Blast cells with nuclear extrusions in the form of micronuclei are associated with MYC amplification in acute myeloid leukemia

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1. Introduction

Micronuclei have been defined as small nuclear-like structures that can be expelled from the nucleus. They can arise from the nucleus by budding during S phase or after mitosis as the nuclear membrane reforms and can contain amplified oncogenes, acentric chromosome fragments, or whole damaged chromosomes [1].

The cytogenetic hallmarks of genomic amplification are homogeneously staining regions (hsr) and double minutes (dmin), which are acentric and usually tiny spherical chromatin particles of few mega-base pairs of size. Dmin are frequently seen in solid tumors but are rare in hematological malignances, present only in < 1% of cytogenetically abnormal acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), plasma cell dyscrasia, and malignant lymphoma.

Fluorescence in situ hybridization (FISH) analysis of dmin in hematological malignances revealed that most cases show MYC (8q24) or MLL (11q23) amplification [2,3]. The incorporation of dmin into micronuclei can lead to their elimination from the cell by extrusion. Elimination of amplified MYC gene via micronuclei has been correlated with loss of tumorigenicity [4].

Herein, we describe three patients diagnosed with AML without maturation (WHO classification) or AML-M1 (FAB classification) who showed micronuclei-associated MYC amplification. Additionally, we review a case previously reported by our group [5]. Morphologically, all cases were characterized by the presence of extrusion of chromatin material and formation of micronuclei excluded from the nucleus.

2. Material and methods

In our institution, the incidence of dmin (containing MYC amplification) in AML from January 2000 to April 2007 was 3/138 (2.17%).

Here, we report four cases with MYC amplification. Cases 1, 2, and 4 showed approximately 10–30 dmin. Case 3 showed a karyotype with an hsr and absence of dmin. The hsr consisted of MYC oncogene. In addition by FISH, we observed MYC aggregation in interphase nuclei illustrating...
the subsequent extrusion from the nucleus and formation of micronuclei expelled from the cell.

Morphologically, we did not detect micronuclei formation or dmin/MYC amplification in other AML cases in our institution.

The morphological and cytogenetic data of the patients are described in Table 1.

2.1. Case 1

A 75-year-old male was admitted to our hospital in July 2006 due to fever of unknown origin. The main laboratory findings on admission were as follows: hemoglobin 113 g/L, hematocrit 29.1%, MCV 92.4 fL, platelet count 38 × 10^9/L, and WBC count 27.58 × 10^9/L. Leukocyte differential count showed 2% segmented neutrophils, 1% bands, 2% lymphocytes, 1% promyelocytes, 1% myelocytes, and 92% blasts. The blast cells were medium to large sized, with immature chromatin, 1-2 large nucleoli, nuclear hernias with extrusion of chromatin material in form of micronuclei, and large basophilic cytoplasm with many azurophilic granules and clasmatosis. Neutrophils disclosed dysplastic changes (hypolobation, hypercondensed chromatin, hypogranulation, vacuoles, and Döhle bodies).

In the bone marrow aspirate, infiltration by 87% blast cells with similar morphology to those observed in peripheral blood (PB) and less than 10% of mature neutrophils were noted (Fig. 1a,b). Blasts were strongly positive for myeloperoxidase (MPO).

Flow cytometry analysis of PB disclosed a blast population with expression of myeloid antigens and an aberrant positivity for CD7. Monocytic, B-cell, and T-cell markers were negative. The diagnosis of AML without maturation (WHO classification) or AML-M1 (FAB subtype) was made. The patient started treatment with oral mercaptopurine and died three months after diagnosis.

2.2. Case 2

A 55-year-old male was diagnosed in March 2003 of AML without maturation (WHO classification) or AML-M1 (FAB classification) with a normal karyotype. He was treated with idarubicine, citarabine, and etoposide and achieved a complete remission (CR).

Four months later, the patient received autologous stem cell transplantation. In March 2007, he relapsed presenting the following laboratory parameters: hemoglobin 127 g/L, hematocrit 37%, MCV 92.6 fL, platelet count 113 × 10^9/L, and WBC count 70.5 × 10^9/L. Leukocyte differential count showed 62% segmented neutrophils, 4% bands, 6% lymphocytes, 5% monocytes, 8% metamyelocytes, 10% myelocytes, 1% promyelocytes, and 4% blasts.

The bone marrow aspirate was hypercellular and showed infiltration by 24% of medium/large sized blasts, with increased azurophilic granulation, presence of Auer rods, pseudo-Chediak-Higashi granules and isolated faggot cells. Blasts were strongly positive for MPO.

