Hematologic Malignancies

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Ayalew Tefferi *Editors*

Myeloproliferative Neoplasms

Critical Concepts and Management

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Hematologic Malignancies

On behalf of all contributing authors who have sacrificed their valuable time, we dedicate this book to all patients with myeloproliferative neoplasms and acknowledge their tremendous courage and tenacity.

Barbui
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Part I

Biology

Update on the Biology of Myeloproliferative Neoplasms

1

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1.1 Introduction

Single cell origin of hematopoiesis is considered to be a hallmark of all myeloid malignancies. In hematological malignancies, the mutations initiating stem cell clonality can have various forms such as translocated chromosomes, chromosomes with deleted or amplified regions, or point mutations in single genes. Once a stem cell clone has been established, it expands and its progeny competes with healthy cells for "habitat" in the bone marrow microenvironment. As the clone expands, more mutagenesis occurs in the next generation of cells. Although vast majority of these newly acquired genomic mutations do not provide any benefit to the clone, some lesions may prove to be useful and provide a selective advantage. Therefore, selection is the main driving force that shapes the cancer genome in the given environment. Different tissues have different selective forces that evolve the cancer genome. In hematological malignancies, the stem cell clone of each patient takes on a unique evolutionary path even though the accompanying genetic defects are often recurrently detected when many myeloid cancer genomes are compared. The mutations acquired in the evolution of the myeloid cancer genome and their combined effects may have different influence on the differentiation dynamics of the hematopoietic progenitors. Some mutations reduce and others may increase the output of the terminally differentiated cells. Each clonal evolution has a certain phenotypic outcome often detectable by differential blood count and histopathologic evaluation of the

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bone marrow. The clinical classification of these different phenotypic outcomes provided the foundations for diagnosis in the past. Inclusion of the genetic defects that associate with certain clinical entity into the diagnosis has brought significant improvements in the diagnostic process. The developments in the field of myeloproliferative neoplasms (MPN) in the past decade are an excellent example of this process.

MPN represent a phenotypically diverse group of hematological malignancies. MPN are characterized by a single or multilineage overproduction of terminally differentiated blood elements and pronounced predisposition to thrombosis, bleeding, and leukemic transformation. There are three major MPN phenotypes characterized by distinct clinical features: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The vast majority of patients have a stable disease often lasting many years. However, the chronic phase phenotype may progress to a stage characterized by secondary (post-PV or post-ET) myelofibrosis, variable degrees of pancytopenia, and accumulation of blasts in the bone marrow and peripheral blood. Further evolution of this stage results in acute leukemia. Although there were certain exceptions described, the majority of myeloid cells in MPN have single cell origin and are derived from a stem cell clone dominating the productive myelopoiesis. MPN is a phenotypic outcomes of clonal stem cell evolution driven by a certain set of somatic mutations. Mutations of the JAK2 kinase gene are found in approximately two thirds of patients with MPN (Baxter et al. 2005; James et al. 2005; Kralovics et al. 2005; Levine et al. 2005). Almost all patients with PV carry JAK2 kinase mutations, whereas only about half of PMF and ET cases test positive for JAK2 mutations. Only a minority of PMF and ET cases carry mutations of the thrombopoietin receptor gene MPL (Beer et al. 2008; Pardanani et al. 2006; Pikman et al. 2006). JAK2 and MPL oncogenic mutations are often preceded or followed by cytogenetic lesions such as deletions or chromosomal gains (Kralovics 2008). Cytogenetic lesions in MPN contribute to clonal outgrowth and have a potential to contribute to the overall clinical phenotype. Despite recent efforts to define the mutation profile of MPN patients, about one third of MPN cases do not carry a detectable cytogenetic lesion or mutations in JAK2 or MPL. Many studies are ongoing that focus on this "gap" in MPN biology. New genetic lesions with diagnostic value will likely emerge in the near future.

