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Review Article

Tumorogensis: The Dual Role of Telomerase

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ABSTRACT

Carcinogenesis is a multistep process characterized by the gradual accumulation of genetic changes that ultimately lead to cancer. These genetic mutations can impart limitless replicative potential to the cancer cells making them immortal. Telomeres are repeat nucleotide sequence TTAGGG that are present at the end of chromosomes. Its functions are to protect the chromosomal ends and to ensure that these ends are not recognized as DNA strand break by the polymerase enzyme. They also act as a clock like mechanism to count the number of times a cell divides. These telomeres are maintained in the cell by an enzyme called as telomerase. The function of telomerase enzyme is to protect the cells from telomere erosion and senescence. Thus, the cell can become immortal and replicate forever. This is called as the canonical function of telomerase. Normally telomerase is present in stem cells, germ cells and blood cells only and the somatic cells usually do not express telomerase. However, a very high concentration of telomerase has been identified in various cancer cells. A few years back it was observed that low levels of telomerase are present in the S phase of cell cycle of somatic cells at levels that are not sufficient to maintain telomeres lengths. Additionally it was observed that ectopic expression of telomerase causes stem cell division, mobilization and migration, increased wound repair and an increased tumor burden. Based on these facts it has been deduced that telomerase has at least one non-canonical and elongation-independent function. Both canonical and non-canonical functions of telomerase are considered to play important roles in development and progression of tumorogensis.

Keywords: Tumorogensis, Telomerase, Carcinogenesis, Non-canonical function, Immortalization.

Introduction

Oral Cancer:

Cancer is a perversion of cellular phenotype. A human cancer represents the end of a long process involving the accumulation of multiple changes in phenotype as well as genotype. However, cancer should be considered as a genetic disease as it arises because of pathological changes in the information carried on by DNA.

Tumorigensis:

The cancer forming process is called as tumorigensis. Tumorigensis is a multistep process with each step representing a genetic mutation, the accumulation of which results in a highly malignant phenotype. A very attractive model of tumorigensis is that the tumor cell clone undergoes a series of successive genetic changes, each of which alters the cell genome in a way to confer an advantageous growth phenotype to the cell. By the accumulation of such changes in the

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critical cellular genes, tumor cells are able to grow increasingly well with in the host tissues (1)(Fig.1).

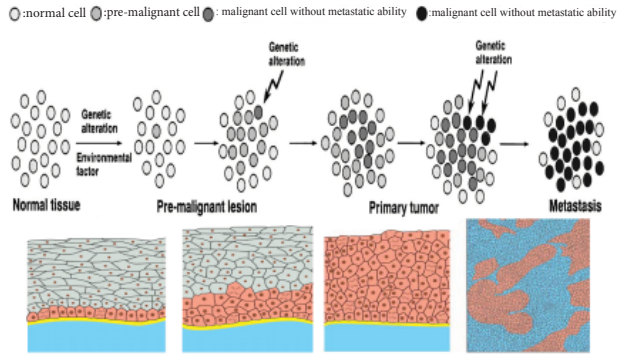

Fig. 1: Multistep tumorogensis. Conventional and Cellular. Adapted from Hittelman w, 2005

Six essential genetic alterations manifesting themselves in cell physiology contribute to the malignant transformation of a cell. These collectively are called as the hallmarks of cancer(1) (Fig. 2). These can be:

Self-sufficiency in growth signals.
Insensitivity to growth inhibitory signals.
Evasion of apoptosis.
Limitless replicative potential.
Sustained angiogenesis.
Tissue invasion and metastasis.

