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ARTICLE

Myelodysplastic syndrome

Recurrent somatic mutations are rare in patients with cryptic dyskeratosis congenita

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Abstract

Dyskeratosis congenita (DKC) is a paradigmatic telomere disorder characterized by substantial and premature telomere shortening, bone marrow failure, and a dramatically increased risk of developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). DKC can occur as a late-onset, so-called cryptic form, with first manifestation in adults. Somatic MDS-related mutations are found in up to 35% of patients with acquired aplastic anemia (AA), especially in patients with short telomeres. The aim of our study was to investigate whether cryptic DKC is associated with an increased incidence of MDS-related somatic mutations, thereby linking the accelerated telomere shortening with the increased risk of MDS/AML. Samples from 15 adult patients (median age: 42 years, range: 23–60 years) with molecularly confirmed cryptic DKC were screened using next-generation gene panel sequencing to detect MDS-related somatic variants. Only one of the 15 patients (7%) demonstrated a clinically relevant MDS-related somatic variant. This incidence was dramatically lower than formerly described in acquired AA. Based on our data, we conclude that clonal evolution of subclones carrying MDS-related mutations is not the predominant mechanism for MDS/AML initiation in adult cryptic DKC patients.

Introduction

The telomere length (TL) shortens with each cell division and reflects the replicative history of a cell or tissue [1, 2]. Telomere shortening (TS) can be slowed down or reversed by the enzyme telomerase—whereas, impaired telomerase function leads to accelerated TS [3]. In analogy to double-

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strand breaks, the critically short telomeres trigger DNA damage checkpoints and as a consequence, the affected cells undergo replicative senescence as long as these checkpoints are intact. Further rounds of replication and/or functional impairment of the respective checkpoints result in increasing chromosomal instability and eventually, clonal evolution [1, 2]. Thus, critical shortening of telomeres can both act as a predisposing factor for malignant transformation or be a part of a tumor suppressor mechanism [4, 5].

Dyskeratosis congenita (DKC) is a paradigmatic disorder to study the effects of critically short telomeres. Classical DKC is characterized clinically by mucocutaneous features, development of bone marrow failure (BMF) and critically short telomeres [6]. In addition, DKC patients are faced with an increased incidence of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and solid tumors, e.g., head–neck squamous cell carcinoma [7]. In DKC patients, the risk of developing secondary AML or MDS is increased 200-fold and 2.500-fold, respectively, compared to the normal population with both malignancies typically occurring at a median age of ~35 years [8]. Premature TS is frequently caused by mutations in genes affecting telomerase or telomerase-related components, leading to functionally reduced telomerase activity. In 30–40% of all clinical DKC cases with short telomeres, an underlying genetic defect cannot be identified thereby complicating the correct diagnosis, especially in BMF patients without further clinical symptoms [9]. While classical DKC is typically diagnosed in childhood, a second late-onset, so-called cryptic subtype of DKC with diverging non-hematologic stigmata often first manifests itself in early or middle-aged adulthood [10].

Detection of short telomeres with recurrent somatic mutations is a hallmark of various acquired clonal disorders, such as MDS [2, 11, 12], CML [13], or others [2]. In addition, in otherwise healthy individuals, clonal hematopoiesis of indeterminate potential (CHIP) with detection of gene mutations originally described in myeloid neoplasms was found to be associated with an increased risk for hematological cancers [14, 15]. Moreover, somatic mutations are observed in up to 35% of patients with acquired aplastic anemia (AA), most likely due to a growth advantage of clonal cells with acquired mutations in the context of an immune escape from the autoimmune-mediated attack against the hematopoietic stem cell (HSC) compartment [16]. This concept was also corroborated by the fact that mutations were preferentially found in patients with premature TS [17, 18].

In this study, we aimed to investigate whether accelerated TS, due to impaired telomere maintenance in adult patients with molecularly confirmed cryptic DKC, was associated with an increased frequency of MDS-related somatic mutations reflecting the first step of the multi-step leukemogenesis, explaining the remarkably increased risk of MDS and/or AML observed in this late-onset hereditary BMF syndrome.

