Structural Rearrangements of Trisomies Are a Risk Marker of Clinical Progression in Hyperdiploid Multiple Myeloma

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Abstract. Background: Hyperdiploid multiple myeloma (HMM) is being characterized by the presence of several trisomies and a low incidence of immunoglobulin heavy chain rearrangements. It has not well defined what specific steps are associated with disease progression. We present two patients that showed some primary trisomies rearranged as a step of cytogenetic and clinical progression. This prompted us to review cytogenetic results from all patients referred to our hospital to assess the importance of this phenomenon in HMM. Patients and Methods: We carried out conventional cytogenetics in all patients. In four cases we also performed spectral karyotype (SKY) and arm-specific chromosome painting (ASP). Results: We demonstrate that in two patients some primary trisomies became along the disease course structurally altered and this coincided with clinical progression. We observed this phenomenon in more than 60% of HMM cases diagnosed at our laboratory. Conclusion: We propose structural rearrangements of trisomies as a biological marker of progression in HMM.

Multiple myeloma is an incurable plasma cell malignancy characterized by a marked genetic and clinic heterogeneity. Multiple myeloma is diagnosed from the presence of paraprotein in serum or urine, infiltration of malignant plasma cells in the bone marrow and bone lesions. A broad classification of multiple myeloma based on the tumour ploidy has been established. Hyperdiploid multiple myeloma (HMM) is characterized by the presence of >48 chromosomes, with several trisomies, and a low prevalence of primary translocations involving the immunoglobulin heavy chain (IgH) locus at 14q32. Non HMM is associated with <48 or >75 chromosomes and the presence of primary IgH translocations (1).

HMM evolves, after the appearance of trisomies, to a DNA loss from other chromosomes, resulting in a DNA complement that is essentially diploid (2). Some studies by array Comparative Genomic Hybridization (aCGH) methodology (3) and gene expression profiling (4, 5) have confirmed that HMM is a heterogeneous disease and there are several subgroups of patients.

Overall survival is favourable for patients with HMM, although in some cases, a more aggressive behaviour has been observed (2). The factors involved in tumor progression of HMM are not well known.

Based on the cytogenetic evolution of two patients, we present structural rearrangements of trisomies as a new mechanism of progression, not described before in HMM. After an accurate analysis of karyotypes, we realized that this phenomenon was present in more than 60% of HMM cases diagnosed at our laboratory.

Patients and Methods

Patients. Fourteen patients diagnosed with HMM were included in this study. Samples were staged according to the International Staging System for Multiple Myeloma (6). Clinical information of patients is shown in Table I.

Cytogenetic analysis. Chromosome analysis was performed on cells obtained by bone marrow aspiration. Cells were cultured for 24-72 h in chromosome synchro M medium (Euroclone, Italy) and processed by standard cytogenetic methodology. The International System for Cytogenetic Nomenclature (7) was used to describe the karyotypes.
Spectral karyotype. The protocol facilitated by Applied Spectral Imaging was used with minor modifications. 4',6-diamidino-2-phenylindole (DAPI) images were simultaneously used to define the chromosome aberrations.

Arm-specific chromosome painting (ASP). The protocol used as a matter of routine in our laboratory (8) was applied using probes labelled with green (short chromosome arm) and red (long chromosome arm) fluorochromes (Q Biogene, USA) for the metaphase slides.

Results

Cytogenetic findings in a series of 14 cases of HMM are shown in Table II. All patients had hyperdiploid karyotypes, with several trisomies and structural chromosome rearrangements. Among them, two patients (case 1 and 2) exhibited cytogenetic evolution at the same time as the clinical progression.

A 61-year-old woman (case 1) was diagnosed with multiple myeloma, stage Ia, in 2002. Cytogenetic analysis revealed a normal karyotype. The patient was treated with bortezomib and melphalan. In 2005, some lytic bone lesions were observed. In 2007, the tumor was staged as IIa and the karyotype was normal. In 2008, there was a clinical evolution toward IIIa stage. At that time the patient showed a hyperdiploid karyotype presenting several trisomies but no structural chromosome rearrangements. Nearly two years later, the patient showed signs of disease progression and at the same time than three structural chromosome abnormalities appeared in the karyotype, two of them involving previous trisomies of chromosomes 6 and 19 (Table II). An additional treatment with melphalan, prednisone and bortezomib was prescribed and the patient is at present alive, with a normal karyotype.

