Positive p53 immunostaining and erythroid maturation in two cases of pure erythroid leukemia with extremely complex karyotypes

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Abstract

Pure erythroid leukemia (PEL) is characterized as a neoplastic erythroid hyperproliferation with maturation arrest, showing highly complex karyotypes, prominent clonal evolution, and a very aggressive clinical course. Here, we describe two cases of PEL that evolved from myelodysplastic syndrome (MDS), focusing on the immunophenotypic, cytogenetic, and molecular features of these cases. Case 1: A 77-year-old woman was diagnosed with PEL that evolved from MDS-refractory cytopenia with multilineage dysplasia. Her disease progressed rapidly despite 5 cycles of azacitidine treatment. The bone marrow (BM) aspirate revealed hypercellular marrow with 92.4% erythroid cells, which expressed CD7 and CD36. Case 2: A 43-year-old woman had MDS-refractory anemia for more than 15 years. When her disease progressed rapidly, the BM aspirate revealed hypercellular marrow with 95.4% erythroid cells, which expressed CD235a. There were several significant findings in our cases. First, flow cytometric analysis of BM cells showed different stages of erythroid maturation. Second, cytogenetics revealed extremely complex karyotypes. Finally, immunohistochemistry showed strong nuclear staining for p53 in BM erythroid cells. We suggest that increased p53 protein expression is correlated with complex karyotypes and worse outcomes, indicating PEL with a high degree of malignant behavior.

Keywords: Pure erythroid leukemia, myelodysplastic syndrome, complex karyotype, double minute chromosome, p53

Introduction

Pure erythroid leukemia (PEL) is classified as “acute myeloid leukemia (AML), not otherwise specified (NOS)” subtype, and is now the only type of acute erythroid leukemia (AEL) according to the updated 2016 World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. PEL is defined as a neoplastic proliferation of immature cells that have an undifferentiated or proerythroblastic appearance representing >80% immature erythroid precursors with ≥30% proerythroblasts in the bone marrow (BM) nucleated cells [1]. PEL is a rare leukemia, comprising less than 1% of all AML cases [2-4]. Approximately 39% of PEL cases evolved from myelodysplastic syndrome (MDS) [2]. Here, we describe two cases of PEL that evolved from MDS, noting the important clinical implications of their immunophenotypic, cytogenetic, and molecular features. Our cases showed different stages of erythroid maturation, extremely complex karyotypes, and increased p53 protein immunostaining in BM neoplastic cells. Strong p53 expression was correlated with complex karyotypes, leading to a highly aggressive clinical course and a dismal prognosis.

Case presentation

Case 1

A 77-year-old woman presented with shortness of breath in November 2013. Her initial blood count profile showed pancytopenia, with a hemoglobin level of 6.3 g/dL, white blood cell count of 3.5×10^9/L, and platelet count of 69×10^9/L. The BM aspirate revealed hypocellularity with trilineage myelodysplasia and 2.2% blasts (Table 1). She was diagnosed with MDS-refractory cytopenia with multilineage dysplasia (RCMD) and received azacitidine treatment. Six months after the initial diagnosis, by which time she had undergone 5 cycles
of azacitidine treatment, she was admitted to our unit with high fever and malaise. The laboratory blood values were as follows: hemoglobin level of 6.7 g/dL, red blood cell count of 1.98×10^{12}/L, white blood cell count of 1.4×10^{9}/L, platelet count of 42×10^{9}/L, reticulocyte count of 0.5%, serum levels of lactate dehydrogenase of 4,324 IU/L (normal range, 119-229), and C-reactive protein level of 9.47 mg/dL. The Wilms’ tumor 1 (WT1) mRNA level increased aggressively from 3,600 to 37,000 copy/µg RNA over a 3-month period (Figure 1). The BM aspirate was hypercellular with 92.4% erythroid precursors. The erythroid cells were predominantly proerythroblasts, and basophilic erythroblasts (Table 1). The neoplastic erythroid cells showed medium to large size with round nuclei, fine chromatin, prominent nucleoli, and basophilic cytoplasm with occasional cytoplasmic vacuoles (Figure 2-1a). Periodic acid–Schiff (PAS) staining revealed globular and block-like positivity in many erythroid cells (PAS staining positive rate, 81%; Figure 2-1b). Prussian blue staining showed a few sideroblasts, but no ringed sideroblasts were observed. The immunohistochemistry of erythroid cells in the BM clot section revealed strong nuclear staining of the p53 protein (Figure 2-1d). Flow cytometry of the BM cells showed positive expression of CD7 and CD36, but was negative for CD235a (Glycophorin A), CD3, CD41, CD56, CD71, CD117, and HLA-DR. Cytogenetic evaluation of BM cells was performed on 20 metaphases and detected a complex karyotype with 18 hyperdiploid (range,
52 to 57 chromosomes) and 2 hypertetraploid (range, 100 to 101 chromosomes) metaphases and an overall karyotype of double minute chromosomes (dmins) (Table 2, Figure 3). Despite further azacitidine treatment, the disease developed and progressed. She died of pneumonia 6 weeks after being diagnosed with PEL.

