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Acute Myeloid Leukemia with *MYC* Rearrangement and *JAK2* V617F Mutation

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Abstract

Little is known about *MYC* dysregulation in myeloid malignancies, and we can find no published studies that have evaluated *MYC* protein expression in primary cases of myelodysplastic syndromes (MDS) or acute myeloid leukemias (AML). We describe the clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic findings in two MDS/AML cases that contained both *MYC* rearrangement and *JAK2*-V617F mutation. We demonstrate *MYC* protein expression by immunohistochemistry in both patients.

Keywords

acute myeloid leukemia; blastic plasmacytoid dendritic cell neoplasm; *MYC*; *JAK2*-V617F mutation

Introduction

MYC dysregulation induces lymphoid malignancies by stimulating proliferation and inhibiting terminal differentiation [1]. Burkitt lymphoma is characterized by rearrangement of *MYC*, on chromosome 8q24, with either the immunoglobulin heavy chain gene (*IGH* on chromosome 14q32) or, less often, one of the immunoglobulin light chain genes in the

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Authorship

All authors approve of the study. Maro Ohanian and Lynne V. Abruzzo analyzed the data and wrote the paper. Carlos Bueso-Ramos, Pei Lin, Chi Young Ok, and Yang O. Huh performed the pathology evaluation. Keyur Patel performed the *JAK2* mutation analysis. Joseph Khoury provided cytogenetics images, Mona Lisa Alattar, Uri Rozovski, Zeev Estrov, and Jorge Cortes provided clinical information.

variant translocations [2]. *MYC* rearrangement occurs in other lymphoid malignancies, e.g. “double-hit” diffuse large B-cell lymphomas or chronic lymphocytic leukemia, where it is associated with an aggressive clinical course [2].

MYC chromosomal translocations in myeloid neoplasms are rare. *MYC* amplification, reported infrequently in AML with double minute chromosomes (dmin) or homogeneously staining regions (hsr)[3], is associated with drug resistance, disease progression, and poor survival [4,5]. Only five cases of myeloid neoplasms with t(8;14)(q24;q32) or one of the variant translocations have been reported previously [6]. Little is known about *MYC* dysregulation or *MYC* protein expression in myeloid malignancies. We describe the clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic findings in two myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) cases containing both *MYC* rearrangement and *JAK2*-V617F mutation, one with concurrent blastic plasmacytoid dendritic cell neoplasm (BPDN). We demonstrate *MYC* protein expression by immunohistochemistry in both patients.

Material and Methods

We reviewed peripheral blood smears, decalcified bone marrow (BM) core biopsy and clot specimens stained with hematoxylin and eosin (H&E), and Wright-Giemsa-stained aspirate smears and touch imprints. Immunophenotypic analysis was performed by flow cytometry, as described previously[7]. Immunohistochemical staining (IHC) for *MYC* was performed on formalin-fixed, paraffin-embedded (FFPE) sections of the bone marrow core biopsy using a pre-diluted rabbit monoclonal antibody specific for *MYC* (Y69, Ventana Medical Systems, Tucson, Arizona).

Conventional cytogenetic analysis was performed on Giemsa-banded metaphase cells prepared from bone marrow aspirates cultured without stimulation for 24 and 48-hour using standard techniques. Twenty metaphases were analyzed and the results were reported using the 2009 International System for Human Cytogenetic Nomenclature (ISCN). Fluorescence in situ hybridization (FISH) analysis was performed on BM cultures using a dual-color break-apart probe for *MYC* (Vysis/Abbott Laboratories, Des Plaines, IL) and a dual-color dual-fusion probe for *MYC/IGH* rearrangement (Vysis/Abbott Laboratories) according to the manufacturers’ instructions. Assessment of *JAK2* mutation was performed as described previously [8].

Results

Case 1 was a 68 year-old woman who presented with a 6 month history of fatigue and weight loss. The physical examination (PE) showed hepatosplenomegaly. The peripheral blood (PB) smear showed anemia, leukopenia, and dysplasia, including giant platelets and micromegakaryocytes. The bone marrow (BM) was inaspirable; the BM core biopsy showed extensive fibrosis with numerous small, hypolobated megakaryocytes and foci of immature cells. The touch-preparation showed trilineage dysplasia and 16% blasts with basophilic, agranular cytoplasm, and cytoplasmic blebs. Analysis for *JAK2*-V617F mutation was positive. Over the next

3 weeks the patient developed fevers, pancytopenia, and circulating blasts. A repeat BM biopsy showed a high-grade myeloid neoplasm transforming to AML, with marked fibrosis and dysplastic megakaryocytes. By flow cytometry the myeloblasts expressed CD34 and CD61, but were negative for CD42b, suggesting megakaryocytic differentiation.

