Telomerase dysfunction and dyskeratosis congenita

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Abstract

Dyskeratosis congenita (DC) is a multi system bone marrow failure syndrome characterized by mucocutaneous abnormalities and an increased predisposition to malignancy. It exhibits considerable clinical and genetic heterogeneity. X-linked recessive, autosomal dominant and autosomal recessive forms of the disease are recognized. The X-linked recessive form is due to mutations in dyskerin, which is a component of both small nucleolar ribonuclear protein particles and the telomerase complex. Autosomal dominant DC is due to mutations in the RNA component of telomerase, *TERC*. As dyskerin and *TERC* are both components of the telomerase complex and all patients with DC have short telomeres it appears that the principal pathology in DC relates to telomerase dysfunction. The gene or genes involved in the recessive form of DC remain elusive, though genes whose products are required for telomere maintenance remain strong candidates. The study of DC has highlighted the critical role of telomerase and the consequences, including premature aging and malignancy, of its dysfunction.

Abbreviations: AA – aplastic anaemia; CR4-CR5 domain – conserved region 4 and conserved region 5; CR7 – conserved region 7; DC – dyskeratosis congenita; DCR – dyskeratosis congenita registry; *DKC1* – dyskeratosis congenita gene 1-mutated in X-linked DC; *Dkc1* – mouse homologue of *DKC1*; GAR1 – glycine-arginine-rich protein 1; H/ACA domain – hairpin-hinge-hairpin-ACA motif; HH – Hoyeraal–Hreidarsson syndrome; IVS – intervening sequence; MDS – myelodysplastic syndrome; NOP10 – novel protein 10Kd; NHP2 – novel high mobility group (HMG)- like protein 2; rRNA – ribosomal RNA; RNP – ribonucleoprotein; SnoRNAs – small nucleolar RNA; SnoRNP – small nucleolar ribonucleoprotein; *Terc* – mouse homologue of *TERC*; hTERT – telomerase reverse transcriptase (human).

Introduction

The telomere is a specialized heterochromatin composed of both repeated DNA elements and specific DNA-binding proteins that cap the termini of eukaryotic chromosomes, thereby protecting the chromosome ends from degradation and fusion, and provides the means for the complete replication of the genome (Blackburn 2000, 2001;

Cong et al. 2002). Telomeres allow cells to distinguish natural chromosome ends from damaged DNA, and as a result, prevent the natural ends from signaling and initiating DNA damage checkpoint cascades (de Lange 2002). A specialized RNA-dependant DNA polymerase, called telomerase, maintains telomeric DNA. This ribonucleoprotein enzyme is a reverse transcriptase, composed of two essential subunits. These are a

reverse transcriptase or catalytic component (TERT) and an RNA component (TERC or hTR) that acts as a template for telomere synthesis (Greider and Blackburn 1989; Nakamura and Cech 1998; Collins and Mitchell 2002). [For more details on the structure/function of the telomere and the telomerase complex, please refer to Dominique Broccoli's review in this issue]. The critical importance of telomeres and telomerase is illustrated by studies of the rare genetic disorder dyskeratosis congenita (DC). In DC, mutations in genes that encode components of the telomerase complex result in telomerase dysfunction, premature telomere shortening, and the development of multi-system abnormalities including bone marrow failure and malignancy.

Dyskeratosis congenita

Classical dyskeratosis congenita (DC, also known as Zinsser-Engman-Cole syndrome) is a severe disorder that is characterized by the triad of abnormal skin pigmentation; nail dystrophy and mucosal leucoplakia (Zinsser 1906; Engman 1926; Cole et al. 1930), with bone marrow failure being a principal cause of death. A variety of other abnormalities have also been reported (Table 1) (Sirinavin and Trowbridge 1975; Drachtman and Alter 1995; Knight et al. 1998; Dokal 2000). The international dyskeratosis congenita registry (DCR) (Hammersmith Hospital, London) has information on 216 families from around the world and provides a valuable resource for studies of the genetics and pathophysiology of this disease.