Methods and patients

Analysis included 15 patients from the "Aachen Telomeropathy Registry" ($n = 13$) and the Freiburg pediatric bone marrow failure registry ($n = 2$). Informed consent was obtained of all patients. All patients had clinical features typical of DKC. No patient had cytologic signs of MDS. The median age of the cohort was 42 years (range: 23–60 y). All patients had confirmed mutations in DKC-causing genes (*TERC* $n = 7$, *TERT* $n = 7$, *DKC1* $n = 1$, Table 1). Additional clinical details of two patients with MDS/AML are shown in Supplementary Table 1. For TL measurement, flow-FISH was used according to previously described protocols, and TL is indicated in kilobases (kb) [19–22]. A self-customized next-generation sequencing (NGS) panel ("telomere-panel") for known DKC-causing genes was used to identify genetic variants [23]. The NGS was carried out on the gDNA of the peripheral blood samples of all 15 patients due to the hypoplastic/aplastic bone marrow, except for patient #4, where sufficient bone marrow for additional analysis was available. In addition, we used a clinically validated self-customized NGS-panel ("MDS/MPN-panel") including genes harboring mutations, which are typically associated with myeloid neoplasms (*ABL, ASXL1, BARD, CALR, CBL, CEBPA, CHEK2, CSF3R, DNMT3A, ETNK1, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NFE2, NRAS, PDGFRA, PTPN11, RUNX1, SETBP1, SF3A1, SF3B1, SH3B2 (LNK), SRSF2, TCF12, TET2, TP53*,and *U2AF1*). For detailed information see Supplementary Material and Methods.

Results

TL was found to be below the first percentile in peripheral blood lymphocytes in 12 out 15 patients (Fig. 1a). the mean TL was 4.78 kb ± 0.63 SD in the lymphocyte and 5.42 kb ± 1.01 SD in the granulocyte (Fig. 1b) subpopulation, respectively. Using the "Telomere-panel", all detected mutations showed a 50/50 ratio between the reference and the mutated allele in line with the anticipated heterozygosity of the detected mutations (Table 1). The "MDS/MPN-panel" sequencing revealed a relevant somatic MDS-related mutation in only 1 out of 15 patients analyzed. This patient (#7) harbored a mutation in the *U2AF1* gene (c.101 C > T, p.Ser34Phe; allele frequency 16%), which has been described in MDS [24], in addition to the known DKC-causing *TERT* mutation (c.2915 G > A; p.Arg972His)

Table 1 Clinical characteristics of the 15 analyzed dyskeratosis congenita patients

Patient	Gender	Age (years)	Telomere length lymphocytes	Telomere length granulocytes	Detected telomeropathy causing mutation	Somatic variant/mutation	Clinical symptoms
1	F	26	<1%	<1%	*TERC: NR_001566.1*:r.128 A > G		Cytopenia, liver cirrhosis
2	M	23	<1%	<1%	*TERT:* NM_198253.2:c.2372 T > C; p. (Val791Ala)		BMF
3	M	34	<1%	>10%	*DKC1*: NM_001363.4:c.1346 G > A; p. (Arg449Gln)		Lung fibrosis
4	M	42	<1%	<1%	*TERC: NR_001566.1*:r.18_31del CCTGGGAGGGGTGG	*ASXL1*: NM_015338.5:c.3244_3246del; p. (Leu1082del)	BMF
5	F	53	<1%	<1%	*TERC: NR_001566.1: NR_001566.1*: r.73 G > A		BMF
6	M	55	<1%	<1%	*TERT: NM_198253.2*:c.2639 C > T; p. (Ala880Val)		Cytopenia
7	M	47	<1%	<1%	*TERT: NM_198253.2*:c.2915 G > A; p. (Arg972His)	*U2AF1*: NM_006758.2:c.101 C > T;p. (Ser34Phe)	Cytopenia, liver cirrhosis, lung fibrosis
8	F	60	<10%	>10%	*TERT: NM_198253.2*:c.3055 C > G; p. (Leu1019Val)		BMF
9	M	46	<1%	<10%	*TERT: NM_198253.2*:c.2372 T > C; p. (Val791Ala)		Cytopenia
10	F	24	<10%[a]	<1%[a]	*TERT: NM_198253.2*:c.1234 C > T; p. (His412Tyr)		Lung fibrosis, AML
11	F	28	<1%	<1%	*TERC: NR_001566.1*:r.303 G > C		Cytopenia
12	F	29	<1%	<1%	*TERC: NR_001566.1*:r.128 A > G		Cytopenia
13	F	50	<1%	<10%	*TERC: NR_001566.1*:r.50 C > A		Lung fibrosis, cytopenia
14	F	42	<1%	<10%	*TERC: NR_001566.1*:r.54_57delAACT		BMF
15	F	46	<10%	<1%	*TERT: NM_198253.2*:c.1234 C > T; p. (His412Tyr)		Lung fibrosis, MDS

