Medical genetics and epigenetics of telomerase

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Abstract

Telomerase is a specialized reverse transcriptase that extends and maintains the terminal ends of chromosomes, or telomeres. Since its discovery in 1985 by Nobel Laureates Elizabeth Blackburn and Carol Greider, thousands of articles have emerged detailing its significance in telomere function and cell survival. This review provides a current assessment on the importance of telomerase regulation and relates it in terms of medical genetics. In this review, we discuss the recent findings on telomerase regulation, focusing on epigenetics and non-coding RNAs regulation of telomerase, such as microRNAs and the recently discovered telomeric-repeat containing RNA transcripts. Human genetic disorders that develop due to mutations in telomerase subunits, the role of single nucleotide polymorphisms in genes encoding telomerase components and diseases as a result of telomerase regulation going awry are also discussed. Continual investigation of the complex regulation of telomerase will further our insight into the use of controlling telomerase activity in medicine.

Keywords: telomerase • medical genetics • epigenetics • miRNAs • TERRA • SNPs • gene variation

Introduction

The ends of linear chromosomes are protected from genomic instability by the DNA-protein complexes known as telomeres [1]. The DNA component of human telomeres comprised a repetitive TTAGGG sequence (and complimentary sequence on the opposite strand), up to 20 kb in length, and a 3’ G-rich single-stranded overhang [2, 3]. The G-rich overhang invades the double-stranded telomeric repeats to form a t-loop, which protects the telomere from end-to-end fusions and unregulated nuclease digestion of the 3’ overhang [4, 5]. The protein components of human telomeres that recognize and bind to the telomeric repeats, such as Telomeric Repeat Binding Factor 1 and 2, Protection of Telomeres 1, TRF-Interacting Nuclear Factor-2, Tripeptidyl Peptidase 1, and Repressor/Activator Protein 1 (TRF1, TRF2, POT1, TIN2, TPP1 and Rap1), are known as the Shelterin complex which is responsible for t-loop formation and telomere protection [6, 7].

Telomerase, the specialized enzyme capable of reverse transcribing DNA, is required for complete replication of telomeres [8]. In human beings, the core enzyme comprises the protein hTERT, which contains a reverse transcriptase domain capable of catalysing the addition of a six nucleotide repeat onto the ends of telomeres [9], and an RNA component, termed hTR or hTERC (Telomerase RNA Component), which contains a template region that is complementary to the human telomere sequence [10]. Telomerase expression is low or absent in normal human somatic cells, but highly expressed in embryonic cells, adult male germline cells, in stem cells of proliferating tissues and over 90% of human malignancies...
Both hTR and hTERT are significantly up-regulated in malignant cells compared to normal cell counterparts [13]. Other telomere/telomerase associated proteins [such as dyskerin, Nucleolar Protein-10 (NOP10), Non-Histone Protein-2 (NHP2) and Glycine Arginine Rich-1 (GAR1)] are required for assembly of a functional telomerase holoenzyme complex [14]. How telomerase is regulated in expression and activity beyond transcription control which is covered by several other reviews, and the consequences of its dysfunction, is the focus of the following sections.

Epigenetic regulation of telomerase: recent findings beyond transcriptional control

Regulation of telomerase is very complex as many factors can effect hTERT expression and telomerase activity. Telomerase may be regulated through such known methods as transcriptional regulation, post-transcriptional regulation, post-translational regulation, localization within the cell, assembly of the subunits, epigenetic regulation and by telomeric proteins and RNAs [15, 16]. Many new forms of regulation may be yet to be uncovered. In this section we review recent studies of telomerase regulation involving methylation of the hTERT promoter and telomeric regions, the possible role of sirtuins and non-coding RNAs including microRNAs (miRNAs) and telomeric-repeat containing RNA (TERRA).

Methylation

Regulation of hTERT transcription generally takes place at the promoter region which has been found to contain an abundance of CpG sites, common targets for methylation [17]. DNA methylation and chromatin remodelling are common regulators of gene activity that alter the binding of transcription factors to gene promoters. The field of epigenetics has become very important in the study of telomerase regulation, and several recent studies have begun to shed light on some of the factors involved.

