Telomeres: structure and function

Complex structures located at the ends of chromosomes, telomeres are primarily made up of multiple copies of a repeating nucleotide sequence, TTAGGG in humans, with a typical length in normal cells of several thousand bases [1]. In addition to the simple repeating sequence, telomeres are also comprised of a number of accessory proteins, collectively called the shelterin complex. These proteins help maintain the stability of the telomere and prevent the chromosome end from being recognised as DNA damage. The overall structure of a telomere is a loop, with a 3’ extension of the telomeric sequence being inserted into an earlier repeat, the structure of which is called a D-loop, while the overall structure is called a T-loop [1] [Figure 1].

The main functions of the telomere complex are believed to be the stabilisation and protection of the chromosome ends from two main hazards. The first is the recognition of the chromosome ends as double stranded DNA breaks in need of repair [1]. The second function of the telomeres is in the abrogation of the “end replication problem”. This is related to the function of DNA polymerase, which is unable to perfectly complete replication of a linear DNA strand at the 5’ end of the daughter DNA. As a consequence of this, every replication cycle results in the loss of DNA from the chromosome ends [1]. Telomeric DNA sequences prevent this by providing a large array of “expendable” DNA at the chromosome ends. This progressive shortening plays a role in cellular ageing and senescence, with cells halting further replication when telomeres reach a critical length. In stem cells, germ line cells and the cells of young growing organisms, the telomeres are maintained and extended by an enzyme complex called telomerase, which is not normally present in somatic cells [1].

Telomerase

Human telomerase is a ribonucleoprotein with two main components, human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT) [2]. These two components are essential for telomerase activity while minor components assist in assembly and stability [1,2]. The components bind together into a single complex in which hTR is used as a primer and template for the addition of telomeric DNA sequences [2]. The hTERT enzyme extends the 3’ overhang of the telomere a variable number of times per cell replication, after which standard DNA polymerase enzymes fill in the single stranded DNA [1,2] [Figure 2]. Not all telomeres are extended in any given round of replication, and short telomeres are preferentially extended by telomerase, with longer telomeres being worked on as the concentration of telomerase in the cell increases.

Without telomerase, cells have limited proliferation potential before they enter senescence or die through genomic erosion. It is a hallmark of cancer cells, however, that they are immortal, with limitless proliferation potential. Because of this and their rapid rates of growth, it is believed that telomerase reactivation in somatic cells is associated with carcinogenesis and is a critical step in the tumour immortalisation process [2]. Indeed, almost all human cancer cells assayed for telomerase activity show increases in telomerase expression and evidence of active telomere maintenance. Those few cells that do not show telomerase expression instead show evidence of one of several alternative mechanisms for telomere extension.

Clinical implications in cancers

Telomeres and telomerase have two main characteristics that make them highly interesting from a clinical point of view. Firstly, somatic cells do not normally express telomerase, making telomerase an excellent marker for the detection of cancer in most tissue types [2]. Secondly, cancer cells require telomerase in order to survive and maintain genomic stability, while most somatic cells do
not use it at all, making telomerase an attractive target for therapeutic intervention. These two factors are enough to ensure the interest in telomerase in the management of cancer, but there are many things that remain unknown about the role of telomerase in cancer. For example, evidence is emerging that telomerase has some influence on apoptosis, DNA damage response and other functions, but it is not known how this impacts on the development of particular cancers [1].

Telomerase has been subject to a great deal of inquiry in the past few years, and despite our lack of proper understanding, some information on potential clinical utility has emerged. Telomerase has been showing potential as a simple diagnostic marker, due to its presence in a majority of cancers, although, because false positives and negatives are both possible, it may need to be coupled to other markers [3]. The activation of telomerase is a function associated with more aggressive types of tumour. This includes general aggressiveness of tumours dependent on the tissue from which they are derived, as well as subtypes within particular cancers [3]. For example, thyroid carcinomas are not normally highly malignant, and few show telomerase activation [4, 5]. In contrast, skin cancers show much higher malignancy, and correspondingly higher rates of telomerase activation [6]. In a similar fashion, basal squamous cell carcinomas and small cell carcinomas of the oesophagus are a rare and highly aggressive forms of oesophageal cancer, and almost all (95 and 100%, respectively) display telomerase expression [7, 8]. Telomerase expression has also been linked to specific pathological features, such as grade and stage, patient survival, proliferative index, recurrence and metastasis risk in a number of different cancer types [3].

