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Telomerase gene screening and telomere overhang detection in Chinese patients with myelodysplastic syndrome

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ABSTRACT

Background: Telomerase disfunction leads to short telometric overhangs, potentially resulting in chromosome instability.
Aims: To better understand the role of overhang length in the progression of myelodysplastic syndrome (MDS).
Methods: Bone marrow samples of 62 Chinese MDS patients were screened for TERT and TERC gene variants. Overhangs length was investigated.
Results: No mutation was identified. MDS patients had shorter overhangs compared to controls. Abnormal karyotype ones had shorter overhang compared to normal. Telomeric overhang length decreased as IPSS/WPSS value increased.
Conclusions: Overhang changes in accordance with IPSS/WPSS in MDS. Short overhang may be an independent factor for poor prognosis in MDS.

Telomeres consist of double stranded TTAGGG repeats with a length of 3–12 kbp and are associated with proteins capping chromosome ends. They prevent telomeres from degradation, loss of genetic information and end-to-end fusion, all of which can lead to senescence and apoptosis [1]. Telomere shortening induces genetic instability [2], which can lead to the development of cancer [2]. At the end of the telomere there is a single stranded 3′-overhang tail which contains a special structure named the t-loop. The t-loop protects against chromosome fusion [3,4]. Unlike the telomere, which shortens with age [1], overhang length remains unchanged over time [5–7]. Telomere length is regulated by the telomerase complex. Genetic mutations in the components of telomerase (the RNA, *TERC*, which contains the template for telomere repeats and the reverse transcriptase *TERT*) have recently been implicated in a variety of bone marrow failure syndromes such as dyskeratosis congenita and aplastic anemia [8–10]. Most of aplastic anemia (AA) patients carrying telomerase gene mutations have prominent erosion of telomeres and critically short telomeric overhangs [6], which may contribute to chromosome instability. Nevertheless, telomerase complex genes mutations which lead to telomere shortening were also reported in patients with myelodysplastic syndrome (MDS) recently and telomere shortening was recognized as one of the mechanisms for MDS progression [11,12]. However, there are very few reports on Chinese people so far.

In this study, we investigated telomerase gene (*TERC* and *TERT*) mutations in Chinese patients with MDS, and their impact on telomere length, telomere overhang length and patients' prognosis. Based on what has been shown in aplastic anemia patients, we also evaluated the role of telomere overhang length in the prognosis of patients with MDS.

1. Patients and methods

1.1. Patients

Sixty-two patients diagnosed with MDS in Peking Union Medical College Hospital (PUMCH), Beijing, China, from Jan 2009 to Dec 2011 were enrolled. The diagnosis was made based on WHO (2008) classification [13]. Bone marrow mononuclear cells from 62 patients with MDS as well as 46 healthy volunteer controls (age and gender matched, 25 male and 21 female, age from 23 to 69 years). The study was approved by the Institutional Research Ethics Committee of PUMCH, and written informed consent was obtained from each subject. The samples from MDS patients were collected during diagnostic procedures before therapy. For MDS patients, their age ranged from 17 to 79 years (median age 56 years). 36 patients were male and 26 were female. Median follow-up time was 12 months (4–36 months). The main clinical features are outlined in Tables 1 and 2.

1.2. Methods

DNA from all 62 MDS patients was extracted from leucocytes of bone marrow samples, using RelaxGene Blood DNA system kit (Tiangen, Co., Beijing). Genes encoding *TERC* and *TERT* (exon 1 and exon 2) were amplified by polymerase-chain-reaction (PCR) on each sample of 62 MDS patients as reported previously [14].

Terminal restriction fragments (TRF) were measured as the length of telomere, using the classic southern blot method. The *Telo* TAGGG Telomere Length

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Table 1
Clinical manifestation of patients with MDS.

Character	Num. of patients
Male	36
Female	26
Median age (range)	56 (17–79)
WHO classification [13]	
RCUD	25
RARS	6
RCMD	2
RAEB I	15
RAEB II	14
Chromosome abnormalities[a]	10
Favorable	0
Intermediate	6
Unfavorable	4
Treatment options	
IST (immunosuppressive therapy)	11
BST (best supportive therapy)	22
Allo-BMT (Allo-bone marrow transplantation)	7
Chemotherapy	18
Unknown	4
Final outcome	
Survival without progression	51
No response to therapy or progression	2
Dead	9

[a] According to 2008 WHO classification cytogenetics abnormalities were stratified into 3 risk status [13].