A recent study in HPV-induced carcinogenesis has suggested that a gradual increase in methylation at the hTERT promoter may coincide with the progression to a tumorigenic phenotype in cervical cell lines, but the effect was not as significant in clinical samples [18]. This discrepancy may be the result of selection for cells with greater methylation or, as the authors suggest, may be caused by the presence of normal tissue in clinical samples [18]. This discrepancy may be the result of selection for cells with greater methylation or, as the authors suggest, may be caused by the presence of normal tissue in clinical samples, although it may be related to another complication: researchers have not been able to agree about whether hTERT methylation follows the expected relationship. Promoter region methylation is commonly associated with gene silencing, but studying the epigenetics affecting the hTERT gene has been difficult. Although some studies have generated the expected results, other researchers have indicated that there is no correlation between methylation and hTERT expression [19]. It has been suggested that methylation status and its relation to hTERT expression is dependent on the cell type [20, 21], but there is some debate.

In 2007, Zinn et al. studied multiple cell types to try to determine if there was some underlying mechanism behind the epigenetic regulation of telomerase in all cancer types [22]. The group tested breast, lung and colon cancer samples using methylation-specific PCR and bisulphite sequencing of the promoter and found that all of the samples maintained at least one allele with less methylation around the transcription start site despite surrounding methylation patterns. They suggest that although much of the hTERT promoter may be heavily methylated in some cancer cell types, a region of about 300 bp around the transcription start site remains unmethylated and following the usual pattern of methylation resulting in gene silencing. Although this is an interesting study providing a possible explanation to the complexity of hTERT promoter epigenetics, only three tissue types were studied, and some research suggests that we are still not seeing the whole picture [20, 23]. Of course, telomerase regulation is complex, and hTERT promoter methylation is just one piece of the puzzle.

Telomerase activity has also been shown to be subjected to regulation by methylation of other regions such as the subtelomeric region [24]. This is the chromosomal region just proximal to the telomeric region which has been found to be highly methylated in mouse and human cells as reviewed previously [24–26]. A recent study has indicated that subtelomeric methylation may influence telomerase activity, and specifically whether a tumour maintains its telomeres with telomerase or through the alternative lengthening of telomeres (ALT) pathway [27]. The specific mechanisms of the ALT pathway are not well known, and it is only utilized by 10–15% of human tumours [28]. Ng et al. analysed sub-telomeric cytosine methylation, first determining methylation state in normal cells, and then comparing cells using the ALT pathway to cells that were telomerase positive. They found that the average percentage of methylated loci across all normal samples was relatively constant: $81 \pm 3\%$. When analysing ALT cells, the group found that the amount of methylation varied dramatically, not just across cell lines, but even across loci. However, when analysing telomerase positive cells, it was found that all loci were heavily methylated with little variation: $97 \pm 1\%$ [27]. This indicates that increased methylation of subtelomeric regions may somehow allow for an increase in telomerase activity, although it is not known if this is somehow affecting hTERT transcription.

Sirtuins

Recently, work has been done by several groups which suggest that sirtuins may have a role in telomerase regulation, but findings have been unclear. Sirtuins are a family of enzymes that have been shown to play a role in increasing overall health and longevity in organisms [29]. A study from 2003 by Lin and Elledge suggested that Sir2 (a yeast protein and the first sirtuin discovered, shown to increase lifespan by guarding against genome instability [29]) may be an activator of hTERT in a small variety of cancer cell lines.
tested [30]. SIRT1 (NAD-dependent deacetylase sirtuin-1, the human analogue to Sir2), however, has been suggested to be an inhibitor of telomerase activity [31]. Likewise, a recent study has shown that an isoform of P63, ΔNP63xx, can induce TERT promoter activity in mice, and it was suggested that this may be due to the down-regulation of SIRT1 [32]. Recent studies with resveratrol have also added to the speculation that SIRT1 may be involved in telomerase regulation. Resveratrol is one of the most commonly used molecules thought to activate sirtuins. It is a small molecule that has been shown to improve health and longevity in many of the same ways as targets of SIRT1 [33]. It has been suggested that resveratrol promotes health and longevity by acting through sirtuins, although this idea is still debated [34, 35]. It has also been shown that resveratrol activates telomerase in endothelial progenitor cells [35] and decreases telomerase activity in breast cancer cells [36]. Although it has not been shown that these modifications of telomerase activity are directly related to SIRT1 activity, it is an interesting area requiring more study. For more information on current studies looking into the pharmaceutical use of resveratrol as a telomerase inhibitor, refer to the section below.

