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Case Report

BCR/ABL1-negative, triple-negative, myeloproliferative neoplasm with a hitherto undescribed, isolated, *SH2B3 (LNK)* gene mutation: A case report

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ABSTRACT

The myeloproliferative neoplasms (MPNs) are chronic myeloid cancers (clonal hematopoietic disorders) that are characterized by the overproduction of terminally differentiated (mature) blood cells, and that may evolve into acute myeloid leukemia (AML). The literature indicates that three known driver mutations currently exist in *BCR/ABL1*-negative MPNs: *JAK2*, *MPL* and *CALR*. A small percentage of *BCR/ABL1*-negative MPN cases lack mutations in all three of these genes and are thus referred to as triple- negative. This case report is of a 48-year-old female with non-chronic myeloid leukemia (non-CML) MPN with increases in all three cell lineages in the bone marrow. No abnormalities were detected in chromosome analysis and FISH studies. Her myeloid molecular profile analyzed by targeted next generation sequencing (NGS) found a frameshift mutation in the *SH2B3* (*LNK*) gene, but no mutations in any other genes typically mutated in MPNs. A few other cases of triple-negative, non-CML MPNs with *SH2B3* (*LNK*) gene mutations have been reported, but we are adding a case of an MPN with a hitherto undescribed frameshift *SH2B3* (*LNK*) mutation between the DD and PH domains of the gene. Currently, the literature is not clear whether *SH2B3* (*LNK*) mutation is a driver mutation or not. More investigation is needed to clarify the significance of *SH2B3* (*LNK*) gene mutations especially in the pathogenesis of MPNs lacking major driver gene alterations.

1. Introduction

Myeloproliferative neoplasms (MPNs) include several different diseases that are identified by overproduction of blood components. Conventional and molecular cytogenetic analyses help distinguish between *BCR/ABL1* (Philadelphia chromosome) positive and negative MPNs. Chronic myeloid leukemia (CML) is a *BCR/ABL1*-positive MPN. Several MPNs are included in the *BCR/ABL1*- negative category: chronic neutrophilic leukemia (CNL), polycythemia vera (PV), essential thrombocythemia (ET), prefibrotic primary myelofibrosis (prePMF), primary myelofibrosis (PMF), chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), and MPN, unclassifiable (MPN-U). These MPNs are distinguished using the 2016 revision of WHO classification [1]. Increases in blood cells have been linked to genetic mutations in component genes in the JAK-STAT signaling pathway. Janus kinase 2 (JAK2) is a key component of this pathway, and if allowed to function in excess, the disease manifests. The majority of MPN cases harbor a crucial *JAK2* mutation, and thus it has been designated as a driver mutation that can cause disease to manifest. Driver mutations have also been identified in two other genes: *MPL* and *CALR*. Despite the crucial nature of these driver mutations, MPN cases have also been identified where none of these driver mutations in *JAK2*, *MPL* and *CALR* genes are present, and these cases are referred to as triple-negative MPNs [2]. Accordingly, driver mutations in additional genes must exist.

The *SH2B3* (*LNK*) gene is located on chromosome 12 (12q24.12) and encodes for LNK, a lymphocyte-specific adapter protein. This protein plays a crucial role in the JAK-STAT signaling pathway by negative regulation of normal hematopoiesis. The *SH2B3* (*LNK*) gene-encoded LNK protein consists of three critical domains. The dimerization domain (DD) allows the protein to dimerize either with itself or with another molecule to modulate its activity. The Pleckstrin homology (PH) domain is necessary for LNK to localize to the plasma membrane so it can interact with other molecules. The third domain is the Src homology 2 (SH2) domain, and is responsible for the interaction with signaling molecules that downregulate hematopoiesis [3]. *SH2B3* (*LNK*)

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mutations are most commonly found in the PH domain, but mutations in other parts of the gene have also been described. If any one of three domains of LNK protein is functionally disrupted due to a loss-of-function mutation in the *SH2B3* gene, LNK can no longer exert a negative effect on hematopoiesis. Without LNK regulation, overproduction of terminally differentiated cells can occur and a MPN results [3–12].