1.2 Diversity of Gene Defects in the Pathogenesis of MPN

Despite a number of newly discovered somatic defects in the MPN pathogenesis, JAK2 and MPL mutations remain the most prominent and show highest specificity for MPN. High-resolution mapping of deletions on chromosome 4 led to the identification of TET2 – an important tumor suppressor gene in most myeloid malignancies including MPN (Delhommeau et al. 2009). In addition to deletions of TET2, loss-of-function point mutations occur even more frequently than deletions. TET2 mutations are detectable in about 13% of MPN patients depending on the studied cohort and MPN entity with highest frequencies observed in PV and PMF (up to 20%) (Delhommeau et al. 2009; Tefferi et al. 2009a, b). TET2 deletions occur in about 3% of MPN patients (Klampfl et al. 2011). Interestingly, most patients carry only heterozygous mutations or hemizygous deletions. Thus, only slight decrease of active gene dosage of TET2 is sufficient to grant clonal advantage to hematopoietic stem cells (Delhommeau et al. 2009). As TET2 encodes an enzyme that converts 5-methylcytosine to 5-hydroxymethylcytosine, loss of TET2 function might alter epigenetic gene regulation. Regulation of gene expression seems to be frequently altered in myeloid malignancies by mutations or deletions of genes involved in transcription. Examples of these lesions include losses/mutations of IKZF1, CUX1, and EZH2, all located on chromosome 7 and found in all myeloid malignancies including MPN (Ernst et al. 2010; Jager et al. 2010; Klampfl et al. 2011). It might explain why monosomy 7 is one of the most severe cytogenetic lesions in myeloid malignancies as at least three tumor suppressor genes are affected at the same time. Another example is

mutations in the ASXL1 gene, a gene encoding a polycomb transcription factor involved in negative regulation of HOX genes (Abdel-Wahab et al. 2010). Cytogenetic studies using high-resolution microarrays implicated additional transcription factors such as ETV6, FOXP1, RUNX1, and CUX2 that are frequently deleted in MPN (Klampfl et al. 2011). It remains unclear what function of hematopoietic stem cells is impaired by decreased functional dosage of transcription factor genes. As most of these proteins are involved in transcriptional repression, their loss might be associated with increased frequency of cell cycle entry and/or alterations of differentiation dynamics within the progenitor compartment.

Another large group of defects in MPN is directly involved in cytokine signaling. In addition to JAK2 and MPL mutations, frequent mutations are found in the E3 ubiquitin ligase CBL (Dunbar et al. 2008; Grand et al. 2009; Sanada et al. 2009). It regulates the stability of proteins involved in cytokine signaling by ubiquitination that leads to proteasome-dependent degradation (Schmidt and Dikic 2005). CBL ubiquitinates cytokine receptors (Epo-R, c-Kit), tyrosine kinases (JAK2, Tyk2, Abl), as well as signaling adaptors (Grb2) (Schmidt and Dikic 2005). CBL mutations act as dominant negative and are found in the RING finger motif of the protein encoded by exons 8 and 9 (Grand et al. 2009; Ogawa et al. 2010a, b; Sanada et al. 2009). Activation of cytokine signaling induces STAT-dependent transcription. Among the genes induced by cytokines are the SOCS proteins that negatively regulate the signaling cascade by binding to the receptors or JAK2 (Nicola et al. 1999). SOCS genes can be impaired in MPN either due to hypermethylation (Jost et al. 2007; Teofili et al. 2008) or due to deletions (Klampfl et al. 2011). In addition to the JAK-STAT signaling, two members of the MAP-kinase pathway NRAS and NF1 have been shown to be targeted by mutagenesis in MPN (Beer et al. 2009; Stegelmann et al. 2010). Activating NRAS mutations have been found in MPN patients that transformed to leukemia, whereas NF1 deletions and mutations are often detected in chronic phase ET and PMF. Mutations in the signaling adaptor LNK (SH2B3) have been recently identified in MPN although they are rare and predominantly present in patients with advanced disease (Gery et al. 2009; Oh et al. 2010; Pardanani et al. 2010a). LNK negatively regulates JAK2 and c-KIT (Simon et al. 2008; Tong and Lodish 2004; Tong et al. 2005), and mice deficient for LNK develop myeloproliferative phenotype (Velazquez et al. 2002). In a different SH2B family member (SH2B2), a single somatic mutation found in a case of post-MPN AML (Klampfl et al. 2011).

The V617F and exon 12 mutations of JAK2 as well as MPL mutations have clearly been shown to have high selectivity for MPN and also induce a myeloproliferative phenotype in mice. Mutations in the rest of the above mentioned genes do not show specificity for MPN and are distributed at variable frequencies across all myeloid malignancies. It remains to be seen if murine models might clarify their potential to induce a myeloproliferative phenotype. Furthermore, more than a third of MPN patients are negative for JAK2 or MPL mutations, and none of the other genes explain MPN phenotype in these patients. Functional studies of individual mutations may not be sufficient as patients often carry several mutations and cytogenetic lesions. As the acquisition order of mutations turned out to be insignificant, the combination of different mutations might be important. It is possible that certain combinations of somatic lesions of weaker phenotypic effect will result in MPN in the absence of dominant lesions such as JAK2-V617F. If this is the case, JAK2- and MPL-negative patients will represent a very heterogeneous population with many types of somatic lesions and their combinations. Whole-genome sequencing will provide some answers, but it is already clear that MPN is a remarkably complex disease both genetically as well as phenotypically.