The second patient, a 56-year-old man (case 2) was diagnosed in 2004 with vertebral plasmacytoma. He was treated with radiotherapy and, one year later was affected with multiple myeloma, stage IIIa. The patient received radio-and chemotherapy and was submitted to an autologous bone marrow transplantation. Three years later, he experienced relapse showing two cell sidelines with complex hyperdiploid karyotypes; several trisomies (including trisomies of chromosomes 5 and 7, alternatively found in the different cell lines) and several structural chromosome abnormalities were observed. Four months later, the patient presented a more evolved karyotype showing, among other abnormalities, +del(5)(q13) and +add(7)(q32) arising from previous trisomies of chromosomes 5 and 7 (Table II). He died seven months later as a result of disease progression.

Discussion

We report the cytogenetic evolution of two patients (cases 1 and 2) with HMM and we compare this with clinical behaviour. We demonstrate that in these patients, some trisomies were structurally rearranged as a secondary event along the disease course and, this coincided with clinical progression.

Apart from these two cases, structural rearrangements of trisomies can also be presumed in 64% of HMM cases (14/22) seen by our laboratory. In all these cases, the typical trisomic chromosomes were structurally altered although no entire trisomy was yet evident (Table II). This is also noted, although it has never been described before, in several cytogenetic published series (9, 10) and also in the Mitelman database (11).

After the appearance of trisomies, structural rearrangements of trisomies were early events in HMM. The only structural
chromosome alteration in patient 3 was a rearrangement of tetrasomy 15. Patients 1, 10 and 13 had only one additional structural abnormality that suggests that rearrangements of polysomies (trisomies and tetrasomies) are early events in progression of HMM.

Patients 9 and 11 had a poor evolution, and patient 12 has just experienced relapse ten years after bone marrow transplantation. In patients 9 and 12, two out of three chromosomes from trisomies 6 and 3, respectively, were structurally rearranged and, in case 11, two out of four chromosomes arising from tetrasomy 3 were also altered. Patients 9 and 11 died one month and one year after diagnosis, respectively. These three patients showed additional structural rearrangements. Such important genomic instability lead to a poor outcome in these patients.

The remaining patients did not show any sign of clinical progression. This can be explained because of differences in the bone marrow microenvironment. It has been suggested that this is essential in development, maintenance and progression of multiple myeloma, given that only a few genomic differences exist between monochlonal gammopathy of undetermined significance and multiple myeloma (12). Changes in the bone marrow microenvironment may modulate the hyperdiploid plasma cells toward structural rearrangements of trisomies that could give rise to an increased expression of oncogenes or a loss of tumor suppressor genes. Alternatively, the microenvironment (stromal cells, extracellular matrix proteins) may be modified as a result of some gene alterations because of structural rearrangements of trisomies. Taking into account the fact that similar combinations of trisomies produce different clinical effects, from smoldering myeloma to a true multiple myeloma, it is reasonable to believe that changes in the bone marrow microenvironment could influence structural chromosome rearrangements, including trisomies.

Trisomies have been considered to be a means of up-regulation of some genes due to a gene dosage effect, promoting transformation by translation of proteins involved in cellular growth control (proto-oncogenes) or control of apoptosis (13). Chng et al. (5) demonstrated in a gene expression profiling study of HMM that many overexpressed genes involved in ribosome/protein biosynthesis were located.