Upon transfer to our facility the PB showed pancytopenia, hypogranular neutrophils, giant platelets, and 4% blasts. A hypocellular BM biopsy (15%) showed increased immature cells and many small, hypolobated megakaryocytes (Figure 1A) in a fibrotic background (Figure 1B). Immunohistochemical staining for MYC demonstrated 10% positive blasts (Figure 1C). The BM aspirate was scanty cellular with micromegakaryocytes and small, agranular blasts, some with nuclear extrusions, which has been associated with *MYC* amplification (Figure 1C inset) [9]. The blasts expressed CD45 (dim), CD34, CD117, HLA-DR, CD13 (bright), CD33 (partial dim), CD38 (partial dim), and CD123; they were negative for CD2, CD3, CD5, CD7, CD10, CD14, CD19, CD42b, CD56, CD61, CD64, myeloperoxidase (MPO), and terminal deoxynucleotidyl transferase (TdT). Expression of CD41 was equivocal due to platelet aggregates. Karyotypic analysis demonstrated 46,XX,t(8;14)(q24.1;q32)[13]/46,XX[7] (Figure 2A). Fluorescence *in situ* hybridization (FISH) analysis using a dual-color break-apart *MYC* probe showed rearrangement in 5% of interphase nuclei (95% confidence limit, 3.4%) (Figure 2C). A dual-color dual-fusion *IGH-MYC* probe showed involvement of both loci (data not shown).” Analysis for the *JAK2-V617F* mutation was positive. The specimen was interpreted as hypoplastic MDS with reticulin fibrosis (MF-2). Despite treatment with 5-azacitidine, the BM blasts increased progressively. The patient developed septic shock and died two months after the diagnosis of MDS.

Case 2 was a 60 year-old man who presented with newly diagnosed AML. He had a history of primary myelofibrosis (PMF) (MF-3) with *JAK2-V617F* mutation, but remained untreated for four years, until developing anemia, leukocytosis, and splenomegaly. Over the next 3 months, he received hydroxyurea and splenic irradiation without improvement. Following treatment with the Janus kinase inhibitor, ruxolitinib, the splenomegaly regressed. However, 2 months later he developed violaceous nodules on his right forearm and right axillary lymphadenopathy; biopsies of both showed BPDN.

Upon presentation to our institution, the PE demonstrated marked splenomegaly and right axillary lymphadenopathy. Imaging studies showed lymphadenopathy (bilateral cervical and axillary, right supraclavicular) and massive hepatosplenomegaly. The PB showed leukoerythroblastosis, 4% blasts, teardrop erythrocytes, and basophilia. The BM biopsy showed blasts, fibrosis, and osteosclerosis; the aspirate showed trilineage dysplasia, basophilia, and small-to-large monocytoid blasts (18%), some with nuclear extrusions. Most blasts were butyrate esterase-positive; a minority was MPO-positive. The blasts expressed CD34, CD117, HLA-DR, CD13, CD33 (partial), CD38 (partial), and CD123 (dim); they were negative for CD2, CD5, CD7, CD64, and CD56. There was a small subpopulation (4%) of cells with an aberrant plasmacytoid dendritic cell immunophenotype (positive for CD4, CD56, and CD123; negative for CD22), that was not apparent morphologically. A diagnosis of PMF transforming to AML was rendered. Conventional cytogenetic analysis demonstrated: 46,XY,del(13)(q12q22)[16]/46,idem,t(6;8)(p12;q24.1),del(14)(q24q32)[cp4].

(Figure 2B). FISH analysis using a dual-color break-apart *MYC* probe showed rearrangement in 12% of interphase nuclei (Figure 2D); 3% also showed an extra copy of 5' end of *MYC*. *JAK2*-V677F mutational analysis was positive (57.6% of PCR products). Immunohistochemical staining for *MYC* demonstrated 5% positive blasts (data not shown). Treatment with hyper-fractionated cyclophosphamide, doxorubicin, vincristine, and dexamethasone (hyper-CVAD) was initiated, but discontinued because the patient developed tumor lysis syndrome, pneumonia, and sepsis. He died one month later with persistent AML. Analyses performed on tissue sections of the BPDN demonstrated *JAK2*-V617F mutation (57.6% of PCR products) and *MYC* rearrangement in 90% of nuclei (data not shown).

