Molecular cytogenetic analysis of Korean patients with Waldenström macroglobulinemia

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Abstract

To compare the molecular cytogenetic characteristics between Waldenström macroglobulinemia (WM) and multiple myeloma (MM), we performed interphase fluorescent in situ hybridization (FISH) in Korean patients with WM and MM. Forty patients with WM and 132 patients with MM were enrolled onto the study. FISH was performed with seven different probes: 6q21, 6q23, CEP4, CEP9, immunoglobulin (IgH) breakapart, RB1 gene, and 1q25. Out of 22 WM patients, 4 (18%) had abnormal karyotypes, mainly structural changes on conventional karyotyping. After performing FISH for the available 29 cases, deletions of 6q23 and 6q21 were newly detected in 3 cases (10%). There was no other anomaly, including trisomy 4 in WM. No 6q deletion was observed in MM patients, but RB1 deletion was the most common change (45%), followed by IgH translocation (42%) and gain of 1q (38%). In conclusion, Korean WM patients had a low rate of 6q deletion (10%) and no trisomy 4. © 2010 Elsevier Inc. All rights reserved.

1. Introduction

Waldenström macroglobulinemia (WM) is a malignant lymphoproliferative disorder associated with infiltration of the bone marrow (BM) by pleomorphic B cells and monoclonal immunoglobulin (Ig) M production [1,2]. WM is a rare malignancy, accounting for approximately 1% of all hematological cancers and 4.7% of plasma cell neoplasms [2,3]. The characteristics of Korean patients with WM have not been well defined [4].

WM has not been clearly differentiated clinically from IgM monoclonal gammopathies, such as multiple myeloma (MM) and monoclonal gammopathies of undetermined significance, because of the production of the same type of immunoglobulin (Ig). However, the analysis of 14q32 by fluorescence in situ hybridization (FISH) has revealed only rare cases with Ig heavy chain (IgH) rearrangement in WM, which differentiates it from MM [5]. In addition, other cytogenetic abnormalities, such as a deletion of the long arm of chromosome 6 (6q) and trisomy 4, have been found to be unique hallmarks of WM [6–8].
In this study, we analyzed the clinical characteristics of Korean patients with WM and compared the molecular cytogenetic changes of WM, including the IgH translocation and deletion of 6q, to those of MM. The incidence of plasma cell neoplasm in Korea was relatively lower than that in Western countries as a result of racial difference. Although this disease has been increasing steadily over the last 25 years in Korea, the incidence and mortality rate of MM is now 1.0 per 100,000 persons per year [9]. Considering the relative scarcity of WM to MM, we have enrolled the major patients with WM in Korea onto our study.

2. Patients and methods

2.1. Patients

We studied 40 patients who fulfilled the criteria for the diagnosis of WM from 11 hospitals in Korea and compared their cytogenetic changes to those of 132 patients with MM from a main research center. This study was approved by the hospitals’ individual institutional review boards. Each patient had IgM paraproteinemia, regardless of the concentration, and clonal lymphoplasmacytic infiltration of the BM [1,2]. Additional diagnostic criteria were: an intertrabecular pattern of BM infiltration by tumor cells, and cell surface markers such as IgM+, CD5±, CD10−, CD19+, CD20+, CD22+, CD23−, CD25+, CD27+, FMC7+, CD103−, and CD138− (variations from this phenotypic profile could occur) [1].

2.2. BM review and conventional G banding

BM aspiration and biopsy samples were obtained at the time of diagnosis. The percentage and pattern of BM infiltration by lymphoplasmacytic cells were reviewed again at the main center. Among the 40 patients, 24 patients had results of chromosome analysis that used G banding [10]. More than 20 metaphase cells per patient were analyzed under the microscope. The definition of clones and the description of karyotypes were in accordance with 1995 International System for Human Cytogenetic Nomenclature [11].