Non-coding RNAs

Little is known about which non-coding RNAs might regulate telomerase. When studying these factors, the complexity of telomerase regulation is compounded by the complex networks of miRNAs. MicroRNAs, also known as miRNAs or miRs, are non-coding, single stranded RNAs that have recently been shown to play critical roles in many biological processes such as development, differentiation, apoptosis and proliferation. They commonly down-regulate the target messenger RNA of protein-coding genes in a sequence-specific manner [37]. More than 1000 miRNAs are predicted to work in human biology in a complex regulation network, and several studies have shown that many miRNAs are deregulated in cancers [37–40]. However, to this day, miRNA regulation of telomerase remains elusive.

In 2008, Mitomo et al. found an association between the down-regulation of miR-138 and overexpression of telomerase in anaplastic thyroid carcinoma cell lines [41]. The researchers selected miRNAs of interest reported to be differentially expressed in thyroid carcinoma when compared to normal thyroid. Each miRNA was studied individually using stem-loop-mediated reverse transcription real-time PCR. miR-138 was found to be significantly down-regulated in anaplastic thyroid carcinoma cell lines compared to papillary thyroid carcinoma cell lines. Using the miR-Base online database (http://microrna.sanger.ac.uk), the authors looked for potential targets of miR-138 and focused on hTERT. The thyroid carcinoma cell lines were transfected with miR-138 precursor molecules, and effects on hTERT expression were studied using Western blotting and luciferase assay of the promoter activity [41]. It is important to note, however, that it has been shown that many commercially available antibodies used for Western blotting are not specific to telomerase and may result in inaccurate conclusions [42]. Although increasing the amount of miR-138 molecules in the cells did not completely turn off telomerase activity in the cell lines studied, this is most likely just the first of many miRNAs to be associated with telomerase activity. Also, miRNA activities may be cell-type specific, and the deregulation of miR-138 may be important in telomerase activity in thyroid carcinomas, whereas other miRNAs and mechanisms may be more important in regulating telomerase in other cells and tissues.

It has proven to be a difficult task to pinpoint which miRNAs target telomerase. Since Motimo et al. 2008 findings, very little headway has been made. Although no other specific miRNAs have been found to date, Miura et al. have found a region of interest that they believe alters telomerase activity and is most likely to contain a miRNA precursor gene [43]. Several studies have indicated that human chromosome 10p may contain a gene involved in regulating telomerase activity [44–46]. To determine which genes in this region may be involved, Miura et al. studied the region by exon trapping using bacterial artificial chromosome clones and studied the effect these segments had on telomerase activity using human hepatocytes and hepatoma cell lines. One of the genes cloned (RGM249-RNA gene for miRNAs, 249 bp in length), located at chromosome 10p15.3 was found to be overexpressed in cancer than in normal liver cells, similar to telomerase expression and was found to inhibit more than 80% of hTERT mRNA expression [43].

Both the Mitomo and the Miura studies shed light on a small portion of non-coding RNA molecules that may regulate telomerase activity. Both studies utilize relatively new techniques and technologies which will need to be continually combined and refined as we continue to study miRNA functions. Telomerase activity is such an important step in development and cancer progression that many complex regulatory pathways play a role. miRNAs have become popular topics of study since their discovery, but even more recently, large non-coding RNAs called TERRA have been suggested to play a role in telomerase regulation.

