HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 3 (2022)

Telomeres and Telomerase in Cancer: Overview and Therapeutic Potential

Taei Kim[1] and Amy Tarangelo[#]

[1]American School in Japan
[#]Advisor

ABSTRACT

Telomeres are specialized structures of eukaryotic chromosomes that protect the tips of the chromosomes. Telomeres prevent chromosomes from losing base pair sequences at their ends, and also stop chromosomes from fusing together. Telomerase is an enzyme that rebuilds telomeres and maintains genomic stability by extending telomeres after each cell division. Telomerase activity prevents cellular aging, also known as senescence. Immortal cell lines can be created by inducing expression of telomerase in cells that normally lack telomerase. Cells need to maintain genomic integrity to prevent mutations that can lead to cancer. Such activation of telomerase mentioned above can often lead to cancer development by preserving genomic stability in rapidly dividing cells. If a cell keeps dividing and overcomes the limitations of telomeres, a cancerous tumor can form. In healthy cells, telomeres achieve the healthy balance between limiting cellular lifespan and keeping cancer growth at bay.

It has been established that telomerase activity is a biomarker of cancer and tumorigenesis. This makes telomerase an attractive target for cancer therapy, and studies indicate that methods of treatment such as oligonucleotides, immunotherapies, and stabilization of G-quadruplexes all hold immense possibilities in the field of oncology. Such discoveries provide insight on the potential of telomeres to play a significant role in the prevention of cancer before tumorigenesis, or the treatment of cancer after diagnosis. Therefore, telomerase is a viable cancer biomarker and a therapeutic target, and an area of interest in the field of cancer study.

Introduction to Cancer

Cancer is caused by mutations, or changes in the DNA sequence of chromosomes. Mutations in genes that are responsible for cell cycle regulation can lead to the unregulated growth that is a hallmark of cancer. Any changes to the DNA, or mutations, will lead to the production of an altered mRNA. This will then lead to the production of a protein that no longer functions properly. Even changing a single nucleotide along the DNA may lead to a completely dysfunctional protein The process by which proteins are made, translation, is based on the reading of mRNA that was produced by transcription. During translation, the mRNA is read and converted into a sequence of amino acids. As mentioned above, if there are any mutations in the DNA, the mRNA is unable to be translated properly, leading to an inability for different proteins to function (Brown, 2002).

In most cases, the transition from a normal, healthy cell to a cancer cell is a stepwise progression that requires many genetic changes that add up to create the cancer cell (Kamps et al., 2017). There are two broad categories of genes, depending on their normal functions in the cell. Proto-oncogenes are genes whose protein products stimulate or enhance the division and viability of cells. This category also includes genes that contribute to tumor growth by inhibiting cell death. Defective versions of proto-oncogenes can cause a cell to divide in an unregulated manner. This can occur in the absence of a normal pro-growth signal, such as those provided by growth factors. Other genes are categorized as tumor suppressors, genes whose protein products can directly or indirectly prevent cell division or lead to cell death (Varmus, 2009).

Each gene codes for a different RNA molecule, which is then used for protein synthesis. Information flows from the DNA, the storage form of the information, to the working form (RNA), then to the final product which is the protein. The process by which the information in a strand of DNA is copied into a new molecule of mRNA is called transcription (S. J. Liu et al., 2016). The process of transcription begins with the transcription factor s recognition of the promoter of the gene that is to be transcribed. Transcription factors are special proteins that bind to the start site, or promoters, of a gene that is to be transcribed. Then, the enzyme that makes the RNA binds to the transcription factor and recognizes the starting site. The enzyme proceeds down the DNA, and makes a copy of each unit until it reaches the end of the gene. Having completed its job, the enzyme falls off and the RNA is released (Solomon et al., n.d.).

p53 is an example of a transcription factor. It is an important tumor-suppressor protein, and plays a key role in cell division and cell death. The p53 gene is important because the protein that it codes for controls the transcription of genes that are involved in causing cells to die. The gene that codes for p53 is mutated in over half of all cancers of any type. It is often called the guardian of the genome." It is the protein most highly involved in activating apoptosis in cells with damaged DNA (Rivlin et al., 2011, p. 5).

Hallmarks of cancer are properties that all cancerous cells have in common. As mentioned before, the cause of cancer is damage to the genes of a cell, in other words genetic damage and mutations. There are six prominent hallmarks of cancer (Senga & Grose, 2021).

Limitless replicative potential is a hallmark of cancer that involves telomeres and telomerase. Every time a cell replicates, the telomeres at the end of the chromosomes get shorter and shorter. Chromosomes are lengths of folded DNA. When a cell divides, it is unable to copy every bit of its DNA– therefore, the DNA that isn t coding for a protein, a telomere, is the bit that is lost at the end of each replication. If the telomere disappears, the coding sections at the end would disappear (Popli et al., 2017). There is a limit to how many times each cell can divide, which is about 40-60 times for human cells, also known as the Hayflick Limit. At this point, cells would begin to self-destruct. However, cancerous cells are able to avoid cellular senescence (escape cell death) by activating an enzyme called telomerase. Telomerase adds DNA bases to the telomeres to make them longer, and this allows cells to continue dividing without eating into the coding portions of DNA (J. Liu et al., 2019).

Overview of Telomeres

Telomeres are specialized structures of eukaryotic chromosomes. Telomeres protect the tips of the chromosomes by preventing them from losing base pair sequences at their ends and stopping them from fusing to each other (Jafri et al., 2016). They protect the chromosomal ends from degradation, DNA recombination, DNA end joining, and the DNA damage response (DDR) (Longhese, 2008). Due to such properties, telomeres are often compared to the protective caps on the end of a shoelace (*What Is a Telomere?*, n.d.).

Telomeres are specialized nucleoprotein structures consisting of chromatin and shelterin protein complexes. The chromatin consists of a repetitive DNA sequence (5 -TTAGGG-3) and a single-stranded 3 overhang found at the ends of chromosomes. The G-overhang plays a key role in preserving telomere integrity and genome stability. In the G-overhang, the guanine-rich strand extends past the complementary cytosine-rich strand. Telomeres with a longer G-overhang are more stabilized and have more growth potential (Doksani et al., 2013). The 3 G-rich overhang assists the telomeric DNA in the formation of a structure in which the 3′ single-stranded overhang folds back and invades the homologous double-stranded TTAGGG region. This forms a telomeric loop, more commonly known as a T-loop, that protects the 3 end of the chromosome by isolating it from recognition by DNA damage response (DDR) machinery (Zhu et al., 2003). These proteins that manage the structure and function of telomeres are recruited to the chromosomal ends by the telomeric DNA and RNA TERRA (telomeric repeat-containing RNA)(Cusanelli & Chartrand, 2015).