2.3. FISH and probes

FISH was performed by the fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasm technique; analysis combined with Ig light chain staining of the cytoplasm, which was used to identify B lineage cells, was performed on BM mononuclear cells or air-dried BM smears. To detect deletions of 6q, we used two commercially available probes: 6q21/ MYC (8q24), dual color (Kreatech Diagnostics, Amsterdam, The Netherlands); and MYB (6q23) (Abbott Molecular, Des Plains, IL). Five other probes (IGH, CEP4, CEP9, RB1, and Ig; Abbott Molecular, Des Plains, IL) were also used to identify the differences between WM and MM samples. We calculated the means and standard deviations of positive signals obtained from FISH analyses using seven kinds of probes for a total of 40 negative controls, and the values of mean ± 3 × standard deviation for each probe were used as reference ranges.

2.4. Statistical analysis

We compared the clinical and laboratory parameters at diagnosis with the 6q deletions identified by FISH. The chi-square test was used to test for significant differences with regard to sex, performance status, B symptoms, hyperviscosity, lymphadenopathy, splenomegaly, hepatomegaly, peripheral neuropathy, and bone involvement. In addition, a t-test was used to detect significant differences in age, blood counts including hemoglobin and platelet, serum levels of albumin, IgM, and β2-microglobulin. The correlation of the 6q deletion and other clinical variables to progression-free and overall survival was analyzed by the log rank test. All calculations were performed with SPSS software, version 15.0 (SPSS, Chicago, IL).

3. Results

3.1. Clinical characteristics of WM patients

Thirty-three men and seven women with a median age of 66 (range, 40–87) years were enrolled onto the study. The clinical characteristics of the WM patients are summarized in Table 1. One patient had a period of IgM monoclonal gammopathy of unknown significance before the diagnosis of WM. Monoclonal IgM in the serum was observed in all patients, with a median level of 5.9 g/dL (range, 0.7–11.5 g/dL). Other significant laboratory abnormalities were as follows: hemoglobin <11.5 g/dL in 34 patients (85%), platelets...
<100 \times 10^9/L in 6 patients (15%), and β-2-microglobulin >3.0 g/dL in 24 of 30 patients (80%). According to the recently reported International Prognostic Scoring System [12], our 30 patients were classified as follows: low risk, 5 patients (17%); intermediate risk, 13 (43%); and high risk, 12 (40%). There was no information in the remaining 10 patients for assessing baseline β2-microglobulin.

3.2. BM histology and conventional cytogenetics

In the patients with WM, diffuse (53%) or intertrabecular (20%) patterns of BM infiltration by plasma cells, plasmacytoid lymphocytes, or small lymphocytes were mainly observed. The results of conventional cytogenetics (CC) were available in 24 patients at the time of the initial diagnosis. Two patients had insufficient numbers of metaphases to be evaluated. Among the remaining 22 patients, 18 had normal karyotypes. Abnormal metaphases were found in four patients, and all of them had structural cytogenetic abnormalities (Table 2, patients 1–4).

3.3. Deletion of 6q by FISH

We analyzed 29 samples from patients with WM for 6q deletions and found that 3 (10%) had abnormalities. These changes were not detected by CC in two patients, and the rest one had no previous cytogenetic result. The three patients had 6q deletions as assessed by both 6q21 and 6q23 probes, and the pattern of deletion was heterozygous (Figs. 1A and 1B). Two patients were found to have deletion of 6q in both small lymphocytes with scant cytoplasm and large plasma cells with abundant cytoplasm. Of note, 6q deletion was observed in only small lymphocytes, but not in plasma cells in one patient (Fig. 1C). Of the 30 MM patients studied, no deletion was observed with both 6q21 and 6q23 probes. No significant difference in clinicolaboratory characteristics such as age, sex, hemoglobin level, platelet count, serum β2-microglobulin, and IgM spike level was observed according to the 6q deletion, but albumin level was lower in patients with 6q deletion than those without (P < 0.05, P = 0.020). Progression-free and overall survival were not different between patients with del(6q) and normal 6q.

