Short telomeres in pulmonary fibrosis: from genetics to clinical significance

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Abstract

Pulmonary fibrosis has been linked molecularly and pathophysiologically by abnormal telomere maintenance. Short telomere lengths are commonly found in both the familial and sporadic forms, telomerase mutations being the most common identifiable genetic cause of the disease. Telomeres are repeated nucleotide sequences that cap the ends of chromosomes and protect them from damage. Telomeres are eroded with cell division and shorten with age. Telomere integrity is mediated by the telomerase complex, a specialized polymerase that adds sequences to the ends of chromosomes. Mutations in the genes encoding telomerase (TERT and TERC) cause pulmonary fibrosis through low telomerase activity, accelerated telomere shortening and exhaustion of lung stem cells. Mutations in TERT or TERC account for only 19% of familial pulmonary fibrosis cases, and it is likely that additional environmental, genetic and epigenetic factors contribute to telomere erosion and to disease phenotype. Identification of short telomeres has potential clinical implications in pulmonary fibrosis: it may be a marker for an increased predisposition toward the development of the disease, it might affect risk stratification as it has been associated with lower survival rates and post-transplant complications that reflect the syndromic nature of this molecular defect.

Keywords: *pulmonary fibrosis, short telomere lengths, telomerase mutations*

Rezumat

Scurtarea telomerilor în fibroza pulmonară: de la genetică la semnificație clinică

Fibroza pulmonară a fost corelată molecular și fiziopatologic cu alterarea biologiei telomerice. Scurtarea semnificativă a telomerilor este frecvent identificată atât în fibroza pulmonară familială cât și în cea sporadică, mutațiile telomerazei fiind cea mai frecventă cauză genetică a bolii. Telomerii, capetele cromozomilor liniari, sunt secvențe repetitive de nucleotide ce protejază materialul genetic în cursul diviziunilor celulare. Integritatea telomerilor este mediată de complexul telomerazic, o polimerază specializată ce extinde capetele cromozomilor prin adăugarea de secvențe repetitive telomerice. Mutațiile la nivelul genelor telomerazei (TERT și TERC) determină fibroză pulmonară prin activitate redusă a telomerazei, accelerarea scurtării telomerilor și epuizarea celulelor stem pulmonare. Mutațiile TERT sau TERC explică doar 18% din cazurile de fibroză pulmonară familială asociate alterării telomerilor, fiind astfel posibil ca factori adiționali genetici, epigenetici și de mediu să contribuie la repararea inadecvată și eroziunea accelerată a telomerilor. Identificarea scurtării telomerilor prezintă implicații clinice potențiale în fibroza pulmonară: pot reprezenta un marker pentru o predispoziție crecută a dezvoltării bolii; pot prezenta semnificație prognostică, telomerii scurți fiind asociați cu o supraviețuire scăzută și complicații rare sistemice post-transplant pulmonar ce reflectă aspectul sindromic al acestui defect molecular.

Cuvinte-cheie: *fibroză pulmonară, scurtarea telomerilor, mutațiile telomerazei*

Introduction

Fibrosing interstitial lung diseases (ILD) comprise a diverse group of diffuse lung disorders characterized by various patterns of inflammation and fibrosis as a result of damage to the lung parenchyma that can occur independent or in systemic diseases.

Although ILD entities share similar clinical, radiological and physiological features, there are important differences in terms of prognosis and management. Idiopathic pulmonary fibrosis (IPF), the prototypic ILD, is a devastating disease that mainly affects elderly adults, with a median survival of 2.5–3.5 years after diagnosis[1]. At present, there are no available treatments for IPF to prolong life, apart from lung transplantation.

Evidence suggests that many genetic factors affect the risk of development of pulmonary fibrosis (PF) in individuals with familial or sporadic disease.

Telomeres are the tandem repeats of TTAGGG that protect the ends of chromosomes during DNA replication and cell division. Mutations in the genes encoding telomerase complex, the enzyme that synthesize telomeres, have been reported in about 19% of kindreds with familial PF and 3% of patients with sporadic PF, making them the most common identifiable genetic cause of the disorder[2,3]. Telomere dysfunction has a role in PF beyond the cases with telomerase mutations, since 21% of patients with familial PF and 22% of patients with sporadic PF have abnormally short telomere length, even when telomerase mutations are not detected[4].