During mitosis, cells do not complete DNA replication to the very chromosome ends. DNA polymerase is not capable of copying the entire strand of DNA– as a result, after the end of each cycle of mitosis, about 20-50 base pairs are lost, leading to gradual shortening of the chromosomes (Barnes et al., 2019). The point when telomeres become too short to allow for successful cell division is known as the Hayflick limit. The Hayflick limit indicates the number of cell cycles that a cell is capable of going through before the cell enters cellular senescence. In mammalian cells, the Hayflick limit is reached after approximately 40-60 cell divisions. Cellular senescence is induced by dysfunctional telomeres. When telomeres reach a certain critical length, they induce a signal of damage to DNA, which results in the cell exiting the cell cycle and entering replicative senescence (Gomez et al., 2012). The period of cellular senescence, also known as mortality stage 1 (M1), is characterized by inhibition of cellular proliferation, probably due to the uncapping of one or a few shortened telomeres (Jafri et al., 2016).

DNA damage is a change in the structure of the DNA that can lead to cellular injury, and a decreased ability for the organism to reproduce (Kaufmann & Paules, 1996). DNA damage response is a mechanism designed to combat DNA damage by detecting lesions, and signaling for the repair of damaged DNA (Jackson & Bartek, 2009). Dysfunctional telomeres in normal cells, due to a severe shortening of telomere length, will induce DDRs, which will consequently prompt cellular senescence (Jafri et al., 2016). If there is detected DNA damage, the cell will undergo mechanisms to halt further progression through the cell cycle at checkpoints that are present in the G1, S, and G2 phases of the cycle (Morgan & Lawrence, 2015). DNA damage response most commonly leads to either cell cycle arrest and DNA repair, or apoptosis (Giglia-Mari et al., 2011). Oftentimes, cancer cells have abnormalities in DNA damage response machinery. Both the activation and inactivation of DNA damage response machinery, and defects of the proteins involved, are commonly observed in cancers (Hosoya & Miyagawa, 2014).

DNA damage response is largely regulated by shelterin is a six-subunit protein complex that allows the DNA damage response machinery to differentiate telomeres from sites of DNA damage, and prevents chromosome ends from fusing and being recognized as double-strand breaks (Brown, 2002). Shelterin regulates telomerase access to the telomere and also allow TERT to synthesize longer telomere tracks (McNally et al., 2019). Shelterin complex consists of six shelterin subunits, three of which are responsible for recognizing the TTAGGG repeats (TRF1, TRF2, and POT1.) TRF1 and TRF2 are responsible for the recognition and binding of the duplex TTAGGG repeats, and POT1 for the single-stranded TTAGGG overhangs. TRF1 and TRF2 both inhibit telomerase activity, therefore preventing the lengthening of telomeres (Gomez et al., 2012). More specifically, TRF1 controls the replication of telomeric DNA, and TRF2 is involved in the formation of T-loops, and prevents non-homologous end joining of telomere pathways, and DDR pathways from being activated (Trybek et al., 2020). TRF1 and TRF2 are involved in two pathways, ATM and ATR, both are which sense DNA damage. After sensing DNA damage, these pathways induce cell cycle arrest by activating downstream kinases, Chk1 for ATM, and Chk2 for ATR. These two kinases induce DDR at telomeres where shelterin is impaired, for instance due to telomerase shortening in senescent cells (Gomez et al., 2012).

Shelterin remodels telomeres into a capped structure to protect its end (Schmutz & de Lange, 2016). However, critically short telomeres lack sufficient binding sites for shelterin, and telomeres become unable to protect the chromosomes. The inability for the shelterin to mediate the telomerase, which maintains the telomeric DNA, exposes the end of the chromosome to degradation leading to false recognition by the DNA damage response proteins (Palm & Titia de Lange, 2008). The uncapped ends are processed by the DNA double strand break repair machinery, which leads to chromosome fusions and rampant chromosomal instability. Most cells are killed in this process, but some of the cells that survive undergo malignant transformation (Soler et al., 2005).

In most cells, when the telomeres become too short, cellular senescence occurs, and cells can no longer replicate. In senescent cells, DNA synthesis is halted, due to a failure to meet the requirements for the G1 and S checkpoints. DDR can sometimes induce senescence, when telomere attrition leads to the uncapping of the

chromosomal ends, which is then detected by the DDR machinery as double-stranded DNA breaks (Ernst & Heidel, 2021). Senescence is also triggered by other internal and external factors, such as oxidative stress. Senescent cells accumulate exponentially in organisms over time, and therefore is thought to be a hallmark of aging (Kumari & Jat, 2021).

Overview of Telomerase

Telomerase is an enzyme made of protein subunits and RNA that act to rebuild telomeres to restore cell division (Trybek et al., 2020). Telomerase maintains the length of telomeres by adding guanine-rich repetitive sequences onto the ends of the telomeres. Telomerase consists of a catalytic subunit with reverse transcriptase activity (TERT-telomerase reverse transcriptase), an RNA template (TERC-telomerase RNA component), and dyskerin (DKC1)(Gomez et al., 2012). TERT polypeptide folds with TERC, a non-coding RNA. TERT wraps around the chromosome to add single-stranded telomere repeats. TERT is a reverse transcriptase, meaning that it is an enzyme that creates single-stranded DNA using single-stranded RNA as a template. TERT binds to TERC and the protein complex. TERT synthesizes telomeric sequences using TERC as a template (Okamoto & Seimiya, 2019). By using TERC, TERT can add a six-nucleotide repeating sequence, 5'-TTAGGG, to the 3 ’of the chromosome. Telomerase maintains the length of telomeres, replenishing them with each cell division. Telomerase is activated to extend telomeres and to prevent senescence. Immortal cell lines can be created by activating telomerase in cells that normally have inactive telomerase (Cohen et al., 2007).

As stated, telomerase elongates the 3 end of the chromosome, thus enabling other polymerase to synthesize the complementary strand. When extending the telomere, the telomerase uses the same short region of its RNA as a template for DNA synthesis, and this creates a cyclic reaction (Nosek, 2008). A single-stranded DNA substrate first base pairs with the RNA template mentioned before, which serves to extend the DNA substrate. The template-DNA hybrid is disrupted and the telomerase complex either disassociates from the DNA substrate or relocates to the new 3 end of the DNA substrate, which then becomes available for another cycle of elongation by the telomerase (Schaich et al., n.d.)/ Continuous rounds of nucleotide addition and enzyme translocation allows reiterative addition of telomeric repeats onto the DNA 3 end. This process is known as repeat addition processivity, or RAP (Wu et al., 2017).