Discussion

The t(8;14)(q24;q32) in myeloid neoplasms is rare, described previously in five AML patients [6,10–12], with *MYC* assessed in only one [10]. Both cases showed rearrangement of chromosome band 8q24 on karyotypic analysis, which contains ~300 genes in addition to *MYC* [11][11][11]. In case 1, the t(8;14) was morphologically indistinguishable from the classic t(8;14) seen in Burkitt lymphoma. Its presence as the sole cytogenetic abnormality suggests that it was an early leukemogenic event. The t(6;8)(p12;q24.1) in case 2 has been reported once previously, as part of a complex karyotype in AML, without additional clinical information or evaluation of *MYC* rearrangement [13]. In our case, the stemline clone, the most basic neoplastic clone, contained a deletion in chromosome 13. Interstitial deletions in chromosome 13, usually in 13q14-21, are common in MPN, MDS, and AML [2]. Presence of the t(6;8) in a subclone suggests that it was a secondary abnormality associated with disease progression, rather than an initiating or early event. In both cases, we confirmed *MYC* rearrangement by FISH analysis, in the first case with *IGH* and in the second case with an unknown partner.

Acquired *JAK2*-V617F mutations occur commonly in MPN, in most cases of polycythemia vera and about half of cases of primary myelofibrosis (PMF) and essential thrombocythemia [14]. This mutation is less common in MDS and *de novo* AML, identified in only ~2.7% of *de novo* AML cases [14,15]. Case 1 presented with MDS and *JAK2*-V617F mutation. The prognostic implications of *JAK2*-V617F mutation in MDS/AML, either transformed or *de novo*, remain unclear [14]. In case 2, analyses of PMF and AML specimens demonstrated that both contained *JAK2*-V617F mutation, presumably because the AML arose through clonal evolution of the underlying MPN. The BPDN also demonstrated *JAK2*-V617F mutation. Further, FISH analyses for *MYC* rearrangement performed on AML and BPDN were positive. These findings suggest that all three myeloid neoplasms were clonally related. Nearly 20% of patients with BPDN have a history of a myeloid neoplasm, including MDS and AML [16]. However, no reports describe BPDN containing *JAK2*-V617F mutation or *MYC* rearrangement.

Immunohistochemical staining for *MYC* on the BM biopsy was positive in a subset of blasts in both cases. In contrast, we have observed rare *MYC*-positive cells in normal BM. Conceivably *MYC* expression may result from mechanisms other than *MYC* rearrangement. For example, expression of *Jak2*-V617F protein in a murine pro-B cell line (BaF3.EpoR)

induced expression of Myc[17]. Regardless, our findings identify an unusual subset of AML cases that demonstrate both *MYC* rearrangement and MYC protein expression.

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Figure 1.

Case 1. Morphologic features in the bone marrow. (A) The core biopsy is hypocellular with increased immature cells and small, hypolobated megakaryocytes in a background of extensive fibrosis (H&E $\times 200$) (B) A reticulin stain is diffusely positive (Reticulin $\times 200$). (C) A subset of the blasts expresses MYC (Immunohistochemistry $\times 500$). (C inset) The blasts have agranular cytoplasm and some show nuclear extrusions (Wright-Giemsa $\times 1000$).

Figure 2.

Cytogenetic findings. **(A)** Case 1. Conventional cytogenetic demonstrates t(8;14) (q24.1;q32). The abnormal derivative 8 and 14 chromosomes are to the right of the normal chromosomes 8 and 14. **(B)** Case 2. Conventional cytogenetic demonstrates t(6;8) (p12;q24.1). The abnormal derivative 6 and 8 chromosomes are to the right of the normal chromosomes 6 and 8. **(C)** Case 1. FISH analysis on a metaphase using a dual-color break-apart probe demonstrates *MYC* rearrangement. The normal chromosome 8 shows a yellow fusion signal. The derivative chromosome 8 shows a red signal and the derivative chromosome 14 shows a green signal. **(D)** Case 2. FISH analysis on interphase nuclei using a dual-color break-apart probe demonstrates *MYC* rearrangement.