Telomere-Related Gene Mutations and Lung Diseases: Pulmonary Fibrosis, Emphysema and Lung Cancer

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ABSTRACT

In this review, we will approach the role of the regulation of telomere length and telomerase activity in different lung diseases. Telomeres are nucleo-protein structures located at the end of chromosomes that protect them from degradation. In the absence of telomerase activity, telomeres are shortened after each round of deoxyribonucleic acid (DNA) replication. When a critical size is reached, there is an induction in cell apoptosis or senescence. Telomere replication is also involved in the acquisition of the unlimited proliferative capacity that characterises tumour cells. Several diseases of lung tissue are associated either with a deficit or overactivation of telomerase activity. Idiopathic pulmonary fibrosis is associated with germinal mutations in telomerase-genes resulting in premature senescence of lung tissue accelerating the onset of the disease. Somatic mutations or polymorphisms in telomere reverse transcriptase (TERT) promoter have also been associated with upregulation of telomerase activity and lung cancer. (BRN Rev. 2019;5(3):184-200)

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INTRODUCTION

Eukaryotic chromosomes are capped at their ends by specialised nucleo-protein structures, named telomeres, that protect them from degradation. Human telomeres are composed of thousands of repetitions of the TTAGGG hexanucleotide[1]. A protein complex, named shelterin, associates to this deoxyribonucleic acid (DNA) region to form the telomere-specific chromatin structure. Telomeres protect the chromosomal ends from degradation, being essential for chromosomal and genome stability[2]. In their absence, chromosomal ends are recognised as damaged DNA by the cell and can be degraded or recombined with other telomeres resulting in the fusion and reorganisation of chromosomes[3]. The maintenance of telomeres is, therefore, of critical importance for the genetic stability of cells and organisms.

Replication of telomeric DNA requires the contribution of specific enzymatic machinery. The DNA polymerases cannot completely synthesise telomeric DNA because they require a primer molecule that covers the 5′ end of the DNA. Therefore, they are not able to complete the synthesis of the lagging strand of linear DNA molecules, such as chromosomes. This end-replication problem results in the shortening of each telomere by 50-100 nucleotides at each cell division[4]. In most eukaryotic organisms, including humans, telomere length (TL) is maintained by the activity of the telomerase complex that elongates telomeres by a replication-independent mechanism[5]. The complex is formed by a protein with telomere reverse transcriptase activity (TERT) and one RNA with a region of homology to the telomere DNA used as a template (Fig. 1, left panel)[6]. Telomerase activity is, therefore, required for unlimited cell proliferation. Telomerase components and, in particular, the TERT gene, are expressed to relatively high levels during embryonic development allowing high cell proliferation rates. However, TERT gene expression is repressed in most human adult cells[7]. It is only found in germinal and stem cells, especially those of highly proliferative tissues such as bone marrow, epithelia and lymphocytes[8]. The rest of the cells express very low TERT levels and their telomeres get progressively shorter after each cell division. When telomeres reach a critical size, they get unprotected and are recognised as damaged DNA. The ataxia-telangiectasia mutated (ATM) and ATR (ATM and Rad3-related) protein kinases are recruited to critically short telomeres activating the p53-dependent pathway that results in cell cycle arrest[3]. Prolonged arrest would finally induce apoptotic cell death or cellular senescence. Actually, most tissue-specific stem cells do not express enough TERT protein to completely replicate their telomeres at each cell division and their proliferative capacity decreases with the age of the organism[9]. With time, stem cell exhaustion impairs tissue renewal. Because of this reason, telomere shortening has been recognised as one of the hallmarks of human aging[10].

Telomere replication is also involved in the acquisition of unlimited proliferative capacity that characterises tumour cells[7]. Telomerase expression and activity are induced in about 85% of tumours, allowing tumour cells to completely elongate their telomeres at each cell division. In the remaining 15% of tumours, TL is maintained by a telomerase-independent mechanism, the alternative
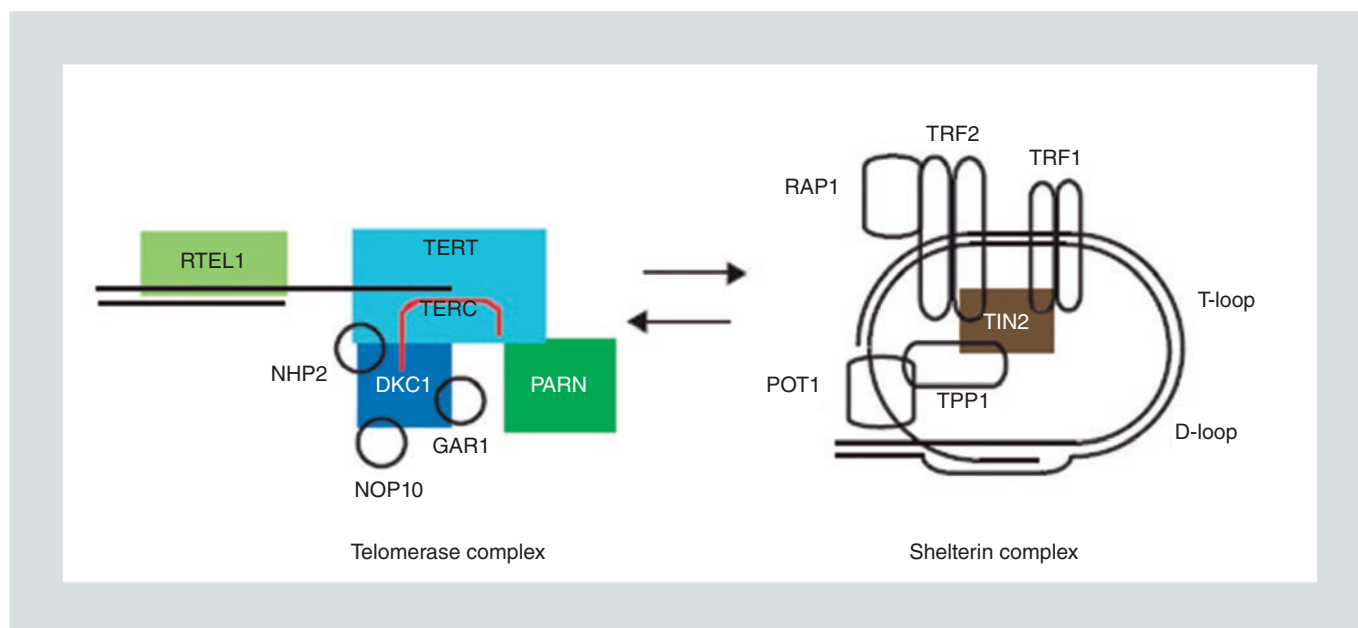
FIGURE 1. Telomere structure. Left panel. Telomerase complex, formed by several proteins and the guide RNA TERC is bound to the telomere repeats. PARN processes the TERC molecule and RTEL1 is a helicase that facilitates access of telomerase to the DNA. Right panel: Shelterin complex bound to telomere. T and D loops at telomere structure are indicated. Genes mutated in lung diseases are colour shadowed.

