Telomere, telomerase, tumorigenesis and therapy: An overview

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The ends of chromosome in higher eukaryote are termed telomere. The DNAs present at that part of chromosome is called telomeric DNA. Telomeric DNA consists of tandemly repeated DNA sequences. The replication of the ends of chromosomes is not controlled by conventional DNA polymerases rather a special kind of enzyme is involved in this process. It is a ribonucleoprotein and known as telomerase. Cells in senescence stage face telomeric crisis that leads to loss of telomeric ends. Surveillance turns to precancer cells with increased telomerase activity which is a later consequence. Based on these facts a key diagnostic approach has been developed for detection of tumour. A novel therapy for tumour repression has been developed using telomerase inhibitors. However, these inhibitors are very much effective for solid tumour therapy and conceptually will not work on hematological malignancies.

Almost all biological events are complex and orchestrated by many regulators, effectors and activators. While discussing basic cellular events most appropriately come replication of DNA in both prokaryotic and eukaryotic lives. In eukaryotic cells, the cellular DNAs are organized in a specialized structure called chromosome. The replication and maintenance of DNA is regulated by the enzyme called DNA polymerases. Till now seven different types of DNA polymerases have been reported. These are α, β, γ, δ, ε, ζ and η. However, the replication of ends of chromosomes which is termed telomere is regulated by a different kind of ribonucleoprotein termed telomerase. Interestingly, telomeric DNA of mammalian chromosomes is composed of several kilobases of tandemly repeated sequences with a terminal 3' overhang in single stranded form. The sequence-(TAGGGT)n is tandemly repeated over the ends of human chromosomes. This sequence varies in different organisms such as in Saccharomyces cerevisiae it is T(G)2-3 (TG)-6, in Giardia-(TAGGG)n, in Drosophila-(CCTAA)n, in Caenorhabditis elegans-(TTAGGC), in Plasmodium falciparum-(TTAGG), in Cryptosporidium parvum-(TTTAGG) and so on23. However, telomerases have been reported from Euploes aciculatus, Saccharomyces cerevisiae3, Schizosaccharomyces pombe4, Caenorhabditis elegans and also from human67. These proteins show sequence and functional similarities with previously known reverse transcriptases (RTs) which is the major catalytic domain of telomerases. Genome database search has provided the understanding that hTERT codes for human telomerase. Interestingly, expression of the human telomerase catalytic component, hTERT, in normal human somatic cells does not appear to influence changes associated with malignant phenotype9. The hTERT was previously known as hESTE, TCS 1 and TP2. Several genes of Saccharomyces cerevisiae have been identified. These are TLC1 which encodes EST1, EST3 and EST4/CDC 13. These genes code for telomerase subunits. Therefore, telomerase is not simply a reverse transcriptase by mechanistic criteria but has a related protein structure with number of functional subunits. In all telomerases, RNA subunit acts as template for the synthesis of telomeric DNA while protein components catalyze the process to make for the conventional DNA polymerase inability to replicate completely the ends of chromosomes. It is of great interest that the heterogeneous nuclear ribonucleoprotein A1 participates in telomere biogenesis in human. A1/UPS is a single stranded DNA binding protein which is involved in mammalian telomere biosynthesis and suggest possible mechanism by which UP1 may modulate telomere length. A1 consensus RNA binding domain which is the A1 consensus RNA binding domain and the sequence is UAGGGU7. It is worthy to note that insects of the order Diptera which includes Drosophila lack functional telomerase and instead maintain telomeric length with non LTR retrotransposons called TART and HeT-A7. However, Bombyx mori has two non LTR elements known as TARS1 and SART1 2. Evolution of telomerase as an enzyme has been discussed nicely by Nakamura and Cech3 and described that many proteins like SIF2, SIR3, Ku etc play key roles in telomeric
functions\textsuperscript{10,11}. Remarkably, another enzyme called Tankyrase has been identified that plays an important function in the telomeric region of human chromosomes\textsuperscript{12}. Telomere and telomerase are in the lime light because of their pivotal roles in tumorigenesis. It should be in our mind that 6.6 million people die of cancer per year in the world\textsuperscript{13}.

