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Extra View

Dyskerin, telomerase and the DNA damage response

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The bone marrow failure syndrome Dyskeratosis congenita (DC), though rare, has attracted a great deal of attention in the last few years because it is caused by mutations in genes whose products are involved in telomere maintenance. The disease presents with a variety of features that can all be due to failure of tissues that require constant renewal via stem cell activity. It is thought this is caused by defects in telomere maintenance leading eventually to cell cycle arrest or cell death caused by critically short telomeres. The most common form of DC is the X-linked form caused by mutations in *DKC1* encoding the nucleolar protein, dyskerin. We recently reported a mouse model of the X-linked form of the disease in which females heterozygous for a mutation that copies a human pathogenic mutation showed a growth disadvantage in cells expressing the mutant dyskerin. This growth disadvantage, which was associated with an enhanced DNA damage response, was dependent on telomerase but appeared to be independent of telomere shortening. Here we discuss these results in terms of the role of dyskerin in telomere maintenance and the possible role that the DNA damage response plays in the pathogenesis of DC.

Introduction

Telomere homeostasis is important in cancer, where nearly all tumors require increased levels of telomerase to counteract the telomere shortening that takes place with each cell division.[1] Telomere maintenance may also be important in aging, since telomere shortening in somatic cells, which express no or little telomerase, means that these cells have a finite number of cell divisions before critically short telomeres drive the cells into senescence in a process known as replicative aging.[2,3] The role of telomere shortening in organismal aging is controversial. In dyskeratosis congenita we have the opportunity to observe the effects of defective telomere maintenance in humans, and the role of telomere maintenance in cancer and aging.

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The Genetics of Dyskeratosis Congenita

Dyskeratosis congenita is genetically diverse with autosomal recessive and dominant and X-linked pedigrees having been described (Table 1). The major X-linked form is due to mutations in the *DKC1* gene which encodes the 57 kD protein dyskerin.[4] Dyskerin is a highly conserved nucleolar protein that, as part of a specialized nucleolar RNP, catalyses the pseudouridylation of specific residues in newly synthesized ribosomal RNAs and spliceosomal snRNAs.[5] Dyskerin also associates with telomerase,[6] and since products of 4 other genes whose mutation causes DC also associate with telomerase it is thought that telomerase defects underlie the DC phenotype. These other genes are *NOP10*,[7] *NHP2* (recessive),[8] *TERC*[9] and *TERT*[10] (dominant). NOP10 and NHP2 are associated with dyskerin in both the RNP complex and in telomerase while TERC is the telomerase RNA template and TERT is the telomerase reverse transcriptase. A sixth gene causing DC, *TINF2*,[11,12] encodes a protein TIN2,[13] that is not part of telomerase but is one of the protein components of shelterin, a protein complex associated with telomere DNA at the ends of chromosomes.[14]

Molecular Pathology

Dyskeratosis congenita, though classically defined by a triad of mucocutaneous features (skin pigmentation abnormalities, leukoplakia and nail dystrophy) usually involves bone marrow failure which is often the cause of death. It is always associated with very short telomeres that are shorter than the first percentile of telomere lengths seen in the normal population.[15] DC mutations have also been found in patients with aplastic anemia[16] and pulmonary fibrosis[17,18] leading to a less restricted definition of the disease. Expression of the disease, which correlates to some extent with the gene mutation, (Table 1) varies between very severe presentation in children below 1 to mild anemia in old age. It is generally accepted that the defects in DC are caused by increased rates of telomere shortening in adult stem cells leading to failure of cell renewal and thereby causing bone marrow failure, pulmonary fibrosis and the other manifestations of DC, which include an increased susceptibility to cancer. In X-linked DC there is good evidence that the mutations in dyskerin lead to destabilization of TERC and a decrease in telomerase activity.[19] In this X-linked condition female carriers of the disease usually show 100% skewing of X-inactivation, at least in white blood cells.[20,21] At an early stage of embryonic development in female placental mammals one X-chromosome is inactivated in every cell. It is random whether in any given cell the

