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Identification of a *HMGA2-EFCAB6* gene rearrangement following next-generation sequencing in a patient with a t(12;22)(q14.3;q13.2) and *JAK2V617F*-positive myeloproliferative neoplasm

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Myeloproliferative neoplasms (MPNs) result from genetically altered hematopoietic stem cells that retain the capacity for multilineage differentiation. The study of genomic mutations identified so far suggests that they occur after a common ancestral event or that different mutations result in similar MPN phenotypes. We report analysis of a chromosomal translocation, t(12;22)(q14.3;q13.2), in a patient with a *BCR-ABL1*-negative, *JAK2V617F*-positive MPN. Comparative genomic hybridization (CGH) array and targeted sequencing detected no mutation in nine genes reported to influence the *JAK2V617F*-driven MPNs (*MPL*, *LNK*, *CBL, TET2*, *EZH2, IKZF1, IDH1, IDH2, ASXL1*). Next-generation sequencing revealed a balanced *HMGA2-EFCAB6* genomic rearrangement. The *HMGA2* breakpoint leads to the loss of seven 3′UTR binding sites for the microRNA (miRNA) *let*-7 tumor suppressor. The breakpoint in the *EFCAB6* gene abrogates transcription of *EFCAB6*. Measurement of expression showed retention of *HMGA2* transcription and no detectable *EFCAB6* transcript. Allele burden comparison in a sample containing the translocation, showed 90% *HMGA2-EFCAB6* versus 50% *JAK2V617F* allele dose, suggesting *HMGA2-EFCAB6* rearrangement plays a more ancestral role, pre-*JAK2V617F*, in the neoplastic process. The pathogenicity of the translocation may rest on collaborations among *JAK2V617F*-induced constitutive activation of JAK2, the oncogenic property of *HMGA2*, and disrupted pathways, such as alteration in *DJ-1* expression, resulting from the impact of *EFCAB6* abrogation.

Keywords Myeloproliferative, *HMGA2*, *EFCAB6*, *JAK2*, *V617F*

Myeloproliferative neoplasms (MPNs) are clinically related forms of neoplastic panmyelosis, seen as the result of genetically altered hematopoietic stem cells that retain the capacity for multilineage differentiation (1,2). In the case of chronic myeloid leukemia, the course of the MPN clinical picture is defined by the Philadelphia (Ph) chromosome translocation, which constitutes a rearrangement of the *BCR* and *ABL1* genes that results in an oncogenic tyrosine kinase (3,4).

The discovery of the *JAK2V617F* allele in the majority of patients with non-*BCR-ABL1* MPNs (i.e., polycythemia vera, essential thrombocythemia, or primary myelofibrosis) indicated that acquisition of somatic mutations of *JAK2* plays a central role in the pathogenesis of these MPNs through gain of function, constitutive activation of non-receptor JAK2 tyrosine kinase (5–9). Experimental models of the *JAK2V617F* recapitulate this critical role, as they capture the features of MPNs (10–13). The presence as well as the dose of the *JAK2V617F* allele is important in the MPN, with both human and murine data suggesting a gene dosage effect,

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since a single copy of the *JAK2V617F* allele does not saturate activation of JAK2 signaling, and because qualitative and/or quantitative differences in constitutive signaling in *JAK2V617F* affect MPN phenotype (5–8,14).

Additional somatic mutations are likely to contribute to the pathogenesis of *JAK2V617F*-positive MPNs. First, clonogenic cytogenetic abnormalities have been observed in patients with MPNs, with reported cases demonstrating cytogenetic abnormalities in the majority of hematopoietic cells, whereas a smaller proportion of cells were *JAK2V617F*-positive (15). These data, as well as clonality analysis in a large MPN cohort, suggest that other mutations are responsible for clonal hematopoiesis beyond the *JAK2V617F* mutated subclone (16–18). In light of such observations, acquired mutations in addition to *JAK2V617F* have been described to be associated with non-*BCR-ABL1* MPNs (2,19). The affected genes reflect a variety of effector pathways, such as signaling or signal regulation (*MPL*, *LNK*, *CBL*), methylation (*TET2*, *EZH2*), transcription (*IKZF1*), metabolism (*IDH1, IDH2*), and chromatin modification (*ASXL1*), and they have as a common theme the potential for cooperation with *JAK2V617F* in the sustenance and progression of the MPN (19). However, these mutations lack clinical specificity and exclusivity, suggesting that, like the *JAK2V617F* allele, they occur after a common ancestral event, and/or that different genomic abnormalities can result in similar phenotypes (2,19).

