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JAK2^{V617F}/TET2 mutations: does the order matter?

Elodie Pronier,[1] Cyril Quivoron,[2] Olivier A. Bernard,[2] Jean-Luc Villeval[1]

[1]INSERM U1009; [2]INSERM U985; Institut Gustave Roussy, Université Paris Sud11, France. Correspondence: Jean-Luc Villeval. E-mail: villeval@igr.fr *doi:10.3324/haematol.2011.042846*

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EP and CQ contributed equally to this article

According to the World Health Organization classification, myeloproliferative neoplasms (MPN) include chronic myelogenous leukemia, also known as *BCR-ABL1*–positive MPN, classic *BCR-ABL1*-negative MPN including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), and non-classic forms (i.e. systemic mastocytosis, chronic eosinophilic leukemia not otherwise specified, chronic neutrophilic leukemia and unclassifiable MPN). All these subtypes are stem cell-derived clonal myeloproliferation, associated with the overproduction of mature blood elements and variable rates of transformation to acute myeloid leukemia (AML).[1]

JAK2^{V617F} activating mutation is the most prevalent abnormality observed in *BCR-ABL1*-negative MPN, found in virtually all cases of PV and in about half of ET and PMF (96%, 55% and 65%, respectively). This mutation lies in the pseudokinase-domain of JAK2 and disrupts its regulatory activity. Another mutation affecting *JAK2* exon 12 is observed in 3% of all PV cases. Mutations affecting W515 of the thrombopoietin receptor MPL are detected in PMF and ET patients. Additional mutations have been identified in MPN (reviewed in [1]). Defects in the control of intracellular signaling involve mutations in *LNK* and *CBL* genes. Genetic abnormalities affecting epigenetic regulation, and possibly responsible for disease initiation, concern the *ASXL1*, *EZH2* and *TET2* genes. Finally, mutation in *IKZF1* and *IDH1/2* may be implicated in MPN transformation. In PV, among these additional mutations to *JAK2*^{V617F}, TET2 mutations are those most frequently reported (16%); the others are only described in small subsets of patients.[1]

TET2 belongs to a family of three conserved genes in mammals: *TET1*, *TET2* and *TET3*. The founding member of the family, *TET1*, has been identified as a fusion partner of *MLL* in the t(10;11)(q22;q23) translocation of acute leukemia.[2,3] The involvement of *TET3* in hematologic disorder has not yet been described. The TET proteins are members of the 2-oxoglutarate (2-OG)- and Fe(II)-dependent dioxygenase that are able to convert 5-methyl-cytosine (5-mC) to 5-hydroxymethyl-cytosine (hmC).[4,5] Recent reports indicate an important role for TET1 and TET2 (and, therefore, hmC) in the control of ES cell self-renewal and differentiation.[6] TET3 might be involved in genome reprogramming following fecundation.[7]

5-hydroxymethylation: a novel major player in the epigenetic field

For decades, the implications and impact of 5-mC in human genome has been extensively studied and it is known to be associated with low gene expression. In contrast, little is known about the recently identified hmC. Indeed, the first study reporting a hydroxylated form of 5-mC in mammalian DNA was described in the early 70s.[8] However, this modified base did not receive full attention until 2 reports demonstrating that hmC accounts for 0.6% and 0.03% of the total nucleotides in Purkinje cells[9] and murine ES cells,[5] respectively.

The function of hmC is not yet clear. Several reports have indicated that hmC prevent the binding of proteins interacting with 5-mC and hmC and may represent a step toward demethylation.[8] Using a novel chemical method, Song *et al.*[10] showed an enrichment of hmC in maturing murine brain cells that increases with age and is associated with gene expression. Contrasting results have been reported in human samples: *TET2* mutation or inhibition of its catalytic activity affect the level of hmC in myeloid malignant samples, but was associated with a decrease of 5-mC in MDS (myelodysplastic syndromes) samples and with an increase of DNA methylation in AML.[11,12]

TET2 function in normal hematopoiesis and MPN

TET2 is expressed in a wide range of tissues, such as kidney, brain and the hematopoietic system.[13] Recent analyses using short-hairpin RNA in murine stem cells from bone

Table 1. TET2 mutations in BCR-ABL1-negative myeloproliferative neoplasms.