Table 1 **Characteristics of genes mutated in dyskeratosis congenita**

Gene	Gene product	Chromosomal location	Function	Types of mutation	Clinical presentation	References
DKC1	Dyskerin	Xq28	Pseudouridine synthase. Component of H/ACA RNP and telomerase complex	Mainly mis-sense. Common recurrent mutation 40% of X-linked cases is A353V. Null mutations probably lethal	X-linked recessive. Dyskeratosis congenita (DC) varying in severity. The more severe variant Hoyeraal-Hreidarrson syndrome (HH) characterized by intra-uterine growth retardation, immune deficiency and cerebellar hypoplasia.	4, 45
TINF2	TIN2—TRF1 interacting factor 2	14q12	Component of shelterin complex at telomeres	Mainly missense clustered at aa 280–291 with two thirds affecting residue 282. Some frameshifts.	Autosomal dominant. Usually sporadic. DC, HH, AA and Revesz syndrome, severe bone marrow failure with retinopathy. Nearly always severe.	11, 12
TERC	Telomerase RNA component	3q21-q28	RNA component of telomerase—provides template for synthesis of telomere repeats	All types, deletions, point mutations etc.	Autosomal dominant. Wide variety of presentation including DC, aplastic anemia, pulmonary fibrosis, MDS. Variable penetrance.	16–18, 45, 46
TERT	Telomerase reverse transcriptase	5p15.33	Telomerase reverse transcriptase—synthesizes telomere repeats	All types, deletions, point mutations etc.	Autosomal dominant. Rarely autosomal recessive. Wide variety of presentation including DC, aplastic anemia, pulmonary fibrosis, MDS. Variable penetrance.	10, 17, 18, 43, 45, 47–49
NOP10	NOP10—Nucleolar protein 10	15q14-q15	Component of H/ACA RNP and telomerase complex	1 missense mutation described in 1 family with 3 homozygous affected siblings	Autosomal recessive. Mild dyskeratosis congenita, low penetrance of aplastic anemia.	7
NHP2	NHP2—Non-histone chromosome protein 2	Chr 5	Component of H/ACA RNP and telomerase complex	2 patients have been described—1 homozygous for a missense mutation and the other compound heterozygous for a missense and a nonsense mutation.	Autosomal recessive. Both patients had classical dyskeratosis congenita	8

paternal or maternal X is inactivated.[22,23] Thereafter in carriers of a *DKC1* mutation there will be 2 types of cell, one with wild type and one with mutant dyskerin, and cells with wild type dyskerin must outgrow those with mutant dyskerin leading to only one type of cell in the adult.

A Mouse Model of X-linked DC

We have made a mouse model of X-linked DC[24] by recreating in the mouse a mutation that causes DC in an X-linked family,[25] a deletion of exon 15 that leads to a truncation of the dyskerin protein by 22 amino acids in human and 21 amino acids in mouse. This deletion had the advantage that we were able to distinguish between the wild type and mutant protein on Western blots. Male mice carrying the deletion, *Dkc1*$^{\Delta 15}$, did not show any obvious phenotype but in female heterozygous mice, *Dkc1*$^{\Delta 15/+}$, expression of the two proteins was unequal in older mice with greater expression of the wild type protein. We reasoned that this was most likely due to a growth advantage enjoyed by the cells expressing wild type dyskerin after random X-inactivation. This was supported by the fact that

the differences in expression at the protein level were reflected in differences in mRNA levels. The growth advantage was greater in hematopoietic tissues such as spleen and bone marrow but was also evident in less proliferative tissues such as liver. Part of the rationale for our experimental approach was that mice would not be expected to show any phenotype due to telomere shortening in the first generation. This was based on the observations that mice that are null for telomerase RNA, (*Terc*$^{-/-}$)[26] or telomerase reverse transcriptase (*Tert*$^{-/-}$)[27] do not show a phenotype at first but after inbreeding for several generations show a phenotype with similarities to human DC. This is because laboratory mice have very long telomeres and several generations in the absence of telomerase are required before telomeres reach a critically short length. Surprisingly, however, when we tested *Dkc1*$^{\Delta 15/+}$*Terc*$^{-/-}$ or *Dkc1*$^{\Delta 15/+}$*Tert*$^{-/-}$ mice no growth advantage was evident. The growth advantage was also partially rescued in *Dkc1*$^{\Delta 15/+}$*p53*$^{-/-}$ mice. Together these results suggested that cells with the *Dkc1*$^{\Delta 15}$ mutation exhibit slower growth than wild type cells and that this was dependent on telomerase, was independent of telomere length and was mediated, at least in part via p53.

