

Reviews

# Telomere, telomerase and digestive cancer

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Recent advances suggest that telomerase is associated with cellular immortality which is a hallmark of cancer.

## TELOMERES

Human telomeres contain an array of tandem DNA repeats. We share the telomeric sequence (TTAGGG)<sub>n</sub> with all other vertebrates. Human chromosomes end in several kilobases of telomeric repeat DNA. These are oriented so that the guanine (G)-rich strand runs out to the 3' end of the chromosome. Despite their monotonous sequence, telomeres fulfil important functions. First, they hide natural chromosome end from factors acting on DNA termini unlike broken chromosome ends which either get degraded or fuse to other DNA. Telomeres are resistant to exonucleases and ligases. They also escape detection by the DNA damage checkpoints. The termini of natural chromosome ends are probably concealed by a complex of specialized proteins that bind telomeric DNA. Telomere length is maintained by a balance between the telomeres-lengthening process (e.g., telomerase)<sup>[1]</sup> and the telomeres-shortening process (end replication). The inability of the DNA to completely replicate chromosome termini (telomeres) leads to the progressive shortening of chromosomes upon continuous cell division. The shortening can ultimately lead to loss of telomeric function and chromosomal destabilization. A DNA polymerase called telomerase is required to overcome the end replication.

## TELOMERASE

Human telomerase is a ribonucleoprotein (RNP) composed of an essential RNA and a few proteins. It synthesizes the G-rich tandem repeats that comprise telomeres [(TTAGGG)<sub>150-2000</sub> in humans]

using a template on the RNA that is complementary to the telomeric repeat. By adding hexameric (TTAGGG) repeats to the telomeric ends of the chromosomes, the continued erosion of telomeres is compensated. The enzyme is expressed in embryonic cell and in adult male germ line cells<sup>[2]</sup>, but is undetectable in normal somatic cells except for proliferative cells of renewable tissues (e.g. haemopoietic stem cells and activated lymphocytes, basal cells of the epidermis and intestinal crypt cells). In normal somatic cells, progressive shortening of telomere leads to a limited replicative capacity. Recently more direct evidence has been found in the role of telomere shortening in aging<sup>[3]</sup>.

## TELOMERIC PROTEINS

Telomeres are essential for the maintenance of chromosomes. Another group of important regulators for telomere function is the telomere binding proteins<sup>[4]</sup>. They were originally described as proteins, binding specifically to telomere DNA (for example, to the hexameric repeat). The RAP 1 protein in budding yeasts and the TRF1 protein in mammalian cells are examples of this class of proteins<sup>[5]</sup>. Another type of proteins interacts with the telomere DNA-binding protein/protein-interactions and comprises a large functional chromosome domain called the "telosome"<sup>[6]</sup>. Examples are the SIR 3/SIR 4 proteins and the RIF 1 protein in *Saccharomyces cerevisiae*<sup>[6]</sup>. These proteins associate with the RAP 1 telomere DNA-binding protein and function to establish telomere silencing effect and regulate telomere length respectively. Some of these proteins negatively regulate telomere length, probably by inhibiting telomerase activity<sup>[7]</sup>. Therefore, the length and function of telomere are not determined simply by the balance between the total number of cell divisions and telomerase activity.

## TELOMERASE IN MALIGNANCY

While all of the steps leading to cancer are still unknown, progression to a cancerous state does require the accumulation of a series of genetic alterations similar to those found in the *in vitro* models of carcinogenesis. For instance, hyperproliferation occurs due to the failure to respond to growth inhibitory signals and the functions allowing cells to divide in the absence of

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specific growth stimulatory signals. Additional mutations must take place for cell to progress to invasive and then metastatic states. To the extent that each of these conditions represents a mutational event, a clonal expansion of the cell is required for the occurrence of mutation. Some of these changes involve recessive events where an initial clonal expansion of cell containing the original mutation must be followed by a second clonal expansion with the remaining wild type allele eliminated. If this series of events require a greater number of cell divisions than permitted for normal cells, potential tumor cells must incorporate a mechanism to overcome this limitation. It appears that in many cases, the reactivation of telomerase serves this purpose, yet it may not be the only mechanism. The presence of telomerase activity only indicates that the cell has the ability to inactivate the telomeric "clock" that limits the proliferative capacity of normal somatic cells<sup>[8]</sup>. The presence of telomerase activity in a cell implies very little about malignancy, but only reflects its potentially immortal state. Cell immortality only gives the cells the proliferative capacity to accumulate the necessary mutations to become malignant. As cancer is diverse, some tumors may need only a few mutations in order to become malignant and may not exhaust the normal limits of proliferation before they cause disease. These types of cancer would be expected to be both immortal and negative for telomerase activity. As proliferative limits can be exceeded at any time during cancer progression, the reactivation of telomerase would be expected to occur early in tumors arising from cells near the limits of their proliferative capacity and late in tumors arising from cells with long telomeres. In some cases of tumors arising from telomerase positive stem cells, the initiating cells may already be competent to be immortal.

