Analysis of Myelodysplastic Syndrome Clones Arising After Multiple Myeloma: a Case Study by Correlative Interphase Cytogenetic Analysis

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Background: A patient with multiple myeloma developed myelodysplastic syndrome (MDS). Chromosomal analysis performed after the development of MDS revealed monosomy of chromosome 9 in all the metaphases. We wished to identify the extent of the clone with the chromosomal abnormality originating from MDS clone.

Methods: A correlative interphase study by fluorescence in situ hybridization (FISH) was performed and we determined whether each lineage of cells obtained the molecular mark. The chromosome 9 classic α satellite region DNA was used as a probe for the FISH analysis in smear specimens stained with Wright-Giemsa stain.

Results: Erythroblasts, granulocytes and myelocytes had only one signal, whereas myeloma cells showed two to four signals.

Conclusions: This study visualized the spectrum of MDS clone. The results suggest that the origin of MDS is different from that of multiple myeloma, at least in this case.

Key words: multiple myeloma - MDS - chromosome analysis - FISH

INTRODUCTION

The cellular origin of multiple myeloma is controversial. Studies have shown the occasional expression of immature markers on the surface of myeloma cells (1–4). Reports of myeloma cases with simultaneously occurring myeloid leukemia (5) suggest a stem cell origin of the tumor. It has also been reported that as many as 17.4% of multiple myeloma cases developed acute myeloid leukemia 50 months after the initial diagnosis (6). These reports suggest that the cells might be more primitive than previously thought and may be able to differentiate into lineages other than B cells. Here, we report a patient with multiple myeloma who developed myelodysplastic syndrome (MDS) 10 years after the initial diagnosis. The concurrent emergence of cytogenetic abnormality and MDS enabled us to study the extent of involvement of abnormal clones by direct comparison of cell morphology in Wright-Giemsa-stained smears with cytogenetic findings by the fluorescence in situ hybridization (FISH) technique.

MATERIALS AND METHODS

Patient

The clinical course of the case has been reported previously (7). Briefly, a 64-year-old woman with multiple myeloma was admitted to our hospital (IMS, The University of Tokyo) because of gradually developing anemia, the appearance of myeloblasts in her peripheral blood and the presence of abnormal karyotype in her bone marrow. She had been diagnosed as having multiple myeloma IgGλ, Durie-Salmon Stage II, 10 years before. Chromosomal analysis performed repeatedly before developing MDS showed 46, XX and after confirmation of MDS showed 44, XX, del(5q), del(7q), -9, add(12p), -21 in a total of 20 cells analyzed, which was suggestive of a secondary MDS due to the cumulative alkylating agents (8). A bone marrow smear at the time of development of MDS revealed 41.6% of myeloid cells with increased myeloblasts(13.6%), 28.8% erythroid cells and 2.4% myeloma cells.
WRIGHT–GIEMSA STAINING

The aspirated bone marrow was smeared on to glass slides, quickly air-dried under a fan, fixed in methanol and stained manually with Wright–Giemsa stain. The specimens were stored at –20°C until the FISH analysis. The stained smears were initially examined by light microscopy under field on an Olympus BX 60 fluorescence microscope (Olympus, Tokyo, Japan). At least 100 cells of each cell lineage were photographed at 1000× magnification, except for lymphocytes, of which only 10 cells were counted because of their limited numbers on the smear. The photographed cells included myeloblasts, myelocytes, neutrophils, erythroblasts, lymphocytes and myeloma cells. The positions of the cells were recorded. The same microscope was used for the subsequent fluorescent microscopy examination.

PROBE

The digoxigenin-conjugated probe D9Z1, for the α-centromeric region of chromosome 9, was purchased from the manufacturer (Oncor, Gaithersburg, MD, USA). The photographed slides were washed in xylene. The slides were then soaked in 95% ethanol and sequentially hydrated with distilled water (90, 70, 50, 30 and 0% ethanol) at room temperature to remove the xylene completely.