In this communication, we report the analysis of an acquired hematopoietic chromosomal translocation, t(12;22)(q14.3;q13.2), that was observed to be the only cytogenetic abnormality in a patient presenting with a *JAK2V617F*-positive, *BCR-ABL1*-negative MPN. Next-generation DNA sequencing, followed by traditional Sanger sequencing confirmation, revealed a reciprocal *HMGA2-EFCAB6* gene rearrangement. Analysis of expression showed retention of *HMGA2* transcription and no *EFCAB6* transcript. The contribution of the *HMGA2-EFCAB6* gene rearrangement to the MPN hematopoietic stem cell pathology can be visualized in context with the *JAK2V617F* mutation. First, 90% of hematopoietic cells were affected by the cytogenetic abnormality encompassing the rearrangement, whereas the *JAK2V617F* allele dose was lower, at 50%, suggesting that the *HMGA2-EFCAB6* gene rearrangement may play a more ancestral, pre-*JAK2V617F* role in the neoplastic process. Second, the contribution of the gene rearrangement can be viewed through the oncogenic effect caused by the loss of the regulatory sequences from the *HMGA2*-3′UTR region, including loss of *HMGA2* binding sites for the miRNA *let*-7 tumor suppressor, and finally, disruption may result from the loss of *EFCAB6* transcription and consequential lower expression of DJ-1/PARK7, the latter an important mediator of numerous cellular responses.

Materials and methods

Case report

A 75-year-old white woman presented in 2003 with an increased white blood cell count (56×10^9 cells/L), mild anemia (hemoglobin 11.4 g/dL, hematocrit 35.4%), normal platelet count (340×10^9 cells/L), and mild splenomegaly. The peripheral blood smear showed a leukoerythroblastic picture. Bone marrow aspirate and biopsy showed limited trilineage cellularity with increased reticulin fibrosis (grade 3 of 4). Cytogenetic studies performed in peripheral blood by a commercial laboratory showed a karyotype with a chromosomal translocation in 90% of metaphases as the only cytogenetic abnormality, which was initially described as a t(12;22)(q13;q13) (Figure 1). Molecular cytogenetics (fluorescence in situ hybridization) revealed no Philadelphia chromosome translocation, and molecular studies revealed no *BCR-ABL1* transcript. Surface antigen analysis of peripheral blood leukocytes, using flow cytometry, revealed no aberrant antigen expression or clonal populations. A diagnosis of a *BCR-ABL1*-negative myeloproliferative neoplasm was made. Additional comorbidities included insulin-dependent diabetes mellitus and compensated hypothyroidism. The clinical course following diagnosis was characterized by progressive leukocytosis, increasing splenomegaly, skin infiltrates suggestive of a vasculitic process by skin biopsy, and a worsening retinopathy. The patient was treated intermittently with hydroxyurea. At 20 months after diagnosis, the patient developed a fatal septic shock syndrome following bilateral lower extremity cellulitis. No autopsy was performed.

Patient sample

All studies were performed on archived tissue samples with the approval and under the guidelines of the Christiana Care Health Systems' (CCHS) investigational review board. Genomic DNA was obtained from tissue stored at −80 °C, whereas RNA studies were performed on material stored at both −80 °C and −190 °C.

Cytogenetic analysis

The presence of the t(12;22) translocation was assessed using traditional cytogenetic techniques, G-banding by trypsin and Giemsa stain (GTG) in bone marrow and peripheral blood metaphase cells by a commercial laboratory at diagnosis and by the CCHS Molecular Diagnostics Laboratory.

JAK2V617F detection

The *JAK2V617F* mutation was evaluated by specific primers and fluorescence resonance energy transfer (FRET) probe technology using the LightCycler 2.0 system (Roche Applied Science, Indianapolis, IN). DNA was extracted from the cytogenetic cell pellet used for karyotyping (20). All primers and FRET probes were obtained commercially (Sigma Proligo Reagents, Sigma-Aldrich, St. Louis, MO). The forward primer (5′-TTCCTTAGTCTTTCTTTGAAGCA-3′) and the reverse primer (5′-GTGATCCTGAAACTGAATTTTCT-3′) were diluted to achieve final concentrations of 0.1 μmol/L and 0.5 μmol/L, respectively. The anchor probe (5′-LCR640-ACGAGAGTAAGTAAAACTACAGGCT-phosphate-3′) and sensor probe (5′-ATGGAGTATGTGTCTGTGG-flourescein-3′) were diluted to achieve a final concentration of 0.2 μmol/L (21). DNA extracted from the HEL60 cell line (Genzyme