DKC1: dyskerin pseudouridine synthase 1; GAR1: GAR1 ribonucleoprotein; NHP2: nuclear protein family A, member 2; NOP10: nucleolar protein 10; PARN: poly(A)-specific ribonuclease; POT1: protection of telomere protein 1; RAP1: rhoptry-associated protein 1; RTEL1: regulator of telomere elongation helicase 1; TERC: telomerase RNA, TR component; TERT: telomere reverse transcriptase; TIN2: TRF1-interacting nuclear protein 2; TPP1: tripeptidyl peptidase 1; TRF1: TTAGGG repeat factor 1; TRF2: TTAGGG repeat factor 2.

lengthening of the telomeres (ALT) based on DNA recombination[11].

The importance of telomere homeostasis is further enforced by the existence of rare hereditary diseases that are caused by the presence of shortened telomeres, collectively named telomeropathies, short telomere syndromes or telomere biology disorders[12]. These diseases are caused by mutations in genes coding for proteins involved in telomere lengthening (telomerase complex and related proteins) or in the maintenance of telomere structure (shelterin complex). Telomerapathies are commonly characterised by the presence of very short telomeres. The molecular pathology of pulmonary telomeropathies, their diagnosis and emerging therapies will be summarised in this chapter. It is

necessary to enforce the importance of telomere homeostasis for healthy life since excessively long telomeres are also causative of disease. Recent reports have associated the presence of long telomeres to increased frequency of cancers such as melanoma or glioma[13]. Mutations in the promoter region of the TERT gene that increase gene expression are frequently found in these and other tumours[14,15]. In addition, mutations in the coding region of genes coding for proteins of the shelterin complex have been found in human tumours[16,17].

TELOMERE STRUCTURE

The nucleotide sequence of telomeres is composed of multiple repetitions of the TTAGGG

hexanucleotide in humans and several other animals. The length of these regions is variable in different organisms. In humans, telomeres have an average size of 8-14 kb in peripheral blood cells in new-born children[18]. The size decreases with age so that the average size in a 90-year-old person is of 3-7 kb[12]. In contrast, most mice strains used in research have an average TL of 50-100 kb, which has made the development of mouse models of telomere biology disorders more difficult[19,20].

Telomere ends are not formed by blunt-ended double-stranded DNA, as might be expected. Instead, the 3′ strand is about 75-300 bases longer than the 5′ end strand forming an overhanging single-stranded DNA fragment (Fig. 1, right panel)[1]. The overhanging strand contains the TTAGGG sequence and is named G-rich strand. The complementary strand contains the complementary CCCTAA repeats and is named the C-rich strand. The overhanging strand turns over the telomere DNA and intercalates in the neighbouring double-stranded DNA forming a loop, named the T-loop, as schematically shown in figure 1, right panel[21]. Looping results in the formation of a triple stranded DNA region known as D-loop, required for the stability of telomeric DNA[22].

Telomeres are further stabilised by the presence of a specific protein complex, the shelterin complex. It is composed of six different proteins: telomere repeat binding factor 1, encoded by the TERF1 gene (TRF1), telomere repeat binding factor 2, encoded by the TERF2 gene (TRF2), repressor/activator protein 1 (RAP1), TRF1-interacting protein 2 (TIN2), POT1-interacting protein 1 (TPP1) and protection of telomere 1 (POT1) protein (Fig. 1, right panel)[12]. The TRF1 binds to telomeric double-stranded DNA as a dimmer[23].

The TRF2 also binds double-stranded DNA as a dimmer and associates with TRF1[24]. The TIN2 protein interacts with TRF1 and TRF2[25] and recruits POT1/TPP1 heterodimer[26]. The POT1 protein binds with high affinity to the G-rich strand overhang[27]. The RAP1 protein incorporates to the shelterin complex via TRF2 interaction[26]. In addition, telomeres and subtelomeric regions are enriched in heterochromatin components including the heterochromatin protein 1 (HP1), the histone 3 Lysine 9 and histone 4 lysine 20 trimethylation, which further contributes to their stability[27,28].