FISH

Fluorescence in situ hybridization slides were baked at 65°C for 3 h, followed by treatment with ribonuclease A (100 μg/ml in 2×SSC, 1× SSC, 0.15 M sodium chloride, 0.015 M sodium citrate) for 1 h at 37°C, peptic solution (0.02% in 0.01 M HCl) and washing twice with 2× SSC at 37°C. After a series of ethanol dehydrations (70, 80 and 95%), the slides were dried, then denatured in 70% formamide–2× SSC at 70°C for 2 min, followed by an identical series of ethanol dehydrations. The slides were prewarmed at 37°C for 10 min and hybridized to the DNA probe at 37°C in a moist chamber overnight. The slides were washed in 50% formamide–1× SSC for 1 min at 42°C twice and then at room temperature twice.

Signals were visualized after treatment with anti-digoxigenin antibody conjugated with FITC (Boehringer, Mannheim, Germany). Nuclei were counterstained with propidium iodine–antifade and covered with a glass coverslip. The number of bright signals was counted and sorted according to the cell lineage under the fluorescence microscope with an absorption maximum of 492 nm and an emission maximum of 520 nm.

SOUTHERN BLOT HYBRIDIZATION

A 10 μg amount of DNA extracted from the peripheral blood was subjected to Southern blot hybridization. The probe used was immunoglobulin heavy chain joining region DNA purchased from the manufacturer (Oncor).

RESULTS

As shown in Table 1, a direct visual correlation was made between the cytology and smears. Control specimens showed two signals in more than 95% of the cells. The peak mode of one signal was shown in the myeloblasts, myelocytes, granulocytes and erythroblasts. In contrast, the myeloma cells contained more

Figure 1. Standard morphological examination. (A) Myeloma cells, erythroid cells and myeloid cells are shown. (B) Mature granulocyte with one signal is shown in the upper left part. (C) Myeloma cells with more than two signals are shown. Compare with Fig. 2C. (D) A lymphocyte with two signals is shown in the upper left part. Compare with Fig. 2D.

Figure 2. Interphase FISH analysis. The same position as in Fig. 1 is shown.
than one signal. As many as four signals were observed in 26% of the myeloma cells. All the lymphocytes showed two signals (Figs 1 and 2).

Southern blot hybridization showed a germ line pattern immunoglobulin gene configuration and the absence of rearranged bands (data not shown).

**DISCUSSION**

We performed a FISH analysis on bone marrow smear specimens and were able to observe clear signals. It has already been shown that FISH can be directly performed on smear specimens. The method was used to detect trisomy 12 clone in CLL cases (9,10), trisomy 17 in acute lymphoblastic leukemia (11) and trisomy 8 in MDS (12). In these studies, trisomy was visible by this method. Here we have shown that a classical satellite probe for chromosome 9 is also available with this direct FISH analysis of smear specimens in cases with monosomy.

We showed monosomy 9 in 81% of granulocytes in addition to myeloblasts and myelocytes. This is a clear indication that MDS clones dominate the myeloid lineage. This result contrasts with those in previous reports using a chromosome 8 probe, in which only a subset of myeloid cell lineage was shown to obtain the chromosome abnormality (12,13). Trisomy 8 has been shown to occur during the evolution of MDS and is thought to be an additional late event seen in MDS (14). Mixed chimerism of clones with and without trisomy 8 are seen (13); therefore, using a chromosome 8 probe alone might easily miss the MDS clone. We used a chromosome 9 probe instead and found that almost all the myeloid lineage cells obtained monosomy, which suggests that all the myeloid lineages have already substituted the myeloid lineage cells with two chromosome 9 alleles. The substitution might have occurred because the case's chromosome 9 clone had a growth advantage, dominating the myeloid cell lineage. Alternatively, but not exclusively, the appearance of a morphological abnormality as MDS was a late event in this case and during the preceding unknown time, a clone with normal karyotype might have disappeared.