The DCR has confirmed that DC is a very heterogeneous disorder. The severity, age of onset and range of somatic and hematological abnormalities varies widely as does the age of death. The inheritance of the disease also varies. The most common form is X-linked recessive (MIM 305000), with autosomal dominant (MIM 127550) and autosomal recessive (MIM 224230) form also recognized. The severity of the muco-cutaneous triad and other non-cutaneous features depends to some extent on the mode of inheritance; the X-linked recessive form is generally more severe compared to autosomal recessive DC, with the autosomal dominant form being less severe, but

Table 1. Somatic abnormalities/complications in patients with DC.

Abnormality	% of patients ^a
<i>Classical presentation</i>	
Abnormal skin pigmentation	89
Nail dystrophy	88
Bone marrow failure	85.5
Leucoplakia	78
<i>Other common features</i>	
Epiphora	30.5
Learning difficulties/developmental delay/mental retardation	25.4
Pulmonary disease	20.3
Short stature	19.5
Extensive dental caries/loss	16.9
Oesophageal stricture	16.9
Premature hair loss/greying/sparse eyelashes	16.1
Hyperhidrosis	15.3
<i>Other less common features</i>	
Malignancy	9.8
Intrauterine growth retardation	7.6
Liver disease/peptic ulceration/enteropathy	7.3
Ataxia/cerebellar hypoplasia	6.8
Hypogonadism/undescended testes	5.9
Microcephaly	5.9
Urethral stricture/phimosis	5.1
Osteoporosis/aseptic necrosis/scoliosis	5.1
Deafness	0.8

^aIn the future, these percentages will change significantly as patients identified to have DC on the basis of mutation analysis are included.

exceptions to this do exist. Heterogeneity is also observed within affected families and is more pronounced in female cases. Generally children are normal at birth; develop progressive disease features in adolescence and life threatening complications usually in the third decade. It is hypothesized that the clinical manifestations of DC reflect aberrant stem cell function with the brunt of the disease falling on rapidly dividing tissues i.e. skin, oral mucosa and bone marrow, giving rise to the classical muco-cutaneous triad and bone marrow failure that characterize this disease.

At least one gene each for the X-linked recessive and autosomal dominant forms of the disease has been identified. These are *DKC1* for the X-linked recessive form (Heiss et al. 1998) and *TERC* (or hTR) for the autosomal dominant form (Vulliamy et al. 2001b). *DKC1*, encodes the nucleolar protein, dyskerin, is highly conserved among

eukaryotes, including yeast, rat, drosophila and mouse (Cadwell et al. 1997; Giordano et al. 1999; Meier and Blobel 1994; Ruggero et al. 2003). It is expressed in all tissues of the body suggesting it is an essential 'house keeping' gene. Like its yeast homologue, dyskerin associates with a class of small nucleolar RNA molecules called H/ACA snoRNA's (Lafontaine et al. 1998; Zebajadian et al. 1999). Four common proteins associated with H/ACA snoRNAs in humans are dyskerin, GAR1, NHP2 and NOP10. The homologues of these four proteins (Cbf5p, Gar1p, Nhp2p and Nop10p, respectively) in yeast are essential for growth, with the depletion of any causing inhibition of 18S rRNA production and defective pseudouridylation of rRNA. (Pogacic et al. 2000). Based on studies on dyskerin homologues it has been suggested that dyskerin functions as the active pseudouridine synthase and as this step is essential for ribosome biogenesis, it was therefore initially suggested that X-linked DC arises probably because of defective ribosome biogenesis (Tollervey and Kiss 1997; Luzzatto and Karadimitris 1998; Filipowicz and Pogacic 2002).

Recent studies have shown that dyskerin is also associated with the RNA component of telomerase (TERC), which too contains an H/ACA consensus sequence (Mitchell et al. 1999a, b). Immunoprecipitation experiments showed that dyskerin associates with both the H/ACA class of snoRNPs and the human telomerase RNP, and that the conserved proteins GAR1, NOP10 and NHP2 were also present in the telomerase complex (Figure 1). The role of these proteins in telomerase is unknown at present but they may be necessary for the efficient assembly of telomerase RNP in the nucleolus (Collins and Mitchell 2002). This association raises new questions as to the role of dyskerin and the pathophysiology of DC. In particular, does the mutated dyskerin found in DC patients lead to deficient ribosomal RNA processing, deficient telomerase activity or defects in both ribosomal RNA processing and telomerase dysfunction? Experiments showed that cells from X-linked DC patients had no detectable deficiency in snoRNA accumulation, pseudouridylation or ribosomal RNA processing, but did have a reduction in TERC accumulation and telomerase activity (Mitchell et al. 1999b). While Vulliamy et al. (2001a) demonstrated comparable telomerase activity levels from peripheral blood mononuclear cells in X-

