

Telomeres and Cancer

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ABSTRACT

Telomere protects the chromosomes in normal cells, and their shortening due to cell divisions and oxidative stress induces telomere shortening causing chromosomal instability. Telomerase is an enzyme that adds TTAGG telomeric repeats at chromosomal ends. The activity of telomerase enzyme plays a significant role in initiation and progression of cancer cells. In cancer cells the telomere length is maintained by telomerase enzyme. Cancer cells survive due to the activity of telomerase enzyme due to which the length of telomere is maintained and cell evades cell death mechanisms. In cancer cells telomere shortening or dysfunctional telomeres suppress cancer progression and development due to the activation of cellular senescence pathway. In this review we summarize telomere structure, function and the role telomere plays in cancer development and progression. Hermen J. Muller and Barbara McClintock identified telomere as a structure present at the ends of the chromosomes. The word telomere is derived from the Greek word “telos” which means ends and “meres” means part. Shorter telomere length or the complete absence of telomere induces end to end fusion of the chromosomes and ultimately cause cellular senescence or cell death. James D Watson in 1970s termed end replication problem in which during DNA replication, the DNA dependant polymerase does not replicates completely at the 5' terminal end leaving small regions of the telomere uncopied. In 1960 Leonard Hayflick and his colleagues identified that the human diploid cell can undergo limited number of cell divisions in culture. The maximum number of divisions that a cell can achieve *in-vitro* is known as Hayflick limit which was termed after Leonard Hayflick. When the cells reaches to a limit where they can no longer divide will eventually go under biochemical and morphological changes that eventually leads to cell cycle arrest, a process known as “cellular senescence. The telomerase is an enzyme that functions to add telomere repeats to the ends of the chromosomes and was identified in 1984 by Elizabeth and her colleague. The presence of telomerase enzyme activity was also identified in human cancer cell lines by Gregg in 1989. Another study conducted by Greider and associates showed the absence of telomerase enzyme in normal somatic cell. Shay and Harley in 1990s detected the presence of telomerase activity in 90 out of 101 human tumor cell samples isolated from 12 different tumor types, whereas they have found no activity in normal somatic samples (n=50) isolated from 4 different tissue types. Since then various studies on 2600 human tumor samples have shown the telomerase activity in around 90% of different tumor cells. The existence of telomerase activity in cancer cells clearly demonstrates a major role of this enzyme in cancer pathogenesis. Telomeres plays a critical role in cancer, aging, Progeria (premature aging) and various other age related disorders due to which telomere and telomerase enzyme are recently an active area of research.

Keywords: Telomerase; Telomere; Cancer; Cellular senescence

INTRODUCTION

Chromosome ends are protected from degradation and irregular DNA repair by non-coding structures called telomeres. These are heterochromatin domains involve various tandem DNA TTAGGG repeats which are bound to a large number of specialized proteins [1-12]. These non-coding structures maintain genomic integrity of normal working cells, and successive cell divisions causes their shortening which result in chromosomal instability [13,14]. Telomeres have a major role in determination of cell fate and

ageing on the basis of previous repetitive cell divisions and DNA destruction happened due to cellular response against growth and stress stimulations [15]. The length of telomeres is maintained by telomerase by adding repetitive Guanine-rich sequences [16,17]. It is an enzyme which is unidentifiable in most human somatic cells. In damaged cells the dysfunctional telomere which is a result of excessive telomere attrition or disruption of telomere structure may cause chromosomal instability through end - end fusion of unprotected chromosomes [18-20]. The activity of telomerase is shown in stem cells, gamete and cancer cells [21]. In somatic

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cells of human beings, proliferation capacity is highly limited and senescence results approximately after 50-70 population doublings [22,23]. In normal cells, the telomeric DNA is not duplicated completely by the replication machinery of the cell, which leads to the shortening of telomeres after each cell division [24,25]. As a result, telomeres become too short thereby blocking any further cell proliferation. This phenomenon is known as replicative senescence - a potential protection mechanism against cancer [26-28]. Telomere progressive length shortening reduce cell proliferations but also responsible for tumorigenesis by causing chromosomal instability [29].

STRUCTURE AND FUNCTION

Cellular chromosomes face wide challenges regarding their fate and survival i.e. how chromosomal ends can be protected from DNA degradation and breakdown and how to avoid double-strand breaks processing and recognition. There are multiple solutions for such complications [15]. The main solution to this problem in diverse organisms as mammals, telomeres consisted of G-rich tandem repeats added by a specialized enzyme named as telomerase. It is a reverse transcriptase enzyme made of proteins and RNA subunits [30].

Telomere

Telomeric DNA structures are typically present at 3' ends of a G-rich single stranded overhang, ranging from 50 to 300 nucleotides. This structure is further folded back on duplex telomeric DNA and form a "T-loop" structure also called as telomeric loop structure [15,31]. The number of G-rich repetitions in telomeric structures varies among different species and also varies in different telomeres present in single organism. In all vertebrates especially humans, the sequence is d(TTAGGG) which is bound to a complex of six proteins called as shelterin [32]. Shelterin includes TRF1, TRF2, interacting factors Rap1 and Tin2 and Pot1-TPP1 heterodimers [33-35].