**Table 1. Results of FISH analysis**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Signals (%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>N.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>2</td>
<td>96</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Blasts</td>
<td></td>
<td>0</td>
<td>95</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Myelocytes</td>
<td></td>
<td>1</td>
<td>94</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td>9</td>
<td>81</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Erythroblasts</td>
<td></td>
<td>0</td>
<td>93</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myeloma cells</td>
<td></td>
<td>0</td>
<td>5</td>
<td>51</td>
<td>9</td>
<td>26</td>
<td>5</td>
</tr>
</tbody>
</table>

N.E., not evaluated. Control is a bone marrow smear obtained from a normal healthy volunteer. One hundred cells were counted in the blasts, myelocytes, granulocytes, erythroblasts and myeloma cells. Ten cells were counted in the lymphocytes.

We have shown in the present study that erythroid lineages are also substituted by cells with abnormal karyotype. Because the monosomy 9 clone dominated in the present MDS case and erythroid series were relatively abundant, we had a good opportunity to examine sufficient numbers of erythroblasts. Our finding that the erythroid cells also had abnormal clones in the MDS is consistent with results using other methods such as an analysis of G6PD isoenzyme (15) and restriction fragment length polymorphism of X-linked genes (16) and confirms the involvement of erythroid series of MDS clones by direct visualization.

Our findings that the multiple myeloma clone did not contain the monosomy 9 MDS clone and that the MDS clone retained the germ line configuration of immunoglobulin gene suggest that the MDS clone and multiple myeloma clone are independent. The origin of the myeloma clone is disputed. In marker studies, Epstein et al. reported that 15 out of 43 cases obtained the immature marker, CD 10 (17). Grogan et al. showed that myelomonocytic markers were present in 16 of 125 cases examined (4). The indirect evidence of these studies suggests a stem cell origin of the tumor. If that is the case, we can speculate that the occurrence of MDS could be a secondary phenomenon that arose from the same myeloma/stem cell clone and therefore one might expect the MDS clone to succeed the genetic marker of multiple myeloma. However, our results of Southern blot hybridization analysis showed that the MDS clone retained the germ cell pattern of immunoglobulin genes in cells of peripheral blood, where myeloid lineage cells dominate and almost all are considered as MDS clones. Because all the B cells including multiple myeloma cells have rearranged immunoglobulin gene and that process is irreversible (18), the germ line configuration of the immunoglobulin gene in cells of MDS suggests that the multiple myeloma clone did not transform to the MDS clone.

Vice versa, in the present case, the multiple myeloma clone did not have monosomy 9. Considering the absence of a rearranged immunoglobulin gene in MDS, the appearance of MDS accompanying monosomy 9 is considered to be a secondary phenomenon, occurring at a different cell lineage level from that of multiple myeloma cells.

We found three or four signals in the multiple myeloma cell sample in spite of the karyotypic analysis of 46,XX, which, when examined, indicated that only multiple myeloma clones existed. This suggests that some multiple myeloma clones might contain trisomy or tetrasomy. It is well known that it is difficult to obtain a correct karyotype of multiple myeloma without B cell stimulating factor(s). Multiple myeloma clones have much more chromosomal abnormality than we can detect by standard karyotype analysis. Dao et al. demonstrated the loss of chromosome 13 or 21 in 14 out of 22 cases (19). Drach et al. showed the loss or addition of chromosome 1, 3, 7, 8, 11, 12, 16, 17, 18, or X in 32 out of 36 multiple myelomas (20). Alternatively, this might be a high fraction of S phase (21) in the multiple myeloma cells; it has been shown that duplicated signals are sometimes observed in cancer cells with an aggressive course reflecting the high proportion of S phase. In either case, we can safely conclude that the myeloma cells did not contain monosomy 9 cells.
In summary, by FISH analysis of smear specimens, we have shown that an additional change that occurred simultaneously with the development of MDS did not appear in the multiple myeloma clone. Our patient developed MDS 10 years after the diagnosis of multiple myeloma. Multiple myeloma patients who simultaneously have leukemia (5) might have a different result. The analysis of more cases is needed to determine whether our present finding is a universal one.

Acknowledgments

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References