Myeloproliferative neoplasms	Mutation frequencies	Patients	Reference
Classic MPN			
Polycythemia vera	9.8%	-	(16-18)
	13%	13 of 100	
	16%	14 of 89	
	4.4%	-	(16-18)
	5%	3 of 57	
Essential thrombocythemia	11%	9 of 84	
	7.7%	-	(16-18)
	17%	10 of 60	
Primary myelofibrosis	present	4 of 16	
Myelofibrosis post-PV or -ET	14%	3 of 21	(17, 18)
	present	1 of 3	
	7.6%	27 of 354	(16-18)
	12%	24 of 198	
All classic MPN	13%	30 of 239	
Non-classic MPN			
Mastocytosis	29%	12 of 42	(19)
Unclassifiable MPN	present	2 of 4	(20)

marrow have shown that depletion of *TET2* promoted an increase in the proportion of hematopoietic stem/progenitor cells with inhibition of normal differentiation of granulocytes and monocytes.[12] Using a similar approach, *TET2* mRNA depletion was also shown to lead to the expansion of monocyte/macrophage cells in the presence of cytokines stimulating granulocytic differentiation (G-CSF or GM-CSF) but not in the presence of macrophage-colony stimulating factor (M-CSF).[11] This study indicates that TET2 may act at many different phases of myeloid differentiation.

Acquired inactivating mutations of the *TET2* gene are frequently observed in human myeloid malignancies (i.e. MDS, *de novo* and secondary AML) suggesting that they affect a very early myeloid progenitor. TET2 mutations are found in subsets of all subtypes of MPN (Table 1). Inherited *TET2* mutations are not responsible for familial MPN,[14] but a germline mutation of *TET2* was recently described in 2 sisters, one suffering from a *JAK2*^{V617F}-positive PV, the other healthy.[15]

In this issue, Swierczek and colleagues[21] have investigated the clonality and allele burdens of *JAK2*^{V617F} and *TET2* mutations in patients with sporadic or familial PV. In contrast to other PV patients described in the initial study,[17] and in agreement with recent reports,[15] they found evidence that *TET2* mutations may follow rather than precede the *JAK2*^{V617F} mutation in some PV patients. Therefore, no strict temporal order of appearance applies to *TET2* and *JAK2* mutations in PV. Furthermore, they show that clonal *in vitro* amplifications of mutated erythroid cells only occur from patient samples having both mutations, suggesting that the presence of TET2 mutations increases the aggression of the PV clone. The questions addressed by this work come down to the respective roles of TET2 and JAK2 mutations in the development of human PV.

Animal models using retroviral delivery, transgenic or knock-in technology have proved conclusively that the sole *JAK2*^{V617F} gene expression in hematopoietic cells is able to induce most of the PV phenotype as soon as the levels match at least that of the *JAK2*^{WT} allele (heterozygous status). However, does it mean that *JAK2*^{V617F} is self sufficient for PV in humans? These mouse models are polyclonal and one can argue that *JAK2*^{V617F} is sufficient for PV as soon as there are enough mutated stem cells. Yet in KI models, the ability of *JAK2*^{V617F} to amplify the stem cell is controversial.[22] Should an additional defect occur for clonal dominance? In human sporadic MPN, other genetic events preceding *JAK2*^{V617F} are suspected from clonal analysis, using the X chromosome activation or the 20qdel chromosomal abnormality,[1] and secondary *JAK2*^{WT} AML progressing from *JAK2*^{V617F} MPN.[23,24] Indeed, *TET2* mutations might be instrumental in amplifying immature myeloid cells, as suggested by several reports on human samples.[17,23] However, is it necessary for PV occurrence? The absence of TET2 mutations is reported in most PV patients and *in vitro* or *in vivo* mouse SCID clonal amplification seems to be missing in those patients lacking TET2 mutations, as suggested by Swierczek and colleagues.[17,21]