Case 2
A 43-year-old woman had MDS-refractory anemia for over 15 years. During the course of her disease, she was treated with oral methenolone acetate with no red blood cell transfusion requirement. In February 2013, her health rapidly worsened and she developed systemic signs of anemia. One month later, she was admitted to our unit because of genitourinary bleeding, high fever, and shortness of breath. The laboratory blood values were as follows: hemoglobin level of 7.8 g/dL, red blood cell count of 2.46×10^{12}/L, white blood cell count of 1.0×10^{9}/L, platelet count of 5×10^{9}/L, reticulocyte count of 0.6%, serum levels of lactate dehydrogenase of 1,749 IU/L (normal range, 119-229), C-reactive protein level of 2.36 mg/dL, and WT1 mRNA of less than 50 copy/μg RNA. The BM aspirate was hypercellular with 95.4% erythroid cells. Examination of the proliferated cells showed that medium sized undifferentiated erythroid cells or proerythroblasts comprised 51.2% of all BM nucleated cells (Table 1, Figure 2-2a). The erythroid cells in this case had a more differentiated form than did those in Case 1. PAS staining revealed positivity in many erythroid cells (PAS staining positive rate, 68%). The immunohistochemistry of immature cells in the BM clot section revealed strong nuclear staining of the p53 protein (Figure 2-2d). The immunophenotypic analysis by flow cytometry showed that BM cells expressed CD235a, but were negative for CD3, CD7, CD41, CD56, CD71, CD117, and HLA-DR. Cytogenetic analysis of BM cells revealed complex karyotype abnormalities in all of the 20 metaphases with 18 hypertriploid or hypotetraploid karyotype (range, 78 to 82 chromosomes) (Table 2). Although azacitidine treatment was started, her condition worsened rapidly. She died of gastrointestinal bleeding 6 weeks after being diagnosed with PEL.

Discussion
In the updated 2016 WHO classification of myeloid neoplasms and acute leukemia, the AEL-erythroid/myeloid type subcategory has been removed from the AML category. As a result, PEL is now the only type of AEL. This change was based on the close biologic relationship of the AEL-erythroid/myeloid type to MDS in terms of its clinical presentation, morphologic features, and genetic abnormalities [1]. PEL is very rare form of leukemia, and some cases of PEL occur as a disease progression stage that has evolved from MDS [2-4]. Morphologic, immunophenotypic, cytogenetic, and molecular analysis are valuable for confirming the diagnosis of PEL. There were several significant findings in our cases. First, in flow cytometric analysis, the erythroid lineages of the two cases showed different immunophenotypic patterns. Second, cytogenetic analysis revealed extremely complex karyotypes, and dmins were found in Case 1. Finally, immunohistochemistry showed strong nuclear staining for p53 in BM cells.

