Dysfunctional telomeres and dyskeratosis congenita

Monica Bessler, Hong-Yan Du, Baiwei Gu, Philip J. Mason
Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA.
E-mail: mbessler@im.wustl.edu

Telomeres are specialized nucleoprotein structures at the end of chromosomes that protect the chromosomes from end-to-end fusion, degradation, and inappropriate recombination.[1,2] In most eukaryotes, telomeric DNA is composed of G-rich repeated sequences that are synthesized by the telomerase ribonucleoprotein complex whose integral RNA component, the telomerase RNA or *TERC* RNA, contains the sequences that act as a template for the synthesis of these repeats. Because DNA polymerase I cannot copy the extreme end of a DNA strand, telomeres get shorter with each cell division.[3] When telomeres become critically short cell cycle arrest or cell death occurs.[4] It is, therefore, thought that telomere length may act as a molecular clock that regulates the life span of a cell.[5] Telomerase activity counteracts the continuous telomere shortening caused by cell replication.[6] However, in humans telomerase activity is not observed after birth in most somatic cells, or occurs only at very low levels. In contrast, germ cells, stem cells and their immediate progeny, activated T cells, monocytes, and notably most cancer cells express telomerase activity, but only in germ cells and cancer cells is telomerase activity sufficient to prevent telomere shortening.[4,7]

Telomere length is highly variable among different species, but within an individual species the number of telomeric repeats is usually maintained within a well-defined range. In humans telomeres shorten with age.[8,9] In peripheral blood cells rapid telomere shortening occurs within the first year of life, followed by a more gradual decline over time.[10] Optimal telomere length setting during stages when telomerase is expressed is crucial for long-term survival of the somatic cells that lack telomerase expression, as telomeres must be sufficiently long to prevent premature cell senescence, but short enough to induce cell cycle arrest in cells that have lost normal growth control before they become cancer cells.

Dyskeratosis congenita

Dyskeratosis congenita (DC) is the first human disease whose pathogenesis has been directly linked to an impairment of telomere maintenance.[11-13] DC is clinically and genetically heterogeneous. Patients with DC typically present with progressive bone marrow failure and the classical triad of mucocutaneous features including abnormal pigmentation, dystrophic nail changes, and leukoplakia of the buccal mucosa.[14] However, other somatic abnormalities may occur, including epiphora caused by the blockage of the tear duct, early graying of the hair, premature tooth loss, enteropathy and diarrhea, pulmonary fibrosis, liver cirrhosis, osteoporosis and avascular necrosis of the bone, testicular atrophy, learning difficulties, mental retardation, and cerebellar ataxia caused by cerebellar atrophy.[15] Mutations in three different genes have been identified in patients with DC - *DKC1, TERC*, and *TERT*. The products of these genes, dyskerin encoded by *DKC1*, the RNA component of telomerase, *TERC*, and the catalytic component of telomerase, TERT, form the catalytically active telomerase (Figure 1).[16]

It is thought that telomere length rather than impaired telomerase activity is responsible for disease in patients with DC. Indeed, all patients with DC and clinically relevant disease have very short telomeres.[13] Interestingly, however, the severity of disease, the age of onset, and the spectrum of clinical manifestations vary with the gene mutated and the nature of the mutation responsible for the disease.

X-linked DC and dyskerin

DKC1 maps to the X chromosome. Pathogenic mutations in *DKC1* cause disease in all male members of the affected family with the mutation, whereas females who carry the mutation show no or only mild disease. Female carriers of *DKC1* mutations nearly always show 100% skewing of X-chromosome inactivation such that the chromosome carrying the mutated *DKC1* allele is inactivated in all cells.[17] Although the age of onset and the severity of disease may vary, by the age of 30 years more than 90% of males with *DKC1* mutations show signs of bone marrow failure and at least one of the cutaneous features typical of the disease. Dyskerin is essential for the nuclear accumulation of telomerase RNA *TERC*.[11] Dyskerin mutant cells have greatly reduced levels of *TERC* RNA and it is thought that the reduction in *TERC* levels leads to the inability of telomerase to maintain telomere length.[18]

Hoyeraal-Hreidarrson syndrome (HH - MIM 300240) is a rare variant of DC that presents in early childhood and is characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, mental retardation, progressive combined immune deficiency, and aplastic anemia. Mutations in the *DKC1* gene have been identified in some, but not all, patients with HH. HH patients have very short telomeres at the time of diagnosis and it is thought that HH is a very severe variant of DC.[19]

