tissues. The Botanist Matthias Schleiden and Zoologist Theodor Schwann proposed the unified cell theory. That states "all living things are composed of one or more cells; the cell is the basic unit of life." Around the 1850s, Robert Remak, Rudolf Virchow, and Albert Koelliker showed that new cells arise from pre-existing cells. Virchow's aphorisms as "Omnis cellula e cellula", thus forming the basis for cell division and tissue formation [1]. It was this statement that changed the scientific approach to life, making Cell division -the basis of life's virtue. The division of normal cells is tightly regulated by multiple genetic and evolutionarily conserved cell cycle control mechanisms which ensure the formation of two genetically identical cells. What if the

* Corresponding author: Prathibha S S; Email: prathibhass97@gmail.com
Department of Biotechnology and Research, K.V.M. College of Science and Technology, Kokkothamangalam P.O., Cherthala, India- 688527.

process of cell growth and division breaks down? Unregulated Abnormalities in the process of cell division lead to cancer. Cancer is one [1] such term given to a wide range of affiliations that can start in almost any organ or tissue of the body. Cancer cells divide repeatedly leading to the formation of tumors. Such abnormal production of a growth factor by the cancer cell leads to continuous auto stimulation of cell division known as autocrine growth stimulation. Cell, being the fundamental unit of life, is also considered the basic element of pathological processes. Diseases lead to an alteration of cells and their processes. Virchow's 'cellular pathologies' was the most important pathogenic concept which paved the path for the theory of molecular pathology. Cell cycle checkpoints operate as DNA surveillance mechanisms that prevent genetic errors during cell division. Cell-cycle checkpoints prevent the transmission of genetic errors to daughter cells. There exist three major cell-cycle checkpoints; the G1/S checkpoint, the G2/M checkpoint, and the spindle assembly checkpoint (SAC). The DNA damage checkpoint is often compromised in cancer cells, allowing continuous cell division despite the accumulation of genetic errors. The p53 protein is a DNA-binding transcription factor with potent tumor suppressor properties. In response to cell stress, p53 protein accumulates and binds to degenerate consensus sequences throughout the genome, activating the transcription of many target genes involved in DNA damage repair and apoptosis. Normal cells become cancer cells due to abnormal changes called hyperplasia and dysplasia. In hyperplasia, there is an increase in the number of cells in an organ or tissue that appear normal under a microscope. In dysplasia, the cells look abnormal in size or number under a microscope. Hyperplasia and dysplasia may or may not become cancer. These abnormal changes lead to Neoplasia. Sometimes, the abnormal cancerous cells invade beyond their usual site and spread to other organs. This invasion of cancer cells causes metastasis, a major cause of death from cancer. Lung, prostate, colorectal, stomach, and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical, and thyroid cancer are the most common among women. These life-threatening disorders are also commonly called neoplasm and malignant tumors. Globally, one in six deaths is due to cancer making it the second leading cause of death [2]. An oncogene is a mutated gene that has the potential to cause cancer. Before an oncogene becomes mutated, it is called a proto-oncogene, and it plays a role in regulating normal cell division. Cancer can arise when a proto-oncogene is mutated, changing it into an oncogene and causing the cell to divide and multiply uncontrollably. Some oncogenes work like an accelerator pedal in a car, pushing a cell to divide repeatedly. Others work like a faulty brake in a car parked on a hill, also causing the cell to divide unchecked. When normal cells lose their ability to divide, differentiate and undergo apoptosis, they become tumor cells. A tumor or neoplasm is a result of the unnecessary abnormal proliferation of cells. The progressive increase in tumor size eventually leads to the breaking of intercellular barriers between the tissues, leading to tumor invasion into the surroundings. Metastasis is defined as the ability of invasive cells to migrate to other locations, where they proliferate to form secondary tumors. A tumor can be either benign or malignant. tumors which remain confined to a specific location such as common skin warts are benign in nature. These Benign tumors can usually be removed surgically. A malignant tumor, however, is capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems [3]. Only malignant tumors are properly referred to as cancers, and it is their ability to invade and metastasize that makes cancer so dangerous. "Most cancer form tumors, but not all tumors are cancerous." Over the years, the incidence of cancer is increasing. Surprisingly, Survival rates are also improving mainly due to accessible early detection, effective clinical research, and constant implementation of new quality treatments and care.

The genetic material of an individual that has been supercoiled to form a structure called a chromosome has ends that have guanine (G) rich repeats. These tandem repeats of guanine in the 5'-3' direction, mark the ends of the chromosomes and these ends are called TELOMERES [4]. Ever wondered how our genetic material is held intact and protected from being disintegrated? Telomeres not only protect DNA from degradation but cause repair and damage control mechanisms and therefore, play a role in the genetic makeup of an organism. The problem arises when the replicative cycles over the various cell divisions during an organism's lifespan, cause the shortening or reducing ends of the telomeres. This occurs in eukaryotes alone as many prokaryotes tend to have circular genetic material which is devoid of the concept of 'chromosomal ends' [5]. The (ribonucleic) protein-rich enzyme with RNA components, called 'TELOMERASE' helps with this frequently repeated loss of telomere fragments and preserves the genetic material. It prevents senescence, genomic stability, stem cell regenerative science, and much more. Stem cell dysfunction and the diseases that arise due to this have been assumed to be key factors in understanding cancer. Scientists have assumed the concept of 'immortality' as true as a fact due to the telomerase study results. So, what does it have to do with cancer and its progression? Telomerase is known primarily to cause elongation of the telomeric ends and prevent them from shortening and therefore increasing their replicating capacity. The amount of telomerase in a normal cell fails to maintain the length at every stage of replication [6]. Regardless, it is known to maintain the chromosomal structure. The removal of the primer causes the telomerase to attach to the 3' end of the lagging strand. The telomerase catalytically adds TTAGGG hexameric nucleotide repeats to the 3'-hydroxyl end of the telomeric leading strand, using a specific sequence in the RNA component as the template as shown in figure 1 [87].

The telomeric complex consists of TERC (RNA component), TERT (telomerase reverse transcriptase), and protein components (dyskerin, NPH2, NOP10, GAR1) which assemble to initiate the extension of telomeric ends. Telomerase

begins with adding dNTPs on the 3' end and elongates the lagging strand using an RNA template. The mechanism here is reverse transcription. Past the extension of the lagging strand, the DNA polymerase works to extend the Okazaki fragments. It is now truly clear that the absence of telomerase causes the loss of telomeres which can cause the expression of silenced genes and even leads to apoptosis [7]. Telomerase is much required in stem cells and germ cells. The fact that somatic cells have a limited concentration of telomerase and show replicative senescence (Hayflick limit) causes the aging factor. Cancer cells' acidic nature tends to cause more telomerase, and this leads to the prevention of the cell from exhibiting senescence The lack of execution of programmed cell death is the actual known reason for the cancer progression [8]. The mutations in a single cell can induce cancer due to the inability to perform apoptosis and cell death by an increase in telomerase concentrations Hence, it is known to be an entry ticket to cancer study and research. Queries like "Can telomerase be used to cause immortality?'," Is cancer purely controlled by suppressing the telomerase activity?", "what sort of mutations cause an increase in telomerase?" are yet to be addressed [9].