The shelterin complex is required for telomere maintenance and function and prevents the recognition of telomeres as damaged DNA. The TRF2 protein inhibits the ATM kinase that induces the canonical non-homologous-end-joining DNA repair pathway that would result in telomere-telomere fusions[29]. In addition, POT1 inhibits signalling by the ATR kinase in response to DNA damage by double-strand and single-strand breaks and alkylating agents[30]. It also inhibits sister-telomere associations[31].

The components of the telomerase complex that catalyse telomere elongation arerepresented in figure 2. Telomerase complex is composed by the TERT, dyskeratosis congenita (DKC), nucleolar protein 10 (NOP10), niobophosphate high-index (NHP)2 and GAR1 ribonucleoprotein (GAR1) and the ribonucleic acid (RNA) molecule TR. The protein TCAB1 (Fig. 1) is required for telomerase recruitment to telomeres. Poly(A)-specific ribonuclease (PARN) is required for telomerase RNA (TR) processing. The regulator of telo- mere elongation helicase 1 (RETEL1) helicase and the conserved telomere maintenance component 1 (CTC1)/
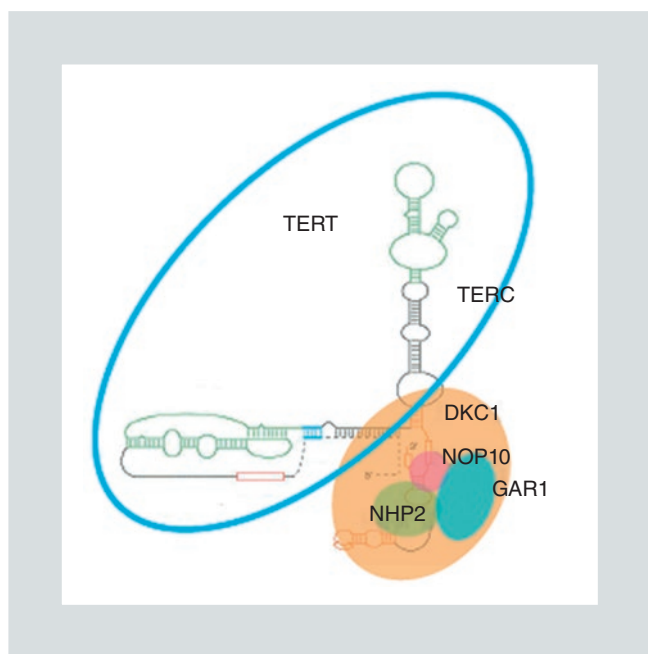
FIGURE 2. Schematic representation of the telomerase activity complex; active telomerase complex is an RNA-protein complex: TERT is the reverse transcriptase, TERC guides RNA to the telomeric sequence, DKC1 binds TERC and gives stability to this complex. NOP10, NHP2 and GAR1 also stabilise the whole complex.
DKC1: dyskerin pseudouridine synthase 1; GAR1: GAR1 ribonucleoprotein; NHP2: nuclear protein family A, member 2; NOP10: nucleolar protein 10; RNA: ribonucleic acid; TERC: telomerase RNA, TR component; TERT: telomere reverse transcriptase.

stress-activated map kinase-interacting protein 1 (SIN1)/teneurin transmembrane protein 1 (TEN1) complex facilitate telomere elongation by disrupting DNA secondary structures (Fig. 1).

TELEMORASE GENES ALTERED IN PULMONARY DISEASES

Telomerase reverse transcriptase (TERT)

The catalytic component of the telomerase complex is an 1132 amino acid long protein that contains three major functional domains

conserved throughout evolution[32]. The telomerase essential N-terminal (TEN) domain, highly conserved among vertebrates, has been implicated in telomere DNA binding upstream of the primer-template interaction[33]. The TERT RNA-binding domain (TRBD) is located next to the TERT N-terminal (TEN) domain and precedes the reverse transcriptase domain, which contains the enzymatic active site. In addition, the reverse transcriptase motif also participates in TR RNA binding ensuring telomerase complex stability[34]. Finally, TERT contains a less-conserved C-terminal extension region (Fig. 3).

