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Telomere Shortening Associated with Disease Evolution Patterns in Myelodysplastic Syndromes[1]

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ABSTRACT

We identified the telomere length at different hematological phases in 16 patients with myelodysplastic syndromes (MDS), showing disease evolution with a conventional Southern blot hybridization using the $(TTAGGG)_4$ probe. The MDS patients studied were classified into three groups according to the pattern of telomere length reduction. The first group had telomere shortening at the time of disease diagnosis. In four of the six MDS patients in this group, the disease progressed within 6 months postdiagnosis and each of them survived for less than 1 year. Moreover, in this group four patients showed a 5q anomaly with or without additional changes, and 50% of patients in this group had complex chromosome abnormalities. The patients in the second group showed reductions in telomere length after disease progression; two of these three patients showed gradual disease progression and had one or two chromosome abnormalities. The third group comprised the remaining seven MDS patients; they showed no telomere reduction by disease evolution. Two patients in this group experienced rapid disease progression. These results may indicate that telomere reduction is linked to disease evolution in some MDS patients, perhaps as a result of genomic instability because patients with complex chromosome abnormalities were clustered in the first group. However, because some MDS patients show disease progression without telomere reduction, genetic changes, including point mutations of certain gene(s), may also contribute to disease progression. We further noted that telomere shortening at the time of MDS diagnosis might indicate a poor MDS prognosis.

INTRODUCTION

The MDS[3] encompasses heterogeneous disorders characterized by cytopenias and dysplastic features in the bone marrow. The French-American-British group proposed a classification system based on morphological criteria (1). Because approximately 30% of patients with MDS progress into AML (post-MDS AML), the disease serves as an extremely useful model of leukemic transformation (2, 3). The delineation of genetic alterations and their interactions has implications for understanding not only their pathogenesis but also the changes occurring in normal and aberrant differentiations of hematopoietic cells. Patients with MDS usually display variable evolutionary patterns in their progression toward AML, *e.g.*, a rapid evolution, an insidious progression with rapid terminal evolution, or a gradual evolution (4). Moreover, some MDS patients with rapid disease progression exhibit multiple chromosome abnormalities (4–8), thus suggesting that genetic instability may play a role in MDS progression (7, 8).

The chromosome termini, termed telomeres, consist of a series of simple tandem repeat *palindromic* sequences that are guanine rich on one strand and are always oriented 5′ to 3′ toward the chromosome terminus (9–11); the repeated sequences have been highly conserved throughout evolution (10). In humans, 10–15 kilobases of TTAGGG repeats are found at the ends of all chromosomes (10); these attach directly to the nuclear membrane (9), thus suggesting that telomeres may protect chromosome ends against illegitimate recombination (9). Because human telomeres progressively shorten with age in blood cells (12), colonic mucosa (13), and fibroblasts (12, 14), telomere shortening may reflect reduced replicative capability. Although the biological significance of this reduction in telomere length is uncertain, shortening resulting from incomplete DNA replication may be related to an increased frequency of chromosome abnormalities (14, 15).

Reductions in telomere repeat length have been found in various freshly obtained solid tumors (13, 16–18) but little is known about a correlation between disease progression and telomere shortening in hematopoietic neoplasias. To determine whether a change of telomere length plays a role in MDS disease progression, we subsequentially examined telomere lengths in 16 patients.

MATERIALS AND METHODS

Patients. The subjects in this study were 16 adult patients with newly diagnosed MDS who had been referred to the Tokyo Medical College Hospital since 1987. Their diagnoses of MDS were made according to the French-American-British classification (1). The 16 patients comprise 5 patients with refractory anemia, 1 with refractory anemia with ringed sideroblasts, 8 with refractory anemia with excess of blasts, and 2 with refractory anemia with excess of blasts in transformation. Two patients (patients 7 and 13) had secondary MDS. An evolution to overt leukemia was observed in 14 of the 16 patients (Table 1).

Cytogenetic Study. We performed chromosome analysis on cells obtained by bone marrow aspiration at the time of MDS diagnosis and subsequentially when the MDS phase changed. The cells were short-term cultured (<48 h) and processed by a standard cytogenetic method as described previously (8). Chromosomes were stained with Hoechst 33258 (Sigma Chemical Co., St. Louis, MO) plus quinacrine mustard (Sigma) (8) and identified and described according to the International System for Human Cytogenetic Nomenclature (19). Cytogenetic classification was used as described previously (8).