Mutant Dyskerin Affects the DNA Damage Response

The involvement of p53 in the reduced growth of cells with a mutant dyskerin implicates a DNA damage responsive cell cycle checkpoint in the phenomenon and indeed male $Dkc1^{\Delta15}$ MEF cells were shown to have an enhanced DNA damage response following etoposide treatment. Both wild type and mutant cells showed an increase in DNA damage foci containing γ-H2AX, a phosphorylated histone that appears rapidly at sites of DNA damage,[28] especially double stranded breaks. Furthermore many of the DNA damage foci co-localized with telomeres in the mutant but not the wild type cells.

These findings, implicating a hitherto unknown pathway, raise many new questions, in particular:- What is the role of dyskerin and telomerase in the DNA damage response? Is the reduction in growth and increased DNA damage response important in the pathogenesis of X-linked DC?

Dyskerin and Telomerase in the DNA Damage Response Pathway?

Telomere maintenance and the DNA damage response are closely connected with many of the proteins involved in the DNA damage response also playing important roles in telomere maintenance.[29] It is well known that critically short telomeres induce a p53 dependent DNA damage response that, if unresolved, may lead to cell cycle arrest and senescence or apoptosis.[30] Indeed the accepted view of the pathogenesis of DC is that defects in telomerase lead to accelerated telomere shortening and eventually to short telomere induced senescence/apoptosis in stem cells. As well as telomere length defective telomere structure may also cause a DNA damage response.[31] Moreover a transient DNA damage response is thought to occur at telomeres during replication.[32] Since telomere elongation and replication are presumably tightly coupled[33] it is likely that defects in elongation may enhance this response. Although dyskerin is thought to be involved chiefly in the assembly of the functional telomerase complex its presence in purified active telomerase[34] may suggest it plays an important role in telomerase action. On this model mutant dyskerin may affect the access of telomerase to its substrate or the efficiency of telomerase activity and lead to a dysfunctional telomere.

Another possibility is that telomerase has a cellular function not dependent on telomere length and it is this function that is affected by mutant dyskerin, leading to the DNA damage response. Suggestions that telomerase has a telomere length independent function in mice arise from observations that overexpression of telomerase can promote tumorigenesis in mice with perfectly long telomeres[35,36] and conversely that lack of telomerase can suppress chemically induced carcinogenesis in long telomere mice.[37] In addition in human cells hTERT was shown to contribute to tumorigenesis in the absence of any effect on telomere length maintenance.[38] The mechanism of this length independent role of telomerase and its connection with DNA damage at telomeres remains to be determined.

Possible Importance of the DNA Damage Response in the Pathogenesis of X-Linked DC

Is the induction of the DNA damage response an important factor in the pathogenesis of dyskeratosis congenita? In the $Dkc1^{\Delta15/+}$ heterozygous female mice the induction of the DNA damage response was associated with decreased proliferation. However no DC like phenotype was observed in male mice suggesting that the lower proliferation rate is not sufficient to impact on tissue renewal, at least in one generation. The assay we use to assess comparative proliferation rate, namely competitive growth of cells in a female mosaic after random X-inactivation may be very sensitive. Indeed if 32 cell divisions are required for the growth[39] of an early embryo into an adult mouse then a 5% increase in the time between cell divisions will lead to an approximately 80% reduction in the number of the slower growing cells. Such a small reduction in growth rate, while easy to see in the competitive situation, may not affect the growth and development of males. Another factor here may be differences between mouse and humans in the detailed mechanisms of both telomere maintenance[40,41] and stem cell function.

Of the six genes so far identified as causing DC, two of them—NOP10[7] and NHP2[8]— have been found to be very rare and present in one or a handful of families. The others fall into two groups in terms of presentation and penetrance. Mutations in TERT[10] and TERC[15] have less than 100% penetrance and generally show anticipation with increased severity and earlier age of onset in later generations.[42,43] This is likely due to the fact that later generations inherit shortened telomeres from the affected parent as well as the pathogenic mutation. Mutations in DKC1,[4] and TINF2,[11,12] however exert their effects in generation 1 where they usually cause severe aplastic anemia and dyskeratosis congenita of varying severity, with TINF2 mutations perhaps being on average more severe. The difference between these two groups may be that TERC and TERT mutations lead to telomere shortening with no consequences until a critically short telomere length is reached whereas DKC1 and TINF2 mutations in addition cause cell death via the p53 DNA damage pathway due to telomere defects independent of telomere length and thus exert their effects in generation 1. Supporting this idea is our finding of a DNA damage response in mice with a pathogenic Dkc1 mutation.[24] While the TINF2 pathogenic mutations have not yet been shown to elicit a DNA damage response at telomeres other TIN2 mutations, that destabilize shelterin components TRF1 and TRF2 do induce DNA damage.[44] All DC patients however have very short telomeres but whether short telomeres are always causative of the disease or are a consequence of increased replicative telomere erosion is not clear. Thus it is possible that the DNA damage caused by these mutations contributes to bone marrow failure by leading to increased rate of recruitment of stem cells and eventual stem cell exhaustion (Fig. 1).