Different investigators have begun to understand the complex mechanisms that transform a normal cell into a cancer cell and subsequent tumor development due to the availability of various precious models. The history of cancer research and the establishment of continuous cell lines are closely related. Tissue culture techniques were first established by Harrison at Johns Hopkins University in 1907. the "growth of cells outside the body" was demonstrated for the first time by the growth of coagulated lymph from frog, and chicken embryo cells. etc., This is an important milestone in biomedical research. Rous sarcoma and carcinoma samples that were obtained from rats, dogs, and humans were cultured in vitro using horse or bovine plasma. In 1951, improvements in defining the biochemical requirements for the growth of physiological and transformed cells permitted Dr. George Otto Gey at Johns Hopkins Hospital in Baltimore to establish the first and well-known human continuous cell line, named HeLa named after Henrietta Lacks, a young black woman affected by cervical carcinoma, from whom HeLa cells were derived. The establishment of the HeLa cell line can be considered another milestone in the history of cell biology and cancer. Starting from their stabilization, the HeLa cells constituted the first example of "human cancer in a test tube" [10]. The establishment of the first human continuous cell line provided a standard model to study the pathophysiology of cancer. In 1963, Robert James Valentine Pulvertaft established, the RAJI cell line, the first human continuous hematopoietic cell line from a Nigerian patient affected by Burkitt's lymphoma. Although the RAJI cell line was successively proven to be a model system that is generated by Epstein–Barr virus infection, the definition of the culture conditions that are necessary for its growth in vitro paved the way for the stabilization of new cell lines growing in suspension. Furthermore, especially during the 1980s and 1990s, the availability of recombinant growth factors helped in the stabilization of a few hematopoietic cell lines that cover all steps of myeloid and lymphoid leukemia [10], [11]. Overall, each new cell line has been necessary, over time, to understand step by step a new feature of cancer disease and to test the efficacy of anticancer drugs. The importance of human continuous cell lines in the development of new drugs has been witnessed. The concept of telomeres was born in the 1930s when McClintock and Muller inferred the existence of a unique structure at the ends of chromosomes in Zea mays and *Drosophila melanogaster* and hypothesized that it was critical for the prevention of chromosome end fusion. In 1980, Elizabeth Blackburn discovered the molecular structure of telomeres. In 1982, together with Jack Szostak, she further proved that this DNA prevents chromosomes from being broken down. Blackburn and Carol Greider co-discovered the enzyme telomerase, which produces the telomeres' DNA, in 1984. These proved to be essential pieces in the puzzle of cellular division and DNA replication and are now in cancer study. Telomeres maintain genomic integrity in normal cells, and their progressive shortening during successive cell divisions induces chromosomal instability. Telomeres, repetitive (TTAGGG) DNA– protein complexes at the ends of chromosomes. Telomerase is the enzyme responsible for the maintenance of telomeres, essential structures that cap and protect the ends of linear chromosomes. Once the shortest telomere becomes uncapped, a DNA damage response is induced that mobilizes the p53 and p16/pRB pathways, inducing senescence. Both telomeres and p53 play important roles in the maintenance of genome integrity and tumor suppression. The presence of recently discovered non-canonical p53 response elements (REs) in the sub telomere region of human and mouse chromosomes plays a vital role. The binding of p53 to these sites stimulated local transcription and enhanced telomere stability in the presence of DNA damage. The p53 recruits its chromatin remodeling factors and DNA damage repair proteins directly to sub telomeres. The DNA damage-induced binding of p53 to chromosome fragile sites such as sub telomeres, therefore, enhances repair and replication of their DNA. Hence, p53 can directly enhance the stability of telomeres in response to DNA damage stress [12]. "The DNA in telomere shortens when cells divide, eventually halting cell division when the telomere reserve is depleted." Recent New results from de Lange's lab in 2020 provide the first evidence that telomere shortening helps prevent cancer in humans, likely because of its power to curtail cell division.

2. The Role of Telomere and Telomerase in Cancer cell growth

According to World Health Organization (W.H.O.), cancer is considered a prominent cause of death all over the world, with nearly 10 million deaths in 2020, or close to one in six deaths. The causes of the disease are varying day by day and many different types of cancers are emerging day by day. Among them, the most common are breast, lung, blood, colon

and rectum, and prostate cancers. Generally, cancer is a common word used for a wide set of diseases that can affect any part of the body characterized by the development of abnormal cells as a result of uncontrolled cell division [13].

As technology is developing in rapid cancer screening, treatment, and prevention, the survival rates of cancer are improving for many types but in most cases, it's very difficult to detect and confirm. An increasing life expectancy prolongs the period over which oncogenes act on cells and increases the threat of cancer progression. The rise and development of cancer are initiated mostly by genetic mutations in cells. Cancer is a genetic result of which normal cells accumulate genomic insecurity and attains the ability to replicate uncontrollably, leading to the phenotype of immortality. Telomerase repression and/or shortened telomeres in human cells are supposed to be a natural evolutionary approach as a defense against cancer; it functions as a strong barrier to tumor transformation and prevents uncontrolled cell proliferation. The foundation of oncogenesis is the vast proliferation of malignant cells, which in most cases is accomplished by the activation of telomerase [14]. The presence of telomerase is expressed in more than 85% of melanoma cells, making it practically a universal cancer biomarker or universal therapeutic agent, while the majority of normal somatic cells are negative for telomerase [15].

Unlike bacterial chromosome eukaryotic chromosome is linear and possess a telomeric region defined as specialized proteins that form a capped end chromosome that protects and maintains the length of the chromosome. Telomeres are the genetic material at the end of the chromosome and are essential for proper chromosome structure and function. Telomeres of humans and all vertebrates contain the tandemly repeated sequence (hexanucleotide) [16] TTAGGG. This protects the DNA from attack by nucleases that degrade the ends of DNA molecules in the cell [16].

The functions of telomeres are as follows

- Prevents the fusion between two chromosomes,
- Prevents loss of genes, and
- It also prevents the shortening of the chromosome during replication (lagging strand).

2.1. Telomerase and Cancer

The telomerase enzyme, otherwise called the human reverse transcriptase enzyme, catalyzes the synthesis of the telomere region. This enzyme is a ribonucleoprotein (RNP). The RNA part acts as the template to synthesize the DNA and the two proteins present in the RNP; p123 and p43. p123 catalyzes the polymerization reaction, which is why it is called TERT (telomere-associated reverse transcriptase). Telomerase replenishes the telomere cap of the DNA. During replication, the removal of the last primer (12 nucleotides) from the lagging strand generates a 3' overhang (two repeats of the telomere sequence). RNA part of the telomerase enzyme pairs with the hanging 3' end and synthesize telomere sequence to the free 3' end. This possesses helicase activity, detaches, and again re-joins to the newly synthesized telomere end. This process continues several times. Again, a primer binds to the 3' end and the polymerase enzyme synthesizes the complementary DNA. Then primer removes and generates a 3' overhang. So, telomerase can prevent DNA loss from the lagging strand end of the chromosome. Telomeres were first discovered in cancer cells as they are saturated with telomerase enzymes.

Maintenance of telomere length is important to evade cell death and apoptosis. Sometimes the shortening of telomere has been seen in relation to chronic stress due to many factors like diseases such as cardiovascular disease and cancer [17].

Many experiments have shown that there is a direct relationship between telomeres and aging, and that telomerase has the ability to prolong life and cell division. Germ cells, stem cells, and cancer cells have the ability to divide indefinitely because they have high telomerase activity. Researches prove that telomerase is the key enzyme for human cells to acquire immortality. Somatic cells have a limited number of telomere sequences, so they can engage only a few rounds of division. This is known as the Hayflick Limit. Senescent cells lack telomeric sequences and enter into programmed cell death or apoptosis [18].

Advances in science and technology have extended our prior vision and theories on the mechanistic underpinnings of telomerase and cancer progression relationship. During the 1980s, people believed that telomeres are something that protects the linear chromosomal end and maintain length whereas telomerase, otherwise called terminal transferase, is a ribonucleotide protein enzyme necessary for the replication of these chromosomal ends in unicellular organisms, i.e., capable of prolonging chromosome ends involving mainly of a protein. However, the concept is still true and today science shows tremendous maturity in various fields especially the fields that are associated with telomerase and cancer. Discovery of molecular components of telomerase, roles of telomerase dynamics in cellular proliferation and

various cancers, aging, immortality, identification, and classification of human genetic disorders as a result of premature telomere shortening, advances in telomerase targeted therapies, and roles for telomeres in regulating gene expression, etc. are significant developments in this field [19].

In 1995, *Schwartz et al.* proved that the Telomere reduction and telomerase activity may be oncogenic sustaining events required to maintain the transformed phenotype seen in giant cell tumors of bone (GCT) [20].

A survey of telomerase activity in human cancer conducted by *J W Shay et al.* in 1997 depicts that almost all the major types of cancer have been screened to detect the presence of telomerase activity. So, the telomerase activity was identified progressively in 85% of malignant tumors. Almost the entire spectrum of human tumors has been exposed to being positive for telomerase [21], [22]. In his comparative study on telomerase and cancer, he discussed the specific association of human telomerase activity with immortal cells and cancer cells [22]. Generally, malignant tumors are categorized by telomerase expression, compared to their ability for abnormal cell proliferation, where the lack of telomerase is described in most benign/ premalignant tumors [21].