Telomeric Repeat Analysis. High molecular weight DNA was extracted from bone marrow cells before and after disease evolution by the urea lysis-cesium chloride method. Ten μg of *Hinf*I-digested DNA was size-fractionated by electrophoresis on 0.8% horizontal agarose gel (20). After electrophoresis, DNA samples were denatured in 0.25 M NaOH and 0.5 M sodium chloride, neutralized in 0.3 M NaCl and 0.25 M Tris-HCl, transferred to a nylon filter (Zetabind), and cross-linked with UV light (20). The filter was hybridized to a $5'$-^{32}P-labeled $(TTAGGG)_4$ telomeric probe. Hybridization was carried out at 50°C for 16 h. The filter was then washed, dried, and exposed to Fuji XR film (Fuji Film Corp., Tokyo, Japan) with an intensifying screen. After removal of the first probe, the same blots were rehybridized with *Alu*I (BLUR-8; Oncor, Inc., Gaithersburg, MD), a human Alu repeat sequence. The telomere length was assessed quantitatively by densitometric analysis. Then we determined the peak of telomere length in kilobases as the average telomere length in each patient.

RESULTS

The average telomere length, determined by the densitometry analysis, is shown in Table 1. We have identified that the shortening of telomere repeats is related to aging of the peripheral mononuclear cells from normal volunteers

$$T = 11.85 - 0.042 \times A$$

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[3] The abbreviations used are: MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; HSR, homogeneously staining region.

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Table 1 *Telomere length and karyotypes in patients with myelodysplastic syndromes with disease evolution*

Patient	Diagnosis	TRA[a] (kilobases)	Expected[b] (kilobases)	Karyotypes [no. of cells observed]	Leukemic transformation[c]	Survivals[d]
				Type A. Short telomere repeat with or without disease evolution		
1	RA	3.2	10.6	46,XY,del(5)(q13q34),+8,t(19;21)(p12;q11),−20[20]	4	5
	RAEB	3.0		46,idem,ace[19]/46,XY[1]		
3	RA	6.5	10.0	46,XY,dup(1)(q21qter)[3]/47,idem,+8[29]	No	12+
	RAEB	6.5		46,idem[17]/49,idem,+8,+13,+16[78]/92,idemx2[3]/46,XY[2]		
6	RARS	5.6	8.7	46,XY,t(5;13)(q31;q21),t(17;21)(p13;q11)[21]	5	8
	AML	4.0		46,idem[21]/44,XY,−11,−17,der(20)t(20;17)(p11;q11)[9]		
9	RAEB	3.6	9.1	46,XY,t(3;4)(q26;q21)[21]/46,XY[1]	36	46
	AML	3.8		46,idem[20]		
13	RAEB	5.8	8.8	46,XX,del(5)(q13q34)[20]	2	3
	AML	6.0		44,idem,−7,−9,−16,+mar[14]/43,idem,−7,−8,−16,del(12)(q21)[5]		
15	RAEB-T	4.3	10.6	43,XY,add(3)(q21),del(5)(q13q34),add(6)(q21),−8,−18, −21[7]/44,idem,+add(3)(q21)[1]/46,XY,−8,+mar[1]	2	3
	AML	4.3		45,XY,del(5)(q13q34),del(7)(p14),−18[10]		
				Type B. Telomere shortening with disease evolution		
2	RA	9.0	10.5	46,XY,dup(1)(q21q42)[38]/92,idemx2[8]/184,idemx4[1]	20	28
	RAEB-T	2.7		46,idem[10]/47,idem,+del(1)(p11)[1]/46,XY[1]		
7	RAEB	10.0	10.2	46,XX[23]	5	6
	AML	4.0		47,XX,+8[2]/46,XX[18]		
8	REAB	9.8	9.3	45,XX,del(5)(q21q34),−9[21]	32	34
	AML	3.6		45,idem[1]/45,idem,6dmin[21]		
				Type C. No reduction of telomere repeat with or without disease evolution		
4	RA	16.0	9.6	47,XY,+8[2]/46,XY[18]	17	42
	AML	16.0		47,idem[2]/46,XY[23]		
5	RA	9.6	9.5	46,XY,der(12)t(1;12)(q24;p21)[23]	No	19
	RAEB	9.6		46,idem[14]		
10	RAEB	11.6	9.0	43,X,−Y,t(3;4)(p21;q12),hsr(6)(q27)add(6)(q27), −7,hsr(11)(q23)add(11)(q23),−20,add(22)(p11)[20]	0.5	2
	AML	11.6		43,idem[19]/46,XY,add(3)(p21)[1]		
11	RAEB	17.5	9.0	46,XY[21]	10	23
	RAEB-T	17.5		46,XY,t(1;7)(p10;p10),del(20)(q11)[3]/46,XY[18]		
12	RAEB	8.2	8.9	46,XY[24]	10	16
	AML	8.2		46,XY[18]		
14	RAEB	10.6	8.6	46,XY[12]	12	14
	AML	10.6		46,XY[22]		
16	RAEB-T	9.8	8.5	46,XY,del(20)(q12)[4]/46,XY[16]	2	12
	AML	9.8		46,XY[20]		