BMF bone marrow failure, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome.

[a]Time point of the telomere length measurement was at the first diagnosis of AML.

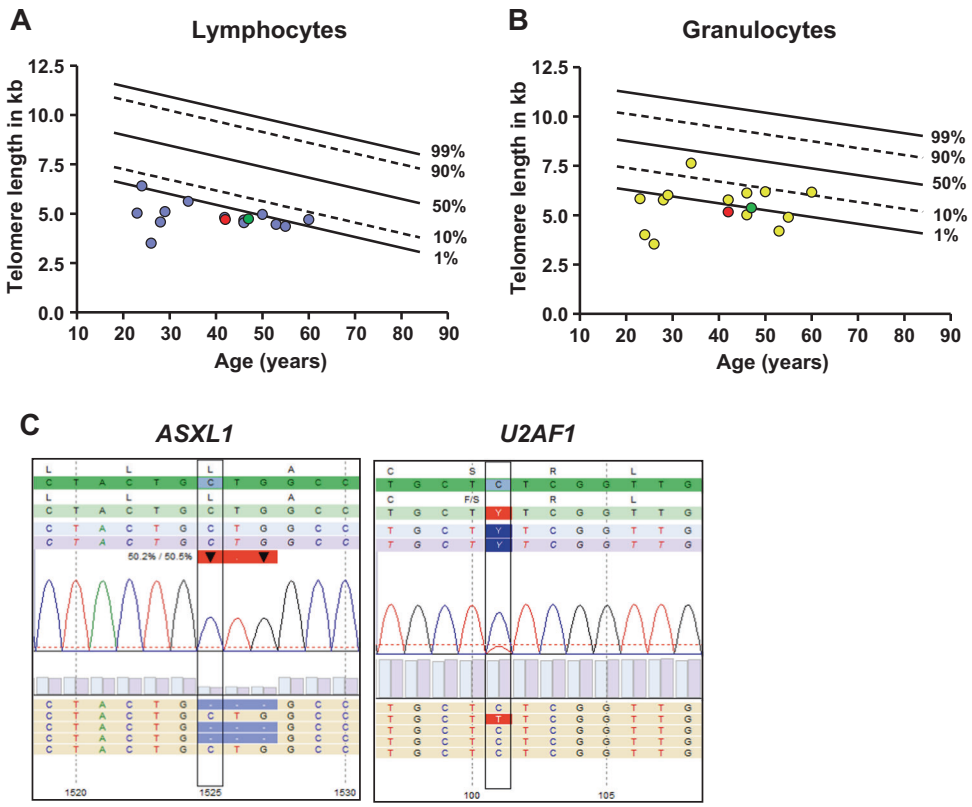

Fig. 1 Telomere analysis and detailed presentation of the observed MDS-related mutations: Lymphocyte (**a**) and granulocyte (**b**) telomere length of 15 dyskeratosis congenita patients, 1%, 10%, 50%, 90%, and 99% percentile are indicated. Patient #4 with the detected ASXL1 mutation is depicted in red. Patient #7 with the detected U2AF1 mutation is depicted in green. **c** Detailed depiction of the detected in-frame ASXL1 deletion and U2AF1 mutation. MDS myelodysplastic syndrome, kb kilobases

(Fig. 1c). In another patient with an inherited *TERC* mutation (patient #4), an *ASXL1* variant (c.3244_3246CTGdel; freq.: 48% [4708/9347]; p.Leu1082del) (Fig. 1c) was detected in the peripheral blood. A similar allele frequency was also observed by analyzing the respective bone-marrow sample (freq.: 50%). Interestingly, TS in granulocytes was not further accelerated in the patients carrying the described variant (patient #4: 5,17 kb) and mutation (patient #7: 5,38 kb), as compared to the remaining 13 patients of the cohort (5.54 kb, $p = 0.84$, *t*-test).

