Dyskeratosis congenita (DC) is an inherited BM failure disorder that is associated with mutations in genes involved with telomere function and maintenance; however, the genetic cause of many instances of DC remains uncharacterized. In this issue of the JCI, Tummala and colleagues identify mutations in the gene encoding the poly(A)-specific ribonuclease (PARN) in individuals with a severe form of DC in three different families. PARN deficiency resulted in decreased expression of genes required for telomere maintenance and an aberrant DNA damage response, including increased levels of p53. Together, the results of this study support PARN as a DC-associated gene and suggest a potential link between p53 and telomere shortening.

Dyskeratosis congenita genes

Dyskeratosis congenita (DC) was first described in 1910 in two brothers who presented with skin pigmentation anomalies, leukoplakia, and nail dystrophy in childhood (1). Anemia later emerged as a common DC feature, and autosomal dominant, recessive, and X-linked recessive forms of inheritance for this disease are now recognized. In the 1990s, the X-linked DC locus was mapped to chromosomal region Xq28 (2, 3), and DKC1, which encodes the 57 kD protein dyskerin, was identified in 1998 as the causative gene within Xq28 (4). Dyskerin is homologous to the well-characterized yeast protein Cbf3p, which is a pseudouridine synthase that acts on ribosomal RNA and spliceosomal small nuclear RNA (snRNA) (5, 6). Following the discovery that dyskerin was also part of the telomerase complex in vertebrates (7) — and the identification of mutations in telomerase RNA (8) and telomerase reverse transcriptase (TERT) (9, 10) as causes of autosomal dominant DC — dysfunctional telomere maintenance was recognized as the basis for the development of DC. Moreover, patients with DC generally have very short telomeres at presentation. In the last decade, seven additional genes have been linked to DC, making a total of ten known DC-causing genes (11), all of which encode products that are involved in telomere maintenance. Some of these products — including TERT, the telomerase RNA component (TERC), dyskerin, and ribonucleoproteins NOP10 and NHP2 — are components of the telomerase complex. TCAB1 is important for telomerase-complex assembly and trafficking. Mutations in TINF2 and ACD — genes that encode components of the shelterin complex, which protects telomeres from degradation or from being recognized by the DNA damage machinery — are also associated with DC (12). Still other DC-associated genes, such as CTS telomere maintenance complex component 1 (CTCI) and regulator of telomere elongation helicase 1 (RTEL1), are necessary for telomere replication. Patients with classical DC and other inherited forms of the disease can present with a wide range of features in addition to the classic mucocutaneous manifestations and BM aplasia. Pulmonary fibrosis, liver cirrhosis, enteropathy, and a variety of cancers — including leukemia — are also commonly observed in these individuals (13).

Poly(A)-specific ribonuclease (PARN) is a new DC gene

In this issue, Tummala et al. identify a DC-associated gene that has no known connection with telomere metabolism (14). Specifically, Tummala and colleagues performed whole exome sequencing on a cohort of patients with severe DC, which is classified as Hoyeraal Hreidarsson syndrome (HHS) and manifests at an early age with immunodeficiency, intrauterine growth deficiency, and cerebellar hypoplasia. Their analysis revealed the presence of mutations in PARN, which encodes a poly(A)-specific ribonuclease (15). In 22 unrelated individuals, biallelic mutations in PARN that were likely to impair function were identified in three families. In these families, the affected children all presented with the HHS phenotype, and statistical analysis suggested that this was extremely unlikely to have occurred by chance, indicating that mutations in PARN underlie these cases of HHS. In one family, a brother and a sister were both homozygous for a missense mutation that results in an A to V substitution at amino acid 393 (PARN<sup>393Val</sup>), which is in a conserved N-terminal domain of the protein that is essential for nuclease activity. In a second family, the individual with HHS was homozygous for a point mutation that abolishes a donor splicing site in the PARN transcript, and analysis of mRNA in blood from this patient revealed an absence of properly spliced PARN RNA and the presence of two different PARN transcripts, one of which was missing one exon and the other two exons. In a third family, the affected child was a compound heterozygote at the PARN locus and harbored one allele with a single base insertion that resulted in a frameshift and one allele with an insertion in a donor splice site that was predicted to abolish splicing. Moreover, compared to healthy, age-matched controls, affected individuals in two of the families had substantially shorter telomeres. Telomere length was not measured in the affected individual from the third family. Together, the identification of PARN mutations in multiple fami-
lies with cases of HHS and the observed decrease in telomere length in affected individuals strongly support PARN as a DC-associated gene.