**Cellular senescence and consequences**—The idea that shortening of telomeres may account for the consecutive loss of replication capability of major DNA polymerases at the ends of chromosomes during cellular senescence. At the early stage of ageing of cells telomerase activity is usually enough to maintain the length of telomere thereby adding to chromosomal stability. This situation is usually changed whenever cells enter senescence stage. It is experimentally derived that somatic cell lacking telomerase activity permit shortening of telomeres with every cell division and a particular point reached when the cell faces crisis. This specific state is termed as 'Hayflick limit or mortality stage one'. At this stage most cells die but in some rare cases the telomere length of the cell stabilizes and cell proliferation is restored which leads to immortalization of these cells thereby acts as initiator of tumourigenesis. More clearly the cells which survive and overcome this crisis work as stem cell for tumour generation. The telomere hypothesis of cellular senescence describes M1, M2 and immortalization stage. The M2 phase is the critical stage which has been described previously\textsuperscript{14-16}.

**Tumorigenesis and later consequences**—Telomere length is maintained by the presence of telomerase activity in the vast majority of primary tumours and procancer cells. In sharp contrast there is strikingly high levels of telomerase activity which indicates telomerase is in a highly activated state during the progression to chronic phase of crisis. As cancer cells rapidly proliferate than their normal counterpart that is the reason cancer cells have a reduced telomere length\textsuperscript{17,18}. In terms of cellular consequences cancer cells should repair the telomeric ends of each chromosome. That is the result of increase of telomerase activity in tumourigenic cells. Important lessons come from clonal origin of cancer cells which led to a model how cancer cells progress.

Before metastasis, the cancer cells grow rapidly to huge numbers. The consequence is the nutritional condition depletion. At that particular juncture, cells resistant to poor conditions or cells able to metastatize have an advantages over cells that simply proliferate. At the beginning, cancer cells show accelerated growth without significant telomerase activity. It is needless to write that cells without telomerases are very unstable. This is, however, best illustrated by a hypothetical model termed 'breakage-fusion-bridge cycle' and same kind of understanding that McClintock described in 1941\textsuperscript{19}. It is important to know that a chromosome having one centromere is more stable than a chromosome having two or many centromeres. Importantly, as long as the telomerase biosynthesis is not activated therefore new healing is not possible. Thus cells undergoing the breakage fusion cycle at that stage cells eventually die. However, reduction of chromosome length by deletion of telomere is the meaningful driving force for clonal evolution and this condition has described as telomerase crisis\textsuperscript{20}. It depicts that cancer patients with high levels of telomerase activity show poorer prognosis compared to those with lower levels of telomerase activity\textsuperscript{21}. Another important factor determining the prognosis of the telomerase positive patients is the high level of clonal evolution which would result in a high probability of the cancer becoming resistant to therapeutic drugs\textsuperscript{22,23}. However, telomere crisis model has implicated in rare chromosome anomaly, jumping translocation, jumping of chromosomal parts to the tips of other chromosomes which are common in leukemia\textsuperscript{24}. Ironically, it is common for telomere to be lengthened instead of shorten. In solid tumours, this is due to increase telomerase activity tumourized cell lines where perfect angiogenesis is very common\textsuperscript{25}. It is important to write that telomerase is only needed for stabilization of chromosome structure. However, in solid tumours but not in hematological malignancies various genetic and epigenetic changes particularly point mutations are of very common occurrence in cancer cells.

**Techniques follow to analyze telomere crisis**—The commonly used technique which is practiced in almost all laboratories in this particular arena of cell biology is TRAP assay. This is the same procedure known as 'telomeric repeat amplification protocol'. This assay has been utilized almost exclusively for assay of telomerase activity\textsuperscript{26}. Excepting this procedure, Southern blot hybridization using telomeric probe- d(CCCTAA)n has also been used. Another important technique is pulsed field gradient electrophoresis for separation of chromosomes which
sequencing for hybridization with specific telomeric probe is also another important technique. However, a recently described PCR based method for the determination of telomerase activity is an effective technique. It is called stretch PCR assay which is highly sensitive and quantitative. It is expected that many more sensitive methods will be available to us in near future.