Discussion

This is the first report of a systematic analysis of somatic mutations in the rare cohort of adult patients with late-onset, cryptic DKC. Until now, no data existed on the exact mechanism for tumor development in DKC patients. The occurrence and potential accumulation of MDS-related clonal mutations might represent a possible mechanism underlying secondary MDS and/or subsequent AML development [25]. However, to our surprise, we observed characteristic acquired mutations in typical MDS-linked genes in only 1 out of 15 patients present in our cohort. The detected ASXL1 variant is most likely a rare germline variant (allele frequency in the sample about 50%) of uncertain significance (see clinvar-including bioinformatical analysis, mean allele frequency /dbsnp150 rs754183801: 0.002%).

Based on our data, clonal hematopoiesis with detection of MDS-related genes is a rather rare event in adult DKC patients, compared to other myeloid diseases with short telomeres, but functional (compared to DKC) telomerase complex (e.g., typical elderly MDS or acquired AA) [11, 12, 16–18]. Therefore surprisingly, evolution of subclones of HSC carrying MDS-related clonal mutations does not seem to be the predominant mechanism for MDS/AML initiation in cryptic DKC patients.

At this stage, the pathophysiology underlying the malignant transformation in (cryptic) DKC remains unclear. Various murine models favor the role of chromosomal instability due to critically short telomeres as the main contributing factor for tumor initiation [1]. In line with this observation, both analyzed patients with MDS or AML (pat. #10 and #15) show chromosomal aberrations but no MDS-related mutations.

We hypothesize that significant replicative potential is a prerequisite of HSCs that can undergo multi-step clonal evolution from genetically normal HSCs via CHIP and eventually toward malignant transformation into MDS and/or AML. In comparison, in patients with DKC, due to the already accelerated TS, the replicative reserve in the HSC compartment is typically limited. Therefore, upon a rather limited number of cell divisions, the DKC stem cells either