Short telomeres or a lack of telomerase can exert a longevity-promoting effect for the organism via prevention of cancer (Hornsby, 2007). Cell division beyond the limitations of telomeres can lead to cancer, and hence, a mechanism called cellular senescence exists to act as an anti-cancer mechanism. Cellular senescence is induced by factors such as telomere erosion, DNA damage, and oxidative stress. Senescent cells are unable to divide, and have a decreased chance of acquiring oncogenic mutations. However, telomerase can be reactivated in some cells to extend telomeres and prevent cells from senescing. Aged cells that re-express telomerase may be more likely to acquire mutations that are necessary for malignant transformation. Telomere shortening, however, is one of many ways in which cells can become senescent. Telomerase is expressed in many cancer cells, but not in most healthy adult cells. Telomerase is also 10-20 times more active in cancer cells than in normal cells, which provides a growth advantage to many types of tumors (*Facts About Telomeres and Telomerase: Shay Lab - UT Southwestern*, n.d.). Because of this, telomerase is a biomarker for cancer diagnosis, as well as a target for new therapies to inhibit tumor growth. Inhibition of telomerase in cancer cells causes telomere erosion in these cells and leads to size reduction of the tumor by arresting the growth of cancer cells or by inducing apoptosis, a form of cell death. Inhibition of telomerase can be achieved by targeting different parts of the enzyme (Hiyama & Hiyama, 2003). hTERT is a common target for telomerase-related cancer therapies, as it is found in approximately 90% of all cancers. It was discovered to be upregulated in cervical carcinomas, hepatocellular carcinomas, kung tumors, breast carcinomas, neuroblastomas– its prominence in tumorigenesis makes it an attractive therapeutic target (Jäger & Walter, 2016).

By introducing telomerase into cells, it may be possible to reverse the effects of aging and treat age-related disease. Furthermore, the rate of aging in patients with premature aging disease may be decreased by activating telomerase in the cells of the patients.(Callaway, 2010) However, the activation of telomerase can prevent cells from senescence, which can result in cells mutating and undergoing malignant transformation. Currently, only GV1001 and imetelstat (GRN163L)– a therapeutic vaccine, and telomerase antagonist, respectively– are being used in clinical trials. (Shay, 2016)

Importance in Health and Disease

It is widely accepted that telomere attrition is responsible for limiting the lifespan of human fibroblasts. In the absence of telomerase, telomeres in fibroblasts shorten accordingly with the number of cell divisions (Harley et al., 1990). Telomeres are essential for regulating the cell cycle and apoptosis. Mutations on telomerase enzymatic activity can result in the development of tumors, aging, and senescence. Understanding telomeres is an important field of research in understanding cell function, as well as tumorigenesis (Chatterjee, 2017). Furthermore, recent discoveries indicate that telomerase may also be crucial in future studies regarding human genetic disorders that result in premature telomere shortening, inhibition of telomerase as a therapeutic strategy, and roles for telomeres in gene expression (Shay & Wright, 2019).

Telomeres have also been found to play a critical role in aging. It was known that telomere shortening is the molecular clock that triggers aging. In a study by… expression of vectors encoding the catalytic subunit of human telomerase (hTERT) in two telomerase-negative normal human cell types extended lifespan and reduced staining for β-galactosidase, a biomarker for aging compared with controls. Furthermore, the life expectancy for the telomerase-positive clones extended by at least 20 doublings, suggesting a correlation between telomere attrition and cellular aging in vitro (Brown, 2002).

The use of telomerase to achieve a therapeutic outcome holds contradictory requirements– while the activation of telomerase is needed for degenerative diseases, the suppression of telomerase is required to battle cancer. Telomere shortening is observed in age-related, or degenerative diseases, and such conditions require the transfer of telomerase-promoting factors. On the other hand, anticancer applications require suppression of telomere lengthening genes, such as telomerase, TERT, ALT, and more. These conflicting conditions mean that any upregulation, or downregulation of telomerase requires close examination to evaluate the consequences of either promoting, or inhibiting telomerase (Hong & Yun, 2019).

As discussed above, telomeres and telomerase hold a significant role in the development of tumors, cellular aging, and senescence. Such discoveries suggest that further investigation of telomerase and its cellular mechanisms may prove vital in future research regarding disorders that result in untimely telomere attrition, gene expression, as well as the use of telomerase as a therapeutic target, including the inhibition of telomerase. Furthermore, it will be interesting to observe how such conflicting requirements, mentioned beforehand, will be reconciled in the future as research continues.

Role of Telomeres and Telomerase in Cancer

As discussed above, telomeres are protective caps at the ends of chromosomes. Telomerase synthesizes new telomere DNA to replenish the telomeres, as they are shortened after each round of cell division (McNally et al., 2019). There are two mechanisms for telomere maintenance:activation of *TERT* , which encodes telomerase; or an alternative lengthening of telomeres (ALT) mechanism. ALT is independent of telomerase, and employs the DNA homologous recombination pathway. The maintenance of telomere length is crucial in normal cells, and the extensive attrition of telomeres, along with other oncogenic factors, can lead to genomic instability, potentially acting as a cause of cancer (Yuan et al., 2019). Telomere shortening is key for tumorigenesis, and

approximately 85-95% of all cancers exhibit telomerase expression, while the remainder show ALT pathway activation (Okamoto & Seimiya, 2019).

Shortened Telomeres in Cancer

In human somatic cells, telomerase activity is usually extremely low or completely inactive. In addition, telomeres of normal somatic cells are generally found to be of longer length than those of cancer cells. This ensures a minimum risk for telomere shortening and malignant transformation in normal cells. Furthermore, cancer cells have shorter telomeres as they have gone through more rounds of the cell cycle than healthy cells (Jafri et al., 2016). As mentioned before, approximately 85-95% of all cancers upregulate TERT, while the remaining 5-15% activate the ALT pathway. After reaching the Hayflick Limit, cells with shortened telomeres go through DNA damage response, and replicative senescence is provoked by the activation of the p53 gene, induction of the RB tumor suppressor pathway, and/or the development of chromosomal instability. Some cells can overcome senescence as a result of genetic mutations in p53 or other checkpoint genes (Okamoto & Seimiya, 2019). Conversely, if p53 or RB were not to be activated due to mutations or alterations in their upstream regulators, this would allow for the extended proliferation of cells with shortened telomeres, and these cells would soon enter crisis.(Roake & Artandi, 2020) Other cells overcome replicative senescence and the crisis stage by upregulating the expression of *TERT*, and therefore telomerase activity. Because *TERT* is upregulated in cancer cells, *TERT* level could potentially be a biomarker for cancer diagnosis (Guterres & Villanueva, 2020).