Clinical significance of telomere dysfunction in PF is reflected by lower survival of this patients subset. The syndromic nature of telomeropathies expose these patients to development of extrapulmonary complications such as bone marrow failure or cryptogenic liver cirrhosis, and contribute to the inferior post lung transplant outcomes through rare complications.

Genetics in pulmonary fibrosis

Evidence of a genetic signature in pulmonary fibrosis comes from familial clustering of the disease[5]. Familial PF is a well-known but poorly understood entity, defined by the

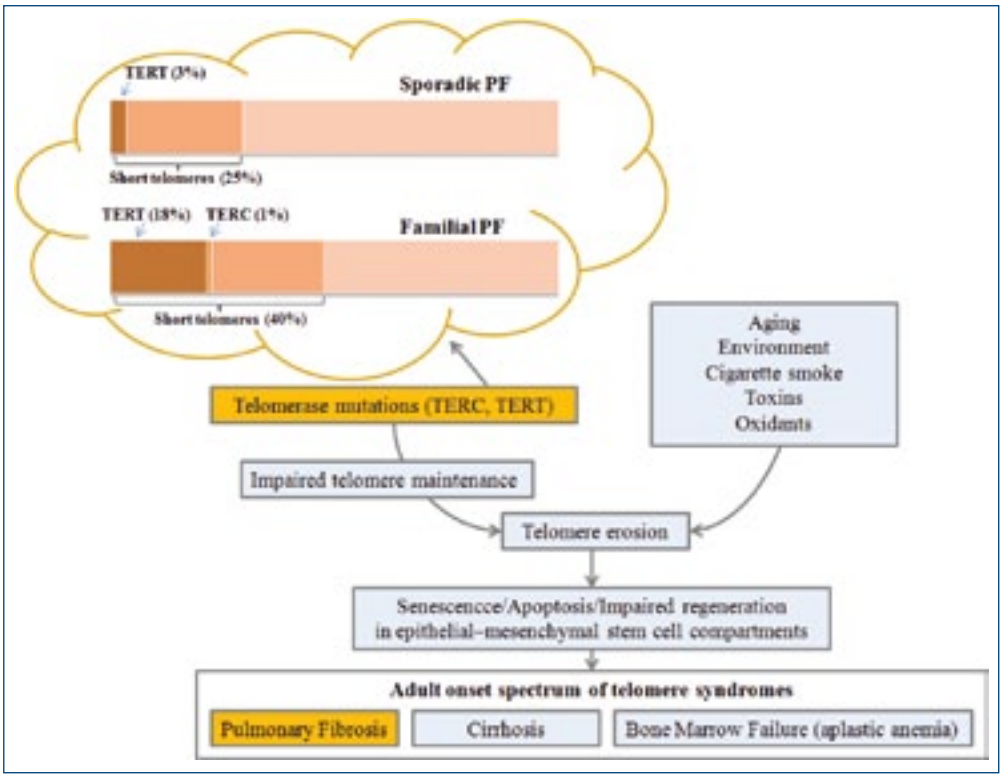

Figure 1. *Telomere shortening may be influenced by genetic, epigenetic, age-related, and environmental factors. Mutation frequencies of telomerase reverse transcriptase (TERT), telomerase RNA component (TERC) and the percentage of patients with short telomeres in familial and sporadic pulmonary fibrosis*

occurrence of the disease in two or more family members, and accounting for 5–10% of ILD cases.

Except for an earlier age of onset, familial forms are clinically and histologically indistinguishable from sporadic cases. Although usual interstitial pneumonia (UIP) dominate the interstitial spectrum and is encountered in the majority of affected families as a uniform diagnosis, about 45% of the pedigrees display radiographic and/or pathologic heterogeneity, with the presence of more than one type of ILD between members of the same family (ILD subtypes reported: usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), cryptogenic organizing pneumonia (COP), unclassified ILD)[6].

To date, genetic factors consistently associated with familial PF are mutations in surfactant protein C (SFTPC), surfactant protein A2 (SFTPA2), telomerase reverse transcriptase (TERT), telomerase RNA component (TERC), mucin 5B (MUC5B) and ELMO domain-containing 2 (ELMOD2)[7].