The purpose of this case report is to describe a non-CML, triple-negative MPN that was found to only have a *SH2B3* (*LNK*) mutation. This particular mutation, which is between the DD and PH domains and results in a truncation of LNK protein with loss of both PH and Src domains, has not been described previously. We also aim to discuss the possibility of a *SH2B3* (*LNK*) mutation as a driver mutation and the possibility of existence of other unidentified driver mutations in triple-negative MPNs.

2. Case report

A 48-year-old female was admitted to the hospital with ascites. Further work-up revealed alcoholic steatohepatitis with fibrosis. Her initial laboratory studies showed increased WBC (34.2 K/uL) and platelets (712 K/uL). Work-up to identify any infectious cause was negative. No other cause of reactive thrombocytosis was found. She had no splenomegaly and her serum LDH level was within normal limits at 227 IU/dL (Reference range: 87–241 IU/L). *JAK2 V617F* mutation analysis and fluorescence in situ hybridization (FISH) for t(9;22) were negative in the peripheral blood. She then underwent bone marrow aspiration and biopsy to rule-out a myeloproliferative neoplasm.

Her complete blood count (CBC) report at the time of the bone marrow aspiration and biopsy indicated WBC 25.1 K/uL, Hgb 9.0 g/dL, HCT 28.1%, MCV 83.4 fL, MCH 26.7 pg, MCHC 32.0 g/dL, RDW 22.0%, platelets 691 K/uL with a differential count of neutrophils 82.9%, lymphocytes 10.40%, monocytes 5.70%, eosinophils 0.80%, and basophils 0.20%. Review of peripheral blood smear confirmed the hemogram findings and no toxic changes were seen in the neutrophils. Of significance were her elevated platelet count (Fig. 1), absolute neutrophilia (Fig. 1) and normocytic normochromic anemia.

Bone marrow aspirate and core biopsy showed hypercellular (90%–100% cellular) bone marrow with moderate increase in megakaryocytes, atypical megakaryocytes with hyperlobated nuclei and large size showing clustering, mild myeloid hyperplasia, mild erythroid hyperplasia, absence of reticulin fibrosis (Fig. 2 A–C), and presence of stainable iron. Immunohistochemical staining for CD61, myeloperoxidase and CD71 confirmed hyperplasia of all 3 cell lineages (Fig. 2 D–F).

FISH on the bone marrow aspirate with 1p/1q, 5p/5q, 7p11/7q31, chromosome 8, t (9;22), *KMT2A (MLL), ETV6*, 13q14/13q34, *TP53*, and 20q-specific probes revealed normal results. Therefore, no *BCR-ABL1* fusion was detected.

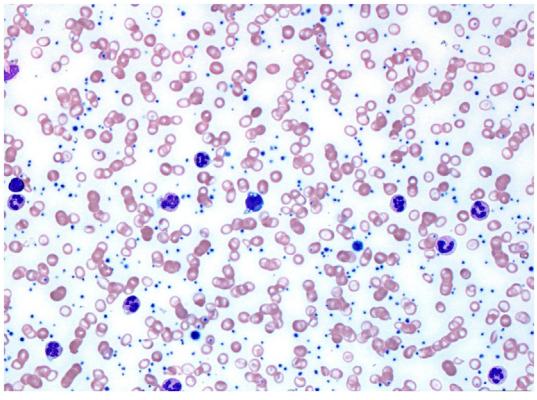

Fig. 1. (Peripheral blood smear; Wright-Giemsa Stain; x400): Peripheral blood smear shows mild neutrophilia and moderate thrombocytosis.

Chromosome analysis of the bone marrow aspirate revealed a normal female karyotype (46,XX) without any apparent clonal aberrations.