Genomic Instability and Apoptosis in Cancer

Two important mechanisms, replicative senescence and crisis, act as barriers against cell immortalization, and therefore, cancer. Mortality stage 1 (M1), also known as the period of cellular senescence, inhibits the cell from further progressing through the cell cycle. Cells usually enter M1 due to the uncapping of one telomere, or detection of a few shortened telomeres. In M1, the cell triggers DNA damage signaling and cellular senescence. However, oncogenic mutations can allow the cell to bypass M1, and continue to proliferate. If the cell bypasses M1, it enters crisis (M2), and the shortened telomeres become even shorter. Most cells that enter M2 will undergo chromosome end-to-end fusions, which then leads to chromosome bridge-breakage-fusion cycles, genomic instability, and eventually apoptosis. However, 1 in 100,000 to 1 in 10 million cells can overcome M2, and gain immortality and progress to become a malignant cell. At this point, either telomerase or ALT must be activated to ensure that the telomeres can be replenished, which is necessary for cell immortality (Jafri et al., 2016; Leão et al., 2018).

Genomic instability describes the increased tendency towards genomic alterations during cell division. Most often, genomic instability occurs due to defects in mechanisms that maintain genomic integrity, including DNA damage checkpoints, mitotic checkpoints, and DNA repair machinery. Genomic instability can make a cell more vulnerable to malignant transformation. The loss of genes that maintain genomic integrity is thought to be the cause for genomic instability, as well as cancer formation. The loss of function of DNA repair genes will lead to increased base pair mutations. Furthermore, chromosomal instability, which is the predominant form of genomic instability, occurs due to chromosome missegregation. This means that during mitosis, the cells complete cytokinesis with an incorrect chromosome number or mutated chromosomal structure. Chromosomal instability is also a hallmark of cancer. Telomeres protect against genomic instability, which is a characteristic of most cancer cells (Guterres & Villanueva, 2020).

Telomerase suppression and short telomeres are both thought to be natural anti-cancer strategies. They act as a barrier against malignant transformation, and prevent rampant cell division. Hence, telomerase is inactive in most cells– in fact, it is only active in cells that require strong proliferative potential, such as germline, hematopoietic, and stem cells (Trybek et al., 2020). However, telomerase or ALT is active in cancer cells, allowing telomere lengthening, further proliferation, and limitless replicative potential, another hallmark of cancer. Through the activation of either telomerase or ALT, the telomeres do not become critically shortened, and therefore, cellular senescence is not induced (Senga & Grose, 2021).

Mechanism of Telomerase (hTERT)

Telomerase is a DNA polymerase made up of two subunits. hTERT, encoded by the *TERT* gene, is a functional catalytic protein subunit. The other subunit is an RNA component called human telomerase RNA component (hTERC). Using TERC as a template, hTERT synthesizes telomerase sequences. Because telomerase is inactive in most human somatic cells, the presence of hTERT acts as a biomarker for cancer. Both *TERT* and *TERC* are expressed at relatively low levels in comparison to other proteins and RNAs in normal somatic cells. Furthermore, telomerase activation is very strictly regulated. In most somatic cells, after initial embryonic development, *TERT*-promoter is suppressed, most often through hypomethylation (McNally et al., 2019). *TERT* expression is upregulated in cancerous cells through various mechanisms, including *TERT* amplifications, structural variants, promoter mutations, and epigenetic modifications by *TERT* promoter methylation (Leão et al., 2018).

TERT is tightly regulated in all cells, but it is disrupted in cancerous cells, most often due to the overexpression of positive regulators, or the silencing of negative regulators. Both increased *TERT* promoter activity and *TERT* expression are both hallmarks of cancer, as they are expressed significantly more in cancer cells with lengthened telomeres, compared to normal cells with shortened telomeres. Various positive or negative factors, and signaling pathways, are responsible for the regulation of the transcription of the hTERT gene. More specifically, the c-MYC, NF-κB, B-Catenin pathways are involved in the transcriptional activation of *TERT*. After translation, the PI3K/AKT pathway amplifies signals which enhance *TERT* activity. One notable example of an oncogenic factor driving *TERT* expression isMyc, a group of proto-oncogenes that encode transcription factors (Trybek et al., 2020; Yuan et al., 2019). Myc is known to activate telomerase by binding to E-boxes (5 -CACGTG-3) on the *TERT* promoter, thereby activating *TERT* transcription (Trybek et al., 2020). Myc is one of the most commonly amplified genes in human cancer, and drives oncogenesis by altering cell proliferation, metabolism, and promoting immune evasion. Other transcription factors, such as Sp1, also regulate the *TERT* promoter by binding to the core transcriptional region of the promoter (Kyo et al., 2008). *TERT* amplification results from a copy number increase, in relation to the overexpression of the amplified gene. The cause of amplification can be attributed to errors in DNA replication, telomere dysfunction, or chromosomal fragile sites. *TERT* gene amplification can occur as a result of telomere dysfunction, chromosomal fusions, and breakage at fragile sites (Leão et al., 2018). As such, *TERT* is amplified during malignant transformation, and therefore, it contributes to the abnormal telomerase activity that is present in tumors (Zhang et al., 2000).

Structural variants can also lead to the activation of telomerase. Genomic rearrangements affecting the *TERT* gene locus can result in the upregulation of *TERT*, and therefore, of telomerase. Such structural variants introduce the *TERT* gene to active enhancers, allowing the enhancers to encourage hTERT expression (Peifer et al., 2015; Valentijn et al., 2015).

Furthermore, mutations in *TERT* promoter also play a key role in the activation of telomerase, and malignant transformation (Jafri et al., 2016). Many cancers increase *TERT* transcription through non-coding mutations in the *TERT* promoter. Two commonly occuring mutations, both of which are located in the proximal promoter upstream of the transcription start site, are C>T transitions. The existence of these mutations in the promoter region allowed for extra binding sites for E-twenty-six (ETS) transcription factors, acting as a poten-

tial cause of genomic alteration and cancer activation. Such mutations in *TERT* promoter activates TERT transcription. This leads to elevated levels of mRNA, increased telomerase activity, and therefore lengthened telomeres. *TERT* promoter mutations are the leading cause of telomerase activation in several types of tumors, including but not limited to: melanoma, glioblastoma, bladder cancer, and hepatocellular carcinoma (Roake & Artandi, 2020).

Epigenetic modifications by *TERT* promoter methylation is also responsible for the increased expression of hTERT. DNA methylation is an epigenetic mechanism that plays an important role in gene expression. DNA methylation occurs mostly in non-coding regions of DNA (Dratwa et al., 2020). In normal cells, the region upstream of the *TERT* promoter core sequence is demethylated, but in cancer cells, it is methylated. Evidence indicates that this demethylated region is responsible for binding to the *TERT* repressor (Trybek et al., 2020). *TERT* transcription is activated by DNA methylation, or histone acetylation at the transcription site of the *TERT* promoter. DNA demethylation and histone methylation invoke histone acetyltransferase (HAT) activity, allowing *TERT* to be transcribed, and telomerase to be activated. The methylation of*TERT* prevents the binding of transcriptional repressors to the promoter, which would block transcriptional machinery from activating telomerase expression. Therefore, the promoter would be able to bind with the transcriptional factors and be activated (Kyo et al., 2008; Leão et al., 2018).