The role of telomerase in cellular proliferation and cancer was studied by *Shay et al.* in 1999. He concluded that telomere repression activity may be an innovative adjuvant therapy for human cancer prevention and that the discovery of telomerase activity may play a vital role in cancer diagnostics [23]. Telomere dynamics in cancer progression and prevention: the fundamental differences in human and mouse telomere biology we studied by W E Wright and J W Shay in 2000. Telomerase-knockout mouse cells were immortalized with an approximately ten-million-fold larger frequency than human cells. They also did a comparison of the differences between mice and humans and their connection to cancer [24]. And in 2001, his journal covers the topical advances in the field of the nature of telomerase in cancer diagnostics, screening, and synopsis of anti- telomerase cancer therapeutic methods [25].

In 2004, *Liu et al.* [26] studied that in somatic cells, the enzyme telomerase remains inactive but is found to be active in germ cells and stem cells. In addition, the revival of the activity of the telomerase enzyme in somatic cells is one of the ways to attain immortal proliferation in cancer.

Agrawal et al. worked on recent patents on anti-telomerase cancer therapy in 2012. They selected nine tumor-specific anti-telomerase methodologies based on their function or target components of human telomerase. They also provided a detailed study of exact granted patents from the viewpoint of various claims and downstream applications for each of the anti-telomerase methods and also delivered a complete list of patents for the various anti-telomerase tactics which involve information about the authors and institutional ownership along with the year of issue of the patent [27].

In 2016 *J W Shay et al*. and their colleagues worked on the role of telomere and telomerase in aging and cancer where they emphases on the recent advances in telomerase, recognizes unresolved queries, and addressed areas and approaches that need improvement. Although science and technology development resulted in significant advances in this field, telomerase remains a competitive target for cancer therapy. Telomerase dynamicity varies day by day; it acts as a defense mechanism of the body by solving the end replication problem and senescence [17] at the same time as a cause of cancer. There are few telomerase-directed treatments, and most of the procedures used to analyze telomeres and telomerase face serious limitations [18], [28].

TERT induction and telomerase activation not only create unlimited cancer cell proliferation potential by controlling telomere length (telomere lengthening-dependent) but also a reason for oncogenic effects freely of the telomere lengthening function. The telomere lengthening-independent functions of TERT, which ominously contribute to cancer initiation or evolution, comprise its role in mitochondrial and ubiquitin-proteasomal function, DNA damage repair, gene transcription, microRNA (miRNA) expression, RNA-dependent RNA polymerase activity, and epithelial-mesenchymal transition [29]. These TERT activities physiologically disrupt the courses that eventually lead to the aging of cells; though, TERT activities drive cancer expansion by conferring survival, spread, motherhood, and aggressive phenotypes. A current report says that the revival of telomerase and efforts to inhibit it in malignant cells give confidence for its possible use in cancer treatment [30].

Liu et al. 2016 reviewed the Cancer-Specific Telomerase Reverse Transcriptase (TERT) Promoter Mutations: Biological and Clinical Implications. He concluded that telomerase is active in 85-95% of carcinogenesis and shows a high telomeric activity that allows for indefinite cell division. As an RNA-dependent DNA polymerase, telomerase creates telomeric DNA at the linear chromosome end and reduces or protects telomere destruction associated with cell divisions. By expanding the telomeres, telomerase lengthens cellular lifespan or encourages immortalization. Reliable with its functional side, telomerase is silent in most human normal somatic cells while active only in germ-line, stem, and other extremely proliferative cells. In contrast, telomerase dynamicity is broadly seen in human cancer and the

enzymatic activity is measurable in up to 90% of malignancies. Recently, point alterations in the controlling region of the telomerase reverse transcriptase (TERT) gene, which encodes the core catalytic component of telomerase, were known as a unique tool to trigger the telomerase enzyme in cancer [31]. TERT promoter mutations are used as cancer-specific biomarkers in diagnostics and screening [14].

The arrival of the latest cancer biomarkers in both gross and molecular levels, improved opportunities for refining cancer diagnostics by rapid detection, prediction, and efficient treatment and also gives great hope for the patients to come back to life. Telomerase activity is prominent in most cancer biopsies but not usually detected in premalignant lesions and normal tissue samples except in germ cells and hematopoietic stem cells. Therefore, its activity can be a promising biomarker for the detection of malignancies, including colorectal cancer [32], and a target for chemotherapy or gene therapy [17]. The expression of RNA subunit (hTR) is also regarded as a diagnostic marker [33]. But the problem here is that the expression of hTR does not always associate with telomerase protein expression in the target cell type. Sometimes it may even express in certain cell types where telomerase activity is not present [33].

In addition to this alteration in telomerase and associated protein genes, they are considered diagnostic and prognostic biomarkers for many genetic aberrations together called telomeropathies [33].

In figure 5, the role of chromosome ends in genome integrity control which depends on telomere functionality and expression is discussed, where (A) During aging and chronic diseases, telomeres lose capping function due to telomere shortening. Dysfunctional telomeres are largely devoid of telomeric DNA and telomere-binding proteins, thus inducing DDR, including DNA repair and checkpoint responses. HDR can contribute to ALT, mediating immortal proliferation capacity in telomerase-negative human cancers. It is currently unknown whether HDR and ALT can contribute to maintenance of non-transformed cells and tissues during aging. Activation of NHEJ leads to generation of chromosomal fusions, thereby initiating chromosomal instability and cancer formation, especially when DDR checkpoint responses are defective. In mammalian cells, chromosome fusions in response to telomere shortening are mediated by alt-NHEJ. This pathway involves microhomology-mediated end-joining and is independent of components involved in c-NHEJ. The choice between different repair pathways is regulated by DNA end resection at dysfunctional telomeres. 5′–3′ end resection generates single-stranded DNA overhangs that inhibit c-NHEJ and activate HDR. 5′–3′ end resection may also promote microhomology-dependent alt-NHEJ, leading to fusion of chromosomes with shortened telomeres; and (B) Young cells and stem cells have long telomeres that cap chromosome ends by forming secondary structures (e.g., G-quadruplexes and T-loops) in concert with telomere-binding proteins. Telomere capping impairs the inappropriate activation of DDR at chromosome ends that would lead to chromosomal instability. However, the same structures that protect the chromosome ends (e.g., G-quadruplexes and T-loops) also represent fragile sites that are difficult to replicate during S phase of the cell cycle. Specific DNA helicases have evolved and cooperate with telomere-binding proteins (e.g., Trf1) to facilitate telomere replication. In both scenarios (telomere shortening and telomere replication stress), telomerase activation can restore telomere function. Thus, telomerase activity in stem cells contributes to stabilize stem cell genomes, but it may also increase the risk of clonal growth when stem cells accumulate mutations [89].

3. Clinical Significance of Telomerase Research in Cancer Therapy

The clinical significance of telomerase in cancer is that it can be used as a prognostic marker. Telomerase is an enzyme that helps to maintain the length of telomeres, which are the protective structures at the ends of chromosomes. When telomeres become too short, cells can no longer divide and die. Cancer cells, however, are capable of producing telomerase, which allows them to keep their telomeres long and continue to divide. This makes telomerase a potential marker for the identification of cancerous cells. Furthermore, telomerase has been implicated in tumorigeneses or the development of cancer. Studies have shown that the expression of telomerase is increased in cancerous cells compared to normal cells. Finally, telomerase RNA (hTR) has been found to be overexpressed in some types of cancer, including ovarian and breast cancer. Telomere activity is considered a useful biomarker for the diagnosis of cancer cells and a prone target for inactivation in chemotherapy and gene therapy. Telomerase is one of the best indicators of cancer, linked only to malignant tumors and not benign growths, making it an ideal target for diagnosis as well.

Telomerase activity has two clinical implications for the conformation of cancer: prior detection of cancer cells for diagnosis of malignancies and prognostic indicator in tumors whose telomerase is activated by the tumor progression. When used to identify telomerase-positive cells against a background of noncancerous cells, immunohistochemical detection of hTERT has crucial implications for telomerase as a marker for cancer diagnosis, as well as for indicators of prognosis and residual disease [34]. Approximately 90% of human tumors sustain the telomere lengths telomerase, with the purpose of indemnifying telomere shortening and guaranteeing the proliferative capacity of tumor cells [35]. In the view of tumor locations, rectal cancers have a worse prognosis than colon cancer and their therapeutic management is also different [36]. Considering the inappropriate expression of telomerase maintains the telomere

length, and allows the cell to overcome senescence and apoptosis, additional molecules related to telomere maintenance and genome instability have yet to be investigated. The molecular basis of the clinical evolution of colorectal cancer suffers from telomere shortening, as previously published data [37], [38]. Telomeres in the most benign type of cancer, Dukes stage A, were shorter than those in the most severe cancer, indicating that there is something about the prevalence of telomere-stabilizing agents. hTERT is involved in the late stages of tumorigenesis [39], [40].