Figure 1. Telomerase holoenzyme – Putative protein associations of TERC and its RNA structure, highlighting the key domain regions within the RNA molecule.

linked DC patients and normal individuals, they additionally found telomere lengths to be much shorter in DC patients with both X-linked and autosomal dominant disease when compared with age matched controls, suggesting that both forms of the disease are associated with rapid telomere shortening in hemopoietic stem cells (Vulliamy et al. 2001a). Taken together these two lines of evidence suggest that DC might primarily be a disease of defective telomere maintenance rather than ribosomal biogenesis, although subtle defects in ribosome biosynthesis cannot be ruled out. This is highlighted by the work of Ruggero et al. (2003) and their study of the hypomorphic *Dkc1* mouse mutant. This mouse model shared many of the features of DC, including increased tumour susceptibility. In the early generation (G1 and G2) the mice showed alterations in rRNA modification whereas defects in telomere length were not observed until the fourth generation. From this they suggest that dysregulation of ribosome function is important for the initiation of DC in mouse and the impairment in telomerase activity may modify or exacerbate the disease in later generations.

Autosomal dominant DC is a rare form of the disease, with less than 10% of families (based on the DCR data) demonstrating this mode of inheritance. Linkage analysis in a large family enabled the assignment for the autosomal dominant DC locus to chromosome 3q (Vulliamy et al. 2001b). As

dyskerin had been shown to be a component of the telomerase complex and *TERC* had been cytogenetically mapped to the same interval on chromosome 3q, *TERC* was investigated as a candidate gene for autosomal dominant DC. Mutation screening of this gene, which encodes an RNA of 451 bases, detected an 821bp deletion in the original large family and additional mutations in other autosomal dominant families. This confirmed that autosomal dominant DC is due to mutations in *TERC*. Subsequently it has been suggested that these mutations appear to give rise to the disease through haploinsufficiency rather than through a dominant-negative effect (Fu and Collins 2003).

As discussed earlier, telomere length in blood cells from patients with X-linked recessive and autosomal dominant DC is reduced compared with age-matched controls. While limited data suggests that this is also true in autosomal recessive DC, this initial observation needs to be confirmed with a larger patient population (Vulliamy et al. 2001a). A recent study by Vulliamy et al. (2004), investigated telomere length in affected individuals with autosomal dominant DC. The disease in these families showed an earlier age of onset with successive generations. This disease anticipation was associated with progressive telomere shortening through the generations suggesting a new mechanism of disease anticipation in humans. This is analogous to the telomerase RNA knockout mice (*Terc*^{-/-}) (Blasco et al. 1997). The parental mice had very long telomeres and in the first generation the *Terc*^{-/-} mice are asymptomatic. Features of telomere shortening, which overlap the clinical features of DC, such as reduced proliferative capacities of hematopoietic cells in the bone marrow and spleen, are only seen from the 4th generation, and by the 6th generation the mice are infertile. Heterozygous mice (*Terc*^{+/-}) appear asymptomatic but they do have difficulty in elongating their telomeres in interspecies crosses (Hathcock et al. 2002).

Mutations in dyskeratosis congenita

X-linked recessive DC is due to mutations in DKC1

To date at least 30 mutations have been identified in *DKC1*. The majority of mutations in *DKC1*

