Case Report

A novel \textit{TET2} mutation in a patient with refractory cytopenia with multilineage dysplasia

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\textbf{ABSTRACT.} Myelodysplastic syndrome diagnosis of karyotypically normal patients may be elusive because it relies exclusively on morphological and clinical data. In routine practice, finding of an acquired mutation or a cytogenetic abnormality provides irrefutable evidence of the clonal nature of that disease. Recurrent deletions and somatic mutations in \textit{TET2}, a gene involved in epigenetic regulation, have been reported in about 20\% of adult patients with myelodysplastic syndrome.
Novel \( TET2 \) mutation in multilineage dysplasia

We report a novel g.95805C>T, nonsense \( TET2 \) mutation, leading to a premature stop codon (p.Gln913*), in an adult patient with refractory cytopenia with multilineage dysplasia.

Key words: Myelodysplastic syndromes; Mutation; Biomarker; Refractory cytopenias with multilineage dysplasia; \( TET2 \)

INTRODUCTION

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of myeloid malignancies characterized by hematopoietic arrest, accounting for the frequently acquired bone marrow failure in adults. The two latest WHO classifications (2001, 2008) discriminate seven MDS categories: refractory cytopenias with unilineage dysplasia (RCUD), refractory anemia with ring sideroblasts (RARS), refractory cytopenias with multilineage dysplasia (RCMD), refractory anemia with excess of blasts-1 (RAEB-1), refractory anemia with excess of blasts-2 (RAEB-2), MDS unclassified (MDS-U), and MDS associated with isolated del (5q), based on cytogenetic abnormalities and presence and number of immature cells in bone marrow and peripheral blood (Vardiman et al., 2009). Cytogenetic alterations are useful prognostic markers for guiding therapy (Sugimoto et al., 2012) but in karyotypically normal patients, the diagnosis of MDS may be difficult, where it is still based on morphological and clinical data.

More recently, deletions and gene mutations revealed by high-throughput methods have been reported in patients with MDS, as is the case of mutations affecting \( TET2 \) (encoding TET oncogene family, member 2, in 4q24) (Langemeijer et al., 2009). \( TET2 \) encodes the ten-eleven-translocation 2 (TET2) enzyme, a methylcytosine dioxygenase that catalyzes the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). TET2 plays a relevant role in myelopoiesis (Pronier et al., 2011) and its loss of function has been observed in different myeloid neoplasms (Mercher et al., 2012). Mutations are often found in heterozygosity, leading to haploinsufficiency, and nonsense, missense and frameshift mutations have been identified in 20% of cases of MDS (Langemeijer et al., 2009).

In MDS patients, \( TET2 \) mutations are associated with a better prognosis, global and event-free survival (Kosmider et al., 2009). Furthermore, the mutational status of \( TET2 \) has been described as an independent, predictive biomarker to response to treatment with the hypomethylating agent azacytidine (Itzykson et al., 2011). This makes \( TET2 \) mutations potential biomarkers for prognosis and therapeutic guidance.

CASE REPORT

A 68-year-old Brazilian woman, former smoker and under treatment for hypertension, was seen at the medical clinic of Hospital da Lagoa (Rio de Janeiro, Brazil) due to 2 month of intense fatigue. On physical examination, the patient appeared pale but without fever, coughing, spontaneous bleeding, dysuria, or weight loss. Serological tests for HIV, HBV and HCV were negative. A low hematocrit with normal levels of B12, folic acid and ferritin excluded B12, folate and iron deficiencies. Hematological parameters were: WBC, 3.8 x 10^9/L; neutrophils, 70%; Hb, 8 g/dL; HCT, 28.8%; MCV, 105; and platelets, 197 x 10^3/mm^3. Bone marrow examination revealed two-lineage dysplasia: increased erythroid precursors and hyposegmen-
tation of neutrophils (pseudo-Pelger-Huet cells). No ring sideroblasts were found. Hematological and clinical parameters were compatible with diagnosis of RCMD with normal karyotype.