Table 1 Significance of telomerase as a biomarker for cancer prognosis

Type of cancer	Significance	References
Bladder Cancer	The negative telomerase activity was identified in exfoliated cells of the patients suffering from stone disease, benign urethral tumor, and benign prostatic hyperplasia.	[42]
Breast Cancer	The pessimistic association between telomere length and age.	[43]
Esophageal Cancer	Oxidative and life stress drastically affect the length of telomerase.	[4]
Colorectal Cancer	Telomere length is smaller than the adjoining mucosa.	[44]
Prostate Cancer	Telomerase activity of prostate cancer is mainly at the post-transcriptional level and not at any threshold level of hTR or hTERT mRNA expression.	[45]
Neuroblastoma	Telomerase activity as a prognostic factor.	[45]
Rectal Cancer	Telomerase activity is lower in rectal cancer as compared to colon cancer. Show the lowest degree of telomere shortening and the highest ratio of telomere length in cancer to non-cancerous tissues. (T/N)	[46]
Lymphocytic Leukemia	There is a negative link between telomere length and telomerase activity in B-cell type chronic leukemia.	[47]–[50]
Pancreatic Cancer	Telomerase inhibition limits the lifespan of pancreatic cancer cells in the human body. Telomere shortening is considered a common genetic modification.	[6], [51]–[53]
Ovarian Cancer	A therapeutic adjuvant and antioxidant Vitamin E is used for suppressing telomerase activity in ovarian cancer cells by improving its cis-platin-mediated cytotoxicity.	[54]
Liposarcoma	Telomerase activation help in maintaining telomeric length.	[3]

As results published by *Tamara Fernández-Marcelo et al*. in their research [37] displayed telomere weakening could be the cause of tumor suppression mechanism in colorectal cancers (CRC). Their efforts to strengthen the clinical prognosis conferred via telomere shortening in CRC sufferers, in a populace of 57 patients with colorectal carcinoma, analyzed by Dr. Gertler and his colleagues [40], an extensively higher overall survival rate became proven within the group of sufferers with the T/N ratio ≤ 0.9. The higher overall survival rate turned into displayed by the organization of CRC patients was analyzed using Dr. Valls and colleagues [5] with a T/N ratio ≤1 and negative lymph node involvement. Since there is no settlement regarding the function of telomeres as markers of disease progression in CRC, it's critical to collect results in order to attain a consensus. With reference to this study, they underline the massive wide variety of patients taken into consideration within the prognosis evaluation as well as using the Cutoff Finder Web Application [41] which allowed a goal difference among groups with a one-of-a-kind clinical diagnosis based on the telomere status.

Regarding the diagnosis of CRC patients, research studies showed a trend of worst clinical evolution in those with telomerase-positive malignancies, and when TERT levels were taken into consideration, no significant prognostic

differences were determined among the two groups of CRC patients defined by TERT expression. However, in different research, telomerase was recognized as an impartial prognostic marker for usual survival in colorectal cancer sufferers: patients with excessive TERT stages confirmed a vigilantly worse ordinary survival than those with lower TERT levels [36], [55]. The consensus has not been reached [56], [57] and similarly, studies are had to confirm the reduce-off value which distinguishes among the populations with exclusive diagnosis. Quantification of TERT mRNA and quantification of telomerase activity are the two essential strategies used to estimate telomerase tiers [56], [58]; furthermore, the telomerase activity predicted by TRAP (telomeric repeat amplification protocol) correlates with the stages of hTERT mRNA [59]. Therefore, the dichotomization of the cancer population considering telomerase activity may be beneficial for figuring out corporations of sufferers susceptible to receiving treatment toward the inhibition of telomerase [37].

A variety of chromosomal experiments have been performed to identify the repressors, but no chromosome areas able to inhibit hTERT expression have been found. The epigenetic regulation of DNA methylation and histone acetylation is inadequate to explain the tumor specificity of hTERT expression. Studies on phosphorylation and subcellular localization of hTERT may possess the ability to provide clearer expertise on telomerase regulation. However, antibodies in opposition to hTERT, which can be indispensable for such studies, have no longer been well characterized [60].

The telomerase-expressing most cancers cells may additionally rise from telomerase-active stem cells concerned with tissue remodeling or from the reactivation of telomerase interest in commonly telomerase-silent somatic cells [42].

In the predictive analysis of patients suffering from acute adult T-cell leukemia, the high telomerase activity and small telomere length show the worst prognosis and best analysis for the low levels of telomerase activity along with small telomere length [49], [50]. Recent studies reported indicate that tumor detection is directly associated with the presence and absence of enzyme activation [48], [61]–[63]. Telomerase activity determination after chemotherapy is an important factor in the clear-cut demarcation of cancer treatment. Despite active telomerase, most people with superior pancreatic tumors harbor quick telomeres and chromosomal ends that lack detectable telomeric repeats [53]. Molecular-targeting therapies play a milestone role in the treatment of cancer [64].

4. Telomerase and the Human Lifespan: The Immortal Cell Lines

Along with its diagnostic and therapeutic aspects, telomerase also plays an important role in fundamental research into life itself. Since the enzyme, when active, renders the cell(s), immortal, it can be used to create transformed immortal cell lines that are useful for all forms of animal cell culture research[65], [66]. It can also be viewed as a method to theoretically eliminate aging, allowing humans to become immortal [67].

The study of telomeres and telomerase has provided insights into the biology of aging and the potential for extending the human lifespan. Telomeres are protective caps at the ends of chromosomes, and telomerase is an enzyme that helps maintain them. Every time a cell divides, the telomeres become shorter [16], [68]. When they become too short, the cells can no longer divide and die. This is one of the reasons why we age [28], [69]. All normal human somatic cells have progressive shortening of telomeres with each cell division. This is also true in proliferative (transit amplifying) adult stem cells. When a few telomeres in a cell reach a shortened state, a DNA damage signal is initiated. This DNA damage signal indicates that the shortened telomere is being sensed as uncapped or broken DNA. In cells that have bypassed the M1 senescent state by inactivation of important cell cycle checkpoint genes (e.g. TP53 and/or pRB), cells ignore the ongoing DNA damage signal and continue to divide until many telomeres are critically shortened. During this extended lifespan period, end associations occur eventually leading to breakage-fusion-bridge cycles resulting in M2 or a state of crisis. During a crisis apoptotic cell death almost universally occurs. However, in a rare human cell (based on fluctuation analyses calculated to be about one in ten million cells) an immortalization event occurs. This cell has two characteristics, expression of telomerase and stabilization of telomeres [69] as shown in Figure 4;

However, some cells express telomerase, which helps to keep telomeres too short. This allows cells to divide indefinitely, which is one of the reasons why cancer cells are difficult to kill [7], [8]. Exploring the biology of telomeres and telomerase could help scientists develop the future of aging and the human lifespan. Aging is associated with the gradual loss of function of various systems in the body. This process is thought to be regulated in part by the length of the telomeres, which are the protective structures at the end of chromosomes [14], [68], [70], [71]. Telomerase can add telomere repeats to the ends of chromosomes, counteracting the effects of telomere shortening. This enzyme is found in some cancer cells, which allows them to divide indefinitely [68]. However, it is not found in most normal cells, which leads to the eventual death of the cell [71], [72]. Some scientists believe that telomerase could be used for immortality. If telomerase could be introduced into normal cells, it would theoretically allow them to divide indefinitely. However, this is still an area of research and it is not clear if this would be possible or safe. Aging is a complex process, and

telomeres are just one part of it. However, understanding how telomeres and telomerase affect aging may help us to develop new ways to combat the effects of aging.

