Analysis of genes encoding epigenetic regulators in myeloproliferative neoplasms: Coexistence of a novel SETBP1 mutation in a patient with a p.V617F JAK2 positive myelofibrosis

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Abstract. In recent years it has been shown that the causes of chronic myeloproliferative neoplasms (MPNs) are more complex than a simple signaling aberration and many other mutated genes affecting different cell processes have been described. For instance, mutations in genes encoding epigenetic regulators are more frequent than expected. One of the latest genes described as mutated is SET binding protein 1 (SETBP1). In silico tools have revealed that there are several human SETBP1 paralogous to nuclear receptor binding SET domain protein 1 (NSD1), NSD2 and NSD3, for example, which are also involved in the development of other hematological malignancies. Therefore, the present study analyzed the mutational status of NSD1, NSD2, NSD3 and SETBP1 in BCR-ABL1 negative MPNs with or without Janus kinase 2 (JAK2) p.V617F mutation. The present study revealed that the NSD genes are not frequently mutated in MPNs. However, a novel SETBP1 mutation was identified in a patient with p.V617F JAK2 positive primary myelofibrosis. These results provide further insight into the genetic complexity of MPNs.

Introduction

Chronic myeloproliferative neoplasms (MPNs) have been considered diseases caused by aberrations in signaling, mainly affecting the JAK-STAT pathway. However, mutations in other genes that affect different cell processes have been described, suggesting that they are diseases with more complex causes. Recent findings suggest that MPNs are likely the result of combined genetic dysregulation and can present mutations in epigenetic associated-genes such as TET2, ASXL1, EZH2, IDH1/2 and DNMT3A (1-3) described also in the related myelodysplastic/myeloproliferative neoplasms (MDS/MPN).

SETBP1 encodes the SET binding protein 1, one component of the multi-functional SET complex in the nucleus that plays a role in histone modifications. In 2013, whole exome sequencing identified recurrent SETBP1 mutations in approximately 25% patients with atypical chronic myeloid leukemia (aCML) and in a small proportion of patients with chronic myelomonocytic leukemia (CML) and chronic neutrophilic leukemia (CNL) (4). SETBP1 was the first gene recurrently mutated in aCML, a disease previously diagnosed by exclusion. All of the mutations described were located in a small region of 14 amino acids (858-871) in the highly-conserved SKI homologous region encoded by exon 4 of the gene.

SETBP1 seems to interact with SET protein, leading to the inhibition of the activity of PP2A and promoting the proliferation of leukemic cells (5). Overexpression of SETBP1 increases the expression of other genes such as HOXA9 and HOXA10 leading to immortalization of murine myeloid progenitors (6), and mutant SETBP1 forms could drive the disease through similar mechanisms (7).

There are three SETBP1 paralogous genes (NSD1, NSD2 and NSD3) that encode proteins with similar functions, so that they could also be mutated in MPNs. Indeed, NSD1 and NSD3 are involved in fusions with NUP98 in myeloid malignancies. Nuclear receptor binding SET domain protein 1 (NSD1) encodes a H3K36 and H4K20 methyltransferase that activates or represses transcription depending on the cellular context. Some rare cases of acute myeloid leukemia (AML) with poor prognosis have a t(5;11)(q35;p15) that fuses NUP98 with NSD1, and show abnormally high levels of H3K36 methylation and activation of transcription of oncogenes such as HOXA9 (8-10). On the other hand, nuclear receptor binding SET domain protein 3 (NSD3) encodes a H3K4 and H3K27 methyltransferase, marks of activation or repression of transcription, respectively. This gene has also been found fused with NUP98 in patients with AML (11) and therapy-related myelodysplastic syndrome (t-MDS) with poor prognosis (12). Finally, nuclear
receptor binding SET domain protein 2 (NSD2) encodes an H3K36 histone methyltransferase that has also been implicated in gene fusions in multiple myeloma (13) and is mutated in pediatric acute lymphoblastic leukemia (ALL) (14). The p.E1099K mutation affects the SET domain and increases H3K36 dimethylation, which may lead to the transforming activity of NSD2 (14).

To date, little is known about the role of individual histone modifications in MPNs, and current research is focusing mainly on changes related to polycomb group proteins (3). These proteins can remodel chromatin; for example, polycomb repressive complex 2 (PRC2) catalyzes di- and trimethylation of H3K27 leading to gene silencing. Addition of sex combs like protein 1 (ASXL1) encodes an important mediator of PRC2 function, and mutations in this gene have been described in MDS/AML (15) and in MPNs (16). Thus, we asked whether SETBP1 related genes could also be mutated in these diseases.