encoding dyskerin cause single amino acid substitutions (Table 2). One mutation causing an alanine to valine substitution at amino acid 353 (A353V) accounts for approximately 40% of all X-linked recessive DC cases, and in many cases was shown to be a *de novo* event. This particular mutation has also been found in patients with Hoyeraal–Hreidarsson (HH) syndrome (Knight et al. 1999b; Yaghmai et al. 2000). Only three other recurrent mutations have been found in multiple families in either DC or HH. Distribution of mutations with respect to exons shows that there are two clusters involving exons 3–6 (43% of all mutations) and exons 9–12 (40% of all mutations). At present, there is very little information regarding the function of the different parts of the protein, and what effects these mutations have. The only coding mutation to affect a known domain is S121G, which is in the TruB domain (Knight et al. 1999b; Zucchini et al. 2003) and is close to the highly conserved catalytic aspartic acid (125D) residue (Ramamurthy et al. 1999). TruB has been shown to catalyse the modification of pseudouridine from uridine in RNA molecules and 125D is essential for pseudouridylation activity. One large deletion removing the last 22 amino acids of dyskerin has been identified in exon 15. The remaining three mutations that have been identified are non-coding, one in the promoter region and the others in intron 1 and intron 2, respectively. The promoter mutation – 142C → G destroys a consensus transcription factor (Sp1) binding site. Although not demonstrated in patient material, in an experimental system the presence of this mutation has been shown to reduce the promoter activity by 60% (Salowsky et al. 2002). One of the intronic mutations, IVS1 592C → G has been shown to affect splicing whereas the other, IVS2 473 C → G alters splice site acceptor motif. Taken together it can be seen that these three non-coding changes may affect the levels of dyskerin produced, and reduced dyskerin levels as well as structurally altered dyskerin may cause DC (Knight et al. 2001).

Autosomal dominant DC is due to mutations in TERC

Fourteen mutations have been found in *TERC* in families with autosomal DC and are detailed in

Table 2. Summary of dyskerin mutations found in DC and HH.

Mutation	AA substitution	Exon	DC or HH	Reference
-142 C → G		promoter	DC	Knight et al. (2001)
5 C → T	A2V ^a	1	DC	Knight et al. (1999b); Safa et al. (2001)
IVS1 592 C → G		IVS1	DC	Knight et al. (2001)
IVS2 473 C → G		IVS2	DC	Knight et al. (1999b)
106 T → G	F36V	3	DC	Heiss et al. (1998)
109-111 ΔCTT	ΔL37	3	DC	Heiss et al. (1998)
113 T → C	I38T	3	HH	Cossu et al. (2002)
115 A → G	K39E	3	DC	Knight et al. (1999b)
119 C → G	P40R	3	DC	Heiss et al. (1998)
121 G → A	E41K	3	DC	Knight et al. (1999b)
127 A → G	K43E	3	DC	Heiss et al. (2001)
146 C → T	T49M ^a	3	HH	Knight et al. (1999a)
194 G → C	R65T	4	DC	Knight et al. (1999b)
196 A → G	T66A	4	DC	Heiss et al. (1998)
214,5 CT → TA	L72Y	4	DC	Heiss et al. (1998)
361 A → G	S121G	5	HH	Knight et al. (1999a)
472 C → T	R158W	6	DC	Knight et al. (2001)
838 A → C	S280R	9	DC	Knight et al. (2001)
949 C → T	L317F	10	DC	Rostamiani et al. (1999)
961 C → G	L321V	10	DC	Knight et al. (1999b)
965 G → A	R322Q	10	DC	Rostamiani et al. (1999)
1049 T → C	M350T ^a	11	DC	Knight et al. (1999b)
1050 G → A	M350I	11	DC	Knight et al. (1999b)
1058 C → T	A353V ^b	11	BOTH	Knight et al. (1999b); Yaghmai et al. (2000)
1150 C → T	P384S	11	DC	Knight et al. (2001)
1151 C → T	P384L	11	DC	Knight et al. (1999b)
1193 T → C	L398P	12	DC	Hiramatsu et al. (2002)
1204 G → A	G402R	12	DC	Knight et al. (1999b)
1205 G → A	G402E	12	DC	Heiss et al. (1998)
Δ Exon 15	Δ493-514	15	DC	Vulliamy et al. (1999)

Δ, deletion

IVS, Intron

^arecurrent mutation^bcommon recurrent mutation

Table 3. They are unique mutations occurring in different regions of the molecule (Figure 1). It is becoming apparent that the degree to which the telomerase activity is affected varies with the precise location of the mutation in the TERC molecule. There are several domains within TERC that are highly conserved: the template domain, the pseudoknot domain, CR4-CR5 domain, H/ACA domain and CR7 domain. Various domains seem to associate with each other further within the TERC protein and have different function; the template domain, the pseudoknot domain and the CR4/CR5 domain are all thought to be part of the catalytic domain, together with TERT, whereas the H/ACA domain and the CR7 domain are thought to form part of the localisation domain (Ueda and Roberts 2004). There are also several conserved regions within these domains that are