4.1. How do telomeres and telomerase affect aging?

As we age, our cells age with us. One of the ways in which they do this is by losing telomeres, the protective structures at the ends of our chromosomes. Telomeres shorten with each cell division, and when they become too short, the cells can no longer divide and die. This is one of the reasons why we age[16], [28], [68], [69]. Interestingly, the length of telomeres is also associated with chronological age. The shorter the telomeres, the older the cell and the person. Telomere length is also associated with the risk of chronic diseases such as heart disease, stroke, and diabetes[56], [73]. When telomeres are too short, cells can no longer divide and die. This is one of the reasons why we age. Fortunately, our cells have a built-in mechanism to address this problem. Telomerase is an enzyme that helps to maintain the length of telomeres. It does this by adding extra DNA to the ends of chromosomes. Some cells can express telomerase, which helps to keep the telomeres from getting too short [28].

As we age, our cells go through a process of wear and tear. This results in a gradual deterioration of tissues and organs. A key component of this process is the telomere. Telomeres help to keep DNA stable as cells divide. As we age, the telomeres become shorter. This leads to cells becoming less effective and eventually dying. Telomerase enzymes help maintain telomere length. It is found in most cells but is inactive in most adult cells [28], [74]–[76]. This means that as we age, our telomeres become increasingly shorter. In some cells, telomerase is active. This leads to the cells becoming immortal, as they can continue to divide indefinitely. This is seen in cancer cells, which is why cancer is difficult to treat; and in developing embryonic cells which allow for the development of the embryo [28], [72], [76].

Evidence suggests that telomeres and telomerase may play a role in the aging process [72]. In particular, telomere shortening may contribute to the loss of cell function as we age. Currently, there is no method to prevent or reverse the aging process. However, understanding the role of telomeres and telomerase may help develop treatments to reduce the effects of aging. How does this affect aging? Cells with high levels of telomerase are immortal [71], [72], [74], [77], [78]. They can continue to divide indefinitely, which means that they do not age in the same way as other cells [74]. Some researchers believe that by boosting telomerase levels, we can slow down or even reverse the aging process. Thus far, these results have been promising [71], [72], [75], [77]–[79]. Telomerase appears to be safe and effective in slowing the aging process in cell lines. However, it is still early days and more research is needed before we can say for sure whether telomerase can have the same effect in humans.

4.2. What is the potential for an extended human lifespan?

There is plenty of evidence to suggest that the human lifespan can be extended. Many species have a long lifespan, including bowhead whales, clams, and tortoises [77]. Humans have shorter lifespans than other primates, but it's thought that diet and lifestyle may have something to do with this. Studies have shown that calorie restriction can help extend the human lifespan by up to 40%. People who consume a nutritious diet, exercise, and get enough sleep tend to be healthier and live longer. One of the problems associated with aging is that the cells do not divide as frequently as they are used when we are younger [74]. In order to slow down the aging process, we need to increase cell division in various tissues and organs. This can be achieved through lifestyle changes, such as eating nutritious foods and getting sufficient exercise. There is no single answer to the question of the potential for an extended human lifespan. Different factors will affect each individual's potential lifespan, from genetic factors to lifestyle choices. However, there are a few different avenues of research that show promise in the quest to lengthen human life.

Using methods such as UV mutagenesis, random mutagenesis, and site-directed mutagenesis, scientists have derived immortal cell lines with upregulated telomerase activity. Published in 2005 in the American Association for Cancer Research Journal, *Zongaro, et al.*, a research article explores the various methods to create immortalized cell lines using nude mice [80]. Since there are a few cell lines that have been made using upregulated telomerase activity like St-TIb (a telomerase-immortalized human endometrial stromal cell line) [81], and Swan 71 (telomerase-immortalized first-trimester trophoblast cell line) in 2009 [82], which were neoplastic or cancerous cell lines, along with BAR-T (telomerase-immortalized, non-neoplastic, human Barrett's cell line) in 2007 [83], and TIGK (telomerase-immortalized gingival keratinocytes) in 2013 [84]. In 1999, a letter published in Genetics Nature, written by *Morales et al.*, discussed the absence of cancer-associated changes in human fibroblasts immortalized using increased telomerase activity by forced expression of hTERT [85]. Telomeres shorten and get damaged due to various intrinsic factors which lead to the loss of tissue/organ regeneration. Cancer cells reactivate TERT and therefore maintain their telomeres and a proliferative state as shown in figure 7. Both scenarios ultimately contribute to or result in death of the organism. Hence, therapeutic strategies aiming at the activation or inhibition are under development [91].

An area of research is the examination of the role of telomeres in aging. Some scientists believe that by increasing the length of telomeres, we could potentially extend the human lifespan.

In theory, by lengthening telomeres, telomerase could help to extend the human lifespan. However, this method is still in the early stages of development and has not yet been proven effective. Telomeres have also been studied as a potential way to extend the lifespan of humans. In one study, mice with shortened telomeres were found to live 20% shorter lives than those of mice with normal telomeres [72]. This suggests that telomeres may indeed have an impact on lifespan; however, more research is needed before any definitive conclusions can be drawn. Gene editing is another promising method for extending the human lifespan. By editing genes that are involved in the aging process, it may be possible to slow down or even reverse the effects of aging [71], [72], [77]–[79]. The same study mentioned above also found that gene editing can extend the lifespan of mice by up to 20% [72]. Although this is a promising result, it is still unclear whether this method is effective in humans. Using gene editing technologies such as CRISPR and in vivo gene therapy, scientists are trying to introduce telomerase activity in a related manner to introduce immortality in cells without carcinogenesis [86]. One example of this is the creation of age-resistant mutant mice by knocking out a pro-aging gene [72], [86]. While this research is still in its early stages, it shows promise for the potential to extend human life. Immortalization may sound like something out of a science-fiction movie, but there are actually a few different ways that scientists are working towards this goal. One way is through the use of telomere lengthening therapies, as mentioned above. Another way is through the use of gene editing to turn off the aging process. While these methods are still in their early stages, they hold promise for the potential to immortalize humans.

Ultimately, the potential for an extended human lifespan remains unknown. However, there are a few different areas of research that show promise for the possibility of lengthening human life. The potential for an extended human lifespan is an intriguing concept. There are several ways that this could be achieved, including through the use of telomerase, telomeres, gene editing, and immortalization. Each of these methods has its advantages and disadvantages, and it is still unclear which, if any, will be successful in extending the human lifespan.

4.3. The Implications of Telomere Research

Telomeres and telomerase have given us insight into the biology of aging and the potential for extending the human lifespan. It's important to note that telomere research is still in its infancy. There are many unanswered questions, and it's likely that there are many more surprises in store for us as we continue to explore this fascinating area of research. However, one thing is certain: This is an exciting time to be involved in aging research. We have made great strides in recent years, and there are plenty more to come.

5. Figures and Images

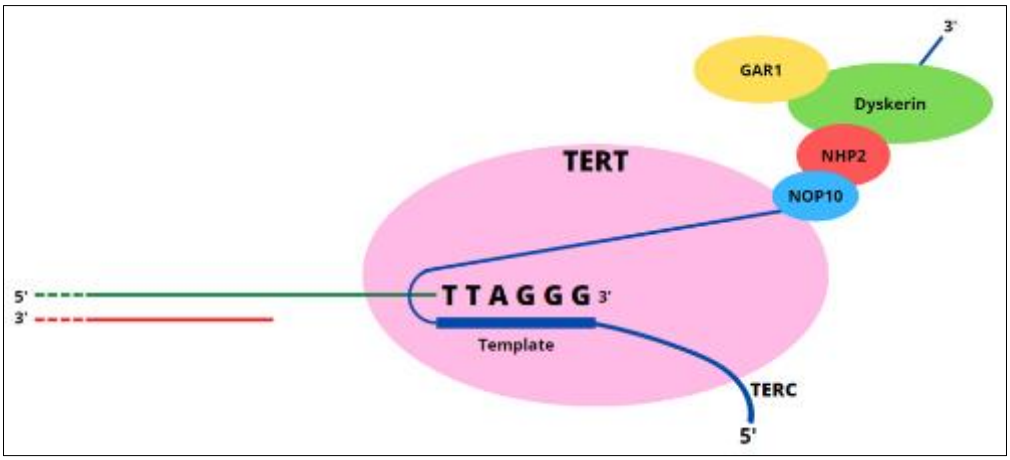

Figure 1 The telomerase complex and its components. The enzyme telomerase reverse transcriptase (TERT), its RNA component (TERC), the protein dyskerin, and other associated proteins (NHP2, NOP10, and GAR1) are shown [87].