The fact that almost all cancers have telomerase activity, despite their shortened telomeres<sup>[2,9,10]</sup> indicate that there is an intense selective pressure for telomerase activation with the progression of malignancy. Indirect support for this view comes from the observations that benign or precancerous lesions (e.g. colonic polyps or adenoma; prostate hyperplasia and fibroids) are telomerase silent<sup>[9]</sup>. As telomeres shorten, accumulated mutations in other genes such as the genes encoding p53 and *RB* (retinoblastoma protein) would result in genomic instability, an extended life-span and progressive erosion of telomeres<sup>[11,12]</sup>. At this point, end to end chromosome fusions are frequently observed, concomitantly with critically shortened telomeres. These events could contribute to the loss of heterozygosity and the expression of recessive mutations, which would result in the reactivation of

telomerase and stabilization of telomere length, as well as fixation of the additional mutations required for invasiveness and metastasis.

#### ***Telomerase activity in colorectal cancer***

The colorectal adenoma-carcinoma sequence is one of the best characterized models for multistep tumorigenesis. Progression may be extremely slow due to the high ratio of adenomas to cancers. Telomere shortening is observed in colorectal adenomas and carcinomas<sup>[13]</sup>. Recent studies have demonstrated that although most adenomas lack telomerase activity, the majority of colorectal cancers express this enzyme<sup>[2,9]</sup> and contain detectable telomerase activity regardless of underlying phenotype (77% of hereditary nonpolyposis colorectal cancers; 81% of sporadic tumors, 88% with mutator phenotypes and 75% without mutator phenotypes)<sup>[14]</sup>. Therefore, telomerase expression appears to be commonly acquired in the progression of both mutator phenotype and sporadic colorectal cancers. These findings in colorectal cancer are consistent with the telomere hypothesis. However a minority of colorectal cancers lack detectable telomerase activity<sup>[8]</sup> and other alternatives remain<sup>[10]</sup>.

#### ***Telomerase activity in gastric cancer***

The pattern of multiple gene changes in gastric cancer varies with the histological type, well differentiated or intestinal type and poorly differentiated or diffuse type. However, activation of telomerase, which is responsible for cell immortality is the most common fundamental event in gastrointestinal cancer<sup>[2,9,15]</sup>. Human telomerase RNA (hTR) is expressed in pre-crisis cell lines and non-neoplastic tissues, as well as in immortalized cell lines or tumor specimens and the expression level is not correlated with that of telomerase activity<sup>[16]</sup>. Telomerase activity is detected in 85%-88% of gastric carcinomatous tissues<sup>[16,17]</sup>. Although all tumor specimens and non-cancerous mucosa expressed various levels of hTR, 81% expressed hTR at a higher level in the tumor than that in the corresponding mucosa. All the 8 gastric carcinoma cell lines also expressed hTR at higher levels. Thirty-five percent of non-cancerous mucosa showed telomerase activity and all of them contained intestinal metaplasia. The degree of *Helicobacter pylori*-infection increased in parallel with the level of hTR expression and telomerase positivity<sup>[17]</sup>. These results suggest that *Helicobacter pylori* infection may be a strong trigger for hTR overexpression in intestinal metaplasia, and this may lead to telomerase reactivation. Tumors with telomerase activity were generally large in size with a high frequency of lymph node metastasis. In the

tumors without detectable telomerase activity, 80% were early stage gastric cancer. In gastric cancer, telomerase activation may occur as a late event of cancer progression as demonstrated previously in non-small cell lung cancer<sup>[18]</sup>. Moreover, the patients with telomerase-positive tumors showed poorer prognosis than those with telomerase-negative tumors, indicating that telomerase-positive gastric cancers may have more malignant potential.