Dyskerin, in addition to its role in telomere maintenance also has a function in ribosome biogenesis in which it is the catalytic component in ribonucleoprotein

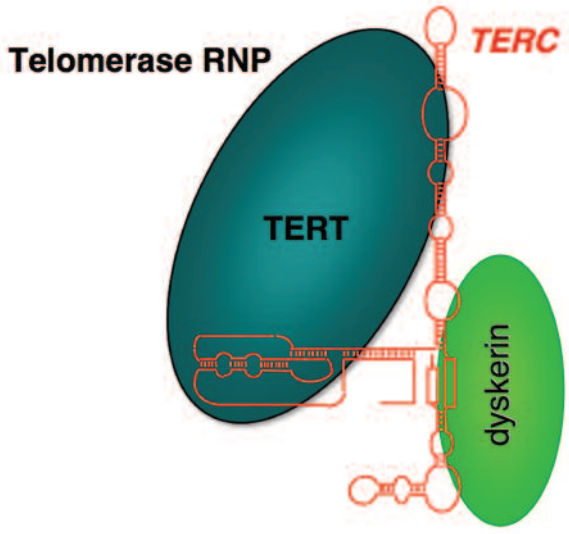

Figure 1. Components of the catalytically active telomerase ribonucleoprotein complex include telomerase TERT, the telomerase RNA TERC, and dyskerin. Mutations in one of the three components of active telomerase lead to the clinical disease of dyskeratosis congenita.

particles responsible for pseudouridylation of uridine residues in ribosomal RNA (rRNA).[20] Pseudouridylation of rRNA is an essential step in the early stages of ribosome biogenesis. Indeed, *DKC1* null mutations are lethal in early embryonic mice.[21] Moreover, defects in ribosome biogenesis have been demonstrated in mouse cells with *Dkc1* mutations that are pathogenic in humans.[22] However, whether and to what extent defects in ribosome biogenesis or ribosome function contribute to the pathogenesis of X-linked DC is controversial.[22,23]

Autosomal dominant DC

In families with DC due to *TERC* or *TERC* gene mutations the disease usually follows an autosomal dominant pattern of inheritance.[24,5] Haploinsufficiency is thought to be the mechanism of disease. Typically, disease in individuals with *TERC* or with *TERT* gene mutations is often much milder than in individuals with *DKC1* gene mutations, and the onset of disease manifestations is often later in life. Furthermore, in some families not all family members carrying the mutation have clinical disease, indicating a variable penetrance of disease in *TERC* and *TERT* gene mutation carriers. Variable disease penetrance in some families results in sporadic disease or mimics the inheritance pattern of autosomal recessive inheritance.[19] In addition to disease penetrance, the expressivity of disease is also highly variable in *TERC* and *TERT* gene mutation carriers. Mucocutaneous manifestations, characteristic of the X-linked disease, are often absent or very mild, whereas bone marrow failure, pulmonary fibrosis, and liver cirrhosis are more prominent and in some families may be the only clinical manifestations of disease.[26,27]

Whether individuals without muccocutaneous manifestations should still be diagnosed as having *dyskeratosis congenita*, a name that refers to the presence of the classic cutaneous features, is currently a matter of controversy. However, because premature telomere shortening is the common pathogenetic pathway of disease for individuals with *DKC1, TERC* or *TERT* gene mutations, clinical disease in the absence of mucocutanous features is usually referred to as *atypical DC*. Interestingly, myelodysplastic syndrome (MDS) and acute myeloid leukemia, which are rare in the X-linked form, appear to be more frequent in individuals with DC due to *TERT* or *TERC* gene mutations, suggesting that a lag period might be needed to allow malignant transformation. Thus, malignant transformation is more likely to occur in individuals with mild disease and a longer survival than in individuals with severe disease and death in childhood or early adolescence.

Anticipation

A unique feature of DC due to *TERT* and *TERC* gene mutation is genetic anticipation,[28,29] which means an inherited disease manifests at increasingly younger ages and/or with increased severity with each succeeding generation. Thus, if the offspring of patients develop the disease, they will tend to do so at an earlier age and display more severe clinical manifestations than their parents. The inheritance of increasingly shorter telomeres with subsequent generations is thought to be the molecular basis of disease anticipation in *TERC* and *TERT* gene mutation carriers. According to this model the disease is caused by the inheritance of a mutated *TERC* or *TERT* gene and the inheritance of short telomeres. Anticipation has been elegantly demonstrated in mice lacking telomerase activity.[30,31] Several generations of inbreeding is necessary to shorten telomeres to the extent that they cause disease. Disease anticipation has also been demonstrated in several families with DC due to *TERC* gene mutations and in one family with DC due to a *TERT* gene mutation.[28,29,32] In all patients the *TERC* or *TERT* gene mutations are always inherited, but the number of generations necessary before the mutations lead to telomeres sufficiently short to cause disease is unknown.