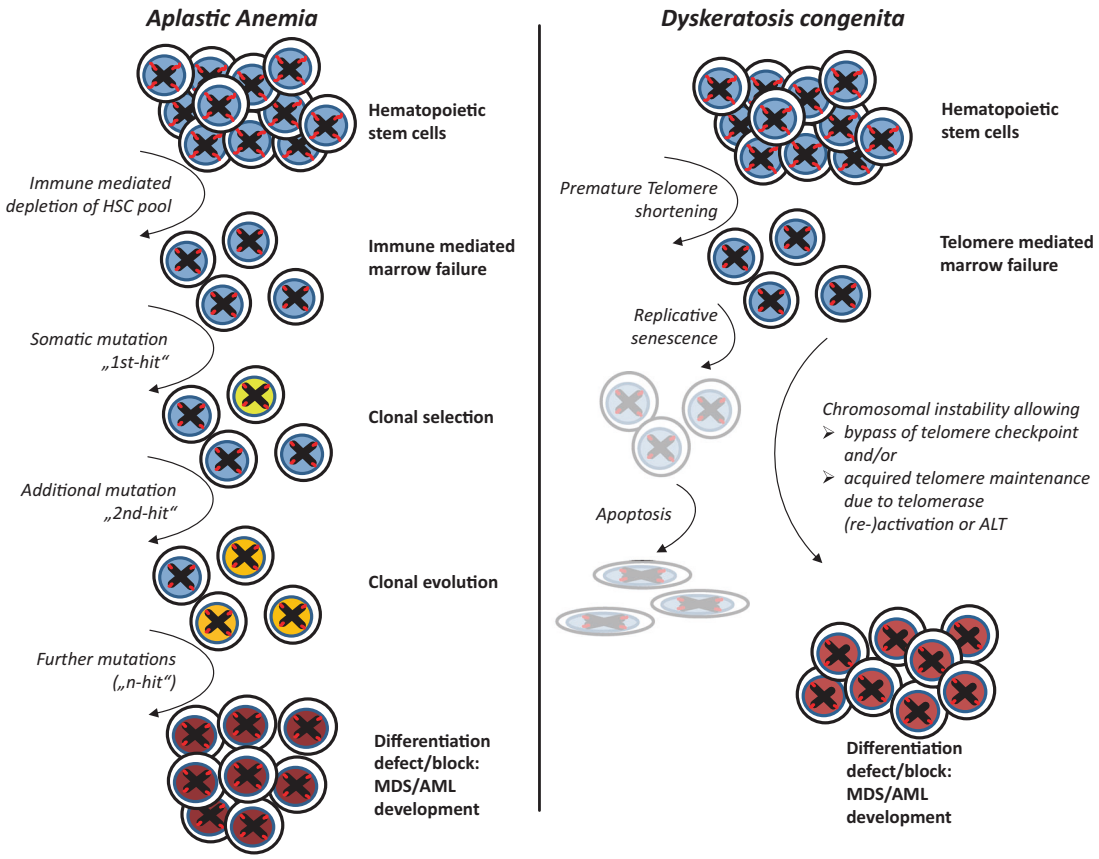

Fig. 2 Schematic representation of possible MDS/AML development in aplastic anemia and dyskeratosis congenita. ALT alternative lengthening of telomeres, AML acute myeloid leukemia, MDS myelodysplastic syndrome, HSC hematopoietic stem cells

undergo replicative senescence/apoptosis, or alternatively acquire the defined genetic events that allow them to either bypass the telomere checkpoint, or maintain or (re-)elongate TL early enough to become clonally selected. The latter is achieved either by reactivating the telomerase activity or alternative lengthening of telomeres (ALT, Fig. 2).

U2AF1 mutations lead to increased proliferation of hematopoietic progenitors [26], thus providing a possible escape mechanism to allow HSC to circumvent the telomere-mediated replicative senescence. Genetic mosaicism, somatic gene reversion [27, 28], occurrence of hTERT promotor mutations, or epigenetic factors (e.g., genomic imprinting) may be the possible alternative mechanisms to allow clonal expansion in the presence of short telomeres in this patient. However, detailed follow-up studies are needed to further elucidate the mechanism of how cells carrying the impaired telomerase activity actually manage to expand to a significant clone size.

Finally, we hypothesize that the absence of typical somatic mutations in NGS screening might potentially help to discriminate the clinically overt DKC patients without DKC-causing mutations from patients with acquired AA typically carrying MDS-related mutations at high frequency. Overall, since correct diagnosis of DKC is of utmost importance for treatment (including donor selection in the case of stem cell transplantation) and management of complications [9], significant clinical benefits can be expected from better identification of particularly late-onset hereditary BMF syndromes, such as cryptic DKC.

We here provide the first data showing that additional somatic mutations in DKC are rare events, possibly arguing against the multi-step clonal evolution based on sequentially acquired MDS-related mutations as the predominant mechanism for the development of secondary hematologic malignancies in adult and late-onset DKC. Due to the uncertain incidence and probable underdiagnosis of this disease subgroup in adults, prospective and cross-registry validation of these results and systematic follow-up of affected patients need to be initiated to further substantiate these findings.

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Author contributions MK: Performed the experiments, analyzed and interpreted the data and wrote the manuscript. AM: Performed the experiments and analyzed the data. MW: Provided patient samples, clinical data and interpreted the data. MSVF, A-SB, IH: Performed the experiments and interpreted the data. WB, MK, MR, SC, JB, MS, JB, MPR, CMW: Provided patient samples, clinical data and interpreted the data. SK, MB, IK, MS: Performed the experiments, analyzed and interpreted the data. THB co-planned the study, analyzed and interpreted the data and wrote the manuscript; FB conceived and planned the study design, interpreted the data, and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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