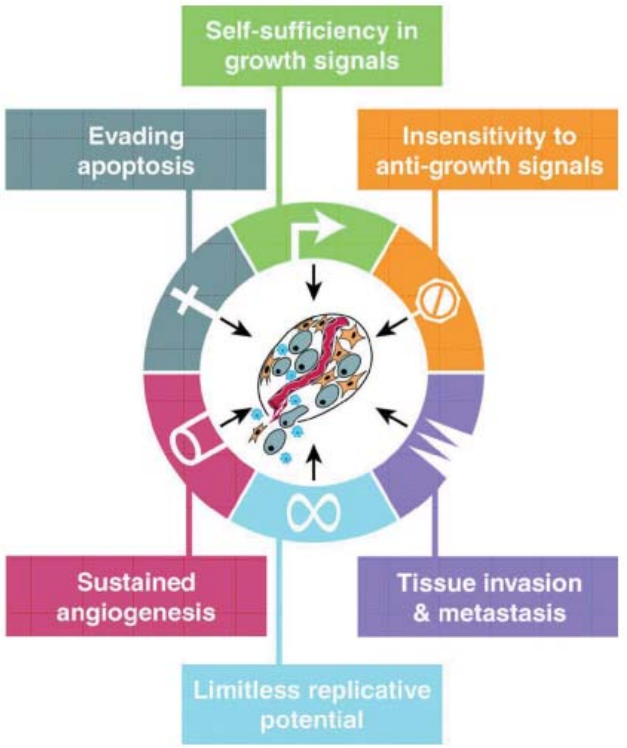

Fig. 2: Six hallmarks of cancer (Adapted from Hanahan D and Weinberg R.A 2000)

Everyone of these genotypic alterations represent a successful contravene of an anti cancer defense mechanism which is an essential part of normal cell physiology. These six hallmarks of cancer are characteristic of most of the cancers, perhaps all. This diversity of resistances may explain the reason of cancer being a relatively infrequent phenomenon during the course of an average human lifetime (1).

It becomes apparent that tumor arises because of successive cycles of mutations, selection and clonal expansion. A decisive step during tumorigensis is the immortalization of the cell (2). Two important genetic changes that significantly modify the immortalization of cells are:

Attainment of unlimited proliferative potential.
Evasion of apoptosis.

Attainment Of Un-Limited Proliferative Potential:

Attainment of unlimited proliferative potential depends on a dominant gain-of-function mutation leading to the activation of an oncogene or a recessive loss-of-function mutation leading to the inactivation of a tumor suppressor gene (3).

Oncogenes:

An oncogene is any gene that encodes a protein able to transform cells in culture or to

induce cancer in animals. Of the many known oncogenes, all but a few are derived from normal cellular genes i.e. proto-oncogenes, whose products promote cell proliferation. Gain of function mutations in oncogenes lead to the aggressively proliferating phenotype of the cancer cell (3). Various oncogenes that can impart un-limited proliferative potential to cells can be named as following:

a. EGFR
b. Cyclin D1
c. Ras (4)

Tumour Supressor Genes:

Tumor suppressor genes generally encode proteins that in one way or the other inhibit cell proliferation. Loss of function mutations in these genes contribute to the development of many cancers3. The main classes of tumor suppressor genes are as following:

a. Intracellular proteins that regulate or inhibit through a specific stage of the cell cycle e.g. p16 and pRb.

b. Receptors or signal transducers for secreted hormones or developmental signals that inhibit cell proliferation e.g. TGF-β.

c. Check point control proteins that arrest the

cell cycle if DNA is damaged or chromosomes are abnormal e.g. p53 which is one of the most commonly mutated gene in all human malignancies.

d.Genes encoding enzymes that are involved in the DNA repair (4).

Telomeres and Cancer:

Telomeres are large nucleoprotein complexes that cap the ends of chromosomes ensuring genomic constancy and cell feasibility and viability (5). The immortalization of cells is the most significant step during tumorigensis (2). Maintenance of telomeres can impart immortal characteristics to a cell. A telomere is an area of repetitive DNA or Nucleotide sequences at the end of linear chromosome that functions as a disposable buffer. The telomeres of humans consist of as many as 15-20 kb of nucleotides TTAGGG sequences (6).

Each time the chromosomes replicate during the S-phase of life cycle the DNA polymerase enzyme is incapable of replicating all the way to the end. The result of this being the fact that the DNA polymerase enzyme can only synthesize DNA in the 5'-3' direction moving along a template (7). As the DNA strands are anti-parallel, and the replication can occur in the 5'-3' direction, the two separated strands of DNA start replicating in opposite directions (Fig.3).