With this aim in mind, we analyzed the mutational profile of NSD1, NSD2 and NSD3 in a selected group of patients with MPNs.

Materials and methods

We performed denaturing high performance liquid chromatography (dHPLC) to analyze exon 13 of NSD1, NSD2 and NSD3, which are homologous to SETBP1 exon 4 in which mutations had been previously described. This SETBP1 exon was also included in the analysis. In addition, we also analyzed exon 19 of NSD1 and NSD2 and exon 20 of NSD3, homologous to the NSD2-mutated exon in ALL (p.E1099K) (primers and conditions are shown in Table I).

We included samples from patients with different types of MPNs (PV, ET, PMF) and MDS/MPNs with and without the p.V617F mutation in Janus kinase 2 (JAK2). Ninety-two samples (34 ET, 37 PMF and 21 MDS/MPNs) were from patients without p.V617F JAK2 mutation, while 50 samples (8 ET, 8 PV, 4 PMF and 30 with an unspecified MPN type) harbored the p.V617F mutation. Finally, we also analyzed samples from 25 different cancer cell lines (Table II), most of them from hematological malignancies. As a control group, we included samples from 13 patients with B-ALL, the disease in which the p.E1099K NSD2 mutation had been described (14). Informed consent was obtained from all patients and procedures were approved by the Ethical Committee on Clinical Research of University of Navarra.

Results

Most of the nucleotide changes that we detected were SNPs already described (data not shown). No mis-sense changes were found in NSD1 and NSD3 so they do not seem to be frequently mutated in these diseases. With regard to NSD2, we did not detect any mis-sense mutations in samples from MPN and MDS/MPN patients, except for the previously described p.E1099K mutation that we observed in one patient with B-precursor ALL.

The analysis of SETBP1 found the mis-sense mutation p.S867G (c.2599A>G according the first nucleotide of the translation start codon of the CDS from NM_015559) (Fig. 1) in the sample from one patient with a p.V617F JAK2 PMF. This change is located in a residue that is highly conserved between different species in the SKI homologous region, the mutational hotspot described previously In silico analysis by several bioinformatics tools like PolyPhen-2, MutPred2, SIFT and FATHMM showed that it is probably damaging (data not shown). No additional material was available to determine the somatic status of this change. This mutation has not been described as a polymorphism or a cancer-related mutation in other databases. In fact, in COSMIC (Catalogue of Somatic Mutations in Cancer, cancer.sanger.ac.uk/cosmic) there are other variants affecting the same codon but not this specific nucleotide. These mutations are p.S867N (c.2600G>A) a somatic change predicted as pathogenic and found in an intestinal adenocarcinoma, p.S867R (c.2601C>A) a non-confirmed somatic change predicted as neutral and found in four samples from MDS and AML associated with MDS and the silent p.S867S (c.2601C>T), a somatic change predicted also as neutral and found in two samples from stomach and intestine with adenocarcinoma but described as a SNP in dbSNP (rs376371660). This is also the only change in ExAC database (Exome Aggregation Consortium, http://exac.broadinstitute.org) affecting this codon, found in 7 of 120746 alleles with a frequency of 0.00005797. This is the only variant also described in 1000 Genomes Phase 3 database (www.internationalgenome.org/category/phase-3) with a frequency of 0.00039936 and in the ESP database (Exome Sequencing Project, evs.gs.washington.edu) with a frequency of 0.00007688, in both cases with only one allele T found. In gnomAD (Genome Aggregation Database, gnomad.broadinstitute.org), that spans 125,748 exome sequences and 15,708 whole-genome sequences from unrelated individuals the mutation p.S867G is also not described.

Discussion

SETBP1 mutations can coexist with aberrations in other genes frequently mutated in myeloid neoplasms such as CBL, ASXL1, U2AF1 or RUNX1 (3,17,18) thus cooperating in the progression of the disease. However, the coexistence of mutations in SETBP1 and JAK2 in MPNs has not been described to date. In the case of ASXL1 and SETBP1 mutations, the former may initially inhibit cell differentiation while mutations in SETBP1 would provide a proliferative advantage (18). Although a direct physical interaction between CBL and SETBP1 has not been demonstrated, it seems plausible that both mutations could cooperate indirectly by reducing cytokine dependence of leukemia cells (17).