1.3 Mutations Associated with Disease Progression

At least three disease stages can be defined in MPN. The chronic phase is characterized by a stable disease with minimal evidence for progression. In ET and PV, disease progression is evident when

patients develop secondary myelofibrosis. Another stage that is clinically recognizable is often referred to as "accelerated" phase characterized by variable degree of cytopenia (most often anemia) and gradual increase of blasts in bone marrow and peripheral blood. The last stage is the leukemic stage where the frequency of blasts increases over 20% in the bone marrow.

To date, MPN is associated with somatic lesions in over 20 different genes; however, their role in the pathogenesis remains unclear. It is important to classify the MPN-associated molecular lesions based on their impact on the severity of phenotype they induce. These studies are often difficult to achieve as large patient cohorts are needed, and many genes due to their shear size are laborious to screen for mutations. For example, TET2, ASXL1, and NF1 combined have a over 19,000 base pairs of coding sequence in over 80 exons, and as their mutation frequencies are relatively low, large patient numbers are needed to test association with clinical features. In certain cases, mutation frequencies are reported only in patients that show disease progression, whereas no data are available on the frequency in the chronic phase of the disease. Thus, rigorous statistical evidence is often missing when a certain lesion is implicated in disease progression.

The first lesion studied in terms of implications for disease progression was the JAK2-V617F mutation. Patients exhibit difference in V617F mutational burden due to variable populations' sizes of myeloid cells with wild-type, heterozygous, and homozygous genotypes for JAK2-V617F. A number of studies addressed the clinical impact of high V617F burden, and a clear association was found with secondary myelofibrosis (Vannucchi et al. 2007). This means that PV and ET patients with high JAK2-V617F burden are more likely to develop secondary myelofibrosis. Similarly, progression to secondary myelofibrosis in PV and ET was confirmed for uniparental disomy 9p associated with high JAK2-V617F burden (Klampfl et al. 2011).

Mutations of the p53 tumor suppressor (encoded by the TP53 gene) have previously been reported in few post-MPN AML cases (Beer et al. 2009). The TP53 mutation frequency in the chronic phase of MPN was unknown, and therefore the significance of TP53 mutations the transformation process was unclear. In recent studies, the TP53 mutation frequency was determined both in chronic phase as well as in post-MPN AML cases (Harutyunyan et al. 2011b; Klampfl et al. 2011). TP53 mutations were found common in the leukemic phase (20%), whereas only few patients in the chronic phase carried a mutation (1.6%). Interestingly, TP53 mutation positive chronic phase patients carried only monoallelic mutations while post-MPN AML patients carried mostly biallelic TP53 mutations. Another p53 pathway related lesion associated with post-MPN AML is gain of chromosome 1q as the MDM4 gene was found within the 1q amplicon (Harutyunyan et al. 2011b). Since MDM4 is a potent inhibitor of p53, 1q gains may increase the MDM4 gene dosage and result in a similar effect as TP53 mutations. Gains of 1q and TP53 mutations never found together in the same patient – an observation made also in solid tumors (Veerakumarasivam et al. 2008). If the frequencies of TP53 mutations and MDM4 gains are combined, they might explain about 40% of transformation event in MPN. As TP53 mutation rate in de novo AML is low (Wattel et al. 1994), the question arises to what degree pharmacologic management of MPN and/or MPN biology influence acquisition of p53 pathway lesions. Long-term treatment of MPN patients with DNA damage-inducing agents might target the p53 pathway for mutagenesis to facilitate clonal progression. However, such link has not been established as yet (Bjorkholm et al. 2011).

The transformation of MPN to post-MPN AML tuned out to share common defects with de novo AML. However, mutations in genes previously implicated in de novo AML are found mutated at somewhat lower frequencies in post-MPN AML. These genes include IDH1/2 (Andrulis et al. 2010; Green and Beer 2010; Klampfl et al. 2011; Kosmider et al. 2010; Pardanani et al. 2010b), RUNX1 (Ding et al. 2009; Klampfl et al. 2011; Taketani et al. 2002), FLT3 (Klampfl et al. 2011; Lin et al. 2006), NPM1 (Klampfl et al. 2011; Oki et al. 2006; Schnittger et al. 2011), and DNMT3A (Abdel-Wahab et al. 2011; Stegelmann et al. 2011).

Mutations of TET2 have been linked to more aggressive disease; however, their leukemic potential remains questionable as TET2 mutation frequencies in chronic phase and in post-MPN AML do not differ dramatically (Tefferi 2010). Moreover, TET2 is often acquired before JAK2-V617F, and thus, TET2 mutations likely play a role in initiation of clonal hematopoiesis. Mutations of CBL and EZH2 seem to have stronger association with leukemic transformation; however, strong evidence is still missing.