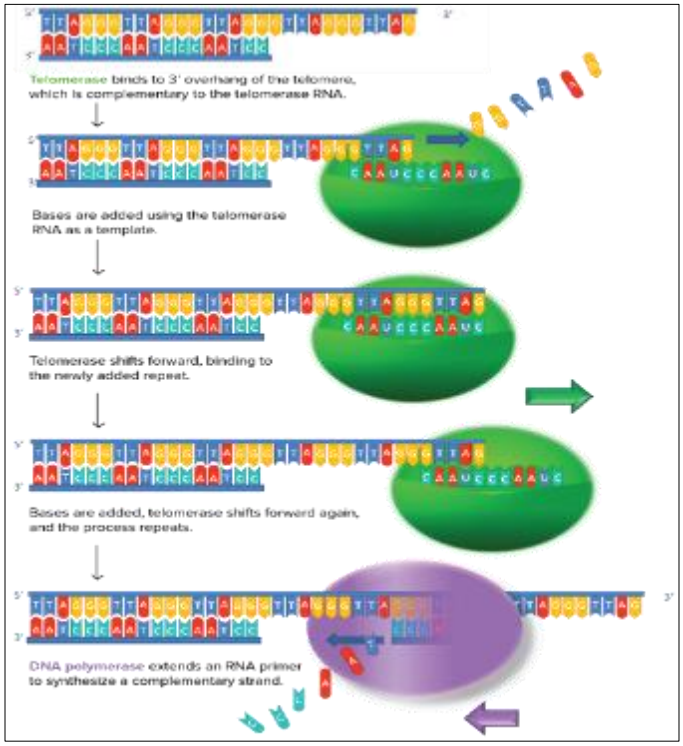

Figure 2 Mechanism of action of telomerase [87].

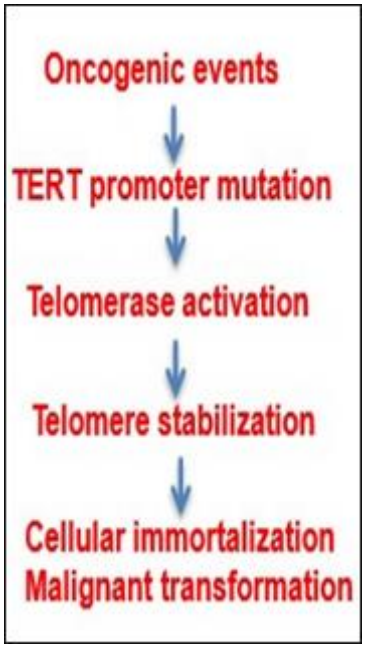

Figure 3 Flowchart showing oncogenic events [88].

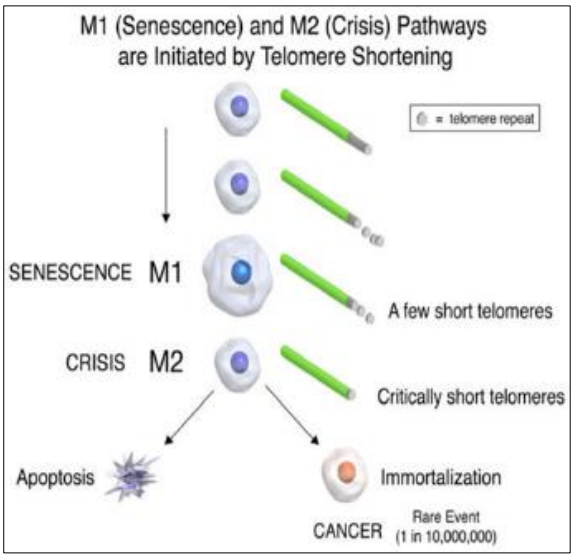

Figure 4 The M1 and M2 models of senescence and crisis [69].

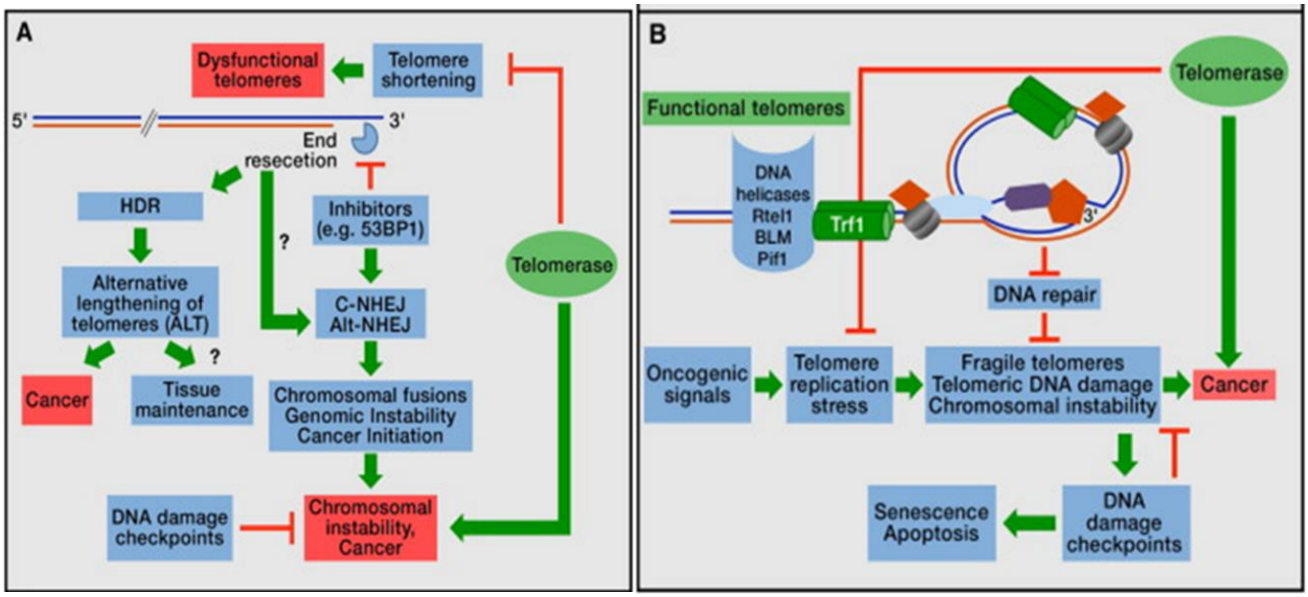

Figure 5 The Role of Chromosome Ends in Genome Integrity Control Depends on Telomere Functionality and Telomerase Expression

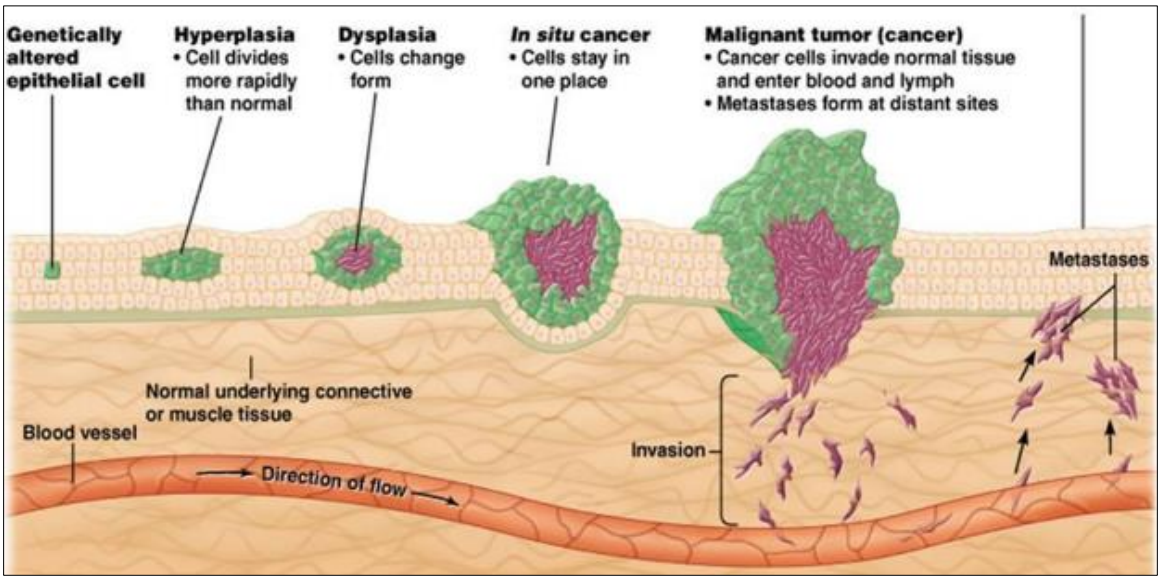

Figure 6 Different stages of Cancer progression [90].