Mechanism of ALT (Alternative Lengthening of Telomeres)

Another maintenance mechanism to fight against telomere attrition is the ALT pathway. With ALT, cancer cells are able to extend their telomeres without the presence of telomerase. ALT is based on homologous recombination through the use of telomeric DNA as a template. It relies on DDR proteins, such as those that are related to the homology-dependent repair (HDR) pathway (Danny MacKenzie, 2021). Activation of this pathway is related to the presence of mutations in genes that code for α-thalassemia/mental retardation X-linked (ATRX) or death-domain associated protein (DAXX) (Trybek et al., 2020). Tumors in which ALT is active often contain somatic mutations in genes coding for ATRX and DAXX. Another marker for ALT is heterogenous telomere length, high levels of telomere-sister chromatid exchanges, extrachromosomal telomeric repeats, and a specialized telomeric nuclear structure called ALT-associated PML (De Vitis et al., 2018). In ALT, the genes coding for ATRX and DAXX are mutated, and are no longer able to remodel the chromatin. Hence, ATRX and DAXX are thought to be suppressors of ALT. In the activation of ALT, there is a strand invasion by the telomere that is being lengthened into the telomere that will be copied from. This donor telomere can either be a sister telomere, another telomere, or extrachromosomal telomeric DNA (Varley et al., 2002). ALT is most commonly found in sarcomas, or cancers of the central and peripheral nervous systems. On the other hand, the activation of the ALT pathway is relatively scarce in more common cancers, such as breast, colon, and lung cancer (Sommer & Royle, 2020).

Identifying activation of the ALT pathway depends on the presence of several of ALT s defining characteristics. These characteristics include: a lack of telomerase activity; telomeres longer than 50kb; telomeres less than 8kb or greater than 50kb of heterogenous length; heightened amounts of sister chromatid exchange; extrachromosomal telomeric repeats; acute promyelocytic leukemia bodies specifically associated with ALT; and foci caused by telomere dysfunction. In general, ALT cannot be identified with one biomarker, so multiple of these characteristics must be observed for a tumor to be deemed as ALT-positive (Danny MacKenzie, 2021).

Telomeres in Different Types of Cancer

hTERT in Glioblastoma

A glioma is a type of tumor that is found in the brain and spinal cord. Glioblastoma multiforme (GBM), or glioblastoma, is the most aggressive type, and is classified as a grade IV glioma. Despite recent findings and advances, it remains incurable. Although it is very rare, affecting fewer than 10 per 100,000 people worldwide, it has a staggeringly low average lifespan of 14-15 months after diagnosis (Hanif et al., 2017). *TERT* promoter mutations are present in approximately 60-75% of glioblastoma (Vuong et al., 2020). *TERT* promoter mutations were the predominant mechanism that allowed for telomerase to be activated and for telomeric length to be maintained, eventually leading to malignant transformation. In glioblastoma, *TERT* promoter (TERTp) mutations generate a 11-base pair sequence that creates a binding site for the GA-binding protein (GABP) that upregulates telomerase activity. GABP does so by forming two protein complexes that bind to the site and activate the mutant TERTp (Killela et al., 2013).

These mutations were also discovered to be clonal events. Clonal alterations are observed in all cells within a specified tumor, and are thought to present themselves early in the process of cancer onset (Aquilanti et al., 2021). In gliomas, mutations in the IDH1 or IDH2 genes are found to occur early during development, and are usually correlated with better chances of survival. It was discovered that mutations in the promoter region of TERT are inversely related to mutations in IDH1, a factor for a more positive prognosis. A study conducted in 2017 indicated that those with mutations in TERTp had a worse overall survival than those with wild type TERTp. Hence, the presence of TERTp may be of prognostic value in glioblastomas, and may also possess therapeutic potential (Lee et al., 2017).

ALT in Osteosarcomas

Meanwhile, the ALT mechanism is commonly observed in sarcomas, a type of cancer of the soft tissue, such as bone or muscle. Generally speaking, ALT is active in tumors of mesenchymal origin. The ALT pathway is present in 62% of bone, 32% of soft tissue, 40% of neuroendocrine, 23% of peripheral nervous system, and 15% of central nervous system cancers. ALT is frequently active in cancers of mesenchymal origin, as cells are forced to choose ALT to lengthen their telomeres, since telomerase is extremely strictly regulated in mesenchymal cells. In most cases, the ALT pathway is usually correlated with a poor prognosis (Dilley & Greenberg, 2015). In a study conducted in 2019, patients who were ALT-positive displayed a significantly higher risk of death than those who were ALT-negative. The ALT-positive group also had both a higher mitotic count and tumor grade (Lawlor et al., 2019).

Osteosarcoma (OS) is a type of bone cancer that arises from cells of mesenchymal origin in the bone. It is a relatively rare cancer most often found in children, with approximately 3.4 cases per million each year worldwide. Studies indicate a strong association between osteosarcoma and ALT. Mutations in ATRX and DAXX genes induce the ALT pathway by depositing histone variants onto the heterochromatin, and eventually increasing chromatin accessibility. Studies showed that if ATRX is lost, ALT is present in osteosarcoma nearly 100% of the time, confirming the strong association between ATRX, ALT, and osteosarcoma. Such a strong association between ATRX/DAXX loss and ALT suggests therapeutic potential, for instance through re-expression of these two proteins to halt ALT activity (Danny MacKenzie, 2021). In 2015, Clynes et. al demonstrated that expressing ATRX in certain OS cells reversed the osteosarcoma phenotype, proving the clinical possibilities that ALT-targeting treatment holds.(Clynes et al., 2015) Furthermore, in 2019, it was proven by Yost et. al that exogenously expressing wild-type DAXX also restored proper DAXX function, and suppressed the ALT phenotypes (Yost et al., 2019). Although more clinical trials have yet to be conducted, evoking tumor differentiation in ALT-positive osteosarcoma that are negative in ATRX and DAXX holds interesting and promising potential (Danny MacKenzie, 2021).

Targeting Telomeres in Cancer Therapy

Telomeres are attractive targets in cancer therapy. Specifically, since telomerase is activated in approximately 85-95% of cancers, telomerase and hTERT have been subjects of interest in the field of oncology.

Imetelstat Sodium (GRN163L)

Oligonucleotides have been demonstrated to have promising telomerase-inhibiting properties forcancer therapy. Oligonucleotides are short DNA or RNA molecules. GRN163L, or imetelstat, is a 13-mer oligonucleotide sequence that is a competitive inhibitor of telomerase. It binds to the TERC template region, where the telomeric DNA binds, and therefore blocks telomerase activity. With no site to bind, the telomeric DNA is unable to activate telomerase (Trybek et al., 2020). Currently, it is the only single direct telomere inhibitor that has progressed to clinical trials (Guterres & Villanueva, 2020). Imetelstat is able to penetrate tissue and cells very efficiently even at lower concentrations, and distributes well into both normal and cancer cells. It has also been shown to prevent cell proliferation, and cause apoptosis in cancer stem cells (Wang et al., 2018). In addition, because it is an oligonucleotide, and is a large polyanionic compound, there is a lower possibility that it will become a substrate for factors such as multidrug resistance, which is important when targeting cancer stem cells. Imetelstat is the only oligonucleotide drug for any disease that is currently being tested in clinical trials (Röth et al., 2010).