Telomerase

Telomeres replicate by a specialized semi-conservative DNA replication mechanism and telomerase is highly responsible for length maintenance [36]. Telomerase a specialized complex of rib nucleoproteins consisted of protein counterpart (Tert) and a RNA component (Terc). If telomerase is absent, DNA polymerase fails to synthesize ends of DNA lagging strands which leads to progressive shortening of telomeres in each round of cell division [37]. Hence, telomeres are responsible for regulating life span of cells through telomerase suppression and telomeric shortening [38]. The telomere is shortened in cancer cells and telomerase activity is very high.

Function

Telomeres perform numerous functions such as chromosome stability, transcription of other genes nearby, chromosomal nuclear localization, segregation during the anaphase, homologous recombination in meiotic cells and DNA double strand breaks repair [39,40]. Various mechanisms and regulatory pathways are linked to the telomeres representing the importance of telomere homeostatic regulation. Most of the mechanisms discussed so far are part of cancer cells, therefore we can say that telomeres play major role in cancer progression [41,42]. Telomeres perform

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MECHANISMS OF CELLULAR SENESCENCE INDUCTION AND THEIR CONNECTION WITH CANCER BIOLOGY

Cellular senescence describes an irreversible growth arrest characterized by distinct morphology, gene expression pattern, and secretory phenotype [44-46]. It has always been found in literature but not yet proven that induction of senescence prevents the production and growth of cancers. Some new experiments show that this hypothesis is partly true but some of the gene functions occurring in senescence are also playing role in cancer development [47]. Recent researches disclose the issues regarding senescence phenotype and unpredicted possible results for organisms [48]. In cancer therapy used currently, the cellular senescence is expected to occur in tumor cells which show that therapy is going well but at the same time the senescence is also induced unwontedly in normal cells (non-tumor cells) which cause inflammation, secondary tumor and cancer. Cancer is a genetic disease and the risk factor of cancer increases with the growing age, so it is also considered to be an age related disorder [49]. When a normal cell over the period of time accumulates genomic aberrations due to which they acquire the ability of replicative immortality. Telomere shortens with every cell division causing genomic instability which induces genomic rearrangements and mutations that ultimately results in tumorigenesis [49,50]. The survival of cancer cells is heavily dependent on Telomere and associated shelterin protein complex [51-53]. The telomere length is maintained by an enzyme named as telomerase in majority of the cancer cells. The mechanism of telomere length and expression of telomerase enzyme involves epigenetic and posttranslational modifications and deep understanding of these mechanisms will provide targets for early cancer prognosis and also provides novel biomarkers for the development of therapeutics [54,55]. Aging may cause by senescence not only due to tissue accumulation of senescent cells but also due to loss of regenerative capacity of stem cell. Hence, these two processes i.e. functional loss of stem cells and senescent cell accumulation causes aging in result [44,56,57]. These cells may occur for a short time i.e. during embryogenesis or the process of wound healing and in these cases these cells either have positive effects such as tissue homeostasis and regeneration or adverse effects such that they may accumulate in tissues chronically which badly effects the microenvironment by loss of function of specific tissues increased secretion of pro-inflammatory and tissue remodeling factors. These factors then lead towards the pro-carcinogenic microenvironment which promotes the formation of aging-associated cancers along with the occurrence of mutations over time [47,58].

TELOMERE HOMEOSTASIS AND CANCER

In order to understand the role of telomere in early stages of

cancer, we need to understand the mechanisms leading to telomere shortening, telomeric proteins and genomic instability associated with carcinogenesis [59]. Telomeric proteins play role in telomere homeostasis. These proteins include TRF1 (TTAGGG repeat factor 1), TRF2 (TTAGGG repeat factor 2) and Pot1 (protection of telomere protein 1) [60]. These proteins are involved in the direct recognition of the TTAGGG tandem repeats. The other three proteins TIN2 (TRF1 interacting nuclear factor 2, TPPI (TINT1, PIP1, PTP1) and RAP1 (Repressor Activator Protein 1) bind indirectly to telomeres via TRF1, TRF2 and Pot1 [61,62]. All these proteins are called Shelterin proteins. As they form Shelterin complex. Shelterin and other proteins linked to it perform several functions involving telomere homeostasis and stabilizing the telomere complex [60,63]. Many of the telomeric proteins play role in various DNA repair mechanisms such as non-homologous end joining (NHEJ), homologous recombination (HR), base excision repair (BER) and nucleotide excision repair (NER) [64-66]. Most of these DNA repair proteins also interact directly with the Shelterin complex. Although telomere maintenance and DNA damage repair are separate entities, but telomere ends must not be recognized as DNA damage [67-69]. Telomerase is composed of two main subunits, human telomerase RNA component (hTERC) and human telomerase reverse transcriptase (hTERT). The (hTERC) serves as a template for replication whereas the (hTERT) catalyzes telomere elongation as it contains a reverse transcriptase domain. Telomere stabilization is essential for cellular immortality which is achieved through the re expression of (hTERT) gene in most of the human cancer cells while the (hTERC) gene is essentially expressed [70,71]. This information indicates that telomere homeostasis play a major role in Cancer progression. Increased telomerase activity has been analyzed in almost all immortalized cell lines and 80-90% of human tumors [72]. Telomere homeostasis depends on structural telomere conformation as well as telomerase activity [73,74].