Furthermore, it is well established that mutations in genes related to epigenetic regulation of gene expression can coexist with mutations in JAK2 and could modulate the progression of the disease (3.19). For instance, TET2 encodes the Tet methylcytosine dioxygenase 2 which catalyzes the conversion of the 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) and plays a key role in active DNA demethylation and it is frequently mutated in patients with MPNs (20). TET2 mutations have been found in 17% of patients with p.V617F JAK2 (20). Although both mutations are present in different clones, data published to date show that TET2 somatic mutations might be associated with increased aggressiveness and
higher frequency of organomegaly in ET patients, but not in
PV or PMF patients (19). Although
SETBP1 mutations have
not been associated with mutations in
JAK2 in MPNs to date,
our finding suggests the possibility of a similar interplay of
mutational mechanisms.

Recently two cases have been reported with Schinzel-
Giedion syndrome (SGS, MIM 269150) and a milder phenotype
with the mutation at this residue p.S867R (21,22). SGS is a rare
dominant developmental disorder characterized by multiple
malformations including midface hypoplasia, cardiac defects,
hydronephrosis and skeletal abnormalities. It seems caused by
germline de novo mutations in
SETBP1 in a hotspot of 12 bp
coding for residues 868 to 871 of the protein, that overlaps to
the somatic mutations reported in myeloid malignancies and
support a gain-of-function effect. This dual role in cancer and
development is not new, there are other genes such as
HRAS,
ASXL1,
EZH2 and
FGFR2 in which germline mutations cause
developmental disorders and the overlapping somatic muta-
tions drive cancer. The recurrently mutated region (868-871)
in SETBP1 is highly conserved and it has been identified
as important to initiate degradation by ubiquitination so
the mutations could increase protein stability (4,21,22). The
milder phenotype shown by both patients with SGS and the
p.S867R would point to a less activating gain-of-function of
this mutant protein.

Table I. Polymerase chain reaction primers used for the mutational analysis of NSD1, NSD2, NSD3 and SETBP1 by dHPLC.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Tm (˚C)</th>
<th>% GC</th>
<th>Sequence (5’→3’)</th>
<th>PCR product (bp)</th>
<th>dHPLC conditions (˚C)</th>
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<td>NSD1-e13L</td>
<td>61.0</td>
<td>29.17</td>
<td>AATTATCCTCTTTTGGCTTCTCA</td>
<td>285</td>
<td>57.3; 59.2; 61</td>
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<tr>
<td>NSD1-e13R</td>
<td>62.8</td>
<td>40.91</td>
<td>TCTGTTGCCAATTAAGACTGAGG</td>
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<td></td>
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<td>NSD1-e13Lm</td>
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<tr>
<td>NSD1-e19L</td>
<td>66.2</td>
<td>54.55</td>
<td>TGCTGCTGACAGTGTTAGAGGT</td>
<td>350</td>
<td>53.1; 56.8; 55.8</td>
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<tr>
<td>NSD1-e19R</td>
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<td>39.13</td>
<td>CAGTGAAAACAGCTTTCCACT</td>
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<td>NSD1-e19Lm</td>
<td>70.3</td>
<td>59.10</td>
<td>TGCTGCTGACAGTGTTAGAGGT</td>
<td>329</td>
<td>57.8; 58.7; 62.7</td>
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<td>NSD2-e13L</td>
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<tr>
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<td>285</td>
<td>61.2; 61.8</td>
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<td>54.55</td>
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<tr>
<td>NSD2-e19Rm</td>
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<td>50.00</td>
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<td>GCTGTTGATGTCCTCTAGCTG</td>
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<td>NSD3-e13Rm</td>
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<td>52.17</td>
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<td>NSD3-e20L</td>
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<td>37.50</td>
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<td>60.7; 62</td>
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<td>NSD3-e20R</td>
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<td>41.67</td>
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<td>NSD3-e20Rm</td>
<td>64.3</td>
<td>41.67</td>
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<td>SETBP1-e4F9L</td>
<td>65.8</td>
<td>40.91</td>
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<td>53.8; 56; 60</td>
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<td>SETBP1-e4F9R</td>
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<td>45.45</td>
<td>CTCTTTCAACCCAAAACCACAAA</td>
<td>394</td>
<td>60.7; 62</td>
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<td>61.2</td>
<td>40.9</td>
<td>CTCTTTCAACCCAAAACCACAAA</td>
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</table>

Forward and reverse primers used to amplify the DNA sequences of interest. The mutations designed to create a control mutant product are underlined. The length of the PCR product is shown in the fifth column. Finally, melting temperature conditions (dHPLC) for each exon are also shown. NSD, nuclear receptor binding SET domain protein 1; SETBP1, SET binding protein 1; dHPLC, denaturing high performance liquid chromatography; PCR, polymerase chain reaction.