Telomeres are usually shorter in tumors than in other non-malignant tissue. This is because tumor cells have generally gone through more processes of cell division, which shortened their telomeres and allowed them to acquire the mutations required for malignant transformation. The length of telomeres has been found to affect the efficiency of telomerase-targeted treatment. Telomerase-targeted treatment usually inhibits telomerase, which then leads to telomere attrition over time. The initial length of the telomere is of great significance when using telomerase inhibitors as treatment, as the length of the shortest telomere within a cell is what determines the start of telomere dysfunction (Guterres & Villanueva, 2020). Hence, this is a challenge that accompanies the potential use of telomerase inhibitors in a clinical setting. Furthermore, telomerase inhibitors may not be immediately effective. Even after telomerase is inhibited, several cycles of cell division are needed before the telomeres are shortened enough that they will go through apoptosis (Röth et al., 2010).

Imetelstat has been studied in myelofibrosis, glioblastoma, bladder, breast, liver, prostate, pancreatic cancer, and more. Myelofibrosis (MF) is a type of rare bone marrow cancer that originates in the hematopoietic stem cell. When imetelstat was used in a clinical trial as treatment for MF, it was found that the use of MF induced reversal of bone marrow fibrosis, and led to morphologic and molecular remissions in some patients. This discovery suggests the ability of imetelstat to selectively target only malignant cells, and sparethe other cells from apoptosis as well (Wang et al., 2018). Similarly, when imetelstat was used in pancreatic cancer, it was found that continuous exposure to imetelstat led to initial rapid telomere shortening, and continued exposure led to crisis and a loss of cell viability. When the drug was removed after long-term use, telomerase was reactivated, and the telomeres were yet again lengthened. Hence, it was concluded that imetelstat could be used to inhibit telomerase activity, and prevent lengthening of telomeres. Such discoveries, as mentioned above, provide promising outlooks on the use of imetelstat in treating cancer (Burchett et al., 2014).

However, the use of imetelstat as a singular agent may be dangerous. Despite its effectiveness, it is hematologically toxic. Moreover, imetelstat also has damaging effects on mesenchymal stem cells. Hence, the use of imetelstat alone is not recommended due to its toxicity However, imetelstat does have potential when used in conjunction with other forms of cancer therapy, or molecularly targeted drugs (Trybek et al., 2020). It was also found that inhibiting telomerase can make cancer cells more sensitive to other methods of treatment, such as radiation and chemotherapy. Therefore, telomerase inhibitors may be more effective when implemented together with other therapies, as mentioned above. Combination therapies are especially powerful, as they can accelerate telomere attrition, or even uncap telomeres (Röth et al., 2010).

Immunotherapies

Immunotherapy can include vaccines, adoptive cell transfer, and oncolytic virotherapy. Currently, several TERT peptide vaccines have been approved to undergo early stage clinical trials (Guterres & Villanueva, 2020). In immunotherapy, tumor-specific immune responses are induced by identifying the correct tumor-associated antigens (TAAs). In cancer stem cells, hTERT is a TAA and is overexpressed, making it a suitable target for cancer immunotherapy. In cancer, major histocompatibility complex (MHC) class I and class II proteins become complexes with short and long peptides from hTERT. MHC molecules play a role in the immune system by coding for proteins on the cell surface that identify foreign substances. These complexes that form as a result of the peptides and MHC proteins then elicit CTL (cytotoxic T lymphocyte) responses, as they are expressed on the surface. CTL responses kill cells that contain a certain antigen but leave other cells unaffected– in this case, they kill cancer cells. hTERT is immunogenic, meaning that it can evoke an immune response. Therefore, it is a universal TAA that can act as a target in cancer immunotherapy (Mizukoshi & Kaneko, 2019).

The majority of vaccines that have been developed using peptides derived from hTERT are highly specific, and their amino acid sequences contain both MHC I and MHCII epitopes (Vonderheide, 2002, 2008). GV1001 is a MHC class II restricted peptide vaccine derived from hTERT, and arguably the most advanced form of cancer immunotherapy so far. It elicits T-cell responses and activates CTL (Brunsvig et al., 2020). GV1001 acts directly on the target cells by penetrating through the cell membrane, which is what makes it so effective as an antitumor therapy. It was shown to induce both CTL and $CD4^+$ T-cell responses, both of which are important in successful immunity to cancer. GV1001 is particularly effective when it is used in conjunction with other peptide vaccines. A phase I/II trial that combined GV1001 with HLA-A2-restricted CTL epitope elicited an immune response in 86% of the patients. Another study involving patients with pancreatic ductal carcinoma showed that combining GV1001 with gemcitabine induced apoptosis, as well as loss of fibrous tissue in cancer cells.

The use of hTERT as a clinical target holds immense potential advantages. First, since hTERT is found in approximately 85% of all cancers, using antigen-specific immunotherapy through hTERT would allow treatment to be extended toward a greater number of patients with common cancers. More than 75% of patients with cancer would become candidates for hTERT-specific immunotherapies. Second, since hTERT plays a key role in the immortalization of cancer, targeting hTERT could potentially evade the possibility of immune escape. Most importantly, the discovery of universal tumor-associated antigens such as hTERT creates the possibility for preventative immunotherapy. Because it does not require human leukocyte antigen (HLA) typing before administration, it is well suited to be a universal cancer vaccine (Mizukoshi & Kaneko, 2019). If further studies were to be conducted on hTERT in immunotherapy, there exists the possibility that people could be vaccinated as an immune prevention strategy for cancer prevention. This may be significant for people who are considered to be at high risk for cancers, based on their familial medical history (Vonderheide, 2002).

G-quadruplex Stabilizers

G-quadruplexes (G4), an alternative structure of DNA, can be stabilized to block the binding of human telomerase RNA (hTR), which then inhibits telomerase action. Consequently, the telomeres are not replenished, and this triggers DDR, and eventually cell death (W. Liu et al., 2018; Tauchi et al., 2006). The G-rich sequences of telomeres can turn into G4 in vivo. G4 is present in most human cancer promoters and at telomeres, making it an interesting mechanism for downregulating hTERT transcription and preventing telomere lengthening in malignant cells. There are three types of G4-interacting proteins relevant– G4 binding, G4 stabilizing, and G4 unwinding. Mutations in these proteins can induce alterations in the conformational structure of G4, thereby leading to changes in transcriptional efficiency, genomic instability, changes in telomerase activity, slowed

DNA replication, and more. These changes in G4 can be caused either by chemical ligands, or by proteins that regulate the formation of G4.

