Mutational spectrum defines primary and secondary myelofibrosis

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Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) belong to the group of Philadelphia chromosome-negative myeloproliferative neoplasias (Ph⁻ MPN). MPNs are clonal bone marrow stem cell disorders involving a multipotent hematopoietic stem cell, characterized by proliferation of one or more lineages of the myeloid, erythroid and megakaryocytic cell lines. This proliferation results in increased numbers of granulocytes, erythrocytes or platelets in the peripheral blood, respectively.

The most prevalent mutation in MPN is the *JAK2-V617F* mutation, discovered in 2005.[1-4] Approximately 95% of PV patients, 50-70% of ET patients and 40-50% of PMF patients possess this specific *JAK2* mutation. The *JAK2*-V617F mutation is located in exon 14 of the gene and abrogates the negative regulatory activity of the pseudokinase domain JH2 of the encoded *JAK2* tyrosine kinase.[5] Therefore, this mutation leads to a constitutive active *JAK2* kinase signaling which is independent of cytokine stimulation.

Hematopoiesis is regulated mainly by hematopoietic cytokines, such as granulocyte colony stimulating factor (GCSF), erythropoietin (EPO) or thrombopoietin (TPO). Mutated genes found in MPNs frequently target these cytokine signaling pathways with mutations in the *JAK2* gene being the most prominent. Myeloproliferative leukemia virus oncogene (*MPL*) encodes the receptor for TPO, which mediates signaling through the *JAK-STAT* pathway and several gain-of-function mutations in exon 10 are seen in *JAK2*-V617F negative ET and PMF patients. Loss-of-function mutations of the adaptor protein LNK (*SH2B3*), which negatively regulates the TPO and EPO cytokine signaling, have been reported at low frequency in *JAK2*- V617F negative MPN patients. Other signaling mutations have also been reported in *SOCS*, and *CBL*, *NRAS* as deletions of *NF1*. Mutations of genes involved in RNA splicing, such as *SF3B1*, *SRSF2*, *U2AF1*, have been identified in myelodysplastic syndrome (MDS) and MPN patients.[6]

Gene expression regulators, such as transcription factors, are deleted or mutated in MPN, suggesting a critical function in MPN pathogenesis. *IKZF1*, which encodes the transcription factor Ikaros, is a target of chromosome 7p deletions in MPNs, and a late event in the clonal evolution from MPN to sAML. Other transcription factors are involved in chromosomal deletions: *FOXP1* and del 3p, *ETV6* and deletions on chromosome 12p, and *CUX1* and chromosome 7q deletions. In addition, the transcription factor *p53* was found to be mutated in a small proportion of patients,[7] and *RUNX1* has been reported to be mutated in AML, post-MDS-AML and post-MPN AML.[8] Furthermore, various genes involved in epigenetic mechanisms can be mutated: *TET2* mutations in approximately 5% in ET, 16% in PV, and 17% in PMF. Mutations in the enzymes isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) which act as a co-factor for *TET2* have also been reported at low frequency and are more often found in post-MPN AML. *DNMT3A*, a *de novo* methyltransferase is mutated in approximately 10% of MPN patients and *ASXL1* (a nuclear polycomb protein) occur more frequently in PMF and secondary MF than in PV and ET.

To date, mutational studies, not just in MPN, have usually focused on one or two mutations. However, the report in this issue of the Journal by Brecqueville *et al.*[9] has used array-comparative genomic hybridization and sequencing of 23 MPN associated genes in a cohort of myelofibrosis patients at primary and acute phases. Array comparative genomic hybridization (arrayCGH) has become a widely used and valuable genome-wide screening tool for the detection of chromosomal aberrations in the form of copy number imbalances or alterations (CNA) in the field of cytogenetics, with the major advantage that it allows a genome wide screen at vastly improved resolution compared to traditional techniques. The sizes of CNAs are variable and range from less than a single gene to entire chromosome changes. Copy number alterations (CNA) were observed in 58% of PMF, 71% of post-PV MF and 18% of post-ET MF cases. In addition, recurrent alterations of 20q, 17q, 7p, 9p, 13q, and 1q were observed. However, no obvious differences for the recurrent abnormalities and the CNA could be determined between three MF subtypes. However, cases with del(20q) were significantly associated with lower leukocyte count, hemoglobin and hematocrit, and the need for red cell transfusion, raising the possibility of phenotypic sub-groups within the PMF patients.