Comparative analysis of the TERT gene in healthy individuals and patients with telomere disorders has shown a high degree of nucleotide variation. Over 75 TERT mutations have been reported in telomere biology disorders diseases[35], including missense, stop gain, frameshift and splice site mutations. However, the existence of a given mutation in a patient does not imply that it is causative of the disease; it might be a mutation that does not affect protein functionality. The existence of a family history showing a strong correlation between the presence of the mutation and disease manifestation would support a causative role. However, if it is a novel mutation or the family history is absent, an experimental assay of the activity of the mutated protein is required to ascertain the possible causal role. For this purpose, mutated TERT proteins are expressed in cells that have low or no telomerase activity. Protein function can be consequently tested using the telomere repeat amplification protocol (TRAP) or primer extension assays (see Collopy et al.[36] for a recent example). Most reported patients with TERT mutations are
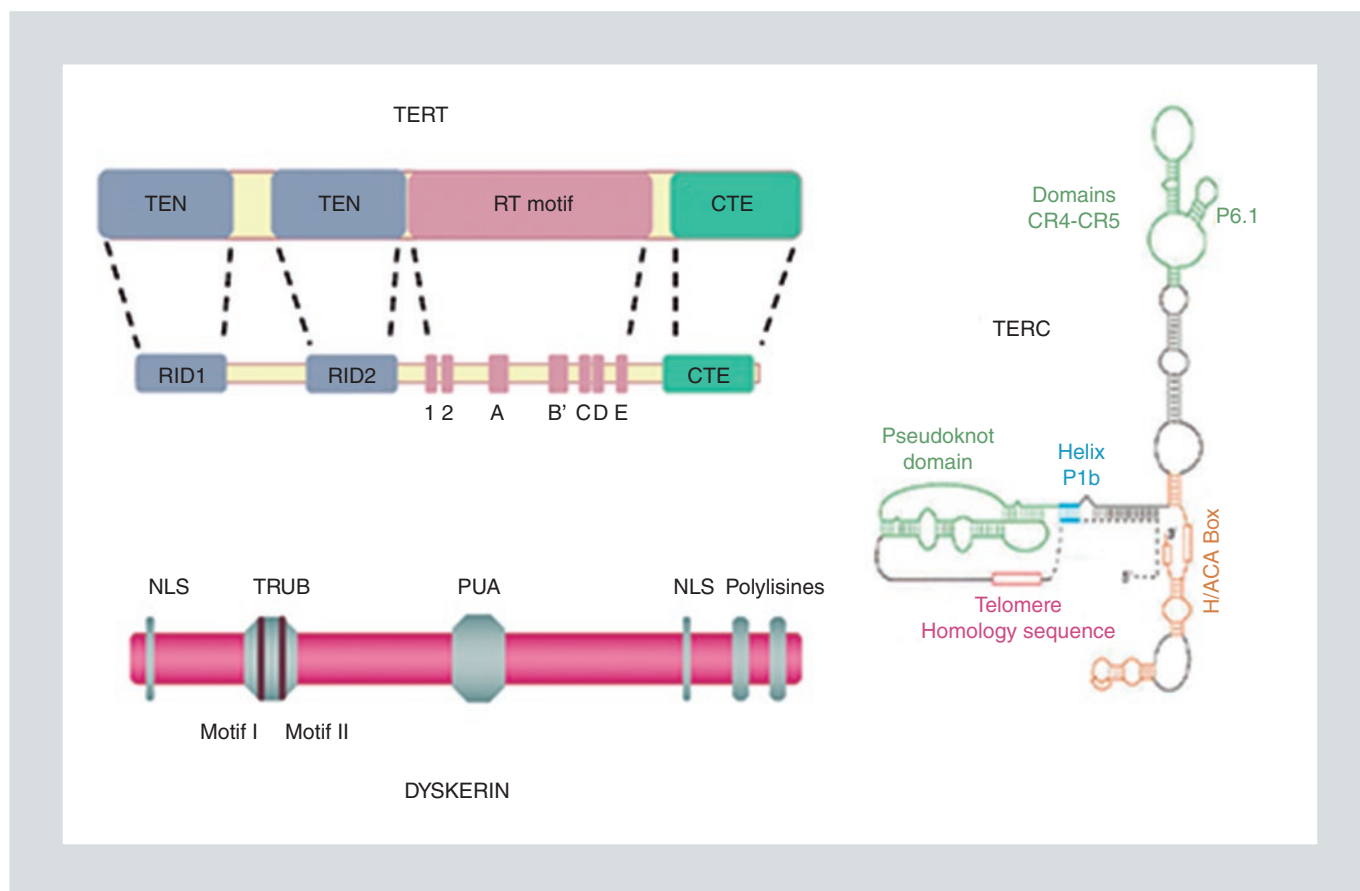
FIGURE 3. Schematic representation of the main components of the telomerase complex. TERT is the reverse transcriptase that synthesises the telomere DNA using TERC as a primer. TERT contains the TEN domain including the RID (RNA interacting domain) and the reverse transcriptase (RT) domain with homology to other reverse transcriptases. TERC contains a complex secondary structure with loops essential for the telomerase activity thorough the interaction with telomerase protein components. Dyskerin is a protein that is part of the telomerase complex, binds TERC and also participates in RNA pseudourydilation. NLS are nuclear location signals, TRUB domain is the pseudouridylation domain and PUA domain the RNA interacting domain.
CTE: constitutuve transport element; NLS: nuclear location signal; PUA: pseudouridine synthase and archaeosine transglycosylase; RID1: RNA interaction domain 1; RID2: RNA interaction domain 2; RT motif: reverse transcriptase motif; TEN: TERT N-terminal; TERC: telomerase RNA, TR component; TERT: telomere reverse transcriptase. TRUB: pseudouridine (psi) synthase domain.

monoallelic heterozygous. The telomerase activity found in cells from these patients is an average of homozygous wild type and mutant cells and might indicate that haploinsufficiency is the cause of the clinical phenotype[37-39].

Telomerase RNA, TR component (TERC)

The RNA component of the telomerase complex is 454 nucleotides in length and is encoded by the TERC gene. This RNA provides the template sequence for reverse transcription and is involved in the assemblage of the telomerase complex[40]. The interaction between TR and TERT regulates the catalytic activity, processivity and telomere-binding activity of the telomerase complex[41]. TR presents domains conserved through evolution that are involved in RNA stabilisation, accumulation, subcellular localisation and telomerase assembly. They are the template/ pseudoknot domain and the CR4/5 motif

(Fig. 3)[42]. These two domains are sufficient to restore telomerase activity when combined with TERT[43-44]. An additional H/ACA domain at the 3' end of TR allows binding of the proteins required for telomerase biogenesis: dyskerin, NOP10, NHP2 and GAR1[45] (Fig. 3).