Because ligands can trigger changes in G4, ligands that are capable of modulating or stabilizing G4 structure were developed with the hope that G4 can be utilized as treatment for cancer by preventing cell replication or oncogene expression. The antitumor effects of G4 ligands are reliant upon alterations in telomere maintenance, changes in oncogene expression, and genomic instability (Kosiol et al., 2021). It has been hypothesized that G4 formation at telomeres blocks telomerase in cancer cells, thereby preventing rampant DNA replication. Somatic cells would remain unaffected by G4, as they do not express telomerase (Tan & Lan, 2020). Most G4 ligands target tumor cells through telomere maintenance factors, and reduce cell growth. For instance, treatment of cancer using the ligand Telomestatin led to telomerase inhibition through stabilization of G4, whereas $RHPS_4$ caused telomere dysfunction by interrupting shelterin. $RHPS_4$ too is a G4 ligand, and is highly potent. It targets the replication forks of telomeres, causing dysfunctionalization. Unprotected telomeres are recognized by the cell as DNA double-strand breaks, which then activates DDR, leading to cell death (Berardinelli et al., 2018). A novel idea is to use G4 as a photosensitizer, a form of treatment that targets specific tumors to induce cytotoxicity (Dratwa et al., 2020; Kosiol et al., 2021).

Another G4 ligand is BRACO-19, an acridine derivative that targets telomeres. It causes cell cycle arrest and DNA damage, as well as apoptosis and senescence. It has been mainly studied in relation to glioblastoma. When used in human glioblastoma cells, BRAC0-19 uncapped the telomeres and exposed the chromosomal ends to DNA damage, thereby inducing DDR. Breakdown of the T-loop structure followed due to displacement of TRF2 and POT1. This resulted in cell cycle arrest, apoptosis, and senescence, as mentioned before, caused by tumor suppressor genes p53 and p21.

G4 also plays a large role in Myc regulation, and attempts have been made to block Myc expression by inducing G4 structures found in the Myc gene promoter. Using certain G4 ligands resulted in decreased tumor growth, which was found to be related to reduced expression of Myc, as well as other oncogenes. Some G4 ligands, such as CX-5461 and CX-3543 have moved to clinical trials (Carvalho et al., 2020). Such ligands have demonstrated positive results as novel therapeutic drugs, but most G4 ligands target more than one G4, and therefore, they affect more than just the target site in the genome. For more G4-targeting treatment to be approved for use in clinical trials, they must be more selective and target sites with high tumor-promoting activity only. Furthermore, G4 structures can also induce genomic instability. They can cause mutations in DNA replication, causing DNA damage. G4 ligands are also responsible for DNA double strand breaks, which is associated with issues in telomere maintenance. This has raised the possibility that G4 formation may be responsible for genetic alterations, such as telomere addition or point mutations. If this were to be true, the use of G4 ligands for anticancer treatment would cause genomic instability, which can induce apoptosis (Kosiol et al., 2021). Furthermore, one of the weaknesses of many forms of cancer treatment related to telomeres is that the length of the telomeres usually affects the effectiveness of the treatment. However, a study by Biroccio and colleagues demonstrated that treatment using G4 ligands were not correlated to telomere length, suggesting a potential advantage of G4 ligands as anticancer treatment (Salvati et al., 2015).

Small Molecule Inhibitors

Small molecule drugs are capable of entering a cell easily, due to ther low molecular weight. Therefore, they can interact relatively easily with the cell surface receptors, and signaling molecules within the cell. They usually disturb the enzymes that regulate cancer cell development, thereby preventing cancer progression. Most small molecule inhibitors target proteins that are crucial in signal transduction pathways, such as kinases, or in stress response pathways, such as heat shock proteins (HSPs). This is because the initiation and progression of cancer is often reliant on signaling pathways and successful responses to stress (Jeswani & Paul, 2017; V. Lavanya et al., 2014).

BIBR1532 is an inhibitor of telomerase, and blocks telomeric activity by binding to the active site of hTERT, thereby initiating senescence. It binds to the conserved hydrophobic pocket of TERT, thereby interfering with the activation domain of TERC (Guterres & Villanueva, 2020). The binding of BIBR1532 acts as a chain terminator as the nucleotides are polymerized, which effectively prevents telomerase catalytic activity (Pascolo et al., 2002). Treatment with BIBR1532 has been shown to be effective in suppressing telomerase activity, eventually causing apoptosis. It also decreases TERT transcription by downregulating the expression of cMyc, its transcriptional activator (Altamura et al., 2021). Multiple studies have shown that BIBR1532 is capable of reducing growth in cancer cells such as brain, breast, leukemia, lung, and germ cell tumors. LN18, a human glioblastoma cell line, was shown to have inhibited telomerase activity when treated with BIBR1532. Overall, it reduce the number of TTAGGG repeats that are added (C. Lavanya et al., 2018).

Exposure of cancer cells to BIBR1532 led to a significant decrease in telomerase activity in glioblastoma cells. Furthermore, it modifies hTERT, which then downregulates telomerase, thereby inducing apoptosis. By limiting the amount of nucleotide repeats that telomerase can use to lengthen the telomeres, BIBR1532 blocks telomerase activity. Studies also indicate that BIBR1532 may work well in conjunction with other models of cancer treatment, such as chemotherapy and radiation. Its best prospects are in its use with other treatments, which will most likely lead to a more positive prognosis (Altamura et al., 2021).

Gene Therapy

The field of telomerase gene therapy includes two main approaches– suicide gene therapy and oncolytic viral therapy. Most gene therapy for cancer focuses on either suicide genes delivered by adenovirus vectors or genes that can directly cause cell death. Adenovirus vectors are especially suitable due to the effective gene transfer property (Chiba et al., n.d.). Oncolytic viral therapy lyses the tumor but not normal cells, through the use of adenovirus vectors. These vectors replicate in cancer cells only, effectively killing them (Shanmugaraj et al., 2020). Adenovirus vectors are highly reliant on the expression of nitroreductase, a bacterial protein that controls TERC and *TERT* promoter (Xu & Goldkorn, 2016).

Adenovirus vectors are being studied as a potential area of interest in the field of oncology as they are also capable of enhancing the activity of the immune system. Furthermore, they can be genetically modified to increase their efficacy, and solve any physical or immunological challenges. Particularly serotypes 2 and 5 are used most often, due to their large packaging capability, smaller risk of illness after infection, and low level of integration into the host genome (Gonzalez-Pastor et al., 2021). In order to make adenoviruses specific to only cancer cells, scientists have genetically modified them to gain transcriptional control of E1A or E1B. E1A and E1B play important roles in adenoviral replication, as adenoviruses cannot replicate in human cells without these two proteins. By deleting 24 base pairs from the E1A protein, it becomes unable to bind to the Rb protein, which consequently prevents adenovirus DNA from replicating in the nucleus of normal cells (Kanerva et al., 2013; Lang et al., 2018; Payne, 2017, p. 3).