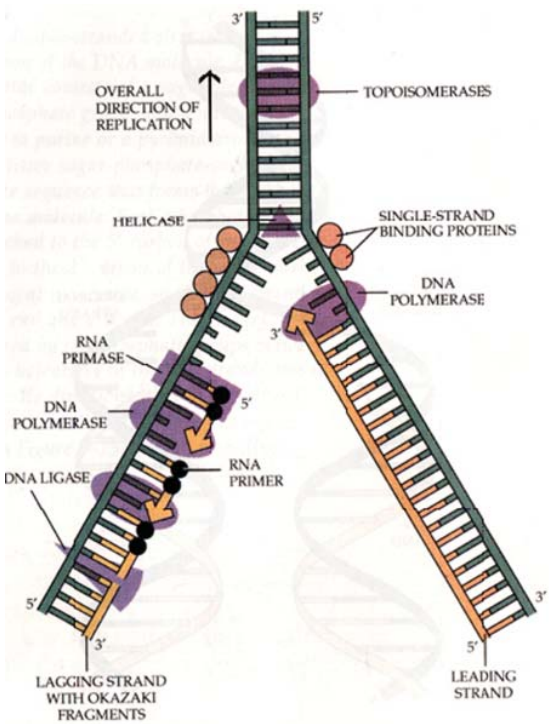

Fig. 3: DNA replication. Leading and Lagging strands.

• One of them i.e. the leading strand replicates continuously from 3' end of the existing strand with the new end of the forming strands facing the replication fork.

• Lagging strand is replicated by a series of Okazaki fragments placed end to end and later ligated by Ligase enzyme(8).

This formation and ligation of Okazaki fragments continues until it reaches close to the end of the chromosomes. Here enough DNA is not present to act as a template. So the 5' end of each newly synthesized DNA strand will not be completed and some genetic information will be lost every time the DNA replicates. That is the reason DNA and chromosomes have telomeres at their ends to prevent this from happening (6).

Functions of Telomeres:

The major functions that telomeres perform are:

1. To allow replication of chromosomal ends.
2. To ensure that the DNA ends at the chromosome termini are not recognized as DNA strand breaks(9).
3. To protect the ends of the chromosomes.
4. To act as an internal clock mechanism to count the number of cell divisions.

It has been suggested that telomeres are influential in the control of proliferative life span of a cell (10). Proof for this comes from the fact that investigational extension of telomere length can extend replicative lifespan in somatic cell hybrids (11). It has also been observed that reconstitution of telomerase enzyme actually lengthens telomeres and blocks senescence in many human fibroblast cell lines (12). The telomere also plays important part in the repair of DNA double strand breaks and telomere dysfunction has been shown to produce radio sensitivity (13).

Telomeres cap at the end of the chromosomes is bound to a complex array of proteins and have a 3' over hang called as the G strand overhang. The current view regarding protective abilities of telomeres is that this higher order chromatin structure physically protects the 3' end from cellular activities. This protective structure could be provided by the ability of 3' overhang to fold back and form the so-called T-loop. Average telomere lengths and telomere binding proteins have been thought to manipulate T loop configuration (9).

Telomerase Enzyme:

The statement that "Telomerase expression is a hallmark of cancer" (14) sufficiently states the imperative nature of the role that telomerase plays in carcinogenesis. Telomerase is a ribo-nucleo protein enzyme that adds the telomere repeat sequence of TTAGGG at the 3' end of DNA sequence by using its snoRNA molecule supplying an AAUCCC template to lead the insertion of TTAGGG. It is composed of two components:

1. A catalytic Reverse Transcriptase protein unit, TERT.
2. An RNA component, TERC5.

Telomerase is transcriptionally silenced in adult cells except for a subset of cells of some tissues with high turn over rates such as:

a.Cells of the germ line including embryonic stem cells.

b. Blood cells.

c. Skin cells.

d. Cells of the Intestines (15).

The fact that adult somatic tissues show very little or no telomerase activity can be a very important tumor suppressor mechanism safeguarding the cells from undergoing tumorigenic changes (5). A model of telomerase is shown in Fig. 4.

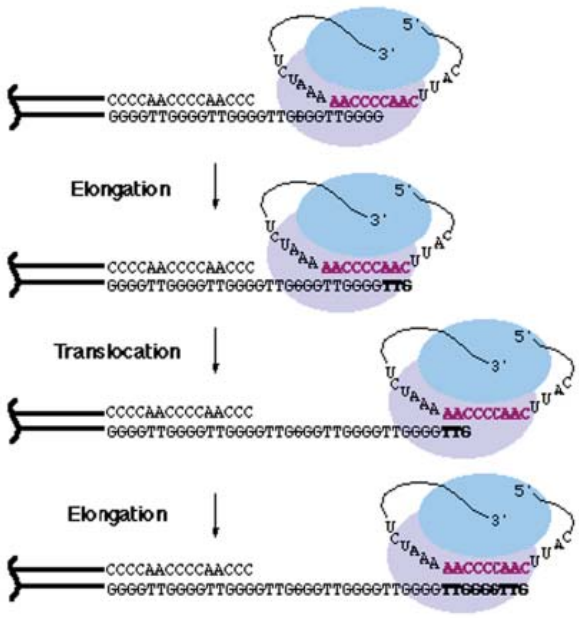

Fig. 4: Telomerase Model. Figure modified & reproduced with permission from Carol Greider