While mutations within SFTPC and SFTPA2 points towards epithelial cell injury by endoplasmic reticulum stress secondary to the accumulation of mutated protein precursors and the activation of unfolded protein response mechanism, mutations within TERT and TERC cause PF through shortening of telomere lengths and probable exhaustion of lung stem cells.

Telomeres are DNA–protein structures that protect chromosome ends and play a critical role in chromosome stability. Telomeres shorten successively with each cell division, and short telomeres ultimately activate a DNA damage response that leads to cell death. This biology has implicated telomere shortening in degenerative age related disease.

Telomerase is a specialized polymerase responsible for telomere elongation. Telomerase has two components that carry out the function of telomere repeat addition: the core telomerase protein, TERT, which contains the telomerase reverse transcriptase domain, and an essential RNA component, TERC, which complexes with TERT and provides the template for telomeric sequence synthesis. Heterogeneous mutations in the components of telomerase cause a loss of function in telomerase enzymatic activity that leads to accelerated telomere shortening, senescence, apoptosis and organ failure.

Mutations in the essential telomerase genes, TERT and TERC, are the causal genetic defect in up to 19% of familial PF cases and are also found in 3% of sporadic PF cases (Figure 1). The pattern of genetic transmission is autosomal dominance with variable penetrance, as some individuals with mutated genes never develop the disease.

Telomere dysfunction has a role in IPF beyond the telomerase mutations, as 22% of patients with sporadic PF have abnormally short telomere length and a small subset show features of a telomere syndrome, even when telomerase mutations are not detected[8,9,].

Telomere shortening can be acquired as multiple non-genetic factors and can impact telomere length and function. Smoking causes telomere shortening in a dose-dependent manner. Telomeres shorten with aging and this process can be accelerated by increased oxidative stress, inflammation, environmental and occupational exposures[10].

This suggests that environmental factors interact with this genetic risk factor, triggering lung injury in a tissue that is more susceptible and explain the incompletely penetrant phenotype. Modification of environmental exposures may prevent or delay the onset of disease for those who have inherited this genetic risk.

Impaired telomere maintenance spectrum disorder

The causal role of telomere maintenance defect has been associated with a range of degenerative disorders and although these disorders seem to be clinically diverse they comprise a single syndrome based on this common genetic background. Their clinical manifestations, age of onset, and severity depend on the extent of the telomere length defect.

In children and young adults, telomere-mediated disease may be recognized in the mucocutaneous syndrome dyskeratosis congenita, a rare constitutional bone marrow failure syndrome characterized by mucocutaneous abnormalities (nail dystrophy, hyperpigmentation, and leukoplakia). Patients with dyskeratosis are at increased risk for malignancies, pulmonary fibrosis, and liver cirrhosis.

In infancy severe telomere dysfunction and mutant telomerase and telomere genes have been linked to severe forms of dyskeratosis congenita: Hoyeraal–Hreidarsson syndrome (characterized by developmental delay, immunodeficiency and cerebellar hypoplasia) and Revesz syndrome (characterized by bilateral exudative retinopathy).

Impaired telomere maintenance most commonly manifests as adult onset disease and comprise a wide spectrum of clinical manifestations associated with pulmonary disease, liver cirrhosis and hematologic abnormalities[11] (Table 1).

Pulmonary fibrosis is the most common manifestation of telomere syndromes. The range of histological and clinical pulmonary manifestations reported to be associated with telomerase mutations is broad, with UIP/IPF accounting for 65% of cases. Different subtypes of ILD histologies are often diagnosed in the same mutation carrier or family[8]. The mutations in telomerase appear to increase the susceptibility to ILD in general and are not associated with one particular clinicopathologic subtype. Heterogeneous patterns of lung disease indicate that mutations in telomerase cause an increased predisposition for PF in reaction with different environmental or occupational exposures.

The majority of IPF patients with telomerase mutations cannot be distinguished clinically because they have a disease that appears limited to the lung. Mutations carriers may exhibit early preclinical signs of lung fibrosis, bone marrow dysfunction, and premature graying. Nearly 16% of self-reported unaffected family members have a preclinical form of familial PF and 8% definitely have disease after histological or radiological examination.