Dyskerin pseudouridine synthase 1 (DKC1)

Dyskerin is a 524 amino acids long protein highly conserved during evolution. It is an essential nucleolar protein expressed in all tissues[42]. Dyskerin participates in two very relevant cellular activities, telomere maintenance and RNA pseudouridylation. The proteins NHP2, NOP10 and GAR1, associated with dyskerin are also involved in telomerase assembly[46]. Small nucleolar RNAs guide snoRNPs to specific uridine residues that are converted to pseudouridines by dyskerin. Pseudouridylation takes place in many cellular RNAs including ribosomal RNAs, small nuclear and nucleolar RNAs and messenger (m)RNAs, as recently described[47] (Fig. 3). This modification is important for folding and processing of these RNAs[48]. The telomerase RNA, TR, assembles as a typical scaRNP, which is important for TR stability and telomerase recruitment[49-50]. DKC1 is encoded in the X-chromosome and dyskerin mutations have X-linked transmission with affected males and carrier mothers.

TERF1-interacting nuclear factor 2 (TINF2)

The gene TINF2 codes for the protein TIN2, component of the shelterin complex that protect telomeres and regulates telomerase recruitment and activity. The TIN2 links DNA binding proteins TRF1 and TRF2 to TPP-1 and POT1 within the shelterin complex; it also interacts with heterochromatin protein 1 gamma (HP1γ)[51,52] which is crucial for sister telomere cohesion (Fig. 1, right panel). Mutations described in TINF2 result in early-onset disease. Familiar mutations are usually inherited in an autosomal-dominant trait[52,53]. All TINF2 mutations reported to date cluster in a segment coding for 34 amino acids centrally located in the gene. The function of this short protein fragment is not clear at the present time.

Regulator of telomere elongation helicase 1 (RTEL1)

The RTEL1 was first discovered as a regulator of TL in mice. It is an essential helicase related to a family of proteins that unwind G-rich DNA secondary structures. The most common mutations disrupt RTEL1 stability (i.e., nonsense, splice-altering, frameshift). There are also missense and short in-frame deletions, but their consequences on protein function are less clear. Even though RTEL1 is known to regulate TL, the exact mechanism by which specific mutations alter TL is the topic of ongoing research. One proposed hypothesis is that RTEL1's helicase activity facilitates DNA replication through G-rich sequences at the telomere (Fig. 1, right panel).

Poly(A)-specific ribonuclease (PARN)

Poly(A)-specific ribonuclease is a widely expressed protein with Poly(A)deadenylase activity that participates in the regulation of global mRNA levels during development[54]. In

addition, PARN also deadenylates small nucleolar RNAs such as TR[40].

Nuclear assembly factor 1 (NAF1)

The NAF1 stabilises H/ACA RNAs, a family that includes more than 100 members[55]. Naf1$^{+/-}$ mice had decreased H/ACA RNA levels but their targeting of rRNA modification was reported to be spared. In contrast, haploinsufficiency for TR causes telomere shortening in late-generation TR$^{+/-}$ mice[56,57]. The intact phenotype of first-generation Naf1$^{+/-}$ mice, despite the decreased H/ACA RNA levels, suggests that they behave similarly to early-generation TR$^{+/-}$ mice. However, in stark contrast to TR$^{-/-}$ mice, which are viable, NAF1 gene is essential in mammals, likely because of its conserved role in rRNA modification, as had been observed in yeast[58-60].

Frameshift mutations in NAF1 have been found in pulmonary fibrosis–emphysema patients[61]. The mutations segregated with short TL, low telomerase RNA levels and extrapulmonary manifestations, including myelodysplastic syndrome and liver disease. Truncated NAF1 was detected in cells derived from patients, and in cells in which the frameshift mutation was introduced by genome editing telomerase RNA levels were reduced and lacked a conserved carboxyl-terminal motif required for nuclear localisation.

Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is the most devastating interstitial lung disease (ILD). Although the pathogenesis remains unclear, there is evidence that IPF is an age-related disease[62]. The mechanisms linking IPF to ageing, including abnormal telomere shortening, are currently under study. Familial forms of pulmonary fibrosis (PF) have been described and might represent up to 20% of the cases[63]. The study of these familial forms identified mutations in TERT and TERC in 8-15 % of the cases[64,65], creating IPF as a telomeropathy. Idiopathic pulmonary fibrosis is inherited in these families as an autosomal dominant trait. This observation is supported by animal models, since TERT-null mice have a decreased number of alveolar epithelial cells[66]. The TRF1 deletion in type II alveolar cells also causes pulmonary fibrosis in mice[67].

Heterozygous mutations in genes coding for telomere-related proteins have been found in 15-20% of IPF families without a history of dyskeratosis congenita (DC)[68] and in 1-3% of sporadic cases of IPF[69]. In addition, 20% of patients with DC develop PF[70,71]. In agreement with these observations, IPF patients have significantly shorter telomeres than age-matched controls. Actually, IPF is a more common manifestation of telomere biology disorders than DC and aplastic anaemia (AA)[72]. Idiopathic pulmonary fibrosis due to telomere dysfunction presents in adulthood, into middle age[66]. Importantly, however, telomere attrition has been identified even in the absence of telomerase mutations. There is also evidence that IPF may be more likely to develop in subjects with the shortest telomeres. Thus, telomere shortening is a risk factor in developing the disease.

The gene most frequently mutated in IPF patients is TERT. but mutations have been also found in TERC (< 1%), DKC1 (< 1%)[73], TINF2 (< 1-2%)[74], RTEL1 (5%)[75], PARN (4%) and NAF1[75] (Table 1). Mutations in these genes

explain the inheritance in at least one-third of families. They are also a risk factor for disease in a smaller subset of IPF patients without a family history. Among the TERC mutations found in PF patients, some deleted large segments affecting functional domains while others are nucleotide substitutions. Mutations are particularly frequent at the pseudoknot domains and present an autosomal dominant inheritance (http://telomerase.asu.edu/diseases.html). As indicated above for TERT, the functional significance of TERC mutations, especially nucleotide substitutions, has to be determined experimentally.