[a] TRA, telomere repeat length of the maximal peak; RA, refractory anemia; RAEB, refractory anemia with excess of blasts; RARS, refractory anemia with ringed sideroblasts; RAEB-T, refractory anemia with excess of blasts in transformation; No, no leukemic transformation.
[b] Expected telomere repeat length calculated by age in years (21).
[c] Duration between the diagnosis and leukemic transformation in months.
[d] Survival periods after the diagnosis of myelodysplastic syndromes in months.

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where T = telomere length in kilobases and A = age in years (21). Thus, we determined that MDS patients with shortening telomeres were those whose telomeres were shorter than expected relative to age (*i.e.*, mean $- 2 \times$ SD). Accordingly, we calculated telomere length in MDS patients at the time of MDS diagnosis and when the disease had evolved, and we attempted to classify subjects into the following three types.

MDS with Short Telomere at the Time of Diagnosis (Type A). Telomere shortening was observed in 6 patients at the time of MDS diagnosis (Table 1). The average length of telomeres was approximately equal before and after disease progression (Fig. 1). Three of the six patients (patients 1, 6, and 15) showed three or more chromosomal changes (*i.e.*, complex aberrations) and they experienced rapid disease progression. Two patients (patients 3 and 9) had abnormalities of two chromosomes (Table 1) and both of them showed no aggressive clinical courses when compared with those having complex chromosomal abnormalities. The one remaining patient (patient 13) had secondary MDS and progressed into the AML phase with multiple chromosome abnormalities. It should be emphasized that no patients with normal karyotypes demonstrated telomeric shortening.

Interestingly, telomere shortening in some patients was evident even when the percentage of blasts in the bone marrow was less than 5%, *i.e.*, refractory anemia or refractory anemia with ringed sideroblasts. This indicates that almost all of the marrow cells derived from abnormal stem cells, and that the morphologically heterogeneous populations had the same genetic alternation.

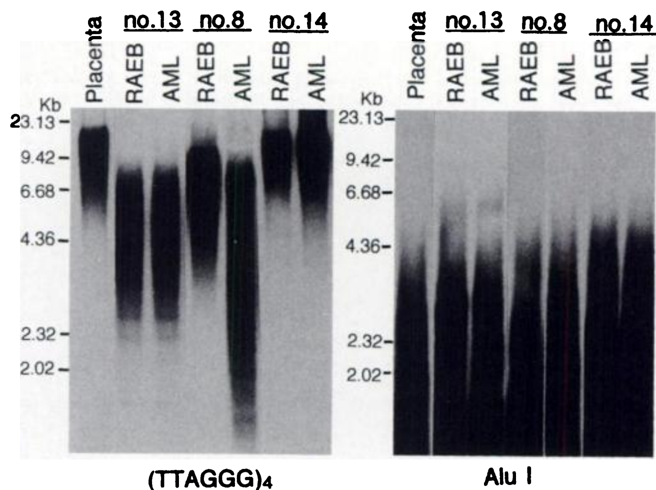

Fig. 1. Southern blot hybridization of the cells of representative MDS patients (patients 13, 8, and 14) using the 5'-^{32}P-labeled $(TTAGGG)_4$ probe (*left*) and the *AluI* probe (*right*). In each patient, different hematological phases are indicated at the *top*. Patient 13 represents pattern A; the telomere length at both the RAEB phase and the AML phase was less than expected. Patient 8 represents pattern B; telomere shortened after the disease has progressed. Patient 14 represents pattern C; telomere length was normal or longer than expected, and this change was noted at the MDS diagnosis (at the refractory anemia with excess of blasts phase) and when the disease had progressed (at the AML phase). *Kb*, kilobases.