believed to be important in the function of TERC. Eight mutations including 58G → A have been described in the pseudoknot domain. Of these, three have been investigated in *in vitro* systems to determine their effects on the function of telomerase. It has been suggested that nucleotides 33-147 and 163-330 contain TERT binding sites and are critical for telomerase activity. Mutations at nucleotides 72 (C → G), 110-113 (Δ GACT) and 107-8 (GC → AT) have been shown to reduce telomerase activity when compared with wild type in a rabbit reticulocyte lysate system (<15, <15 and ~50%, respectively). 72 C → G disrupts a conserved stem structure within the pseudoknot and studies have demonstrated that it is the secondary structure rather than the primary sequence that determine the effect of this particular mutation (Fu and Collins 2003; Marrone et al. 2003).

Table 3. Summary of mutations and polymorphisms found in *TERC*

Mutation	Domain	Disease	Reference
~2kb Δ to 317 nt	Template	DC	Vulliamy et al. (2004)
58 G $\rightarrow$ A ^{a, b}		AA	Vulliamy et al. (2002)
		AA	Fogarty et al. (2003)
		Controls	Wilson et al. (2003)
72 C $\rightarrow$ G	Pseudoknot	CAA	Vulliamy et al. (2002)
96,7 Δ CT	Pseudoknot	DC	Vulliamy et al. (2004)
107,8 GC $\rightarrow$ AG	Pseudoknot	DC	Vulliamy et al. (2001b)
110-3 Δ GACT	Pseudoknot	CAA	Vulliamy et al. (2002)
116 C $\rightarrow$ T	Pseudoknot	AA	Fogarty et al. (2003)
143 G $\rightarrow$ A	Pseudoknot	MDS	Vulliamy et al. (2004)
204 C $\rightarrow$ G	Pseudoknot	AA	Fogarty et al. (2003)
228 G $\rightarrow$ A ^c	Hypervariable region	AA, Controls	Wilson et al. (2003)
305 G $\rightarrow$ A	CR4-CR5 domain	AA	Yamaguchi et al. (2003a, b)
322 G $\rightarrow$ A	CR4-CR5 domain	MDS	Yamaguchi et al. (2003a, b)
Δ 378-3' end	Δ H/ACA box domain & CR7 domain	DC	Vulliamy et al. (2001b)
408 C $\rightarrow$ G	CR7 domain	DC	Vulliamy et al. (2001b)
450 G $\rightarrow$ A	H/ACA box domain	AA	Yamaguchi et al. (2003a, b)
467 T $\rightarrow$ C	16bp down stream of 3' transcription region of <i>TERC</i>	AA	Yamaguchi et al. (2003a, b)

AA, aplastic anaemia; IAA, idiopathic aplastic anaemia; CAA, constitutional aplastic anaemia; MDS, myelodysplastic syndrome.
 Δ , deletion

^aPolymorphic site in African-Americans (frequency of A allele 4%)

^bpresent in more than one family with AA – the role in disease pathogenesis remains unclear

^cPolymorphic site in African-Americans (frequency of A allele 2%)

107-8 GC $\rightarrow$ AT has been shown to destabilise the pseudoknot conformation by disrupting the P3 helix within the pseudoknot, which compromises the catalytic function (Theimer et al. 2003). This P3 helix helps *TERC* to form homodimers and mutations within this region lead to loss of enzymatic activity but do not appear to prevent binding of *TERC* to *TERT* (Ly et al. 2003). It has been demonstrated in mutagenesis studies that the deletion of nucleotides 303 to 315 interferes with the proper *TERC* and *TERT* interaction and abrogates telomerase activity (Ueda and Roberts 2004). Yamaguchi et al. (2003a) demonstrated the presence of a 305 G $\rightarrow$ A mutation in a patient with aplastic anaemia. Although not confirmed, this mutation could interfere with the correct interaction of *TERC* and *TERT* in the telomerase complex thus leading to the pathology observed in this patient. This is supported by the work of Keppler and Jarster (2004) who found that the CR4-CR5 domain and specific pairing regions within the pseudoknot domain interact autonomously with *TERT* and targeted disruptions of these interactions before the holoenzyme assembly disrupts telomerase activity. It is thought that the Δ 378-3' end mutation will result in inefficient