Features identified in asymptomatic carriers are significantly lower diffusing capacity of lung for carbon monoxide (DLCO), impaired recruitment of DLCO with exercise, radiographic signs of lung fibrosis (ranging from mild reticulation to multilobar radiographic densities and even UIP pattern), and increased fractional lung tissue volume quantified by high-resolution chest CT scan[11].

Mutations within TERT and TERC are risk factors for developing bone marrow failure of variable severity. Short telomeres are identified in about one-third of acquired aplastic anemia patients, 8% of these cases being associated with TERT or TERC mutations that cause low telomerase activity, accelerated telomere shortening, and diminished proliferative capacity of hematopoietic progenitors. Short telomeres may also cause malignant progression through genomic instability, a family history of myelodysplastic syndrome and acute myeloid leukemia often being identified in telomerase-mutant acquired aplastic anemia patients[12].

Telomere dysfunction also causes stem cell failure phenotypes in other high-turnover tissues: premature hair greying (skin), villous atrophy (intestinal epithelium), immunodeficiency and opportunistic infections (immune system)[11].

IPF, aplastic anemia and liver cirrhosis occur frequently in the same patient at different times[12] and some patients with mutations in TERT present with liver cirrhosis and IPF concurrently[11].

The co-occurrence of IPF and bone marrow failure, along with liver cirrhosis, is specific to and highly predictive of a germline telomere maintenance defect. The shared underlying telomere defect in aplastic anaemia and IPF brings together clinical entities that were previously considered to be disparate and defines a recognizable syndrome complex.

Affected individuals often have subclinical disease concurrently in other organs, even when symptoms related to a single disorder predominate. Patients with IPF who have mutant telomerase genes are at an increased risk of developing bone marrow failure and liver disease. Conversely, individuals with telomere-related aplastic anemia have an increased incidence of fatal pulmonary fibrosis when they are exposed to pulmonary toxic drugs in the bone marrow transplant set-

Table 1 Adult onset spectrum of lung, liver and bone marrow disease associated with impaired telomere maintenance

Pulmonary disease
Asymptomatic restrictive defects
Premature onset-emphysema
Interstitial lung disease
Usual interstitial pneumonia (UIP/IPF)
Nonspecific interstitial pneumonitis (NSIP)
Cryptogenic organizing pneumonia (COP)
Interstitial fibrosis (nonclassifiable histology)
Liver disease
Normal or mildly elevated transaminases
Atrophic nodular liver on imaging studies
Splenomegaly
Cryptogenic liver fibrosis/cirrhosis
Hematologic features
Macrocytosis
Elevated hemoglobin F
Isolated cytopenias (most commonly thrombocytopenia)
Clonal hematopoietic malignancy
Acquired aplastic anemia
Myelodysplastic syndrome
Acute myeloid leukemia

ting, even though they may have previously had no symptoms.

Clinical significance of short telomere lengths in pulmonary fibrosis

An important area of research concerns whether telomerase mutations and telomere lengths are associated with worse progression or with severity of the pulmonary fibrosis.

A strong association between shorter telomere length and reduction in survival has been found for patients with IPF in a recent study[13]. The association was independent for age, sex, forced vital capacity, or diffusing capacity of carbon monoxide. Short telomere length identifies a subphenotype of IPF that has an accelerated progressive course and points to the possibility that telomere length might be a determinant of IPF progression and not only a risk factor.

Interestingly, the association with differences in survival was not seen for non-IPF interstitial lung diseases, such as other idiopathic interstitial pneumonias, connective tissue disorders, sarcoidosis, hypersensitivity pneumonitis, drug-related ILD, radiation-associated ILD or sarcoidosis. This suggests a specific interaction between short telomeres and the natural history of IPF.

Lung transplantation in telomerase mutation carriers with pulmonary fibrosis

The prognostic relevance of mutations in telomerase for IPF patients who undergo lung transplantation is an active area of research.

Lung transplantation is considered for patients with severe IPF, being the only therapy that has been shown to prolong survival. After recent changes in allocation algorithms, IPF/ILD has emerged as the leading indication, accounting for one-third of lung transplantation cases.

Unless contraindications exist, patients with severe functional impairment (DLCO <39% predicted, a decrease in pulse oximetry <88% in 6-MWT), oxygen dependency, and a deteriorating course (a 10% or greater decrement in FVC in 6 months) should be listed for transplantation[14].