In this issue of the Journal Marrone and colleagues describe two patients with two different *TERC* gene mutations and demonstrate that these have most likely occurred *de novo*.[33] Both mutations are likely to be responsible for disease as both significantly impair *in vitro* telomerase activity and *in vivo* are associated with short telomeres. Why a *TERC* mutation leads to telomeres short enough to cause disease in these two patients is unclear. In both cases the unaffected parent has telomere length within the normal age-dependent distribution, which excludes the inheritance of preshortened telomeres. It is possible that both individuals experienced some sort of injury either during embryonic

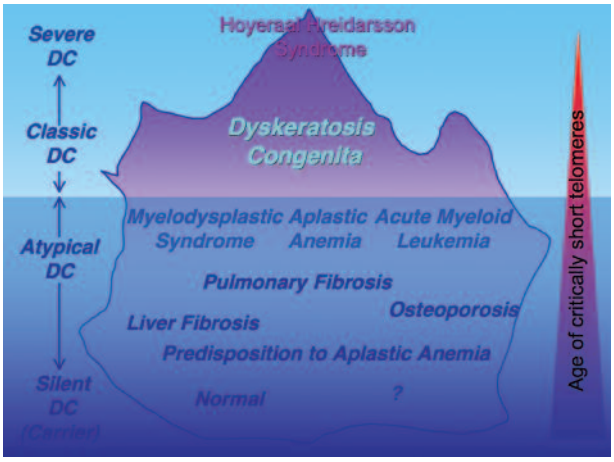

Figure 2. Telomere hypothesis in dyskeratosis congenita. Penetrance and expressivity of disease are highly variable in patients with DC. The severity of disease may vary from causing death in early infancy to a clinically normal appearance. Premature telomere shortening is thought to be the underlying mechanism of disease. We propose that the time point when telomeres become critically short greatly determines the clinical picture of disease. According to this model, in HH telomeres become critically short early in life, thus affecting intrauterine development of the entire fetus, in particular also the brain, bone marrow, immune system, and gut. In classic DC telomeres become critically short during childhood and adolescence, thus clinical features present in the bone marrow, skin and fingernails. In atypical DC, telomeres become critically short in adults, thus mainly the bone marrow and alveolar epithelium are affected.

development or during the development of definitive hematopoiesis necessitating an increased replication of the surviving cells, which, due to an impaired telomerase activity, would result in a shorter telomere length setting in cells that become telomerase negative and a more rapid telomere exhaustion in these cells compared to that in individuals with only the *TERT* gene mutation but no additional injury. Alternatively, despite the lack of a dominant negative effect of the *TERC* mutation on telomerase activity *in vitro*, the mutations that occur within two base pairs of each other (nt 178 and nt 180, see Marrone *et al.*)[33] may be associated with accelerated telomere shortening by a mechanism that remains to be determined. Indeed there may be a dominant negative effect on telomere maintenance that is not detected in the *in vitro* assay. Rapid disease progression would suggest accelerated telomere shortening, whereas a more protracted progression would support the hypothesis of prenatal injury. Longitudinal monitoring of telomere length would provide further valuable insights into the dynamics of telomere length and the impact of the environmental and genetic factors that modulate the disease manifestation and disease severity in individuals with DC.

With the availability of molecular diagnostics our perception of DC has changed dramatically. Previously, the diagnosis was dictated by the clinical appearance and restricted to individuals with severe disease and the classical clinical manifestations. Today, we know that these individuals only represent a small proportion of patients with DC, the tip of an iceberg whose size is now beginning to be appreciated. Patients with atypical DC are probably much more frequent than currently realised due to the atypical presentation causing misdiagnosis. We propose that the time point when telomeres become critically short strongly affects the clinical picture and severity of disease (Figure 2). Most of what we know about DC comes from previous studies that are biased towards patients with more severe disease and the classic clinical presentation. To what extent the course of disease, prognosis, and response to treatment differs between patients with classic DC and patients with atypical DC will be the focus of future studies.

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