MDS Showing Telomere Shortening with Disease Evolution (Type B). Reduced telomere length accompanying disease progression was noted in 3 patients (patients 2, 7, and 8) (Table 1). As shown in Fig. 1, two separate peaks were observed in the leukemic phase of patient 8. The higher peak corresponded closely in size with the sample obtained at the refractory anemia with excess of blasts phase, suggesting that a new population of abnormal cells with short telomere repeats appeared at the leukemic phase. It is noteworthy that the telomere shortening in patient 8 was associated with an appearance of double minute chromosomes. This suggests that the cells with shorter telomeres may be derived from a new clone that appeared after leukemic transformation as a result of genomic instability. In patient 2, prominent telomere shortening was also noted in the refractory anemia with excess of blasts in transformation phase, but no karyotypic evolution was evident. These patients showed insidious disease evolution until leukemic transformation (20 and 32 months, respectively) with terminal rapid progression of the disease (Table 1).

In patient 7, MDS developed after chemotherapy for previous gastric cancer. Cytogenetic analysis revealed trisomy of chromosome 8 at the leukemic phase, and both telomere shortening and AML transformation were evident.

MDS without Telomere Shortening (Type C). Seven patients (patients 4, 5, 10, 11, 12, 14, and 16) showed no significant reduction of telomere length before and after disease evolution. Among them, six patients showed normal karyotypes and/or simple chromosome changes at the MDS diagnosis (Table 1). The one remaining patient (patient 10) showed complex chromosomal abnormalities including HSRs at the 6q27 and 11q23 regions with an unidentified large marker chromosomal segment at the distal site of the HSRs (22). This patient progressed into the AML phase 2 weeks after the initial diagnosis of MDS.

DISCUSSION

Many lines of evidence suggest that the pathogenesis of cancer proceeds by sequential steps from normal to malignant cells (23, 24). In some hematopoietic malignancies, discrete steps in cancer progression have been associated with chromosomal abnormalities but specific chromosomal translocations are not common in post-MDS AML (25). It is uncertain how many mutations are required for disease progression in MDS patients, although an increased mutation rate is important in tumor progression. Multistep genetic alterations also may be necessary for disease evolution in MDS, as has been reported in the presence of colon cancer (23).

Because the reduction of telomere length at the time of MDS diagnosis is frequently associated with complex chromosomal abnormalities, as reported here, telomere shortening may possibly represent a genomic instability of the neoplastic cells. In the current study, however, approximately one-half of the MDS patients (type C) maintained constant telomere lengths both at the time of MDS diagnosis and during the leukemic phase. This suggests that in some MDS patients, changes in certain gene(s) rather than overall genomic instability play a role in the early stage of AML transformation. Candidates for these early molecular events are mutations of *RAS* and *FMS* genes. In 20–40% of MDS patients, point mutations of activating *RAS* family genes (mainly N-*RAS*) have been found (26, 27), and mutations of *FMS* genes also are observed in some 5% of MDS patients (28). Because *p53* mutation does not occur frequently in MDS, it is believed that the mutation does not play a critical role in MDS development (29–31). Moreover, neither amplification nor rearrangement of the MDM_2 gene, a regulator of *p53* function, is identified in MDS patients (32, 33), indicating that inactivation of *p53* is a rare pathogenic event in MDS. Because a 5q anomaly is one of the nonrandom chromosome abnormalities in MDS, interferon regulatory factor 1 (mapped at 5q31.1) may play some role in those MDS patients with 5q abnormality (34).

Genomic instability may induce an increased mutation rate and result in chromosomal instability, which may then induce further mutations. These sequential steps might occur during the disease evolution in MDS. Thus, telomere shortening, one example of genomic instability, is of prognostic and clinical importance in MDS. One patient (patient 10) with HSRs at the 6q27 and 11q23 regions retained telomere length after AML transformation, perhaps because telomerase activity in the neoplastic cells induces an inappropriate DNA replication at the HSRs.

The average telomere lengths do not change during the course of disease in most patients with MDS (13 of 16 cases). These findings are quite different from those observed in *de novo* AML cells; telomere shortening was observed only in leukemia cells, and length was restored in the remission state (17). These results suggest that hematopoietic cells in MDS undergo the same genetic alterations, although the blast cells are capable of differentiating into morphologically mature cells. Evolution to acute leukemia may result from an impairment of cellular differentiation.

In conclusion, terminal repeat assay combined with cytogenetic study is a useful tool for predicting disease evolution patterns. MDS patients with telomere shortening at the time of diagnosis should be followed carefully, regardless of the percentage of blasts, because currently available chemotherapy or cytokines have limited power to promote prolonged survival once the disease has progressed.

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