Flow cytometry analysis of blast population showed expression of myeloid markers. At that time there were 14% of micronuclei (not found at diagnosis). Dyshemopoiesis trends were observed in more than 40% of elements.

At the present time, the patient is in CR after treatment with chemotherapy and waiting for an unrelated donor to bone marrow (BM) transplantation.

2.3. Case 3

A 69-years-old male was referred to our hospital in June 2007 due to malaise and cutaneous necrotic infiltrative lesions. The main laboratory findings were as follows: hemoglobin 7.1 g/L, hematocrit 20.7%, MCV 102.5 fL, platelet count 12 × 10^9/L, and WBC count 82.2 × 10^9/L. Leukocyte differential count showed 2% segmented neutrophils, 2% lymphocytes, and 96% blasts.

Table 1

<table>
<thead>
<tr>
<th>Case 1</th>
<th>75</th>
<th>92</th>
<th>Present/Absent</th>
<th>Strongly positive</th>
<th>27.5</th>
<th>47,XY,del(1)(q32q44),del(16)(q22q24),add(17)(q?),+mar, -20dmin[15]/46,idem,-mar[5]</th>
<th>Present (&lt;5%) Amplified (dmin) /deleted in one 8 chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2a</td>
<td>57</td>
<td>24</td>
<td>Present*</td>
<td>Strongly positive</td>
<td>70.5</td>
<td>46,XY,3-17dmin[4]/46,XY[16]</td>
<td>Present (15%) Amplified (dmin) /deleted in one 8 chromosome</td>
</tr>
<tr>
<td>Case 3b</td>
<td>69</td>
<td>78</td>
<td>Present</td>
<td>Positive</td>
<td>82.2</td>
<td>45,XY,-5,-8,+hsr[20]</td>
<td>Present (1%) Amplified (hsr) /deleted in one 8 chromosome</td>
</tr>
<tr>
<td>Case 4</td>
<td>86</td>
<td>92</td>
<td>Present</td>
<td>Strongly positive</td>
<td>18</td>
<td>45,XX,-8, dic(8;16)(8q10-&gt;8q25::16p13.3-&gt;16q22), del(17)(q12q22),+20, del(20)(q11), -21,7~30dmin[20]</td>
<td>Present (&lt;5%) Amplified (dmin) /deleted in one 8 chromosome</td>
</tr>
</tbody>
</table>


* Morphological and cytogenetic data at relapse.

† Patient chemoresistant.

‡ Presence of pseudo-Chediak-Higashi granules.

§ Micronuclei detected after first induction course.
Bone marrow smears showed 78% of blasts, some of them with thin Auer rods and with occasional pink cytoplasmatic inclusions. Ninety-seven per cent of blasts were strongly positive for MPO.

Flow cytometry analysis of PB blasts showed expression of myeloid markers and an aberrant positivity for CD7. The patient received a first induction cycle with idarubicine, citarabine, and etoposide without achieving a response. In the last follow-up, PB exhibit 82% of medium/large blasts with increased azurophilic granulation, Auer rods, pseudo-Chediak-Higashi granules, and 1% of them showed extrusion of nuclear material and micronuclei.

3. Results

3.1. Case 1

G-banding cytogenetic analysis on bone marrow cells revealed the following karyotype: 47,XY,del(1)(q32q44), del(16)(q22),add(17)(q?),+mar,~20dmin[15]/46,idem,-mar[5].

FISH analysis using the MYC/IGH/CEP8 probe (Abbott Molecular, Inc.) showed MYC amplification. White arrows denote two micronuclei are labeled with the MYC probe (case 1); (d) Meta-phase spreads of case 1 showing dmin; (e) FISH with the MYC/IGH/CEP8 probe in case 1 showing three chromosomes 8 and multiple copies of the MYC oncogene in form of dmin; (f) FISH with the MYC/IGH/CEP8 probe revealing an hsr with MYC amplification (case 3).

3.2. Case 2

Cytogenetic analysis revealed a 46,XY,3~17dmin karyotype. The morphological evidence of micronuclei, in accordance to the cytogenetic finding of dmin, led us to study the MYC status by FISH and confirmed that the dmin contained the MYC oncogene (Table 1). The diagnostic sample was then analyzed by FISH, but no evidence of MYC amplification was found.

3.3. Case 3

The karyotype of this patient was 45,XY,-5,-8,+hsr. FISH with painting probes for chromosomes 5 and 8 showed that the hsr was composed of material from both chromosomes. Due to the cytological findings (extrusion of nuclear material and micronuclei), we performed FISH with the MYC probe and found that the marker chromosome contained extra copies of MYC in the form of an hsr (Fig. 1f).