<table>
<thead>
<tr>
<th>Case</th>
<th>D/PE/E</th>
<th>Results</th>
<th>Primary trisomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2002/2010/-</td>
<td>52.X,-t(6)(q15),+del(8)(p21),+9,+11,+15,+der(19)(t(1;19)(q12;13),+21[4]/46,XX[14]</td>
<td>+6,+19</td>
</tr>
<tr>
<td>3</td>
<td>2007/2007/-</td>
<td>56.XY,+4t(6)(q31),+5,+7,+9,+11,+15,+t(15;20)(q21;q22),+19,+19,+21[9]</td>
<td>+15</td>
</tr>
<tr>
<td>5</td>
<td>2009/2009/-</td>
<td>53.XX,+3,+6t(6)(q31),+5,+7,+9,-13,del(14)(q22),+15,+15,-16,+19,+20,add(21)(q22), +7(12t)[3]/46.XY[21]</td>
<td>+4</td>
</tr>
<tr>
<td>6</td>
<td>2006/2009/-</td>
<td>51~59.XY,+5[2],+del(6)(q21)[2],+7[2],add(9)[p21][2],+del(11)[p11][2],+15[2],+18[2], +21[2][cp2]</td>
<td>+6,+11</td>
</tr>
<tr>
<td>7</td>
<td>2006/2008/-</td>
<td>48.X,add(X)(p22),-2,+del(3)(p21),+4t(6)(q24),-7,-8,add(8)(q24),+9,-14,-17,+19,-20,+21, +mar[1]/46.XX[46]</td>
<td>+3</td>
</tr>
<tr>
<td>8</td>
<td>2001/2006/-</td>
<td>52.XX,add(3)(p24),-4,+add(5)[p15],-13,-16,-17,+18,+20,+6,mar[5]/46.XX[9]</td>
<td>+5</td>
</tr>
<tr>
<td>10</td>
<td>2010/2010/-</td>
<td>52.XX,+3,+add(7)[p21],+11,+15,+19,+21,del(22)(q11)[3]/46.XX[18] +7</td>
<td>+3,+11,+19</td>
</tr>
<tr>
<td>11</td>
<td>2009/2010/2010</td>
<td>42~56.X,+del(1)(q32)[3],del(1)(t;17)(p13;7)[4],del(2)(q21)[2],+add(3)(q26)[4], +add(3)[p23][2],-4[4],add(5)[p15][4],+7[3],+9[4],+add(11)[q23][4],+14[2],+15[2],+15[12, +der(19)(t;19)(q21,q13)[4],+20[2,0][46.XX][4]</td>
<td>+3</td>
</tr>
<tr>
<td>12</td>
<td>1991/2009/-</td>
<td>50.X,Y,+der(1)(t;1;8)(p11;q12),+3,+6,+8,der(9)(t;9;9)[p11],+11,+15,+19[3]</td>
<td>+9</td>
</tr>
<tr>
<td>13</td>
<td>2008/2008/-</td>
<td>50.X,Y,+der(1)(t;1;8)(p11;q12),+3,+6,+8,der(9)(t;9;9)[p11],+11,+15,+19[3]</td>
<td>+9</td>
</tr>
<tr>
<td>14</td>
<td>2007/2007/-</td>
<td>48.XX,(t;5;5)(p11;11;1q35),del(4)(p11),der(6)(t;X;6)(?;q21),+7;17)(p15~p21;q23), t(8;15)(q24;q24),+9,+13,del(16)(p11),+19,der(21)[3]/46.XX[6]</td>
<td>+7</td>
</tr>
</tbody>
</table>

D, Year of diagnosis; PE, year of analysis; E, year of death; -, alive.
in the trisomic chromosomes. Our findings suggest that the role of trisomies is not only in triggering the disease but also in taking part in tumor progression of HMM through their own structural rearrangements. The time at which rearrangements of trisomies become clinically apparent may be related to the bone marrow microenvironment.

On the basis of our findings, we suggest that all patients presenting structurally rearranged trisomies should be followed up closely because of the risk of clinical progression.

In agreement with Fonseca et al. (1) it is likely that some of the best prognostic markers for HMM will come from the complete understanding of secondary (progression) events.

Strikingly, in the era of arrays, conventional cytogenetics and molecular cytogenetics methodologies are still essential to unmask some aspects of the biological progression of this disease.

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References


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