Canonical Function of Telomerase:

The canonical function of telomerase is the most well understood function of this ribonucleoprotein enzyme, which is to act as a reverse transcriptase by adding TTAGGG nucleotide repeats at the ends of chromosomes thus compensating for the gradual reduction of telomeric repeats that occurs after every cell division (16). Thus, the enzymatic activity of telomerase is dependant on hTERT, which is the catalyst reverse transcriptase moiety of the telomerase holoenzyme (17). The functional RNA component of telomerase is utilized as a template for initiating the DNA addition at the telomeres (18).

It has been estimated that the human telomeres loose approximately 30-150 base pairs at the end of each mitotic cell division (10). Taking the average out this represents about 16 TTAGGG repeats, which conclude that after 125 divisions the telomeres will be completely eroded. This is the reason behind the normal somatic cell's limited life span (6). Thus, these telomere extension and stabilization functions of telomerase enzyme impart regulatory functions to it, controlling the cell regulatory potential and life span. These molecular functions of telomerase are essential in:

a. Genesis

b. Growth

c. Degeneration

d. Regeneration of human body on the whole (17) .

Activation of telomerase is related to:

a. Proliferation of embryonic cells during development.

b. Activation of immune cells

c. Expansion of bone marrow progenitor blood cells

d. Immortalization of neoplastic cells (19-22).

It has been suggested that during the course of normal cell development telomerase is inactivated (11) and when this happens, the telomeres start to erode with each subsequent cell division thus acting like an intrinsic clock like mechanism to count the number of times a cell divides (23). When this intrinsic replicative senescent clock becomes exhausted then signals sent to the inner cell control machinery causes the cell cycle to arrest in G1(24). Inactivation of telomerase is intimately associated with

Cellular senescence.

Apoptosis.

Premature aging.

Degenerative diseases (19,22).

Telomerase in Carcinogensis:

a. 85-90% of cells derived from human cancers express telomerase (25,26)

b. Squamous cell carcinomas (27,28)
c. Salivary gland tumours (29)
d. Oro-pharyngeal tumours (30)
e. Tongue cancer (31)
f. Oral Cancers (32,33)
g. Increased telomerase activity in oral lichen planus (34)
h. Cancers of Epithelial origin including urinary bladder, oesophagus, large intestines, oral cavity and uterine cervix (30)

Thus, it is apparent that more then 90% of all human tumours reactivate telomerase, signifying that telomerase is crucial to maintain cancer cell viability. Telomerase is able to restore telomere length, thereby preventing DNA-damage responses and enabling the viability of cancer cells, which under normal circumstances would have either arrested in division or undergone apoptosis (35). As a result telomerase is considered to have wide spread implications in the field of tumorogensis not only in terms of diagnosis and prognosis but treatment as well.

A close relationship between high telomerase activity and loss of p53 tumour suppressor gene in producing tumours has been established in studies involving mouse models. In addition to this mutant Ras and T antigen expression working in collaboration with telomerase activity appears to be sufficient to transform cultured cells (5,19). It has been shown that telomerase co-operates intimately with the oncogenes to transform primary human cells into neoplastic ones *in vitro*, signifying that telomerase activation contributes to malignant transformation (36). Further supporting this is the fact that inhibition of telomerase using dominant negative versions of TERT in human tumour cell lines leads to telomere shortening and cell death(19,37). It has also been shown by a separate set of studies that over expression of hTERT in breast epithelial cells and basal keratinocytes enhances mammary carcinogenesis as well as carcinogen induced epidermal tumours. Recently it was demonstrated that the oncogenic transformation of human fibroblasts by telomerase can be independent of its capability to preserve telomere lengths. Thus, telomerase can effect proliferation of epithelial cells by a different telomere length independent mechanism consisting of altering the expression of growth promoting genes(38). This brings us to the non-canonical function of telomerase.