An additional case previously reported by our group (case 4) with documented MYC amplification was reevaluated with MYC/IGH/CEP8 probe to determine MYC extrusion [5]. Thus, the ISCN (2005) formulation was rewritten as follows: 45,XX,-8,del(8;16)(q24;p13.3),del(17)(q12q22),+20, del(20)(q11),-21,7~30dmin. This case was also reported by Storlazzi et al [3].
4. Discussion

In this study, the outstanding findings were micronuclei anomaly and MYC amplification in the form of dmin or hsr in four patients diagnosed with AML-M1. Dmin, frequently seen in solid tumors, are rare in hematological malignances. AML-M2 is the most common hematological malignancy associated with amplification of MYC gene in the form of dmin. In a previous series published by Asker et al., eight out of 10 cases with MYC amplification in the form of dmin were classified as AML-M2, and only one case as AML-M1.

The cytogenetic findings in the majority of reported cases exhibit complex aberrant karyotypes, and a poor clinical outcome [6].

Patients 2 and 4 showed pseudo-Chediak-Higashi granules in the blasts cells. Simultaneous occurrence of this anomaly with dmin involving MYC has been reported in three patients with AML-M2, suggesting a pathophysiological role of MYC expression in the formation of pseudo-Chediak-Higashi granules, but no evidence of micronuclei was found either by morphology or by the FISH study [7].

In all cases, blasts showed many azurophilic granules, even pseudo-Chediak-Higashi type and Auer rods, and were strongly positively for MPO. PML-RARA fusion gene was ruled-out by FISH and real time PCR. In a previously AML-M1 case reported by our group (case 4), no PML-RARA rearrangement was detected by FISH, although only one RARA signal was observed. Frater et al. reported a case of acute promyelocytic-like leukemia lacking either t(15;17)(q22;q21) or the RARA rearrangement and having MYC amplification in the form of dmin [7].

Regarding the prognostic value of MYC amplification in AML patients, Frater et al. suggested that the excessive exclusion of dmin (20–25%) could predict a better prognosis [8]. In our study, in cases 1, 3, and 4, we only observed about 1–5% of micronuclei, and case 2 showed 15% of nuclear extrusion (percentage evaluated by FISH technique with the MYC probe). The prognostic impact of dmin has not been well elucidated because few cases of AML with dmin have been reported.

Dmin seem to be associated to chemotherapy resistance and disease aggressiveness, and it has been suggested that they represent a sign of disease progression rather than a primary malignant event. It has also been suggested that AML patients with a normal karyotype and numerous dmin might respond better to treatment and have a better prognosis than patients with complex karyotype and few dmin [3].

Although we report a small number of AML with micronuclei-associated MYC amplification with a heterogeneous treatment, our results are in accordance with those previously reported. Patient 2 presented few dmin (3–17) as the sole aberration at relapse and acquired a complete remission. Patients 3 and 4 showed a complex karyotype (case 2 with ~30 dmin) and did not respond to standard induction treatment.

Cases 1, 2, and 4 are in agreement with the “episome model”, which suggests that 8q24 region is deleted in one of the chromosome 8 homologues due to the excision of this DNA segment prior to its amplification [3]. In case 2, MYC integration in the marker chromosome (hsr) does not involve deletion of 8q24 locus since normal signals were detected on normal chromosome 8 and in the marker chromosome.

The elimination of dmin containing amplified genes through the formation of micronuclei was previously reported in neuroblastoma tumor cells with MYCN as the target oncogene [9]. We report on the same phenomenon of oncogene elimination via micronuclei in AML without maturation in which MYC (8q24) was the target gene.

The expulsion of amplified genes has been shown to reduce the tumorigenicity and revert to the phenotype of malignant cells. These authors suggested that drugs such as hydroxyurea have been shown to promote elimination of extrachromosomally amplified MYCN gene from the cell and could be a therapeutic option.

In conclusion, morphological evidence of cytoplasmic micronuclei and extrusion of chromatin material in blast cells (May-Grünwald Giemsa stain) could be correlated to the expulsion of dmin observed with the FISH technique. The simultaneous observation of the appearance of chromatinic extrusion and dmin should draw the attention of cytologists to this association. Further studies are required to determine if this phenomenon is a cytological marker for the presence of MYC amplification in form of dmin and to analyze its prognostic impact.

Acknowledgments

This study was supported by the Spanish Ministry of Health, Networks of Centers of Clinical and Molecular Genetics (C03/07, PI050747), and the Centers of Cancer (C03/10).

References