Telomeres have long been thought to be transcriptionally silent regions of the genome. Recently, this notion has been challenged with the discovery of TERRA, or telomeric repeat-containing RNA, also called TelRNA [47, 48]. Interestingly, these molecules have been found to have regulatory effects on telomerase. Mammalian TERRA molecules are large, non-coding RNA containing UUAGGG repeats and range in size from 100 bases to about 9 kb [47, 48]. At least some of the TERRA also contain subtelomeric-derived RNAs as well as the telomeric UUAGGG repeats suggesting that transcription of these elements begins at different starting points in the subtelomeric regions and moves towards the chromosome ends [47–49]. Relatively little is known about the functions of TERRA, but they have been shown to be an integral part of telomeric heterochromatin [47, 48], and possibly help maintain telomere architecture [49], provide epigenetic protection of telomeres from DNA repair mechanisms and regulation of telomerase activity [50]. It is thought that TERRA block telomerase activity at the telomeres in at least two ways: by blocking the RNA component with its sequence complementarity [27, 48] and by recruiting heterochromatinizing activities to the telomeric regions [48].

Several recent publications have supported the notion that TERRA molecules may function as telomerase inhibitors.
Researchers have found that TERRA are capable of duplexing with hTERC [48, 50], and it has been shown that there are lower levels of TERRA molecules in tumours than in corresponding normal tissue [48, 51]. It has also been found that shorter telomeres have lower levels of TERRA molecules [48, 50]. Shorter telomeres may transcribe lower amounts of TERRA because of the decrease in template space. This would cause less impairment of telomerase activity and help explain why telomerase has been shown to be selectively active at critically short telomeres [48, 50].

To further elucidate how transcription of telomeres is regulated and how it might be linked to telomere length, Caslini et al. have shown that histone 3/lysine 4 (H3/L4) histone methyltransferase and the transcription regulator Mixed-Lineage Leukemia (MLL) may contribute to telomere methylation and their transcription [52]. Local combined activity of H3/L4 and MLL are thought to promote transcription elongation and chromatin maintenance, but MLL had not been previously associated with telomeres. Using ChIP, they found that TTAGGG repeats were capable of pulling down both the N- and C-terminal domains of MLL in human haematopoietic cell lines and human ovarian surface epithelium. The same methods also showed that telomeres were acetylated at H3 and H4 and to be methylated at H3/K4. It was also shown that MLL binding positively correlated to H3/K4 methylation and transcription of telomeres, and that knocking down MLL resulted in histone modifications at the telomeres [52].

Interestingly, it has also been shown that there are lower levels of TERRA transcripts in telomerase positive cells than in cancer cells that maintain their telomere lengths through the ALT pathway [27]. Ng et al. compared the levels of TERRA in normal cells, ALT cells and telomerase positive cells by hybridizing RNA from each sample set to probes against the UUAGGG TERRA sequence and the antisense CCCUAA sequence and normalizing to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). They found greater amounts of TERRA molecules in the ALT cells than any others. One possibility is that these amounts were higher because ALT cells have longer telomeres and therefore can transcribe more TERRA molecules. Taking this into account, the group corrected for the additional telomeres by measuring telomere lengths in each sample. ALT cells were still found to have more TERRA transcripts. The group had already shown that telomerase positive cells have increased methylation at the subtelomeric region compared to normal and ALT cells. It is possible that these two findings are connected and indicate a mechanism in which TERRA molecule transcription is dampened to allow for more telomerase activity without hindrance from TERRA in telomerase positive cells, whereas normal TERRA transcription is allowed in ALT cells.

Consequences of genetic dysfunction of telomerase: medical genetics of telomerase

Dyskeratosis congenita

DC is a rare multisystem disorder defined by three clinical characteristics: oral leukoplakia, nail dystrophy and hyperpigmentation of the skin [78]. Patients also display signs of premature aging including hair greying and hair loss, osteoporosis and poor dentition [14]. The vast majority of DC patients (80–90%) develop bone marrow abnormalities by the age of 30 [79] and bone marrow failure is the principal cause of early mortality [56]. Individuals with DC also have a predisposition to cancer with an 11-fold increase in all cancer types, an 1154-fold increase in frequency of tongue cancer, and a 196-fold increase in frequency of acute myeloid leukaemia (AML) compared to the general population [80].