TELOMERE LENGTH IN MULTISTEP CARCINOGENESIS

Telomere length deformities are universal in preinvasive stages of Carcinogenesis in human epithelial cells. Certainly, telomere shortening occurs mostly in early stage of bladder, colon, cervix, esophageal and oral cavity cancer [75,76]. This phenomenon is also observed in prostate cancer [77-79]. These observations indicate the role of telomere shortening in pre-invasive as well as invasive cancer. Therefore, we can say that deformity in the telomere length is the earliest and most frequent genetic alteration involved in the malignant transformation. Several (NHEJ) proteins present at telomeres play significant role in the telomere length homeostasis. These proteins are specifically involved in the telomeric structure maintenance, telomerase regulation and play a collective role in chromatin telomere structure by interacting with HP1 in human cells [80-81]. The significant role of these NHEJ proteins and DNA damage proteins present at telomeres integrate a highly regulated nucleoprotein complex [82,84]. This complex stabilizes the telomeres and induces cell cycle arrest [59,85].

ROLE OF TELOMERES AND TELOMERASE IN COLORECTAL CANCER

The third most common cancer is colorectal cancer and it is the major cause of deaths despite of its available treatments [87,88].

CRC arise from a multistep process of genetic and epigenetic events. Along with the heterogeneous characteristics in the molecular and biological aspects of CRC, the chromosomal instability is a trademark of tumorigenesis. These cancer cells restrain apoptosis thus adopt the ability to maintain unlimited proliferation [88]. In human somatic cells, telomeres are shortened at each cell division as a result of end replication problem. At the point when telomere length is reduced underneath a critical value, cellular senescence takes place. If this check and balance is skipped through inactivation of p53, cells may escape from this barrier and continue to divide, resulting in broad telomere attrition. Finally, its dysfunction cause genomic instability and cell death [89-91]. The degradation of telomeres due to the cell proliferation can be enhanced by specific alterations in the genes involved in CRC. Telomerase reverse transcriptase TERT plays catalytic role in telomerase complex, and activation of this TERT promotes the growth of cancer cells by conserving the length of telomeres thus promoting tumor formation/progression. TERT itself increases as the disease progresses [49,92,93]. Several examinations indicate that telomere shortening and telomerase activation play a vital role during cancer progression. Thus, the telomere length has developed as a clinical marker for risk, progression, and prognosis prediction for patients with malignant disorders, particularly with colorectal cancer CRC [94-96].

THERAPEUTICS STRATEGIES BASED ON TELOMERES AND TELOMERASE

The straightforward therapy is direct inhibition of telomerase activity (TA) [97]. This therapy aim is to destabilize the telomeres, telomere shortening and senescence. Another methodology is to utilize the TERT promoter to drive the expression of suicide genes or the replication of infections in cancer cells. Another tumor remedial approach is to straightforwardly target the telomere integrity promoting telomere dysfunction and cancer growth inhibition [98,99].

CONCLUSION

Until now cellular senescence was observed as an *in-vitro* phenomenon and its impact on human aging was very arguable, but now it has been proposed that senescent cells contribute to aging associated diseases and eventually lead to organism's life and health span. Telomere length acts as an intracellular timer, restricting cell replication, this phenomenon is widely accepted nowadays as it is understood that by critical shortening or capping deficiency, telomeres restrict the cell proliferation. This occurs because critically shortened telomeres, which have become dysfunctional, play a key role in oncogenesis. They induce genetic rearrangements that disturb the oncogenes or tumor suppressor pathways. In recent years, the focus on senescence has been increased with respect to cancer research, as senescence is induced by tumor therapies on one hand and induced in other cells thereby enhancing secondary tumors on the other hand. Along with this, the accumulation of senescent cells can explain the increase in incidence of cancer with age. Cellular senescence also provides a model system for protumorigenic microenvironment that can be useful for drug screening. Pharmaceutically targeting the senescent cells will not only prove to be a novel tool in challenging aging-associated pathologies, but also a counterpart to cancer therapy

to eradicate senescent cancer and non-cancer cells and alleviate the side effects. Repetitive domains, as well as two polyglutamine domains, which are intragenic microsatellites at the level of DNA are characteristic of genes encoding gliadins of the β -type.

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