Most adenovirus vectors are genetically modified versions of adenovirus type 5 (Wold & Toth, 2013). The adenoviruses attach to the receptors on the surface of the tumor cell. Then, the virus is internalized into the cell by the virus penton proteins. Consequently, the virus DNA enters the nucleus of the tumor cell, which disrupts the cell and infects newly forming cells. The immune system eventually puts a stop to this process. This is the mechanism of cell lysis, a highly immunogenic process. Adenoviral infection causes immunogenic cell death by activating the immune defense. It lyses the cell, and releases TAAs and damage-associated molecular pattern molecules and pathogen-associated molecular pattern molecules (Nattress & Halldén, 2018). Considering that cancer appears to be unaffected by normal immune system mechanisms, the ability of adenovirus vectors to trigger an immune response is significant (Hemminki et al., 2020).

Factors that Impact the Effectiveness of Cancer Therapy

Telomere length has a significant impact on the effectiveness of cancer therapy that targets telomeres. In the case of telomerase inhibitors, the initial length of the telomeres influenced the time taken for the inhibitor to demonstrate its effects. This is because the beginning of telomere dysfunction can be determined by the length of the shortest telomere within the cell (Hemann et al., 2001). In a phase II trial involving imetelstat, patients with short telomeres showed heightened survival, suggesting that telomerase inhibitors are most effective when the telomeres are shorter (Salvati et al., 2015). Therefore, when observing responses to telomerase inhibitors, it is recommended to look at the distribution of telomere lengths, rather than the average.(Lai et al., 2017)

Cancer and Aging: Conflicting Interests

As telomeres get shorter after each round of cell division, and DDR occurs at the chromosome ends, cells usually become senescent and sometimes develop age-related diseases. In addition, telomere shortening or uncapping of chromosomes can lead to tissue degeneration, organ failure, inability of tissues to repair properly, stem cell depletion, and more. Therefore, telomeres and telomerase have been investigated as a means to fight age-degenerative disease (Jaskelioff et al., 2011).

Whereas using telomerase to fight aging requires upregulation, using it to fight cancer requires suppression. Therefore, these contradictory provisions for these two therapeutic goals means that any upregulation or downregulation of telomerase, while acting as a cure for one malady, may prove to aggrevate or cause another. This must be taken into consideration for any clinical application using telomeres, as such may be safety hazards.

Short telomere length was found to be associated with cardiovascular risk factors, including smoking, obesity, and hypertension. This is due to oxidative stress, which causes both telomere shortening and DNA damage. Oxidative stress is found often in cardiovascular diseases (Hong & Yun, 2019). A clinical study in 2017 by Rozman et al. indicated that in patients with cardiovascular disease, higher levels of TERT in cell therapeutics improved the effect of the treatment. Therefore, using vectors to transfer *TERT* may hold therapeutic potential as a way to improve the effectiveness of treatment for cardiovascular disease (Rozman et al., 2017). On the contrary, cancer therapy requires telomere shortening, induced by telomerase suppression, thus demonstrating the conflicting requirements of cancer therapy using telomerase and of treatments involving telomere attrition. In addition, since cancer therapy involves the shortening of telomeres, it can also lead to accelerated aging. Chemotherapy was shown to shorten telomeres in hematopoietic stem cells. Radiation has also been proven to lead to telomere shortening, which eventually leads to cellular senescence (Hurria et al., 2016).

It was discovered that telomerase could be introduced to accelerated-aging mice to slow their aging, without increasing the occurrence of cancer. This could be attributed to the fact that AAV9, the vector used in this particular experiment, is non-integrative, meaning that it prevents the expansion of clones that show elevated levels of telomerase. However, the same discovery has not been found true for humans. Still, this suggests that expressing telomerase could potentially be a reasonable solution to reversing tissue dysfunction, or a way to extend one's lifespan without increasing the risk of cancer (Bernardes de Jesus & Blasco, 2013).

Because the mechanism of telomerase operates differently in humans and in mice, this discovery cannot be transferred directly to humans as justification for the use of pro-telomerase therapy in humans (Fujiki et al., 2010; Weise & GüneŞ, 2009). There are also other reasons as to why the same result from mice does not translate to humans. For one, humans have a significantly greater lifespan than do mice. Therefore, humans have a greater chance of developing cancer when administered with pro-telomerase treatment. However, the counterargument that humans are less likely to develop cancer as a result of telomerase activation can also be

made, since humans are more resistant to cancer than are mice (Collins & Mitchell, 2002; Prowse & Greider, 1995).

Studies indicate that the optimal use of telomerase in an anti-aging application would be through transient telomere induction to restore reserves of telomeres and cause healing, while evading the possibility of oncogenesis that entails upregulation of telomerase. In addition, activating telomerase during early onset of disease, before entering the crisis stage, could also stop malignant transformation (Chakravarti et al., 2021).

Conclusion

As discussed in this review, the connection between telomeres and cancer has been well established. Telomeres are protective caps at the end of eukaryotic chromosomes that protect them from degradation, and telomerase is an enzyme that extends telomeres to maintain genomic integrity. Telomeres are most often lengthened by telomerase by hTERT, but there is also a less frequent, alternative pathway that does not involve telomerase (ALT). Activation of telomerase prevents cellular aging, but it can also lead to cancer development by allowing a cell to overcome the limitations of telomeres. In healthy cells, telomeres achieve the healthy balance between limiting cellular lifespan and keeping cancer growth at bay. This balance is somewhat difficult to navigate, but there is no doubt that this field of study holds great potential as treatment for both age-related diseases and cancer.

Telomerase activity is a marker of cancer, which has made telomerase a field of interest for cancer therapy. Treatments utilizing oligonucleotides, immunotherapies, and stabilization of G-quadruplexes have all been investigated as a means of targeting telomerase expression in cancer. Although very few of these novel therapeutic models have moved to clinical trials, the possibilities for these drugs to be used as treatment are immense. Studies on these models have provided great insight into the role of telomeres and telomerase in tumorigenesis, as well as how they can be used as treatment for various diseases. Indeed, much more research must be conducted in order for therapy targeting telomerase to become a viable method of treatment. However, it is evident that telomerase is a viable cancer biomarker and a therapeutic target, and therefore an area of interest in the field of oncology.

Acknowledgments

I would like to thank my mentor Amy Tarangelo, and my AP Biology teacher, Beth Crissy for mentoring me in this review paper as well as for proof-reading this paper.