Mutations in patients suffering from DC were initially discovered in the DKC1 gene (See Table 1) located on the X chromosome, which encodes dyskerin, a protein involved in stabilizing
Table 1 Known mutations in DKC1, TERT and TERC involved in human diseases

| Disease | Gene mutated | Domain          | Type of mutation | DNA or protein change | Telomerase activity (%) | References |
|---------|--------------|-----------------|------------------|-----------------------|-------------------------|------------|
| DC      | DKC1         | N-terminal      | Missense         | A2V                   | NA                      | [54]       |
|         |              |                 |                  | P10L                  | NA                      | [55]       |
|         |              |                 |                  | Q31K                  | NA                      | [55]       |
|         |              |                 |                  | Q31E                  | NA                      | [55]       |
|         |              |                 |                  | F36V                  | NA                      | [56]       |
|         |              |                 |                  | I38T                  | NA                      | [55]       |
|         |              |                 |                  | K39E                  | NA                      | [54]       |
|         |              |                 |                  | P40R                  | NA                      | [56]       |
|         |              |                 |                  | E41K                  | NA                      | [54]       |
|         |              |                 |                  | K43E                  | NA                      | [55]       |
|         |              |                 |                  | T49M                  | NA                      | [55]       |
|         |              |                 |                  | R65T                  | NA                      | [54]       |
|         |              |                 |                  | T66A                  | NA                      | [54]       |
|         |              |                 |                  | T67I                  | NA                      | [55]       |
|         |              |                 |                  | H68Q                  | NA                      | [55]       |
|         |              |                 |                  | L72Y                  | NA                      | [56]       |
|         |              | Deletion        | Missense         | L37del                | NA                      | [56]       |
|         |              | TruB domain     | Missense         | S121G                 | NA                      | [56]       |
|         |              |                 |                  | R153W                 | NA                      | [56]       |
|         |              | Middle Region   | Missense         | S280R                 | NA                      | [56]       |
|         |              | PUA domain      | Missense         | K314R                 | NA                      | [55]       |
|         |              |                 |                  | L317F                 | NA                      | [57]       |
|         |              |                 |                  | L321V                 | NA                      | [54]       |
|         |              |                 |                  | R322Q                 | NA                      | [57]       |
|         |              |                 |                  | M350T                 | NA                      | [54]       |
|         |              |                 |                  | M350I                 | NA                      | [54]       |
|         |              |                 |                  | A353V                 | NA                      | [54]       |
|         |              |                 |                  | T357A                 | NA                      | [55]       |
|         |              |                 |                  | D359N                 | NA                      | [55]       |
|         |              | C-terminal region| Missense        | P384L                 | NA                      | [56]       |
|         |              |                 |                  | P384S                 | NA                      | [56]       |
|         |              |                 |                  | A386T                 | NA                      | [55]       |
|         |              |                 |                  | L398P                 | NA                      | [55]       |
|         |              |                 |                  | G402E                 | NA                      | [56]       |
|         |              |                 |                  | G402R                 | NA                      | [54]       |
|         |              |                 |                  | T408I                 | NA                      | [55]       |
|         |              |                 |                  | P409L                 | NA                      | [55]       |
|         |              |                 |                  | S420Y                 | NA                      | [55]       |
|         |              | Deletion        | Missense         | Δ from 493            | NA                      | [55]       |
| hTERT   | RT domain    |                 | Missense         | P702S                 | 13                      | [58]       |
|         |              |                 |                  | P721R                 | 20–100                  | [59]       |
|         |              |                 |                  | Y846C                 | 10                      | [58]       |
|         |              |                 |                  | H876Q                 | 50                      | [58]       |
|         |              |                 |                  | K902N                 | 0                       | [59]       |