Flow cytometry of bone marrow cells was carried out to determine the following parameters: presence of myeloblasts, B cell progenitors, CD45 expression and side scatter using the following monoclonal antibody panel: CD71, Glycophorin A, CD61, CD42b, CD13, CD33, CD10, CD16, CD11b, CD15, HLA-DR, CD64, CD36, CD14, CD2, CD4, CD8, CD10, CD19, CD56, CD34, CD117, and CD45 (pan leukocyte). Data were analyzed with Paint-A-Gate (BD Biosciences, San Diego, CA, USA). These analyses showed 1.21% myeloblasts. Phenotypic changes in other cellular lineages were: absence of B lymphoid precursor cells, asynchronous maturation of granulocytic series with increased metamyelocyte and aberrant expression of CD56, monocytic cell depletion and loss of CD14 expression. This profile was consistent with the immunophenotypic characterization of MDS. According to the Revised International Prognostic Scoring System (IPSS-R), this condition accounted for low risk, with an overall survival estimate of 5.3 years.

To evaluate the mutational status of TET2, direct sequencing of coding exons 3 and 5-11 was performed from bone marrow and buccal swab DNA, as previously reported (Martínez-Avilés et al., 2012). Approximately 50 ng of purified amplified products for PCR was labeled with Big Dye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems) and run in an ABI3130XL sequencing platform. Data were analyzed with Mutation Surveyor (Softgenetics). Identified mutations were compared and analyzed with the following databases: dbSNP Short Genetic Variations (NCBI) (http://www.ncbi.nlm.nih.gov/SNP/), UniProtKB (ExPASy) (http://www.uniprot.org/), Gene Cards Database (Weizmann Institute of Science) (http://www.genecards.org/), and Catalogue of Somatic Mutations in Cancer (COSMIC) (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).

Sequencing analysis showed the heterozygous status of p.Gln913* (Gln913X) mutation, located in exon 3 of TET2 (Figure 1). This mutation was found in bone marrow cells but not in buccal swab DNA. No variants were found in other exons.

Initial treatment consisted of transfusions of red cell concentrates and administration of 20,000 IU/week erythropoietin (EPO). After 2 months following treatment, the patient showed no improvement in blood cell count, and therefore, she was given gradually increas-

Figure 1. Partial DNA sequence of TET2. A. Wild-type TET2 in buccal swab DNA. B. p.Gln913* mutation resulting from g.95805C>T in one allele of bone marrow cells.
ing doses of EPO up to 80,000 IU/week. Following this treatment, there was no need for new transfusions; hematocrit was normalized and remained stable. Ten months after diagnosis the patient presented leukemic transformation and is currently being treated and followed up in the same hospital.

DISCUSSION

_TET2_, located in 4q24, spans 141 kb and encodes two alternative isoforms, A and B, with 2002 and 1165 amino acids, respectively. Hundreds of _TET2_ mutations have been described, mostly resulting in loss of function, but their association with specific cellular phenotypes is not fully understood. These mutations, spread along this gene, mainly affect coding exons 3-11 and their splicing regions. Point mutations are most frequent, although insertions and deletions have also been reported as well as those leading to a premature stop-codon. _TET2_ has two conserved domains (1 and 2) shared with other members of the TET family, encoded by exons 4-10 and the terminal portion of exon 11. A high proportion of nonsense and insertion/deletion (indels) mutations are frequently found in these regions.

The frequency of _TET2_ mutations differs for each myeloid malignancy. Mutations in exon 11 are uncommon in chronic myelomonocytic leukemia (CMML) and more recurrent in myeloproliferative neoplasms BCR-ABL negative (MPN), acute myeloid leukemia (AML), and MDS, while mutations in exon 3 are more common in MDS than in other exons (Cimmino et al., 2011). In MDS patients, _TET2_ mutations are associated with a better prognosis (Kosmider et al., 2009), while in AML, CMML and MPN, the prognostic value of these mutations is still uncertain. In MDS, the majority of mutations in exon 3 consist of indels, while nonsense mutations are more frequent in other myeloid malignancies (Cimmino et al., 2011). The role of _TET2_ in the genesis and clinical modulation of myeloid malignancies is still discussed. However, some studies show that the presence of mutations in this gene may be associated with transformation into AML in patients with MPN (Abdel-Wahab et al., 2010; Zhang et al., 2012).

The novel g.95805C>T (p.Gln913*) mutation reported herein predicted the appearance of a premature stop codon resulting in a truncated protein. This was found in a patient with RCMD and normal karyotype whose MDS diagnosis was based on morphological, immunophenotypic and clinical profile. At the molecular level, the presence of this mutation allows the identification of the clonal nature of the disease. Although _TET2_ mutations are not very frequent in MDS, they can be most useful for tracing the molecular events underlying this clinical condition.

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