A recent exome sequencing study linked PARN mutations with IPF and telomere shortening[75]. A subsequent study, also based on exome sequencing, identified biallelic mutations in the PARN gene in three families with individuals exhibiting severe DC[54]. Two of the families were homozygous for one missense variant and one splicing-altering variant, respectively. The third affected patient was a compound heterozygous. The patients exhibited reduced TERC, DKC1, RTEL1 and TERF1 mRNA levels. Cells from these patients showed activated DNA-damage response associated to nuclear p53 regulation, cell-cycle arrest and reduced viability upon ultraviolet (UV) treatment[54]. These results supported a potential link between PARN, the p53-dependent pathway and telomere shortening[75]. A subsequent study using cells derived from these patients has shown that PARN is required for the 3'- maturation of the telomerase RNA component[76] (Fig. 1, right panel).

Patients with IPF that carry mutations in telomere-related genes can also present extra-pulmonary manifestations related to telomere biology disorders, such as bone marrow failure including red blood cells, single lineage cytopenias or AA[70]. Actually, the complex syndrome of IPF and bone marrow failure predicted the presence of TERT or TERC mutations in ten families that presented these diseases in consecutive generations[65]. Short TL is a common finding in IPF patients, even in those without mutations in telomere-related genes[71]. These results could indicate that IPF may be more likely to develop in those individuals who naturally present shorter telomeres in the general population. These individuals might also have increased incidence of other telomere-related disorders such as cryptogenic liver cirrhosis and diabetes[70-77].

Within families with autosomal dominant inheritance, genetic anticipation, defined by an earlier and more severe onset of disease in successive generations, may be seen. The change in onset across generations depends on the extent of the loss-of-function caused by a mutation, with more deleterious mutations

causing obvious genetic anticipation in two or three generations, while hypomorphic mutations show more subtle changes in the same timeframe[78]. The progressive shortening of telomeres across generations underlies this earlier onset of disease[79]. Identifying this subset of patients is increasingly recognised to have implications for patient care. One of the most important examples is in the lung transplantation setting, where recognising patients with telomere-mediated lung disease is critical because it predicts potentially avoidable complications related to myelosuppression, infection and renal failure[80].

Emphysema and pulmonary fibrosis–emphysema

Emphysema is estimated to affect three to four million individuals in the United States alone; it is a leading cause of disability and mortality worldwide[81,82]. Chronic obstructive pulmonary disease and IPF have been hypothesised to represent premature aging phenotypes. At times, they cluster in families, but the genetic basis is not understood. Although IPF was the first lung disease causally associated to telomere gene mutations, their connection to emphysema risk was first documented in mouse models[83]. The TERC-null mice model with short telomeres share features of human diseases such as bone marrow failure[13], but do not develop *de novo* lung disease[83]. However, after chronic exposure to cigarette smoke, mutant mice develop airspace destruction that recapitulates all the hallmarks of emphysema[83]. Concurrent with this observation, severe young-onset emphysema was reported in two sisters who carried a deleterious mutation in the TR gene. These early observations suggested that telomerase mutations and short telomeres may cause susceptibility to cigarette smoke–induced emphysema in humans.

In the Genetic Epidemiology of COPD (COPD Gene) and Lung Health Studies, the frequency of deleterious TERT mutations was 1% in severe, early-onset disease. Severe, early-onset disease was defined as Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades 3 and 4 that occurs in individuals younger than 65 years; the COPDGene Study included additional radiographic criteria for emphysema[84]. Some of the mutations found had been previously reported in families with IPF. To date, three telomerase genes TERT, TR, and NAF1 have been linked to familial emphysema risk[61,85,86] (Table 1). The TERT mutations have been found in smokers with severe emphysema at a frequency of 1%[83]. Telomere dysfunction due to these genetic mutations can originate irreversible alveolar stem cell failure that would be at the origin of PF and emphysema[83].

Importantly, the pedigrees of telomerase mutation carriers with familial emphysema risk showed that their relatives, who also carry mutations have IPF. A pattern could be established showing that within a single family, never smokers develop IPF, while smoker mutation carriers are at risk for emphysema. This pattern highlights a unique gene–environment interaction in which the lung disease phenotype is determined by an environmental exposure in the presence of the genetically determined short telomeres. Notably, the gene–environment interaction seems to be particularly evident in females[83]. In these small series of patients with telomerase-associated emphysema, 90% are female even though the populations studied have been equally divided between males and females[83].

WHAT ARE THE MECHANISMS UNDERLYING THE SHORT TELOMERE-MEDIATED LUNG DISEASE PHENOTYPE?

Studies in the telomerase knockout mice model suggest that abnormally short telomeres lower the threshold to genotoxic stress from cigarette smoke[87]. Supporting this model, only the telomerase-null mice with short telomeres one develop emphysema, while telomerase-null mice with long telomeres are protected[87]. The short telomere–dependent defect is intrinsic to the lung, as normal bone marrow cells transplanted before cigarette smoke exposure are not protective[87]. The experimental data support a model of "two-hits", in which the first is the genetically determined short TL and the second the acquired cigarette smoke–induced damage. The additive effect provokes the telomere-mediated emphysema phenotype. One candidate cell type in which the additive damage accumulates in the alveolar space is the alveolar epithelial stem cell. In that milieu, at least a subset of type 2 alveolar epithelial cells (AEC2s) function as a facultative progenitor for new AEC2s as well as type 1 cells[88]. The role of stem cell failure in lung disease susceptibility has been addressed experimentally[89].