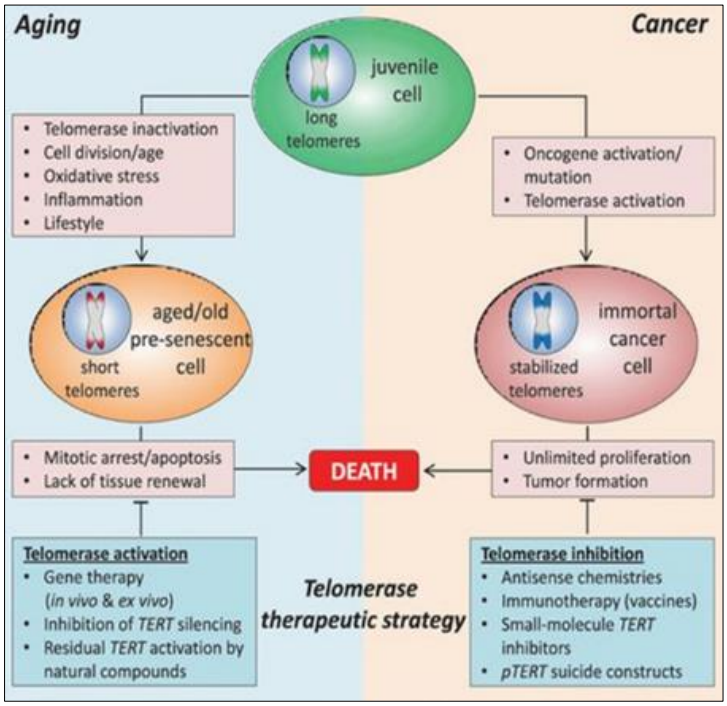

Figure 7 Telomeres and telomerase in aging and disease [91].

6. Survey- Opinions on Biological Immortality

While researching the literature connecting telomerase, cancer, and aging, we conducted a survey of the general public to understand their views and opinions on the concept of biological immortality- and both the scientific and moral aspects of humans attaining the same. The survey was anonymous and was conducted over a period of three weeks. The total number of responses came out to be 100 and a sample of the survey form is attached below

Figure 8 Survey Page 1

Figure 9 Survey Page 2

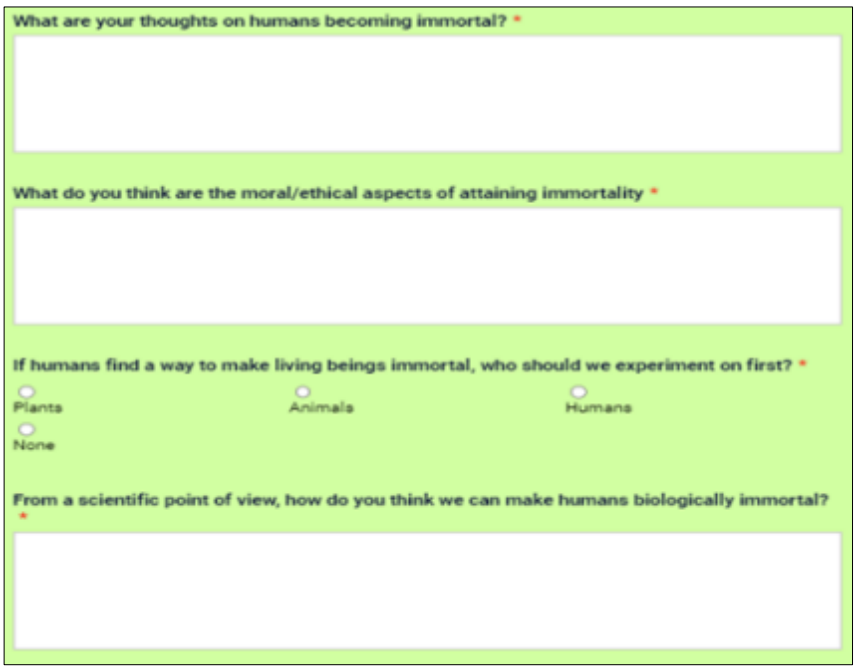

Figure 10 Survey Page 3

6.1. Analysis of the responses

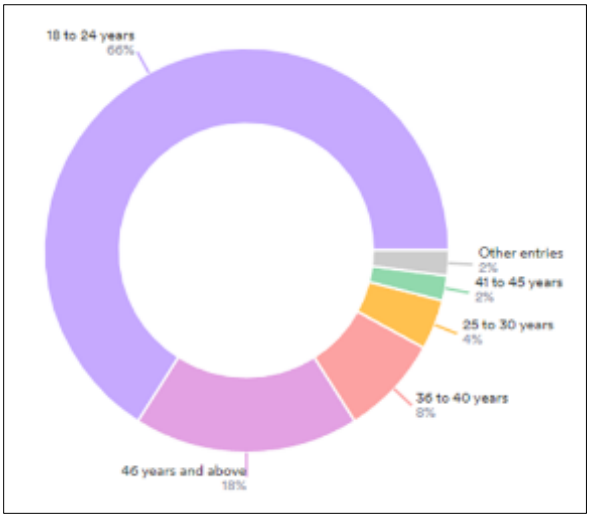

Figure 11 Age Demographics of the survey respondents

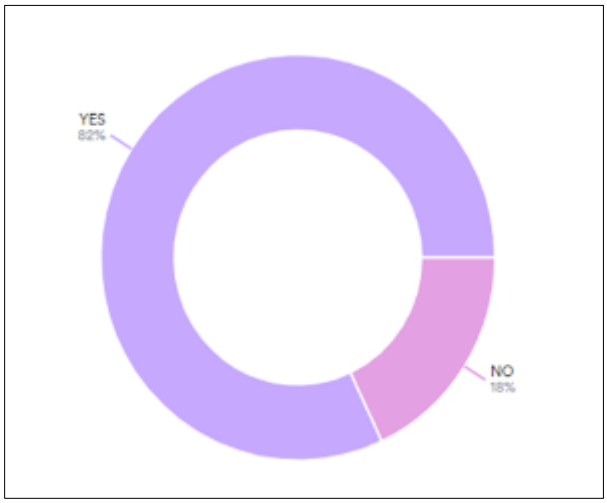

Figure 12 Percentage of respondents with some knowledge or experience in the field of biology

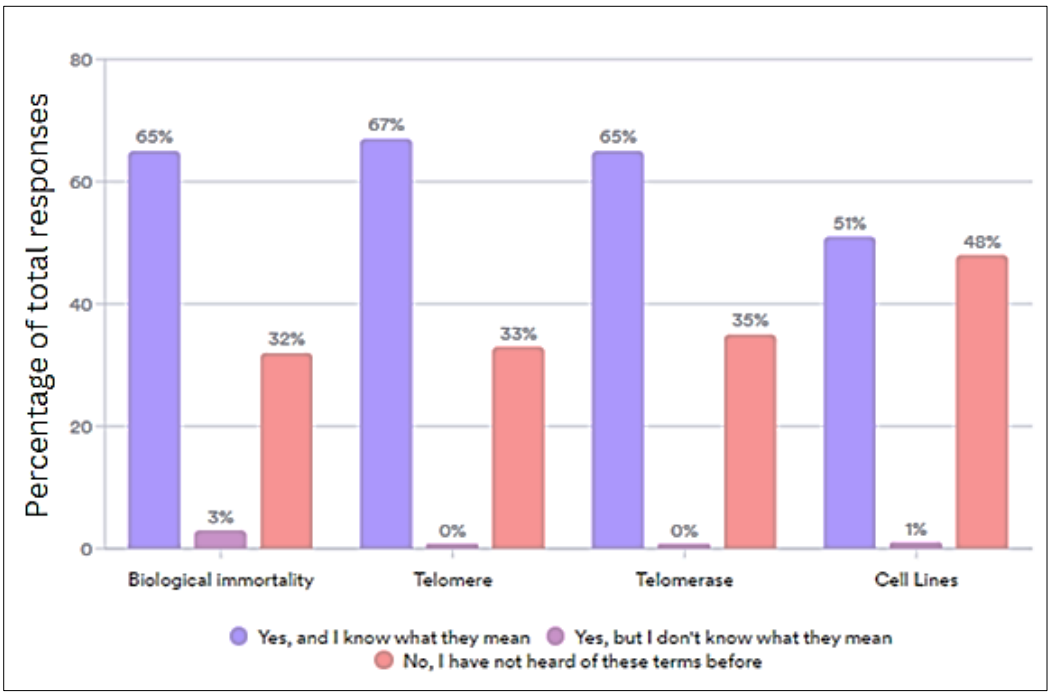

Figure 13 Percentage of the respondents familiar with relevant keywords to the article

When asked about their thoughts on humans attaining biological immortality in the foreseeable future, most of them rejected the idea, saying that aging is inevitable and that it would be against the natural order, and citing other religious or ethical arguments. They felt that immortality could make humans even more despondent to the issues like climate change and could create issues for future generations.