Non-Canonical Telomerase Functions:

While the two core components of telomerase are generally alleged to toil in co-operation to extend telomeres, there has been mounting evidence concerning the role of TERT protein independent of telomerase RNA and telomere length. It has been shown by some studies that TERT protein has an intrinsic terminal transferase activity (TT) (39). Some recent evidence has indicated the presence of telomerase at telomeres even when it was not able to increase their lengths (40). In another study done by Eva Gonzalez-Suarez, a two-stage model of mouse tumorigensis was developed to evaluate the role of telomerase in tumorigensis. Late generation mice deficient in TERC RNA encoding for telomerase as well as normal mice, were used in this study and the two stages of tumour initiation and promotion were carried out to provoke papilloma formation. While the normal mice not only showed papilloma initiation but its progression to carcinoma the telomerase deficient mice having short telomeres were resistant to tumour development, which maybe a direct consequence of these short telomeres. However, early generation mice with long telomeres still showed a small reduction in papilloma yield (36). Other researches have shown an enhancement in telomerase activity during mouse tumorogensis despite the fact that mice already have very long telomeres (35). Interestingly increased expression of TERT in cells that already have long telomeres leads to their malignant transformation (41) and enhanced wound healing (36,42). TERT over expression has been demonstrated to causes stem cell proliferation and mobilization as well as hair growth in a telomere-length independent fashion (43). The non-canonical TERT pathway has also been implicated in promoting the proliferation of resting stem cells (44) and appears to be concerned with chromosomal healing and enhanced cell survival (45). It is prudent to mention here that the telomerase RNA component is considered essential for these tumour promoting effects of TERT over expression (42)

Interestingly it has been observed that TERT is up regulated in the S-phase of cell cycle of normal somatic cells where it induces chromatin reorganization during DNA replication (41). Further studies have described a TERT-mediated alteration in transcription of a subset of genes leading to enhanced genomic stability and increased DNA repair (46). Various researchers have shown that telomerase has an anti-apoptotic and pro-survival role in telomere length independent fashion in various cell types (17) as well as having a

telomere capping functions (47) and interactions with other signalling pathways (48).

Recently a study involving the mouse models demonstrated that mTERT expression interferes with TGF-β signalling by repressing TGF-β mediated growth inhibition (49). Thus it can be deduced that TERT inhibits TGF-β signalling. Another study showed the attainment of resistance to TGF-β mediated growth arrest in cultured human mammary epithelial cells as a result of ectopic expression of hTERT . Thus it can be deduced that telomerase maybe enhancing epithelial cell proliferation by interacting with growth promoting genes (50). Up-regulation of Epiregulin, a member of the EGF family growth promoter has also been observed in fibroblasts immortalized by activation of telomerase(51). hTERT expressing epithelial cells have been shown to have activated oncogene c-Myc (52), and show down regulation of p16 gene (53). Inhibition of p53 induced apoptosis in a manner, which does not not require telomerase has also been reported (54).

All these studies and the discussions derived from them indicate that hTERT confers an additional function that is required for tumorogensis, Cellular proliferation and survival but does so in a telomere length independent fashion (45). Therefore TERT is now believed to have at least one non-canonical or length independent function. Some of the researchers, however, do believe that TERT has various growth and survival promoting functions some of which require telomere length maintenance while others do not (54).

The Two Roles of Telomerase in Carcinogensis:

Thus it can be concluded from the above discussion that telomerase can contribute to tumorigensis by two different mechanisms. Firstly, by rescuing critically short telomeres and maintaining their lengths thus giving them survival advantage. Secondly by maintaining telomere lengths and promoting growth of cancer cells which already have long telomeres. There can be many possible explanations for this. Telomerase may interact with survival and growth signals when present at telomeres, and it may play important role in processing and repairing DNA damage in the genome. Thus, the Absence of telomerase can have a negative effect on the tumour cells even before the erosion of telomeres below a critical level. On the other hand, the erosion of telomeres beyond the critical level in the absence of telomerase would cause the cell to undergo apoptosis or to become arrested, as mediated by the p53 DNA damage signalling pathway35. The dual role that telomerase plays is depicted in Fig. 6.

Conclusion

As described, the activation of telomerase may have wide spread biological consequences in addition to its essential functions and thereby it plays a much more important role in immortalization and tumorigensis then previously thought. Although the activation of telomerase remains an established fact in the progression of tumor, the timing and context of this activation is still undetermined. In most of the cancers, this activation is a late event occurring after significant telomere shortening; however, certain other studies have revealed its role earlier on in cancer development, in certain tissues (40). A better understanding of the different roles of telomerase is required to have an understanding of the basic processes of tumorigensis and to unable scientists to modify the ever-evolving field of cancer therapeutics to our advantage.

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