accumulation and localisation of the molecule as the H/ACA domain and CR7 domains are removed as these two domains are necessary for nucleolar localisation, 3' end processing and RNA stability. Finally in a study of the mutation 408 C $\rightarrow$ G in the CR7 domain, Marrone et al. (2003) showed that the activity of this particular mutation is less than 25% of wild type, suggesting that it interferes with assembly or stability of the telomerase complex. This particular mutation has been shown to alter a single-stranded internal loop in this domain (loop J7b/8a), which acts as a specific *TERC*:*TERC* interaction site implicated in the multimerisation of *TERC* molecules (Ren et al. 2003).

DC gene mutations in other diseases

There are several lines of evidence that suggest mutations in the DC genes may underlie other bone marrow failure syndromes, particularly aplastic anaemia (AA), myelodysplastic syndrome (MDS) and the Hoyeraal-Hreidarsson syndrome (HH). Firstly, as in DC patients, patients with idiopathic and constitutional AA have short telo-

meres compared with age-matched controls (Ball et al. 1998; Brummendorf et al. 2001; Vulliamy et al. 2002). Telomere erosion has also been suggested to be relevant to the pathology of MDS (Boulton et al. 1997). Secondly, in some of the DCR families, affected members have died of severe AA before the age of 10 years and a diagnosis of DC was made subsequently, only when other members of the family survived long enough to develop the classical muco-cutaneous features. If it were not for the presence of subsequent members, these individuals would have been characterised as idiopathic AA. These observations have led to the analysis of the 'DC' genes, and *TERC* mutations have subsequently been detected in some patients presenting with AA or MDS (Table 3). Of the mutations described, 58 G → A was detected in several individuals with AA (Vulliamy et al. 2002) and 228 G → A was detected in a single patient with AA (Wilson et al. 2003). Subsequently, these variations have been shown to be present in the general population in African Americans (frequency of the rare A allele was 4 and 2%, respectively; Wilson et al. 2003; Yamaguchi et al. 2003a, b). These findings suggest that these variations are polymorphisms, which may also be functioning as susceptibility loci rather than disease causing mutations.

The HH (MIM 600545) syndrome is a severe multi system disorder that can present in the neonatal period and infancy. It is characterized by severe growth retardation neurological abnormalities, immunodeficiency and bone marrow failure. The overlap of the features between HH and DC led to the analysis of *DKC1* in HH. It has been demonstrated that HH is a severe variant of X-linked DC where death from bone marrow failure and immunodeficiency occurs before the appearance of the classical diagnostic features of DC usually in the first years of life (Knight et al. 1999a; Yaghmai et al. 2000). These observations highlight the heterogeneity of DC. Several mutations in dyskerin have been confirmed in HH patients (Table 1). One mutation, T49M, has been observed in more than one family and another is the common A353V mutation that is present in many X-linked recessive DC patients. Female cases of HH are also recognised and it is likely that they represent a severe variety of the autosomal form of DC, the genetic basis of which presently remains unknown. The identification of mutations

in *DKC1* and *TERC* in HH and AA patients, respectively, further highlights the heterogeneity of DC.

Autosomal recessive DC

No genetic mutations to date have been found to cause autosomal recessive DC. Since dyskerin and *TERC* have been shown to interact specifically with the H/ACA class of snoRNPs, possible candidate genes could be the molecules involved with this complex (see Figure 1), or molecules involved in rRNA biosynthesis and/or telomerase RNP assembly. *TERT* itself remains a possible candidate, as no mutations have been found in this gene in recessive DC. However, heterozygous mutations in *TERT* have been reported in patients with aplastic anaemia (Yamaguchi et al. 2003b), and telomere lengths in these patients have been demonstrated to be markedly shorter than in age matched controls. This finding suggests that *TERT* mutations might represent a genetic risk factor for marrow failure in adults. In a recent study by our group, no coding mutations in three candidate genes (*GAR1*, *NHP2* and *NOPI10*) were detected in a selection of DC families showing autosomal recessive inheritance (Marrone et al. unpublished data). A similar study was undertaken with hStau, a double-stranded RNA binding protein associated with the telomerase complex *in vivo* (Le et al. 2000). Again, our results revealed no mutations in the coding region of hStau that were associated with the disease (Walne et al. unpublished data). Results from these studies suggest that none of these genes are commonly mutated in autosomal recessive DC. Although the core proteins within the H/ACA snoRNP are known, not all the molecular components of the telomerase complex have yet been identified. Chaperone proteins such as p23 and hsp90 that are known to interact with *TERT* could also be involved (Holt et al. 1999). Using the consanguineous families within the Hammersmith Hospital DCR, this laboratory is currently using linkage analysis to identify chromosomal regions of interest. This is being used in conjunction with denaturing HPLC (Transgenic Wave technologies) analysis for screening of candidate genes identified to be associated with the telomerase complex or identified by linkage.