**Therapy, classics in combating and concluding thoughts**—A class of understanding thus evolved to use telomerase inhibitors for treatment of tumours. These inhibitors are now in use for treatment of tumours. These inhibitors are anticipated to form a new class of anticancer drugs. It is, therefore, explained in that way cancer cell will lose its viability after treatment with telomerase inhibitors. It is unrealistic how these inhibitors will work only on cancer cells instead the drug may go to healthy cells and activate new cancer cell in body system. Therefore, possibility should be the use of targeted drug delivery system. This type of treatment can be performed only in solid tumours rather than hematological malignancies. However, many pharmaceutical companies have been actively engaged in designing telomerase inhibitors. This is perhaps a novel way to treat cancer and exclusively useful for the treatment of solid tumours.

Tremendous efforts have been made to encounter cancer. However, a potent and efficient therapy to combat this disease is yet to be introduced even though lot of approaches have been undertaken to treat cancer. Telomeric crisis and consequences of generation of stem cells of cancer in mammals and particularly in human is a clue to understand tumourigenesis. However, cause of cancer is multiple. Therapy using telomerase inhibitors is a new pharmacological approach which needs proper targeting to only deregulated cells. As a matter of fact telomere repair and stabilization of structure of chromosomes is not the only way for cancer remission. Tumorigenesis perhaps can be best understood form the point of oncogenic regulations and their interactions. Currently tumour suppressor genes are now in lime light for cancer therapy. These proteins are p53, p21, p73, p40 etc. Many cellular regulators are now in trials for therapeutic use such as antibody for the treatment of breast, kidney, prostrate, head and neck tumours and antibody which works on HERS growth factor and EGF receptor. Tyrosine kinase inhibitors are used for the treatment of Glioma and various solid tumours. These inhibitor work on PDGF receptor and EGF receptor. For mesyl transferase inhibitor which prevents RAS activity is used for treatment of many tumours. CDK inhibitors have been used as a therapeutic agent which block cell cycle. Bcl 2 antisense which restores apoptosis is now being used for lymphoma and solid tumour treatment. Virus with p53 which restores tumour suppression function is effective for the treatment of lung, head, neck, ovarian, liver and uterus cancers. However, modified adenovirus which selectively kills p53 lacking cells has great impact in cancer therapy. Other cellular regulators such as interferon γ and interferon α etc are now being used as a potent drugs. It is also important to mention that many angiogenic inhibitors are now in therapeutic use.

Hundreds and hundreds of chemically synthesized and naturally isolated drugs are available of which about 50 have been evaluated and approved by U.S. Food and Drug administration (FAD). Worth mentioning are cisplatin, mitoxanthrone, streptozotocin, camptothecin, cytarabine, hydroxyurea, tamoxifen, raloxifene, 13-cis retinoic acid, atocopherol, MF-tricyclin, ricin, epothilone A, epothilone b, taxol, discodermolide, resveratrol etc. Another drug is now available which is called ONYX-015 that work selectively on tumour cells deficient for the p53 tumour suppressor pathway. However, combination therapy is now in practice for many clinical cases. A goal of cancer research is to develop therapies that can selectively kill tumour cells without affecting normal cells that is crucial for both the short term comfort and long term survival of cancer patients. When a cell is under crisis in course of time that cell overcomes the crisis and works as a stem cell for cancer. In that specific stage that cell should be sorted out from the body and a specific drug should be used to restore healthy state. Genes regulating apoptosis and their regulation such as survivin, a protein which is synthesized in rescued cells should be utilized for therapy. Treatment with survivin inhibitor and acceleration of apoptosis in these cells is the class of understanding for novel therapeutic evolution. Other advancement are use of inducible gene disruption technique, ribozymes and many useful antisense molecules are future avenues to explore.
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