Detection of Monoclonal Hematopoiesis Using Restriction Fragment Length Polymorphism (RFLP) of Phosphoglycerokinase (PGK) Gene in a Patient with Hypoplastic Myelodysplastic Syndrome (MDS)

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A clonal analysis of hematopoiesis in a hypoplastic MDS patient was performed. Monoclonal utilization of PGK gene on her two X chromosomes was detected by Southern blot analysis of the bone marrow cells using a PGK probe after methylation-sensitive restriction enzyme treatment. This is compatible with monoclonal construction of her hematopoiesis, confirming MDS nature with a progression of her morphological dysplasia in bone marrow cells. RFLP of PGK gene on X chromosome is thus useful to detect a monoclonality in hematopoietic diseases. (*Internal Medicine* 32: 255–256, 1993)

Key words: diagnosis of MDS, PGK gene analysis

Introduction

MDS is a monoclonal disease derived from a disorder of the hematopoietic stem cell, which usually has normo to hyperplastic marrow with dysplastic features (1–3). But in some cases the bone marrow is hypoplastic and morphological dysplasia is mild, thus it is difficult to distinguish MDS from aplastic anemia (AA) (4). Recently, RFLP of the PGK gene has been utilized to detect the monoclonality of many hematopoietic diseases (5). This method should likewise be useful in proving a monoclonal hematopoiesis in hypoplastic MDS.

Here, we report the analysis of monoclonality in the bone marrow cells of a female hypoplastic MDS patient, as performed by RFLP of the PGK gene.

Case Report

A 17-year-old Japanese female suffered from easy fatigability, gum bleeding and genital bleeding in October 1986. She was well before this episode and visited our hospital and a physical examination showed anemic conjunctiva, bleeding spots in skin and oral mucosa. Hematological examination revealed pan-

cytopenia, and hypoplastic marrow with decreased megakaryocytes (Table 1). Mild to moderate morphological dysplasias, such as karyorrhexis of mature granulocytes and megaloblastic change of erythroblasts, were detected in her bone marrow smear. The blast percentage in the bone marrow was less than 5%. The diagnosis of hypoplastic MDS (refractory anemia) was suspected. Chromosomal analysis revealed the normal karyotype of 46,XX. Corticosteroids, androgen and erythropoietin were administered, but were ineffective. She became dependent on red blood cell and platelet transfusion because of progressive anemia and bleeding tendency. In 1991, hemochromatosis developed with abnormal liver function, diabetes mellitus, skin pigmentation and arrhythmia. She was occasionally admitted to our hospital with infection and/or bleeding episodes. She had no HLA-identical siblings.

Bone marrow aspiration was performed in June 1991, and high molecular weight DNA was obtained from bone marrow cells by standard phenol-chloroform method with informed consent. DNA (20 µg) was treated with restriction enzyme Pst I and Bst XI. Southern blot hybridization was performed using one-half of this DNA with a PGK probe (Fig. 1). Two bands, 1.05 Kb

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Table 1. Hematological Data on Admission and Five Years Later

	Oct. 1986	June 1991
Peripheral blood		
RBC ($\times 10^4/\mu\text{l}$)	146	243
Hb (g/dl)	5.6	7.9
Ht (%)	15	26.4
WBC ($/\mu\text{l}$)	1700	1400
Stab (%)	3	8
Seg (%)	29	41
Ly (%)	63	44
Mo (%)	5	7
Plat ($\times 10^4/\mu\text{l}$)	3.7	0.1
Bone marrow		
Cellularity	hypocellular*	hypocellular
M/E ratio	0.9	0.8
Blast (%)	< 1	< 1

* Lower than 30%, confirmed by bone marrow biopsy

Fig. 1. Southern blot analysis using PGK probe. High molecular weight DNA was obtained from bone marrow cells as described in the text. Left lane: DNA treated with Pst I and Bst XI. Right lane (+ H): DNA treated with Pst I, Bst XI and Hpa II.

and 0.9 Kb were detected, and it indicated that she had a polymorphism of Bst XI site in 5' region of PGK gene. Additional cutting of another half of DNA with methylation-sensitive restriction enzyme, Hpa II, was performed to detect the monoclonality of her hematopoiesis. Hpa II enzyme cuts non-methylated DNA; in this case, the active PGK gene on her two X chromosomes was cut into some small fragments. After this treatment, Southern blotting with the same PGK probe showed only one band at 0.9 Kb. This suggested monoclonal bone marrow cell construction. On the bone marrow smear, distinct dysplastic changes such as Pseudo Pelger-Huët anomaly with agranular neutrophils, could be detected. The blast percentage was also below 5%. The monoclonal construction of her bone marrow and a progression of dysplastic features strongly supported the diagnosis of MDS although her bone marrow was still hypoplastic.

Discussion

MDS is a monoclonal hematopoietic disease with cytopenias due to ineffective hematopoiesis, although bone marrow usually shows hypercellularity (1). The diagnosis of MDS is usually made by detecting morphological dysplasia on the bone marrow and peripheral blood smears when a patient has normo to hypercellular bone marrow. However, in some MDS patients, the cellularity of the bone marrow is hypoplastic but with mild dysplastic change. In these cases, differential diagnosis from AA is often difficult (4). In such cases monoclonal analysis facilitates the determination of a diagnosis of MDS. If it reveals monoclonal hematopoiesis, the diagnosis of MDS becomes more likely. However, monoclonal hematopoiesis has recently been reported in some AA patients (6, 7). Therefore detailed clonal analysis is necessary to reveal clonal and non-clonal hematopoiesis in AA. The relationship of clonal AA to MDS must be further clarified. But some other findings such as obvious morphological dysplasia, chromosomal abnormality and point mutations of oncogenes (ex. ras gene) strongly support the diagnosis of MDS. In the present case, the dysplastic features progressed in the patient's bone marrow cells during her clinical course.

Although polymorphism of the PGK gene is detected in about 40% of Japanese women, clonal analysis using RFLP of the PGK gene is very useful to detect clonal hemopathy in various blood diseases.

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