Disease evolution occurs in approximately 20% of patients and overall survival can be predicted by several different prognostic scoring systems, such as IPSS,[10] DIPSS[11] and DIPSS-plus.[12] DIPSS-plus takes into account unfavorable karyotypic abnormalities. In the study, the authors reported scores for primary MF and showed that the presence of CNA was associated with intermediate-2/high risk DIPSS and DIPSS-plus scores.

However, the mutational analysis of the 23 selected genes in the current study[9] has highlighted some important and significant differences in the mutational spectrum across the primary and secondary MF and between those patients who have progressed to MF from PV or ET. The authors have compared their data with that previously published[13] by the same group, and using the same technology panel, on mutations in ET and PV. The striking difference is the number of genes that were mutated in MF patients when compared to those identified in PV and ET. All the PV patients had *JAK2* mutations and only 23% of patients had additional mutations in one of the *AXSL1*, *TET2*, *SUZ12* or *DNMT3A* genes. In ET patients, *JAK2* mutations also dominated, with again a proportion of these co-occurring with additional mutations in ASXL1, TET2 or CBL, although ASXL1 or SF3B1 mutations could also occur without mutated *JAK2*.[14,15] Although as reported in their earlier study, 30% of ET patients did not exhibit any of the studied mutations. However, very recent data from two independent groups in Austria and the UK have identified a previously unreported mutation in the *CALR* gene in the majority of ET or PMF patients who did not have *JAK2* or *MPL* mutations (approx. 65-70%).[16,17]

The study by Brequeville *et al.*[9] in this issue highlights some very interesting data emerging when the overall data from MF is directly compared to PMF, and also on disease progression on the evolution to ET or PV post PMF. A high number of mutated genes were seen in PMF compared to acute phase MF. Moreover, 24% of PMF patients have a del(20q) chromosomal abnormality. In each case, this co-occurs with a *JAK2* mutation; del(20q) has

previously been shown not to be a predisposing event for *JAK2* mutations.[18]

The comparison of MF evolving from PV or ET is also very illuminating. Post-PV MF patients all have *JAK2* mutation, as is seen in PV, but the proportion of patients with co-occurring mutations increases from 23% to 40%. The genes involved also include those seen in MF, with the exception of *SUZ12*, and also *EZH2*, *LNK* and del(20q) abnormalities. This might suggest that those patients with *JAK2* / SUZ12 may have a very low rate of progression whilst *EZH2*, *LNK* and del(20q) abnormalities are associated with clonal evolution. In a comparison of MF *versus* Post-ET MF, a similar proportion of patients had *JAK2* mutations (67% *vs.* 63%, respectively). However, within these patients, two-thirds of the Post-ET MF patients also had co-occurring mutations compared to only 18% of MF patients. The Post-ET MF patients, in common with Post-PV MF patients, had an increased proportion of patients with mutations of *ASXL1* and *TET2*. The proportion of *SF3B1* / *JAK2* mutations was 8% in the patients who progressed, but this combination was not observed in ET patients, whereas *LNK* mutations were only seen in patients who progressed in combination with *JAK2* mutations. The proportion of unmutated patients remained the same at ET and Post-ET MF. Interestingly, no patient with *CBL* / *JAK2* co-occurring mutations were observed in Post-ET MF, a situation mirroring those *SUZ12* / *JAK2* patients in Post-PV MF.

This study has highlighted the mutational diversity of phenotypically diverse sub-types of MPN. The comparative analysis has demonstrated that PMF involves a larger number of mutated genes whilst MF progressing from either ET or PV more closely mirrors the original disease than MF or PMF. Moreover, the involvement and interaction between *JAK2* and other genes has given an intriguing insight into both clonal evolutions. This study has suggested that those patients with mutations in more than one gene in the initial disease type have an increased chance of progression, in addition some mutations or abnormalities were only detected after progression from ET or PV. Follow-up studies whether ET patients with *JAK2* / *SUZ12* or PV patients with *JAK2* / *CBL* have a low rate of disease progression. Several of the cooperating mutations and those appearing during progression are also epigenetic associated genes. Mutations in these genes are also associated with higher age and leukocyte count. Therefore, the use of demethylation or histone deactylase inhibitors should be more actively pursued as a therapy for MPN in elderly patients.

The use of the new sequencing technologies is now allowing not only the association of gene mutations with different disease phenotypes but also the role of mutational combinations to be associated with disease progression, evolution prevention and disease classification. The identification of the number, type and depth of mutated clones present at diagnosis may be able to guide therapeutic decisions, the effect of which can then be monitored through gene mutations, although the effect and appearance of novel progression related mutations should not be ignored.

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