It is not clear how PARN would affect telomere maintenance. PARN is a poly(A)-specific deadenylase that regulates gene expression by shortening poly(A) tails on transcripts, thereby decreasing their stability. Recent studies indicate that PARN activity is differentially regulated under various cellular conditions (16, 17). Tummala and colleagues demonstrated that cells from individuals with PARN mutations had reduced deadenylation activity, which should affect transcript stability. While PARN has been extensively studied at the biochemical level, the range of mRNA substrates and the physiological relevance of PARN-targeted transcripts has not been fully characterized. Tummala and colleagues compared expression of genes involved in telomere maintenance in the blood of healthy controls and individuals with PARN mutations. They found decreased expression of several key regulators of telomere metabolism, including DKCI, TERFI, RETL, and TERC. The same transcripts were also decreased in HEK293T cells treated with PARN siRNA (14).

p53: guilty by association?

Tummala et al. also observed that PARN-deficient patient cells exhibit an abnormal DNA damage response, including increased cell death and an increase in p53 following UV exposure (14). PARN has recently been shown to participate in positive and negative feedback loops that mediate p53 and mRNA processing (18). In nonstressed conditions, PARN deadenylates and destabilizes p53 mRNA through recognition of a sequence element in the 3′UTR. Under DNA damaging conditions, such as UV exposure, p53 accumulates, associates with, and activates PARN by forming a complex that includes cleavage stimulation factor 1 (CSTF1). Based on this model, a DNA damage–associated increase in p53 would subsequently be kept in check by PARN-dependent destabilization of p53 mRNA; therefore, loss of PARN activity would lead to an increase in p53 mRNA levels. Activation of p53 has been observed in a variety of BM failure syndromes, including Fanconi anemia (19, 20), Diamond Blackfan anemia (21), and even some forms of DC (22, 23). Recently, Simeonova et al. (24) described a strain of mice that is homozygous for a Trp53 allele that results in production of a truncated p53, which lacks 31 C-terminal amino acid residues. In these animals, both the truncated p53 and transcripts of p53 targets Cdkn1a, which encodes p21, and oncogene Mdm2 were increased. Most of these mice died 14–43 days after birth and exhibited DC-associated features, including BM failure, dark skin, cerebellar hypoplasia, and pulmonary fibrosis. Transcript analysis in embryonic fibroblasts from these animals also revealed a decrease in transcripts associated with telomere maintenance, including Dkci, Retl, Tinfl2, and Terfl, which overlap with some of the telomere-associated genes Tummala et al. (14) determined to be downregulated in PARN-deficient cells.

Together, these studies suggest a potential mechanism for the development of DC-like phenotypes in the chronic absence of PARN (Figure 1). In rapidly dividing PARN-deficient cells, p53 levels would rise in response to an accumulation of DNA damage. The lack of PARN deadenylase activity would allow accumulation of p53 mRNA and further increase protein levels. Elevated p53 would, in turn, decrease transcription of genes required for telomere maintenance, with a subsequent shortening of telomeres. A novel feature in this model is the regulation of p53 at the mRNA level, whereas p53 is typically regulated at the level of protein stability. Such a mechanism would result in a cycle that enhances telomere shortening and increases p53, thereby inducing failure of tissues that are renewed via stem cell expansion. More work will need to be done to validate the involvement of the p53 pathway in aberrant telomere maintenance in PARN-associated DC; however, if correct, this model would provide an alternative pathway by which aberrant p53 activation contributes to the development of disease manifestations such as BM failure. Further investigation of the relationship between telomere metabolism, p53, and PARN may provide crucial information about BM failure and the prevalence of leukemic transformation and other forms of cancer in these disorders (13).
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