The sample available in our case did not allow us to deter-
mine whether SETBP1 and JAK2 mutations coexist in the same
clon. Lack of access to clinical data of this patient prevents us
from drawing definitive conclusions about the putative effects
of their presence in the progression of the disease. SETBP1
mutations usually have poor prognosis and are frequently
acquired as secondary events in other myeloid neoplasms (PMF
can arise on its own or as a progression of PV or ET) (18), so the
p.S867G mutation in
SETBP1 could be relevant for the progres-
sion of the disease. In addition, additional experiments would
be required to demonstrate the pathogenicity of the p.S867G
mutation. These experiments should be performed on cell
lines that stably express p.S867G
SETBP1 and this mutation
in combination with the p.V617F
JAK2 to test their possible
cooperation for the transformation of the disease.

In this study we show that
NSD1, NSD2 and NSD3 genes,
that are paralogous to
SETBP1 and encode also epigenetic
regulators, are not frequently mutated in MPNs in the
SETBP1 homologous region. However, we show that the p.V617F
JAK2 can coexist with mutations in
SETBP1, a gene mutated in some
myeloid neoplasms. This fact adds support to the notion that
these diseases are genetically complex and shows the need to
identify additional oncogenic mechanisms that could promote
progression or phenotypic diversity, in order to design new
targeted therapies.
Acknowledgements

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Funding

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Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Authors’ contributions

LEA, FJN and JLV designed the study. LEA and CH performed the experiments. LEA, DNH, DC and JLV analyzed the data. LEA, FJN and JLV wrote the manuscript. All authors have read and approved the final manuscript.

Table II. Human cancer cell lines tested.

<table>
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<th>Cell line name</th>
<th>Repository (number)</th>
<th>Origin</th>
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<td>A-549</td>
<td>DSMZ (ACC-107)</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>BK-006</td>
<td>ECACC</td>
<td>p.V617F JAK2 positive PV</td>
</tr>
<tr>
<td>BK-013</td>
<td>ECACC (98100924)</td>
<td>p.V617F JAK2 negative ET</td>
</tr>
<tr>
<td>BK015</td>
<td>ECACC (99092421)</td>
<td>p.V617F JAK2 negative ET</td>
</tr>
<tr>
<td>DAUDI</td>
<td>DSMZ (ACC-78)</td>
<td>Burkitt lymphoma</td>
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<td>EOL-1</td>
<td>DSMZ (ACC-386)</td>
<td>Acute myeloid eosinophilic leukemia</td>
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<td>F-36P</td>
<td>DSMZ (ACC-543)</td>
<td>Acute myeloid leukemia secondary to myelodysplastic syndrome (MDS)</td>
</tr>
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<td>HCC-1937</td>
<td>DSMZ (ACC-513)</td>
<td>Breast carcinoma</td>
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<td>HEL</td>
<td>DSMZ ACC-11</td>
<td>Erythroleukemia</td>
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<td>HL60</td>
<td>DMSZ ACC-3</td>
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<tr>
<td>HU3</td>
<td>Dr Morgan, USA</td>
<td>Acute megakaryoblastic leukemia</td>
</tr>
<tr>
<td>K-562</td>
<td>DSMZ ACC-10</td>
<td>BCR-ABL1 positive chronic myeloid leukemia (CML) in blast crisis</td>
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<td>KARPAS-299</td>
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<td>KARPAS-422</td>
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<td>Acute megakaryoblastic leukemia</td>
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<td>Osteosarcoma</td>
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<td>MOLT-16</td>
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<td>T cell acute lymphoid leukemia (T-ALL)</td>
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<td>MOLT-4</td>
<td>DSMZ ACC-362</td>
<td>Acute lymphoid leukemia (ALL)</td>
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<td>MV-411</td>
<td>DSMZ ACC-102</td>
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<td>RAJI</td>
<td>DSMZ ACC-319</td>
<td>Burkitt lymphoma</td>
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<td>REH</td>
<td>DSMZ ACC-22</td>
<td>B-ALL (t(12;21) (p13;q22) (fusion ETV6-RUNXI))</td>
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<td>SET2</td>
<td>DSMZ ACC-608</td>
<td>p.V617F JAK2 positive ET</td>
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<td>UKE-1</td>
<td>Dr W. Fiedler, Hamburg, Germany</td>
<td>p.V617F JAK2 positive ET transformed to AML</td>
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</table>

ECACC, European Collection of Cell Culture; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

Figure 1. Electropherograms of the sequence surrounding the mutation c.2599A>G (p.S867G). Up, wild-type sequence; down, sequence from the patient. SETBP1, SET binding protein 1.
Ethics approval and consent to participate

Written informed consent was obtained from all of the patients and the procedures were approved by the Ethical Committee on Clinical Research of University of Navarra (Pamplona, Spain).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References