Summary and Future Prospects

In summary the finding of an increased DNA damage response in mice with a pathogenic mutation in Dkc1 has important implications for the role of dyskerin in telomere maintenance. Coupled with the recent finding of DC due to TINF2 mutations it suggests that failure to maintain telomere integrity in DC may arise by more than one pathway. It will now be important to work out precisely how dyskerin and TIN2 interact with other components of telomerase and of shelterin to bring about the exquisite control of telomere homeostasis needed to strike the essential balance between aging and malignancy.
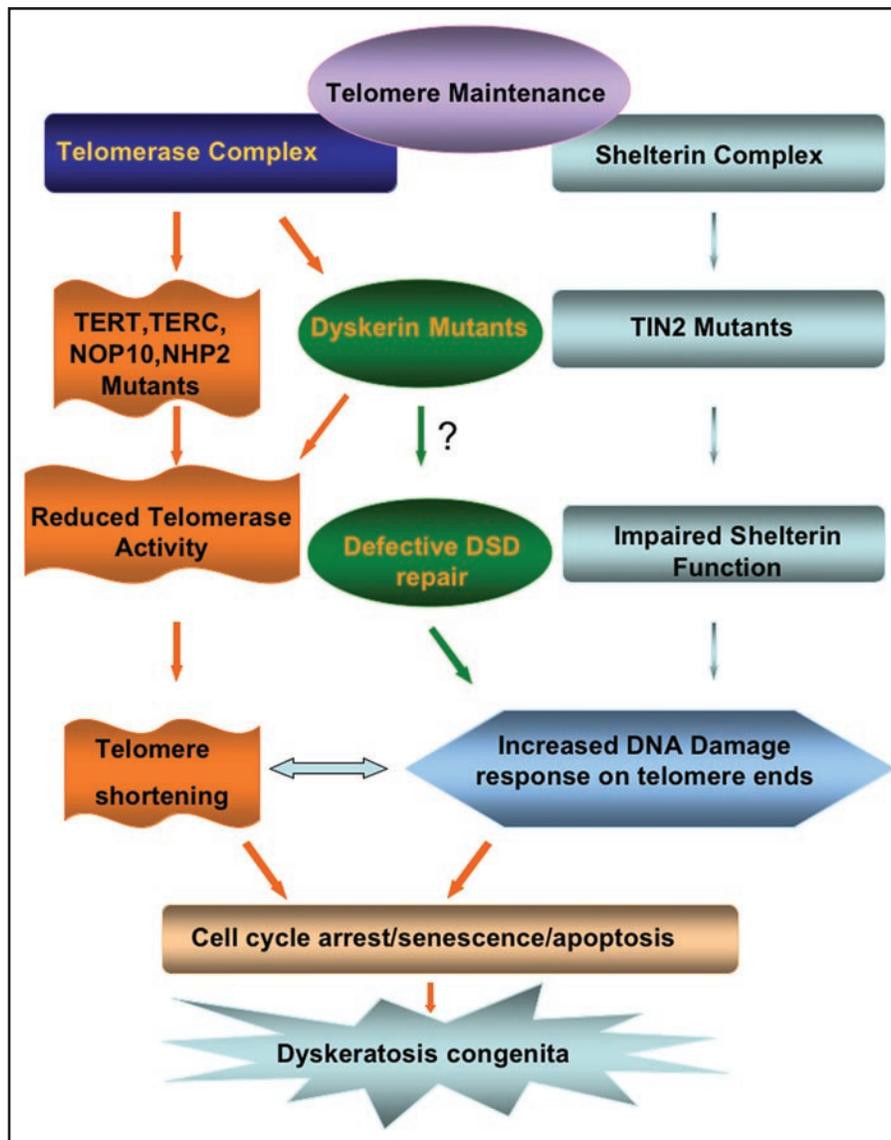
Figure 1. Model of pathogenesis of dyskeratosis congenita.

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