#### ***Telomerase activity and telomere length in HCC and CLD***

It is well known that almost all HCC are preceded by chronic hepatitis (CH) and/or liver cirrhosis (LC). These two conditions are regenerative lesions in response to repeated liver damage induced by hepatitis B or C virus (HBV/HCV) infection or other factors. However, the role of these lesions in HCC carcinogenesis remains unclear, except for the possible involvement of transforming activity of the HBVX protein<sup>[19]</sup>. There was progressive shortening of telomeres during hepatocellular carcinogenesis from normal liver to CH to LC to HCC. The average telomere length of HCC was significantly and consistently shorter than that of adjacent CH or LC<sup>[20]</sup>. The possible role of telomere shortening in regenerative, noncancerous liver lesions (CH and LC) is that it may eventually lead to reactivation of telomerase, which may then contribute to the malignant conversion to HCC. Thus telomere shortening in the regenerative lesions is not merely representative of cellular aging, but may also be a prerequisite for the development of malignancy. A subpopulation of cells in such lesions with telomeres shortened to a critical length, may suffer genetic changes due to chromosome instability<sup>[3]</sup>. These genetic changes would make most cells senescent, but allow a small number of cells to undergo additional mutations, including those activating or upregulating telomerase which then clonally develop into immortal cancer cells. Telomere length was shorter in chronic liver diseases compared with that in normal liver<sup>[21]</sup>. This indicates that senescence of hepatocytes occurs in patients with advanced liver diseases probably as a result of degeneration and the following regeneration of hepatocytes. Although the mechanisms of carcinogenesis in type C chronic liver diseases are not known, the incidence of HCC increases as the stage of the disease advances<sup>[22]</sup>. Telomerase activity was measured in various tissues and cell lines<sup>[2,9,15]</sup> including HCC<sup>[23]</sup> and was shown to be positive in 85%-100% of various malignant tissues and negative in almost all non-malignant tissues, except for reproductive and haemopoietic cells. Telomerase activity was negative in 15% of the HCC specimens<sup>[21]</sup>. One

possible reason for this undetectability might be false negativity due to the possible presence of polymerase chain reaction inhibitor<sup>[24]</sup>. However the presence of such inhibitor was not confirmed. In another previous report the telomerase activity in malignant tissue was not positive<sup>[23]</sup> and a recent report has clearly shown that several tumor cell lines keep their telomere length without telomerase activity<sup>[25]</sup>. Consequently, some unidentified mechanism for restoring the telomere length must exist<sup>[26]</sup>. Thus, it seems that a small number of HCC do not possess telomerase activity.

#### **TELOMERASE—A TARGET FOR CANCER TREATMENT**

Telomeres and telomerase play a role in signalling cellular senescence and in the progression of tumorigenesis, anticancer therapies could be targeted at telomerase. The gene encoding the human RNA component of telomerase has been cloned<sup>[27]</sup>. Following strategies are available.

A: Obstructing telomerase RNA activity through an antisense oligonucleotide targeted to the template region. Encouragingly, expression of antisense to hTR in an immortal telomerase-expressing cell line resulted in a gradual reduction of telomere length, leading to death of the cells<sup>[27]</sup>.

B: Generation of mutant telomerase RNA.

C: Protein components of telomerase present another viable target for inhibition.

Telomerase inhibitors may thus provide an effective cancer therapy with no side effects of general cancer therapy in normal somatic cells that lack telomerase expression. A future treatment regimen may include surgical removal of the tumor, followed by combined antitelomerase therapy with conventional radiation and/or chemotherapies. As most cancer cells have much shorter telomeres than the stem cells of renewal tissues, the treatment period could be designed to end prior to stem cell attrition by preserving the replicative abilities to divide stem cells. Thus the effects of inhibiting telomerase activity are likely to eliminate the cancer cells long before telomere lengths in stem cells become limited.

#### **CONCLUSION**

Clearly, telomerase regulation is complex and further studies are needed on the multiple mechanisms regulating telomerase activity with respect to the addition of telomeric repeats and its relation to cellular growth. Telomerase fulfills many of the criteria for an ideal cancer target and has a nearly ideal developmental and tissue-expression pattern, what remains is to show that tumors require telomerase for growth and that loss of telomerase function will be clinically useful.

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