In this context, *TET2* mutations following or preceding *JAK2*^{V617F} could, therefore, be seen as paradigmatic of events possibly predisposing to PV occurrence and/or accelerating PV evolution. Establishing the importance of *JAK2*^{V617F} and other TET2-type of mutation orders, their association with transformation and prognosis value, and the precise contribution of each mutation in PV occurrence/evolution will need extensive, in depth clinical, cellular and molecular analyses of a large number of human samples, and animal models demonstrating the precise effect of the identified mutation(s) in a controlled genetic background and their cooperation with *JAK2*^{V617F}.

Elodie Pronier is a PhD student in unit 1009 of INSERM and works in the search for TET2 function in human cells. Cyril Quivoron is a PhD student in unit U985 of INSERM and works in the search for TET2 function in mouse models. Olivier A. Bernard, PhD, is Director of Research and Head of unit U985 of INSERM « Genetic of tumors », and works in the search for the occurence and roles of genetic abnormalities, especially TET2 mutations, in human malignancies. Jean-Luc Villeval, PhD, is Director of Research in unit 1009 of INSERM «Normal and Pathologic Hematopoiesis» and works in the development of MPN animal models and translational research.

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Impact of molecular prognostic factors in cytogenetically normal acute myeloid leukemia at diagnosis and relapse

Alison Walker and Guido Marcucci

Division of Hematology and Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; E-mail: guido.marcucci@osumc.edu; alison.walker@osumc.edu
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While morphological evaluation of bone marrow and blood remains a cornerstone for the diagnosis of acute myeloid leukemia (AML), it is clear that the presence or absence of specific cytogenetic and molecular abnormalities is not only useful for determining overall prognosis, but is also used to guide treatment. However, while cytogenetic abnormalities present at diagnosis enable prediction of outcome and, in turn, stratification to risk-adapted treatments, clonal chromosomal aberrations are not detected in 40 to 50% of patients.[1] It is within this cytogenetically normal (CN) group that the presence of acquired mutations, in addition to the expression of deregulated genes and non-coding RNA (i.e. microRNA), allows for molecular-risk classification of what has hitherto been a clinically heterogeneous subset of patients.[2-5] Indeed, the relevance of recurrent molecular abnormalities in CN-AML has been recently acknowledged by the inclusion of these markers within both the World Health Organization (WHO) and the European LeukemiaNet (ELN) classifications as a complement to cytogenetics.[6,7]

Molecular analysis of markers that have been incorporated in both the WHO and ELN classifications (i.e., *NPM1*, *FLT3* and *CEBPA*) is now routine. However, other mutated genes (e.g. *WT1*, *IDH1/IDH2*, *TET2*, *RUNX1*, *MLL*) or aberrantly expressed ones (e.g. *BAALC*, *ERG*, *EVI1*, *miR-181a*) will likely become useful in refining molecular risk in CN-AML.[8-21] Furthermore, as these molecular markers are not mutually exclusive, the prognostic impact of the different combinations of mutated and/or aberrantly expressed genes present within the same patient should be carefully evaluated to construct a molecular-risk score for practicing hematologists.

The *NPM1* gene encodes a protein that functions as a nucleus-cytoplasm chaperone and is involved in intracellular processes including transport of pre-ribosomal particles, response to stress stimuli and DNA repair, and regulation of the activity and stability of tumor suppressors such as p53.[22] Acquired mutations in the *NPM1* gene are found in 45-60% of patients with CN-AML, and result in aberrant cytoplasmic expression of the protein.[23] The presence of an *NPM1*