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>FISH del(6q)</th>
<th>del(13q)</th>
<th>IGH Gain of 1q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45,X,-X,add(1)(p32p34),t(3;7)(q27q21),t(8;22)(q24.1;q11.2),del(9)(q22),add(12)(p11.2)(16)[46,XX][4]</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>46,XX,14pdel(18)[46,idem,i(6)p10][2]</td>
<td>24%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>46,XX[t(1;14)(p11;q32)][5]/46,XX[4]</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>46,XX,del(20)(q13.1)[29]</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>46,XX,inv(9)(p11q13)[9]</td>
<td>18%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Not done</td>
<td>28%</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviation: FISH, fluorescent in situ hybridization; IGH, immunoglobulin heavy chain.

σGH indicates any translocation of immunoglobin heavy chain gene; a dash, negative findings for each probe.

3.4. Other cytogenetic abnormalities

The studies for detecting numerical changes of chromosome 4 and 9, any IgH rearrangement, deletion of 13q, and trisomy 1q were performed in 29 WM patients. No additional abnormality was detected (Table 2). In MM patients, the 13q deletion (45%, 59 of 132) was most common, followed by the IgH translocation (42%, 55 of 132), and gain of 1q (38%, 50 of 132).

4. Discussion

Both WM and MM are neoplasms of mature B cells. The normal counterpart of WM is presumed to be the postgerminal center of the B cell, whereas the normal counterpart is the plasma cell for patients with MM [13–16]. In MM, the oncogenic event occurs during plasma cell differentiation, after postgerminal antigenic stimulation, while the tumorigenesis develops in the memory of B cells after postgerminal antigenic stimulation in WM [17]. The frequency of IgH rearrangement was much lower in WM than MM [5]. The rarity of Ig gene rearrangement in the patients with WM was likely due to an isotype switch by deletion recombination, which does not contribute to the pathogenesis of WM [18]. The hallmark of WM has not been identified; the only recurrent abnormality noted to date is a deletion of the long arm of chromosome 6 and trisomy 4; however, the oncogenic mechanism associated with those has not been elucidated. Suspected mechanisms of del(6q) include the deletion of a tumor suppressor gene on chromosome 6q, possibly the PRDMI gene, which is predominantly expressed at the plasma cell stage, where it directly represses PAX5, and indirectly through XBP1 [19]. PAX5 is a gene essential for B cell development, and continuous expression of PAX5 is required throughout the B cell lineage to maintain the functional identity of B cells. The repressed expression of PAX5 prevents the development of plasma cells [20,21]. The known tumor suppressor genes on 6q include gravin, known to be involved in chronic myeloid leukemia, myelodysplastic syndrome and acute myeloid leukemia [22], and the ID4 gene, involved in malignant lymphoma [23]. Trisomy 4...
might accelerate tumorigenesis by the mutational C-KIT gene, which is located on chromosome 4q11–q12 in acute myeloid leukemia [24].

The use of CC is limited as a result of the low mitotic activity of mature B cells. A normal karyotype does not rule out the presence of malignant clones in patients with WM. In this study, we used FISH to overcome the limitations of CC. The reported frequency of 6q deletions and trisomy 4 in Western patients with WM by FISH is 32–54 and 18%, respectively [7,8,17,21]. But those were 10 and 0% in our study. The low frequency of the 6q deletion and absence of trisomy 4 might reflect small number of patients or ethnic differences. Therefore, a multinational collaborative study should be performed in the future. One previous study revealed that the del(6q) was associated with poor survival [25]. Our study failed to show such an association, perhaps as a result of the small number of patients and low frequency of del(6q). The common cytogenetic abnormalities associated with MM, such as del(13q), IgH translocation, and trisomy 1q, were not detected in patients with WM. These cytogenetic changes help differentiate MM from WM.

A recent study that used gene expression profiling of WM found that interleukin 6 was upregulated in WM. Interleukin 6 is currently being considered as a possible therapeutic target, and its upregulation can explain the elevated serum C-reactive protein and anemia in many patients with WM. After unsupervised hierarchic clustering, WM samples segregated with MM. Future studies are needed to better understand the genomic alterations found in patients with WM [26]. These results may help therapeutic decisions in patients with WM and improve patient outcomes with novel targeting agents.

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References