Telomeres, cancer and aging

The maintenance of telomeres regulates many crucial functions and may participate in the control of complex phenotypes such as cancer and aging. In normal tissue function, the rate of cell loss is matched by the rate of cell renewal. Aging is hastened by forces that either accelerate cell loss or prevent tissue repair. When the rate of loss exceeds the rate of repair, cellular attrition eventually leads to a decline in organ function and eventually organ failure. Studies in both humans and mice support the view that physiological erosion of telomeres with successive rounds of cell division suppresses tumour formation by limiting cell life span. However many groups are now arriving at the hypothesis that it is probably not the actual telomere length, in terms of the number of double-stranded hexameric repeats units, but the structure of the telomere itself, the nucleoprotein cap for the chromosome end, that is important in limiting the cell life span and causing the cells to enter senescence (reviewed by Ben Porath and Weinberg 2004; Sharpless and DePinho 2004). The majority of human cancers (85%) and cancer cell lines possess active telomerase and maintain stable telomeres with proliferation and when this telomerase activity is inhibited; telomere maintenance is disrupted leading to senescence or cell death. The observations relating telomere maintenance to the regulation of replicative life span strongly imply that alterations in telomere biology play an important role in malignant transformation. This in turn leads to a paradigm. In DC it has been well documented that telomeres are shorter than in age-matched controls, and there is an increased predisposition to malignancy in these patients (approximately 10%). This could be due to increased chromosomal instability, which in turn is a result of critically shortened telomeres. This could also explain the increased rates of cancer in an older population (who have shorter telomeres than younger persons), where the risk of developing a malignancy at 70 years of age is 10 times the risk three decades earlier. An increased understanding of telomere biology and telomerase will help with the understanding of the aging mechanisms in cells as well as the development of cancers and may in time give rise to therapeutic intervention to control the develop-

ment of cancers in humans. Recent data on DC has given us a glimpse of the critical role of normal telomerase (and telomeres) and of the dire consequences, including malignancy and premature aging, of its dysfunction.

Conclusions and future prospective

The study of DC has tremendously increased our understanding of the key components and complex regulation of telomerase. It has also led to major findings in both basic biology and insights into the pathogenesis of the more common AA. Mutations in genes encoding two telomerase holoenzyme components (dyskerin and TERC) reduce the maximal level of telomerase activity and dramatically compromise the proliferative renewal of haematopoietic and epithelial tissues. These observations suggest a much greater requirement for telomerase in the process of human growth and development than previously thought. It is becoming increasingly apparent that the location of various mutations particularly in *TERC* itself may give rise to the differences in the phenotypes observed as some mutations influence the catalytic activity of telomerase whereas others affect its localisation and stability. Disease anticipation is now also observed in autosomal dominant DC and is associated with progressive telomere shortening. The similarity in the structure of telomerase RNP and H/ACA snoRNP raises questions about the evolution and the function of these complexes, as well as the relative importance of the two known functions of dyskerin (rRNA processing and as part of the telomerase complex). More detailed analysis of the different dyskerin mutations and their effect on pseudouridylation and/or telomerase function is needed to answer questions pertaining to the role of dyskerin in DC.

From a clinical perspective the discovery that rapidly shortening telomeres is fundamental to the development of DC, possibly AA and other bone marrow failure diseases suggest possible avenues for research into therapies aimed at preventing or repairing telomere shortening. The identification of genes known to cause DC and in some cases AA and MDS are already facilitating the development of screening strategies of patients presenting with an abnormal blood count.

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