The current working model that synthesises these findings is that short telomere–mediated stem cell senescence upregulates the expression of pro-inflammatory cytokines, which in turn drives inflammation[89]. In effect, the inflammation in this form of emphysema is a secondary bystander caused by an upstream defect in stem cell senescence rather than a primary driver per se[89]. Such a paradigm predicts a modest benefit for anti-inflammatory approaches in subsets of patients in whom telomere shortening is the primary driver of disease.

Lung cancer

Activating mutations in the TERT promoter lead to increased telomerase expression, which sustains TL and genomic stability, thereby allowing cancer cells to continuously divide, and preventing senescence or apoptosis[90]. The TERT promoter mutations (TPM) were primarily established in melanoma and subsequently discovered in a number of other common types of cancer, including hepatocellular cancer, bladder cancer, glioblastoma, and thyroid cancer[91,92]. In addition, a previous study reported that TPM with ultraviolet signatures are frequent in skin cancer, suggesting that telomerase overexpression serves an important role in the pathogenesis of these tumours.

The TPM were detected in 2.2% (4/188) of patients with non-small cell lung carcinoma (NSCLC), which was consistent with previous studies[9] (Table 1). All cases with TPM exhibited a C228T mutation and one case exhibited the novel mutation, G247T (–143G>T) (Fig. 4). Certain studies have reported novel mutations of the TERT promoter region; however, the G247T mutation has not been reported in any other type of cancer. In addition, TPM were associated with poor differentiation and lymph node invasion, which may predict a poor prognosis. Consistent with these characteristics, survival of NSCLC patients with TERT mutations was significantly decreased compared with patients without TPM, following univariate and multivariate analyses.
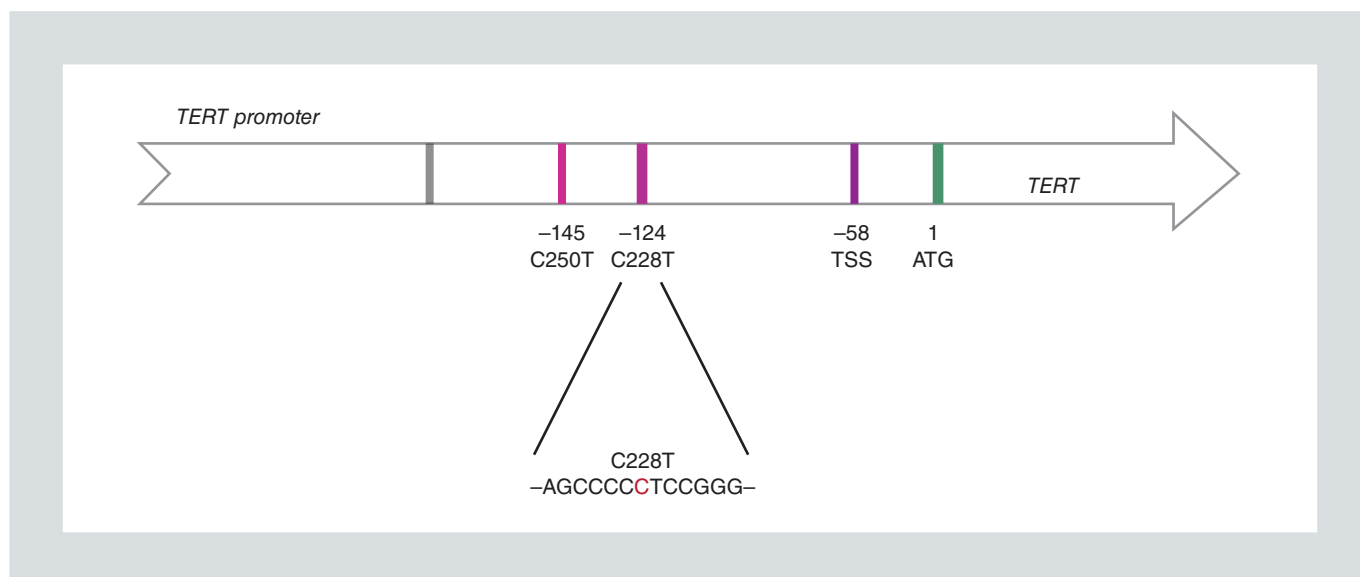
Figure 4. Schematic representation of the TERT promoter with the localizations of the TERT transcription (TSS) and translation (ATG) start sites, and the two 'hotspot' mutations (in pink): referred to as C250T and C228T, respectively and at the bottom the sequence of the mutated site C228T. TERT: telomere reverse transcriptase.

The relationship between TPM and TERT gene expression has shown significantly higher levels of mRNA expression in mesothelioma indicating that genetic alterations of the TERT promoter are a mechanism of TERT mRNA upregulation in mesothelioma[94]. Recent results of a meta-analysis suggested that the TERT rs2853669 polymorphism is associated with a significantly increased risk of cancer, particularly lung cancer; in Asian populations[95], the TERT rs2853669 T>C polymorphism (SNP), located upstream of the TERT promoter region, has been shown to affect telomerase activity and TL.

Finally, while several lung cancer susceptibility loci have been identified, much of lung cancer heritability remains unexplained. By studying genotypes of 14,803 cases and 12,262 controls of European descent, 18 susceptibility loci achieving genome-wide significance were identified, including 10 novel loci. Some of these loci include genes such as the telomere-related genes, OFBC1 and RTEL1[96]

Further exploration of the target genes needs to be performed in order to explain their role in the aetiology of lung cancer.