Colorectal cancer and telomerase activity

In colorectal cancer, conflicting results have been reported as regards telomerase activation and its correlation with clinical parameters. Despite the absence of telomerase in normal mucosa, these studies have demonstrated that the activation of telomerase occurs in adenomas, and telomerase level is further increased in carcinomas [9]. Other reports have revealed a detectable level of telomerase activity in normal mucosa [10]. Some studies in other cancers have also found this trend, indicating that telomerase

Figure 2. Extension of telomeres by telomerase.
may be expressed at extremely low levels in normal tissue, that the rapid turn-over of colorectal epithelium is derived from stem or semi-stem cell populations or that some samples are partially contaminated with immune cells, which are known to express telomerase. A few studies have investigated the use of telomerase expression in tissue or blood to identify the presence of primary, metastatic or recurrent tumours [11]. Results have been mixed, but promising. The detection of normal levels of telomerase from stem cell populations is a barrier that needs to be overcome, but significant correlations between detected levels of telomerase and the presence of malignant cells has been noted.

In the realm of clinical implications of telomerase in colorectal cancer, the controversy continues. A few studies have shown a significant association between telomerase and clinicopathological features during cancer progression, whereas others have failed to find such relationships. Although telomerase activity has been detected in colorectal cancer, much remains unknown about hTERT expression and its correlation to tumour progression and clinopathological features. However, evidence that telomerase does play a role in the development of colorectal cancer has been emerging. Several studies have identified associations between telomerase activity and expression as well as a number of prognostic and clinicopathological features in colorectal cancer. These include: relationships to survival (directly for metastatic cancers, indirectly via survivin in primary tumours); other cancer markers (p21, aurora kinase and survivin); Duke’s staging; tumour site (increases in telomerase in distal tumours); presence of metastases; recurrence and the development of metachronous cancer [9, 12-17]. While many studies in colorectal cancer find some, but not all, of these relationships, the overall pattern is one of deep involvement of telomeres in the pathogenesis and progression of colorectal cancer.

It is possible that some of the conflicting results obtained to date in colorectal cancer studies may be due to the different ways used to assess telomerase activity. There are four main methods of detection used; terminal restriction fragment length analysis (TRF); real time polymerase chain reaction (real time PCR); immuno-histochemistry (IHC); and telomeric repeat amplification protocol (TRAP). Each of these methods [Figure 3] detects a different aspect of telomerase activity and the use of all four is not homogenous in research, meaning that different studies are detecting relationships between specific telomerase behaviours and clinicopathological characteristics.

While the picture emerging from research is that the detection of telomerase activity has excellent value as both a diagnostic and prognostic marker, more work needs to be done clarifying the role of telomerase in colorectal cancer as well as determining the most efficient and relevant way to detect it. IHC and TRF are both relatively simple methods, and IHC in particular is highly familiar to clinical workers, but these methods lack a degree of precision which may be necessary for developing appropriate management strategies. TRAP provides the best analysis of enzymatic activity, but it is an involved method, and some cancer-related functions of telomerasers may not be directly related to the ability of the complex to synthesise telomeric repeats. Real time PCR is the most sensitive and accurate method, but it is prone to contamination if not performed correctly, and it does not detect finished protein, so may have limited clinical value.

Conclusions
Treatments based on disrupting telomerase or telomeres have been in development for some years. These are based on the suppression of hTERT activity by various chemicals, as well as the selective targeting of cancer cells using hTERT as a marker. Both chemical and vaccine based treatments are now entering clinical trials and viral vectors have also showed promise in animal models. In this context, it is extremely important to improve our understanding of telomerase activity in colorectal cancer, as our ability to detect appropriate measures of telomerase activity could mean a huge improvement in the management of patients.

In the future, telomerase is likely to become a common marker for the management of colorectal and other cancers on multiple levels. However, there still remains a great deal of work to be done in determining the best methods to approach telomerase detection. Future studies will help to pin down precisely how the different levels of telomerase activity play into the cancer phenotype and behaviour and further refine our ability to manipulate the telomeres for cancer detection and treatment.
References


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