The cytogenetic lesions have been extensively investigated using single-nucleotide polymorphism (SNP) array technologies. These microarrays allow the measurement of copy number and provide simultaneous assessment of SNP heterozygosity across the whole genome (Klampfl et al. 2011; Stegelmann et al. 2010; Thoennissen et al. 2010). Marker densities used depended on the microarray platform ranging from 50,000 to 1.8 million. In the most recent study using SNP arrays with 1.8 million marker/genome resolution, over 400 MPN patients were evaluated in various disease stages (Klampfl et al. 2011). Cytogenetic complexity of MPN patients did not differ among the three MPN entities, and JAK2 mutations status and disease duration did not associate with increased number of cytogenetic lesions. The cytogenetic complexity considerably increased with disease progression. Among the 25 recurrent aberrations, only 8 showed association with leukemic transformation including gains of 1q (MDM4) and 3q, deletions of chromosomes 7q (CUX1), 7p (IKZF1), 5q, and 6p, as well as uniparental disomies on 19q and 22q. Post-MPN AML patients carrying these chromosomal aberrations also had other mutations in TP53, RUNX1, or IDH1/2; however, one third of patients had not detectable somatic lesion and had a normal karyotype (Klampfl et al. 2011).

As more data are available on the leukemic association of individual lesions, we might have come closer to assemble a set of molecular markers with prognostic value. Those lesions that are strongly inducing leukemic transformation are of less prognostic value as they are almost never observed in the chronic phase of MPN, and the time between acquisition of the lesion and transformation is short. Mutations in TP53, CBL, and perhaps EZH2 and LNK might be of some value, but their usefulness needs to be examined in prospective studies.

1.4 Hereditary Factors Influence MPN Pathogenesis

Existence of familial clustering of MPNs is considered to be the strongest evidence that germline mutations may cause an MPN like phenotype. This concept was further strengthened when a clear molecular distinction of true familial MPN from other familial syndromes such as familial erythrocytosis and hereditary thrombocythemia has become possible using clonality markers, cellular studies, and JAK2 mutation analysis. Familial MPN remains clinically indistinguishable from sporadic MPN, and this applies also for the presence of somatically occurring JAK2, MPL, TET2 mutations. Only MPL mutations were found germline in some pedigrees with an ET-like phenotype. Germline TET2 mutations/variants were reported, but they were not segregating in familial MPN cases, and thus their role remains elusive.

Another example of germline genetic factors influencing MPN pathogenesis was the discovery of the GGCC (also known as 46/1) haplotype of the JAK2 gene (Jones et al. 2009; Kilpivaara et al. 2009; Olcaydu et al. 2009a, b). Somatic mutations of JAK2 in MPN do not distribute equally between the two most common JAK2 gene haplotypes in Caucasian populations. The GGCC haplotype acquires over 80% of all V617F mutations as well as exon 12 mutations. The molecular reason why this deviation from random mutagenesis exists remains unclear. The GGCC haplotype predisposes carries for JAK2 mutation positive MPN, and thus its major role is in the disease initiation. The hypothesis that the GGCC haplotype might account for familial clustering of MPN has recently been disproved in a study showing equal haplotype frequency in sporadic and familial MPN cases (Olcaydu et al. 2011). The reason why the GGCC haplotype has negligible role in familial MPN is its weak ability (low

penetrance) to initiate the disease phenotype. An as yet unknown, germline mutation(s) in familial MPN has an estimated three orders of magnitude higher penetrance than the GGCC haplotype (Olcaydu et al. 2011).

Another interesting way how germline mutations or polymorphisms may influence disease course in cancer has recently been described (Harutyunyan et al. 2011a). Harutyunyan et al. studied a polycythemia vera patient who was a germline carrier of a rare Fanconi anemia nonsense mutation (FANCM) on chromosome 14. The patient acquired uniparental disomy on chromosome 14 that switched the FANCM mutation from heterozygosity to homozygosity and caused the shift from polycythemia to anemia. Rare recessive mutations each patient carries have no phenotypic effect in heterozygous state (when the wild-type gene copy is present). However, loss of heterozygosity (LOH) often occurs in the cancer tissue that may expose recessive mutations. This way cancer cells may tap into a resource of germline recessive mutations from which some may prove advantageous for cancer growth; others may have unpredictable phenotypic effects.

1.5 Future Directions

With the advances of whole-genome analysis, more somatic lesions are expected to be discovered in MPN. Thus, genetic complexity of MPN will increase. To sort out the role of these, molecular lesions in the disease pathogenesis and their clinical significance will require coordinated multicenter studies that ensure large cohort size and access to high-throughput genome analysis. Genotypic stratification of patients may also be the key to successful treatment as well as to proper clinical management of patients.

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