EMERGING THERAPIES RELATIES TO TELOMEROPATHIES

Currently, IPF treatment is mainly supportive of pulmonary rehabilitation therapy and the administration of supplemental oxygen. Recently, two pharmacological agents, pirfenidone[97] and nintedanib[98] were shown to reduce lung function decline in IPF patients. Danazol administration has also been described to slow down the progression of IPF in DC patients[99]. However, lung transplantation is the only curative strategy available. Lung transplantation was successfully used in a patient after hematopoietic stem cell transplantation (HSCT)[100]. The study of a small series of IPF patients with TERC or TERT mutations showed a favourable short-term output with 7 of 8 patients

alive after a median follow-up of 1,9 years. However, frequent haematological, renal and infectious complications were observed[101].

Because of the lack of curative therapies, telomere biology disorder's management is presently based on supportive measures and close follow-up for medium- and long-term complications[102]. Regular clinical review to monitor organ-specific disease progression, such as haematological analysis and pulmonary function testing must be performed. Since cells with mutations in telomerase genes are more susceptible to DNA damage, preventive measures such as avoidance of potential carcinogens (tobacco smoke, sun exposure) and adequate dental hygiene are also very important.

Important efforts for the development of mice models of telomere biology disorders aimed at the development of novel therapies have been made in the last years[103]. However, the existence of very long telomeres in the mice strains used for experimentation (50-100 kb) has made this a difficult task. Mice strains with defective telomerase activity have been generated[104-106] but they have to be crossed for 4-5 generations before their telomeres are sufficiently short to manifest telomere-associated defects[106]. The use of mice with short telomeres to generate telomerase-deficient strains provided a better experimental model[107]. More recently, tissue-specific inactivation of genes related to telomere biology has been used to generate mice models of these diseases. For example, mice models of bone marrow failure and PF were generated by deleting the TRF1 gene in the hematopoietic compartment and type 2 alveolar cells, respectively[66,107]. However, these mouse models did not completely reproduce the human disease and telomere size was not reduced[106].

Telomere biology disorders are caused by mutations in a single gene in most patients and could be, therefore, amenable for gene therapy strategies. An important caveat is that telomere length is narrowly controlled and excessively long telomeres increase the probability of developing some cancers such as melanoma[108]. Transient expression of TERT extends telomeres in human cells[109]. Recent reports indicate that TERT plays a role beyond telomeres and contributes to stem cell maintenance and cell reprogramming which might offer new therapeutic targets for telomere biology disorders[110]. Recently, it has been reported that reintroducing TERT by delivering tert-AAV9 virus rescues PF in a mouse TERT$^{-/-}$ model presenting short telomeres[111].

A new therapeutic opportunity came from the observation that GSE24.2, peptide corresponding to the pseudouridine (psi) synthase domain (TRUB) of dyskerin-reactivated telomerase activity in DC patients and human telomerase-deficient cells[112]. This peptide activated human TERT promoter and rescued DC-fibroblasts from premature senescence, increased the telomerase RNA, TR, expression trough stabilization of the molecule[113]. Dyskeratosis congenita cells presented increased DNA damage at the telomeres and increased levels of oxidative stress. Expression of GSE24.2 decreased both DNA damage and oxidative stress of dyskerin mutant cells[114]. Subsequent studies demonstrated that a shorter fragment of GSE24.2, named GSE4, maintained the same biological activity and induced telomerase activity and cell proliferation of DKC-mutant cells, decreased DNA damage, oxidative stress and cell senescence[115]. GSE24.2 and GSE4 could be delivered to cells using surface modified biodegradable polymeric nanoparticles, which

might facilitate their administration to patients[116]. Finally GSE4 has proven to decrease DNA damage, reactive oxygen species, activate TERT expression and telomerase activity in a secondary telomeropathy ataxia telangiectasia[117]. More interestingly, GSE4 was able to increase TL and decrease oxidative damage at telomere and mitochondrial DNA, suggesting that this could be an interesting approach for cell or gene therapy in telomere defective diseases. These results open a new therapeutic opportunity for the treatment of telomere biology disorders and GSE24.2 was recently approved by the European Medicines Agency as an orphan drug for DC treatment (EU/ 3/12/1070-EMA/OD/136/11).

CONCLUSIONS

Regulation of telomerase activity has a high impact on different lung diseases. Germinal mutations in some telomerase-related genes such as TERT, TERC, DKC1, TIN2, RTEL1. PARN and NAF1 are associated as a cause of many cases of FPF, some cases of IPF and emphysema/pulmonary fibrosis. In all these conditions there is a decrease in TL that is below the 10% percentile of the control population. The biological bases of the consequences of short telomeres are an increased susceptibility of environmental conditions and premature aging of lung tissue stem cells. The TERT promoter mutations described in cancer result in increased expression of TERT and increased telomerase activity that supports cancer cell division. They have also been described in a low proportion of lung cancer patients and are always related with a bad prognosis. The presence of polymorphisms in TERT promoter has also been described in lung cancer and

reported to results in increase of telomerase activity and telomere length.

In summary, decrease in TL and the presence of telomerase-gene germinal mutations have a high impact on the biology and progression of ageing-related lung diseases such as IPF and emphysema. On the other hand, the impact of somatic mutations in TERT promoter results in bad prognosis of NSCLC and mesothelioma patients. These results reinforce the need to study the telomere biology in several diseases of lung tissue in order to improve the diagnosis and future treatments of such diseases.

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DISCLOSURES

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