Assay kit (Roche, Mannheim, Germany) was used. Images were analyzed with software Telomeric 1.2 after background subtraction, according to *Telo* TAGGG kit's instructions. Telometric overhang length of leucocytes was measured by the nondenaturing southern blot method, in which double stranded DNA was not denatured, leaving only the single strand overhangs to hybridize to the probe [14,15]. Briefly, 1 μg DNA samples, digested by Hinf I and RsaI, were separated on 0.8% agarose gel in 1× TAE at 90 V for 4 h. DNA fragments were then transferred to a nylon membrane and fixed on it by drying oven at 120 °C for 30 min. The membrane was then incubated with DIG-labeled (CCCTAA) 3 probes in hybridization buffer (50 mM NaCl, 50 mM Tris–HCl pH 8.0, 1 mM EDTA) and incubated at 50 °C overnight. After washing, blocking and developing, the membrane was exposed to autoradiography film.

1.3. Statistical analysis

Data were analyzed through the *SPSS 18.0*. The average telomere lengths and overhang lengths were shown as values ± SEM. Student's *t*-test and *Chi-Square test* was used for differences between different patient cohorts. The COX regression analysis was explored for independent prognostic factors. P value < 0.05 was considered as statistically significant.

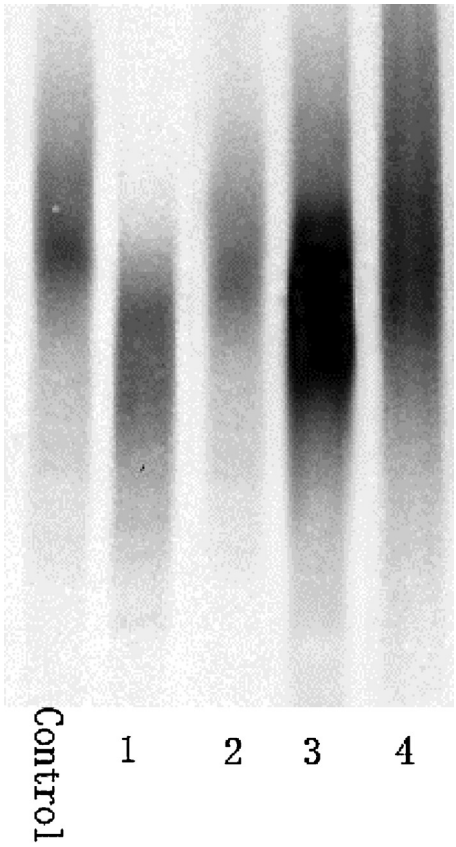

Fig. 1. Nondenaturing solution hybridization analysis of the overhang length. Samples were all hybridized to (CCCTAA) 3 probes, separated by electrophoresis, and detected by autoradiography. Control and lanes 1–4: nondenaturing hybridization of 1 μg Hinf I and RsaI restriction fragments from immortal IMR-90 cell and 5 different samples of leucocytes from bone marrow of MDS patients, respectively. By comparing the hybridization signal of probes combined to patients' sample DNA (lanes 1–4) with control DNA, the overhang length of each sample was estimated. For example: overhang length of the DNA in Lane 1 was 135.2 nt, as the nondenaturing hybridization signal for lane 1 was 1.04 times greater than to the same amount of control DNA with 130 nt overhang. The overhang length of lanes 2–4 was 69 nt, 345.2 nt and 257.9 nt, respectively.

2. Results

No mutations in telomerase gene were found in the MDS patients screened.

2.1. Telomeric overhang length in patients with MDS

Different from telomere length, which shortened with age, the telomeric overhang length in 46 healthy controls was 223 ± 42 nt and remained consistent in different age groups (data not shown). MDS patients had a shorter telomere overhang length (176 ± 62 nt) compared to normal controls (*t*-test, $P = 0.001$) Fig. 1. Those with abnormal karyotypes ($n = 10$) had shorter telomere overhang length (137 ± 72 nt) compared to those with normal karyotypes ($n = 52$, overhang length 184 ± 58 nt, $P = 0.027$). Telomeric overhang length decreased as IPSS or WPSS value increased (Tables 3 and 4). Patients with higher risk IPSS or WPSS had shorter telomere overhang length compared to those with lower risk.

Table 2
Cytogenetic aberrations of the MDS patients.

Patient ID	WHO classification [13]	Gender	Age (y/o)	Cytogenetic aberrations
1	RCUD	F	24	45,XX,−8 [5]/46,XX [15]
2	RCUD	M	52	46,XY,−2,+mar [6]
3	RCMD	M	28	47,XY,+8 [5]/46,XY [20]
4	RAEB-1	M	43	46,XY,−13,+mar [5]
5	RAEB-1	F	64	45,XX,−7 [20]
6	RAEB-1	M	17	46,XY,−11,+mar [8]
7	RAEB-1	M	19	47,XY, −?8,+21,+mar [7]
8	RAEB-1	F	72	46,XX,del(7q) [20]
9	RAEB-2	M	43	47,XY,+13 [7]/46,XY [13]
10	RAEB-2	M	71	48,XY,dup(1)(q21q32)×2,+8,+16 [20]