Continued
Table 1

| Disease   | Gene mutated | Domain       | Type of mutation | DNA or protein change | Telomerase activity (%) | References |
|-----------|--------------|--------------|-------------------|-----------------------|-------------------------|------------|
| C-terminus| Missense     | R979W        | Missense          | 20–100                | [59]                    |            |
|           |              | F1127L       |                   |                       |                         |            |
| hTERC     | Template     | 48A→G        | Deletion          | 0                     | [55]                    |            |
|           |              | Δ52–55       |                   | 0                     | [55]                    |            |
|           | Near template| 58G→A        | Deletion          | 100                   | [60]                    |            |
| Core pseudoknot | Point | 37A→G        | Point             | 70                    | [61]                    |            |
|           |              | 72 C→G       |                   | 5                     | [62]                    |            |
|           |              | 116C→T       |                   | 0                     | [63]                    |            |
|           |              | 143G→A       |                   | 0                     | [62]                    |            |
|           | Deletion     | Δ96–97       |                   | 0                     | [62]                    |            |
|           | Double Point | 107–108G→AG  | Deletion          | 5                     | [64]                    |            |
| Hypervariable | Deletion     | Δ216–229 |                   | 0                     | [61]                    |            |
|           | Point        | 228G→A       |                   | 1                     | [60]                    |            |
| CR4-CR5   | Deletion     | Δ316–451     | Deletion          | ND                    | [62]                    |            |
| H/ACA box | Deletion     | Δ378–451     | Deletion          | 5                     | [64]                    |            |
|           | Point        | 408C→G       | Deletion          | 25                    | [64]                    |            |
| AA hTERT  | N-terminus   | V96L         | Missense          | ND                    | [65]                    |            |
|           |              | V119L        |                   | ND                    | [65]                    |            |
|           |              | A202T        |                   | 0                     | [66]                    |            |
|           |              | A279T        | Missense          | 20–100                | [59]                    |            |
|           |              | H412Y        |                   | 36                    | [58]                    |            |
|           |              | K570N        |                   | 0                     | [59]                    |            |
| RT domain | Missense     | G682D        | Missense          | 0                     | [67]                    |            |
|           |              | V694M        |                   | 0                     | [66]                    |            |
|           |              | T726M        | Missense          | 20–100                | [67]                    |            |
|           |              | Y772C        | Missense          | 0                     | [66]                    |            |
| C-terminus| Missense     | V1090M       | Missense          | 0                     | [66]                    |            |
| hTERC     | Near template| 58G→A        | Missense          | 100                   | [68]                    |            |
| Core pseudoknot | Deletion | Δ28–34       | Deletion          | 20–100                | [59]                    |            |
|           |              | Δ79          |                   | 0                     | [55]                    |            |
|           |              | Δ110–113     |                   | 0                     | [68]                    |            |
|           | Point        | 72C→G        | Missense          | 0                     | [68]                    |            |
|           |              | 116C→T       |                   | 0                     | [69]                    |            |
|           |              | 117A→C       |                   | 0                     | [69]                    |            |
|           |              | 204C→G       |                   | 0                     | [69]                    |            |
| CR4-CR5 domain | Point | 228G→A       | Missense          | 100                   | [69]                    |            |
|           |              | 305G→A       |                   | 1–2                   | [69]                    |            |
| H/ACA box | Point        | 450G→A       | Missense          | 20–100                | [69]                    |            |
|           |              | 467T→C       |                   | ND                    | [70]                    |            |
telomerase RNA and forming the enzyme complex [12]. The most common mutation found in DKC1 is a C→T nucleotide substitution, which results in the A353V missense mutation in the protein sequence. This mutation can be inherited or arise de novo [54]. Approximately half of DC patients have a mutation in the genes of the telomerase holoenzyme complex, hTERT, hTERC or DKC1 [81]. It is likely that the remaining 50% of patients have mutations in other telomere maintenance genes, such as the mutations found in TINF2, NHP2 and NOP10 [82]. The most common form of the disease shows X-linked inheritance (DKC1 mutations), with 90% of patients being male, although both autosomal dominant (hTERT, hTERC and TINF2 mutations) and autosomal recessive (NHP2 and NOP10 mutations) inheritance patterns are recognized [83, 84].