The minority that supported the idea said that if possible, this could help humans go leaps and bounds ahead in the field of science and technology, especially in the sector of space exploration. They also mentioned that if humans attained immortality, people could die of their own accord; albeit by losing their lives in accidents, making peace with everyone in their lives, and making sure that the ones they leave behind do not feel saddened by their loss. They also encouraged research in this sector to help humans lead longer, more fulfilling lives.

Analyzing the various methods, the respondents felt could help humans become immortal, most cited technologies like artificial intelligence and the computer mind, with some even suggesting the fabled hypothesized sci-fi megastructure- "The Matrioshka Brain" proposed by Dr. Robert J Bradbury in 1997 [92]. Some other respondents leaned towards biological research in an attempt to achieve the feat with methods like genetic engineering, gene therapy, induction of telomerase activity in a regulated manner, and even nanorobots that would repair cells on the verge of senescence by repairing their telomeres and continuously checking these cells for mutations and DNA damage. They also mentioned that repairing the damage that the thymus undergoes during aging could help increase human lifespan by emboldening the human immune system. While this may not be a method to achieve immortality, it could increase the life conditions and vitality of humans.

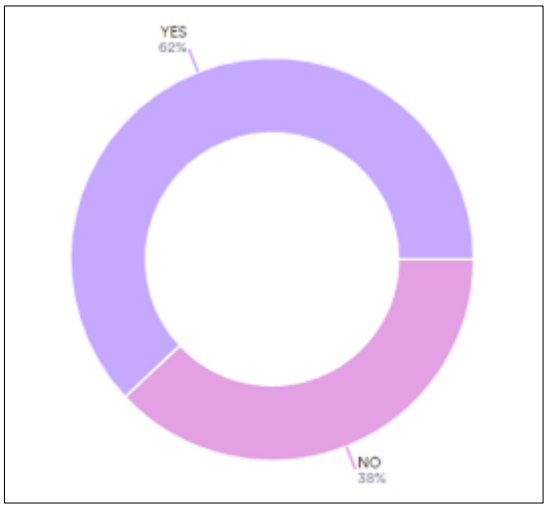

Figure 14 Percentage of respondents familiar with biologically immortal organisms

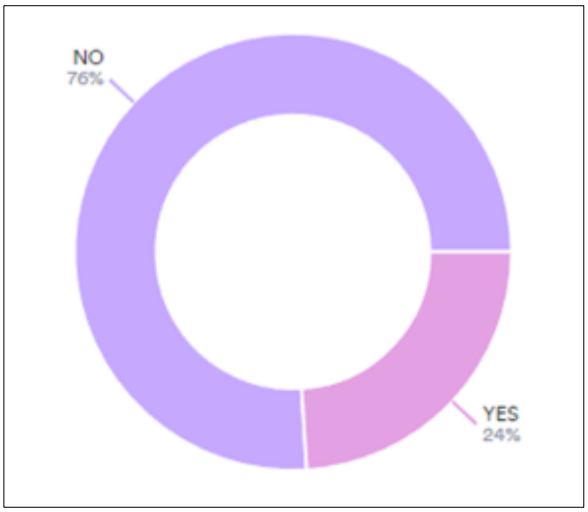

Figure 15 Percentage of respondents who feel that humans should attain immortality

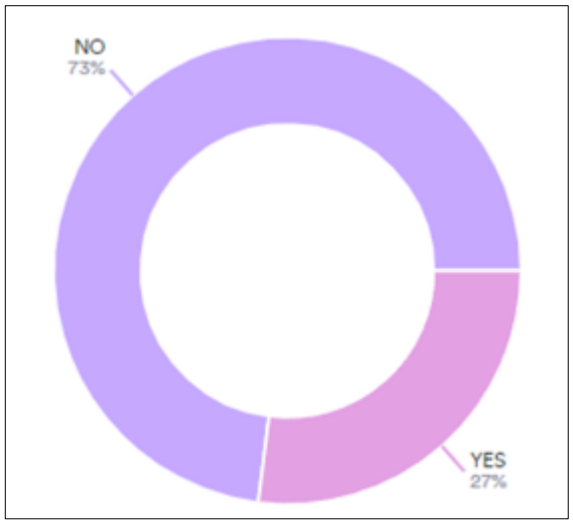

Figure 16 Percentage of respondents who would like to attain immortality of possible

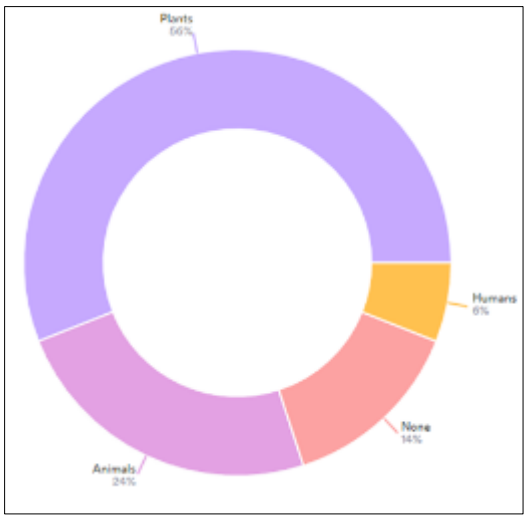

Figure 17 Which organisms do the respondents feel should be experimented on first for testing technologies and therapies for immortality

Upon analyzing the survey as a whole, our team concluded that while most people feel that immortality is something that humans should never aspire to, some people are in its favor and have some interesting ideas on how to achieve it. During our interactions, one thing was certain- All of them felt that while this was something we as humans should research, our focus should also not get diverted from the problems we face today. Many of them were hopeful that

maybe someday it is possible for humans to live a long, endless life without worrying about the issues that we currently face.

Abbreviations

- DNA, deoxyribonucleic acid;
- SAC, spindle assembly checkpoint;
- RNA, ribonucleic acid;
- mRNA, messenger ribonucleic acid;
- hTERT, human telomerase reverse transcriptase;
- REs, response elements;
- WHO, World Health Organization;
- RNP, ribonucleoprotein;
- GCT, giant cell tumor;
- miRNA, micro ribonucleic acid;
- CRC, colorectal cancer;
- TRAP; telomeric repeat amplification protocol;
- TIGK, telomerase-immortalized gingival keratinocytes;
- CRISPR, clustered regularly interspaced short palindromic repeats;

7. Conclusion

After reviewing the last 25 years of literature on the topic, we have reached the conclusion that telomerase has been used as a prognostic factor for various types of cancer. It is also now understood that the same telomerase can be used as the basis for therapy to treat various types of cancers. By regulating telomerase activity, research in the field of immortal cell lines is being conducted- a prospect of which is biological immortality for humans. Understanding the mechanism of action of telomerase and developing technology to control and regulate it could give way to anti-aging or age reversal therapies that could boost our immune system and make humans biologically immortal (theoretically speaking). While research in the former is an ongoing process, the latter is something we look forward to in the future.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declare that there no conflict of interest between them and the topic of the research conducted by them.

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Author's short biography

Prathibha S S

Master's graduate, independent research explorer to improve research and theoretical skills through constant learning and endeavor so as to become an integral part of synthetic biology.

3 Presentations, 1 publication (Assessment of physicochemical and microbiological parameters in surface water of Ooty lake, a tourist hot spot in South India — Published in International Journal of Advanced Research in Biology Sciences, Vol. 6(8) – 2019)

Area of Research: Molecular cloning, Cancer Biology.