The International Society for Heart and Lung Transplant (ISHLT) Registry data for recipients with IPF cited lower survival rates at 3 months post lung transplantation among patients with IPF (84%) compared to cystic fibrosis (90%) and chronic obstructive pulmonary disease (COPD) (91%). Among patients surviving to 1 year, IPF and COPD had the lowest long-term survival, most likely reflecting older age and comorbidities. Most deaths following lung transplantation are due to chronic allograft rejection or complications of immunosuppressive therapy[15].

The telomere defect may contribute to the inferior post-transplant outcomes in IPF relative to other lung transplant recipients. In the lung transplant setting, patients with IPF who have telomerase mutations are at increased risk for rare toxic effects from drugs (thrombocytopenia, acute tubular necrosis) reflecting the syndromic nature of their disease, with vulnerable stem cell reserves[16]. Pre-transplant identification of telomere molecular defect may facilitate risk assessment and inform post-transplant management for this subset.

Conclusions

Short telomere lengths have pathogenic significance in a subset of patients with both familial and sporadic forms of pulmonary fibrosis. In addition to mutations in the telomerase genes, multiple non-genetic factors can impact telomere length and function such as drugs, environmental air pollution and smoking. Recognising this genetically definable subset of patients has important implications for clinical care in several settings.

Patients with pulmonary fibrosis who have short telomere defect can manifest a syndromic pattern with features that include bone marrow failure and liver disease. In the lung transplant setting, vulnerable stem cell reserves expose the patients to risk of rare toxic effects and contribute to inferior post-transplant outcomes.

Short telomeres and telomerase mutations have been proposed as biological markers to identify individuals who are susceptible to pulmonary fibrosis. Association with the worst progression of the disease suggests that short telomere length could also be a biologically relevant disease modifier. Improved prognostic information might help to guide the timing of lung transplantation referrals or allow for appropriate life planning. ■

References

1. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011; 183: 431-440.
2. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci USA* 2007; 104: 7552-7557.
3. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 2007; 356: 1317-1326.
4. Christine Kim Garcia. Idiopathic Pulmonary Fibrosis Update on Genetic Discoveries. *Proc Am Thorac Soc* 2011; Vol 8. pp 158-162.
5. Hughes EW. Familial interstitial pulmonary fibrosis. *Thorax* 1964; 19: 515-525.
6. Steele MP, Speer MC, Loyd JE, et al. Clinical and Pathologic Features of Familial Interstitial Pneumonia. *Am J Respir Crit Care Med* 2005; Vol 172. pp 1146-1152.
7. Spagnolo P, Grunewald J, Roland M du Bois. Genetic determinants of pulmonary fibrosis evolving concepts. *The Lancet Respiratory Medicine* 2014; Volume 2, Issue 5, Pages 416-428.
8. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med* 2008; 178: 729-737.
9. Alder JK, Chen JJ, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci USA* 2008; 105: 13051-13056.
10. Zhang X, Lin S, Funk WE, Hou L. Environmental and occupational exposure to chemicals and telomere length in human studies. *Occup Environ Med* 2013; Oct; 70(10): 743-749.
11. Armanios MY. Syndromes of Telomere Shortening. *Annu Rev Genomics Hum Genet* 2009; 10: 45.
12. Parry EM, Alder JK, Qi X, Chen JJ, Armanios MY. Syndrome complex of bone marrow failure and pulmonary fibrosis predicts germline defects in telomerase. *Blood* 2011; 117: 5607-5611.
13. Stuart BD, Lee JS, Kozlitina J, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *The Lancet Respiratory Medicine* 2014; Volume 2, Issue 7, Pages 557-565.
14. Silhan LL, Orens JB. Update to the guidelines for selection of lung-transplant candidates. *Curr Respir Care Rep* 2014; 3:103–111
15. Christie JD, Edwards LB, Aurora P, et al. Registry of the international society for heart and lung transplantation: twenty-fifth official adult lung and heart/lung transplantation report-2008. *J Heart Lung Transplant* 2008; 27(9): 957–969.
16. Silhan LL, Shah PD, Chambers DC, et al. Lung transplantation in telomerase mutation carriers with pulmonary fibrosis. *Eur Respir J*. 2014; Jul; 44(1): 178-187.