### Aplastic anaemia

AA is another bone marrow failure disease associated with telomerase mutations. The disease is rare with an incidence of one to five per million and defined by hypocellular bone marrow and low peripheral blood cell counts [63]. A few patients who initially presented with AA have been found to develop DC, suggesting that AA is a marker of more severe phenotypes [68]. The observation that AA patient leukocytes had significantly shorter telomeres than controls led researchers to screen patients for mutations in telomerase components [53]. In acquired AA, approximately 2.5% of patients have mutations in hTERC and 3.5% have mutations in hTERT (See Table 1 for list) [66]. The majority of hTERC mutations cluster in the pseudoknot region, which is essential for hTERT binding and telomerase enzymatic activity [53]. The identified hTERT mutations are spread throughout the gene and act by haploinsufficiency, not a dominant negative mechanism. hTERC mutations decrease telomerase activity through haploinsufficiency as well [66].

### Idiopathic pulmonary fibrosis

IPF is a much more common disease, with a prevalence of at least 90,000, than DC or AA, although the aetiology of the disease is unclear, as implied by idiopathic [85]. The clinical course of IPF is progressive and predictable, beginning with massive fibrotic changes and alveolar wall thickening in the lungs and patients ultimately succumbing to respiratory failure [72, 73]. One in five patients with IPF has a family history of the disease and inheritance looks to be autosomal dominant with variable penetrance [53]. In patients with a documented family history of IPF, hTERC and hTERT mutations account for 8–15% of disease inheritance [86]. Sporadic IPF has detectable telomerase mutations in 1–3% of cases. Although the percentage of patients with telomerase mutations is lower in IPF than dyskeratosis congenita, the higher incidence rate makes pulmonary fibrosis the most common clinical manifestation of telomerase gene mutations [85]. There are fifteen known mutations in hTERT and only two in hTERC (See Table 1); moreover, the mutations do not appear to cluster in any region or domain of either gene. IPF patients without telomerase mutations also have short telomeres when compared to controls, suggesting that telomere length, rather than telomerase mutations, is a better predictor of disease onset [86].

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**Table 1 Continued**

| Disease | Gene mutated | Domain       | Type of mutation | DNA or protein change | Telomerase activity (%) | References |
|---------|--------------|--------------|------------------|-----------------------|-------------------------|------------|
| IPF     | hTERT        | N-terminus   | Missense         | P33S                  | 80                      | [71]       |
|         |              |              |                  | L55Q                  | 40                      | [72]       |
|         |              |              |                  | V144M                 | 78                      | [71]       |
|         |              |              |                  | R486C                 | 45                      | [71]       |
|         |              | Deletion     |                  | Δ112C                 | ND                      | [72]       |
| RT Domain | Frameshift del | V747fs       | Missense         | R865C                 | 20                      | [71]       |
|         |              |              |                  | R865H                 | 28                      | [71]       |
| C-terminus | Missense   | T1110M       |                  | 50                    |                         | [72]       |
| Intron  | Frameshift del | E1116fs      | Missense         | 6                     |                         | [71]       |
| Intron  | Point        | Intron 1 + 1 G→A | Missense   | 2                     |                         | [72]       |
| hTERC   | Core pseudoknot | Point        | 37A→G             | 70                    |                         | [71]       |
|         |              |              | 98G→A             | 10                    |                         | [12]       |

For further review, see http://telomerase.asu.edu/diseases.html.
Acute myeloid leukaemia

Due to AA patients with telomerase mutations often having a family history of AML and patients suffering from AA and DC having a predisposition to AML, researchers looked for and found telomerase mutations in AML patients [87]. AML is a heterogeneous disease due to an acquired somatic mutation in the myeloid lineage of a haematopoietic progenitor cell [88]. Nearly half of AML patients have abnormal karyotypes and genomic instability plays a crucial role in leukemogenesis [87]. Calado et al. recently reported that 6.8% of patients with AML had mutations in hTERT and no mutations were found in hTERC. The A1062T hTERT mutation was found to be the most common variant between cases and controls and constituted 60% of all hTERT mutations found in AML patients [87]. Kirwan et al. have expanded on this work and found another hTERT mutation and two hTERC mutations in familial forms of AML [89]. The A1062T mutation has been found in multiple haematopoietic cancers and may serve as a surrogate marker for mutations throughout hTERT as a screening tool to identify other malignancies with telomerase as a predisposing factor [12].