Early erythroid cells were identified at three differentiation stages by flow cytometry. During the first stage, erythroid differentiation is characterized by expression of CD36, CD105, CD173, and CD238. At this stage, CD71-positive cells consist of both erythroid cells and myeloid cells. With ongoing differentiation, at the second stage, CD36, CD71, CD105, CD117, CD173, and CD238 can be observed, whereas CD45
expression is diminished and HLA-DR is no longer detectable. The third stage is defined by the appearance of CD235a and the disappearance of CD117 \[5,6\]. In Case 1, neoplastic cells were positive for CD36, but negative for CD235a, exhibiting the early stage after commitment to the erythroid lineage. On the other hand, neoplastic cells in Case 2 were positive for CD235a, indicating a more differentiated form than was observed in Case 1. In addition, neoplastic cells were positive for CD7 in Case 1. Although CD7 is not usually expressed in erythroid lineages, the expression of CD7 has been reported in 12-42% of AML cases \[7\]. Abnormal erythroid maturation may be due to changes in the intensity of antigen expression and maturation arrest, as well as gains and losses or modifications of antigen expression. Immunophenotypic analysis revealed that neoplastic cells in Case 1 were early erythroid progenitor cells, while those in Case 2 were later erythroid cells. Morphologically, the percentage of undifferentiated erythroid cells or proerythroblasts in Case 1 was higher than that in Case 2. Based on these results, it appeared that the rapidly aggressive clinical course was not related to the erythroid differentiation stage of the neoplastic cells.

Cytogenetically, no specific chromosome abnormality has been described in PEL, but complex karyotypes with multiple structural abnormalities are common, especially with -5/del(5q), -7/del(7q), and trisomy 8 \[2,4\]. Chromosomal abnormalities have been found in approximately 55% of adult AML cases. Additionally, complex karyotypes with ≥3 aberrations account for 10-19% of adult AML cases \[8\]. During the progression of MDS to AML, cytogenetically complex karyotypes are often acquired, especially in cases of PEL \[2,4\]. Interestingly, dmins were only detected in Case 1, and were not observed in PEL previously. Dmins are small, paired chromatin bodies that represent a form of extrachromosomal gene amplification. They are frequently found in various solid tumors, but appear in less than 1% of AML and MDS. They are often associated with complex karyotypes, and are generally associated with a poor prognosis. Dmins in myeloid neoplasms commonly harbor MYC or MLL gene amplification and manifest as micronuclei within leukemic cells \[9,10\].

Although DNA sequencing was not performed in our cases, immunohistochemistry showed strong nuclear staining of the p53 protein in the BM erythroid cells. p53 is a tumor
suppressor protein that is encoded by the TP53 gene and regulates the cell cycle in response to cellular stress, leading to DNA damage. TP53 mutations are detected in 5–10% of MDS and AML cases, and 53–72% of AML that have complex karyotypes [11-13]. In case of del(5q) MDS, a strong accumulation of p53 protein was found in erythroid progenitor cells; in contrast, myeloid or megakaryocyte lineage cells did not have increased p53 expression. Therefore, the p53 pathway may play an important role in the erythroid lineage, improving the efficiency of erythropoiesis and preventing malignant transformation [14,15]. Significantly, immunohistochemical analysis of p53 expression is a clinically useful tool that does not require expensive gene sequencing techniques and is readily available for routine clinical use [12,14,15]. In our cases, strong positive immunostaining for p53 might have led to resistance to treatment and a short survival time. Taken together, it appears that TP53 mutations in MDS and AML are correlated with complex karyotypes and dismal outcomes.

Conclusions
In the updated 2016 WHO classification, PEL is now the only type of AEL. The reported cases showed increased p53 protein expression and illustrate the potential importance of this increased expression to future disease progression and management. Our cases suggest that morphologic, immunophenotypic, cytogenetic, and molecular evaluations of leukemic cells are needed to improve the current understanding of the pathogenesis of PEL. Such evaluations ultimately have clinical importance because leukemic cells play important roles in disease development and/or progression.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

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References

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