Subsequent molecular genetic studies on the bone marrow aspirate did not show mutations in *JAK2*, *MPL* or *CALR* genes. Of the 44 genes (*ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DDX41, DNMT3A, ETNK1, ETV6, EZH2, GATA2, GNAS, GNB1, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PHF6, PPM1D, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, TET2, TP53, U2AF1, WT1, ZRSR2*) that were analyzed using the next-generation sequencing (NGS) method (using Illumina NexSeq 500 platform at Genoptix Medical Laboratory, Carlsbad, CA), *SH2B3* (*LNK*) was the only gene that indicated any genomic alteration. This mutation was a1bp insertion frameshift alteration (c.393dupG; p.P132Afs*40) in exon 2 of the *SH2B3* gene (Fig. 3) with 35% mutant allele frequency.

Based on these studies and by applying 2016 revision of WHO classification criteria, a differential diagnosis of prePMF and MPN-U was raised. She was not put on any treatment for her MPN and her 4-month follow-up CBC showed WBC 36.0 K/uL, Hgb 11.1 g/dL, HCT 34.1%, MCV 84.2 fL, MCH 27.2 pg, MCHC 32.3 g/dL, RDW 21.0%, platelets 663 K/uL with a manual differential count of neutrophils 80%, lymphocytes 12%, monocytes 6%, eosinophils 1%, and basophils1%. At follow-up, she had no splenomegaly and her serum LDH level was within normal limits at 180 IU/dL (Reference range: 87–241 IU/L).

The patient died of decompensated cirrhosis a few days after this testing, so no further CBC and differential count is available to confirm or refute the persistence of leukocytosis. Therefore, the.

differential diagnosis of prePMF and MPN-U could not be resolved.

3. Discussion

Before discussing the significance of the *SH2B3* (*LNK*) gene mutation and its relevance to our case, it is necessary to discuss the differential diagnosis of prePMF and MPN-U. Our patient had alcoholic steatohepatitis with fibrosis, and no splenomegaly. Laboratory work-up indicated normal serum LDH, moderate persistent normocytic-normochromic anemia, mild persistent absolute neutrophilia without toxic changes in the neutrophils, moderate persistent thrombocytosis, hypercellular non-fibrotic bone marrow with trilineage hyperplasia and atypical clustered megakaryocytes. FISH and sequencing studies indicated absence of *BCR-ABL1* fusion, absence of mutations in *JAK2*, *CALR* and *MPL* genes, and a mutation only in the *SH2B3 (LNK)* gene in a 44-gene NGS panel targeted for MPN. Persistent normochromic-normocytic anemia, leukocytosis and thrombocytosis along with increased megakaryocytes showing clustering and atypia do look like a MPN. When we considered a differential diagnosis of ET and pre-PMF and applied the 2016 revised WHO criteria for the diagnosis of ET and pre-PMF (Tables 1 and 2), the criteria for ET were not met. Trilineage hyperplasia and triple-negative status and criteria for pre-PMF can be met if we consider that her persistent leukocytosis was not due to her co-morbid condition and also if we use *SH2B3* mutation as a clonal marker of MPN. As the WHO criteria have listed only some of the most common mutations (e.g. *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, SRSF2, or *SF3B1* gene mutations) that can be used as markers of clonal disease, it is not the intention of WHO classification to exclude other clonal markers of MPN. We are using *SH2B3* mutation as a clonal marker of MPN, but we are not sure about using persistent leukocytosis as a minor criterion to meet the criteria for the diagnosis of pre-PMF. Due to the patient's death, a more current CBC and differential count could not be obtained, so we are unable to rule-out or rule-in the diagnosis of prePMF, are, therefore, calling it MPN-U.

As previously stated and as seen in our case, a small percentage of *BCR/ABL1*-negative MPN cases do not have any of the known driver mutations in three genes (*JAK2*, *CALR* and *MPL*) and are referred to as triple-negative [3,4]. Some of these triple-negative MPN cases, as in our