TERT and TERC variations and disease predisposition

Although mutations in telomerase have been found to have important implications in growth and development, naturally occurring variations in hTERT and hTERC remain to be fully understood. These SNPs are limited and telomerase genes express less heterozygosity compared to other genes further demonstrating the overall importance of these genes [80, 90]. hTERT is located within a locus at chromosome 5p13.33 and hTERC is located within a locus at chromosome 3p26. Given the presence of telomerase activity in haematopoietic stem cells, it is likely that variations and/or mutations in the telomerase components would have significant implications for haematopoiesis. In fact, defects in telomerase function contribute to disease pathogenesis particularly in bone-marrow failure syndromes [91]. Studies have also identified an association between telomerase mutations in both hTERT and hTERC with familial myelodysplastic syndrome and AML as mentioned previously [12, 89]. In addition to the haematopoietic cell compartment, tissue based stem cells might also be affected by variations in telomerase.

Variations in the genes required to form the active telomerase complex may contribute to cancer risk. The correlation between telomerase function and cancer is most likely due to the fact that tumour formation can be driven by telomerase dysfunction. Immortalization of tumour cells can occur through hTERT gene rearrangement which may contribute to cancer risk if variations in telomerase promote gene rearrangement and telomerase activation [92]. It is also probable that failure to stabilize telomeres prior to significant shortening could contribute to an increased genomic instability capable of promoting increased tumour development and progression [80]. Variations in hTERC and hTERT have been found to contribute to overall telomere length implying a potential role for these variations in cancer risk [93, 94]. Recent genome-wide association studies have been performed in order to define whether there is an association between telomerase SNPs and cancer risk.

There is conflicting data regarding the role of hTERT variations and increased risk of breast cancer [80]. Although some studies have found an association between breast cancer risk and telomerase SNP [90], the most compelling data involve studies performed on a total of 1656 breast cancer samples and 2019 matched controls [95]. These compelling, high power studies did not find an association between increased breast cancer risk and the SNPs of the telomerase promoter [95, 96]. In line with these findings, Pooley et al. did not observe a strong association of the SNP rs401681 in the TERT-CLPTM1L locus with cancer risk, related to telomere length [97].

Although breast cancer risk does not appear to be associated with telomerase SNPs, other studies have demonstrated a change in cancer risk associated with hTERT and hTERC variations. For example, variation in the TERT-CLPTM1L genes was found to be associated with a decreased risk of squamous cell carcinoma of the head and neck [98]. Although a decreased risk was observed in this study, other studies have found an association for this region (5p15.33) and increased cancer risk. Additional genome-wide association studies found this region to be associated with increased risk for both pancreatic cancer and lung adenocarcinoma [99–104]. Unlike the studies involving breast cancer, an increased risk of prostate cancer was found to be associated with at least one hTERT variation that influences telomerase expression [105, 106]. Other studies have linked cancer risk and telomerase SNP for several cancers including the following: bladder cancer [106, 107], ovarian cancer [108] and cervix cancer [106].

But, what do these reported associations mean? What is the functional consequence of a SNP/variant in hTERT or hTERC? It is known that telomerase is tightly controlled and complex; mutations or haploinsufficiency of hTERT/hTERC drive telomere dysfunction and bone marrow failures that are detrimental to the organism’s survival. More research is needed to determine whether a variant in the hTERT promoter could alter the ability of a repressor to bind and maintain its tight control over its transcription and ultimately activity. Loss of such repression could impact telomere maintenance, immortality, and progression of precancerous lesions. Furthermore, could a SNP in hTERC slightly alter structure of the RNA which in turn affects telomerase activity and telomere maintenance? These are questions arising by the observations of the medical geneticists for the telomerase/telomere biology field to address in the laboratory.

Summary and perspectives

Telomerase regulation is complex and has important consequences not just for telomere length, but also for the development of disease.
Obtaining further insight into the role of telomerase gene regulation and therefore telomerase activity in disease development will serve to assist in the generation of enhanced therapies. Understanding how telomerase is regulated will also lead to improved targeting of telomerase as a therapy for genetic diseases and cancer.

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Conflict of interest

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