Stem Cell Involvement in Myeloproliferative Neoplasms

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Myeloproliferative neoplasms (MPN) are clonal disorders that originate at the level of hematopoietic stem cells. Somatic mutations such as JAK2^{V617F} can be found in purified hematopoietic stem cells and their progeny. Nevertheless, large inter-individual differences in the mutant allele burden in the patient's mature blood cells and in the number of hematopoietic lineages that are involved can be found. Not all patients with MPN show presence of clonality markers in B lymphocytes and T cells are almost always excluded. In many MPN patients more than one somatic mutation can be found and the order in which these mutations occur can vary between individual patients. Mouse models have shown that MPN can be initiated by JAK2^{V617F} without the presence of additional mutations. However, all mouse models to date are based on polyclonal disease. Understanding the early steps in disease initiation will be important for designing better strategies for the treatment and ultimately cure of MPN.

Key words: myeloproliferative neoplasms, Janus kinase 2, stem cell hierarchy, disease initiating cell, clonality.

Postižení kmenových buněk u myeloproliferativních neoplázií

Myeloproliferativní neoplázie (MPN) jsou klonální poruchy, které vznikají na úrovni hematopoetických kmenových buněk. Somatické mutace, jako např. JAK2^{V617F}, lze nalézt u purifikovaných hematopoetických kmenových buněk a jejich potomstva. Nicméně je možné objevit výrazné interindividuální rozdíly v mutantní alelické zátěži u zralých krvinek pacienta a v počtu hematopoetických linií, které jsou zasažené. Ne všichni pacienti s MPN vykazují přítomnost klonálních markerů u B lymfocytů a T buňky jsou téměř vždy vyloučeny. U mnoha pacientů s MPN je možné objevit více než jednu somatickou mutaci a pořadí, v němž se tyto mutace objevují, se může u jednotlivých pacientů lišit. Myší modely ukázaly, že MPN může být iniciována JAK2^{V617F} bez přítomnosti dalších mutací. Všechny dosavadní myší modely jsou však založeny na polyklonálním onemocnění. Za účelem navržení lepších léčebných postupů a potažmo i vyléčení MPN bude důležité pochopit časné kroky při iniciaci choroby.

Klíčová slova: myeloproliferativní neoplázie, Janus kináza 2, hierarchie kmenových buněk, onemocnění iniciující buňky, klonalita.

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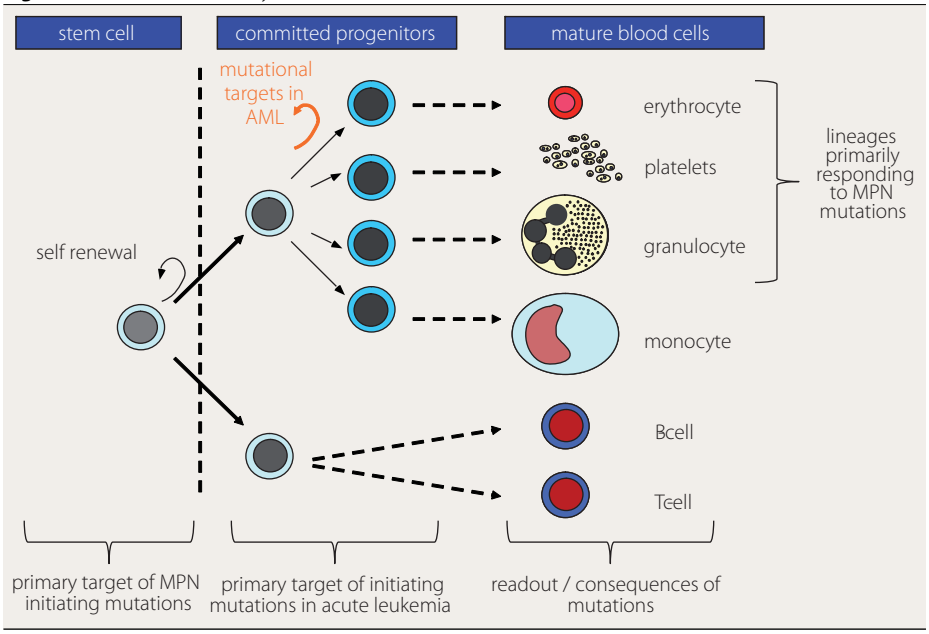
The clonal origin of myeloproliferative neoplasms (MPN) has been first implied by work of John W. Adamson, Philip J. Fialkov and colleagues (1), who in 1976 performed X-chromosome inactivation pattern (XCIP) studies in patients with MPN using restriction fragment length polymorphisms in the X-chromosomal gene glucose-6-phosphate dehydrogenase *(G6PDH)* (2). They demonstrated that peripheral blood cells in two female patients with polycythemia vera (PV) expressed the *G6PDH* derived solely from one of the two parental X chromosomes, indicating that hematopoiesis is clonal (1). Later the same authors showed that lymphoid cells can also be part of the MPN clone in essential thrombocythemia (ET) and PV, implying that the MPN disease initiated at the level of a multipotent hematopoietic stem cell (HSC) (3).

For many decades XCIP was the only methodology to study clonality in MPN. The major advantage of XCIP is that the analysis can be applied in the absence of any knowledge about the molecular and genetic alterations. Among the disadvantages are that only female patients can be studied and that the clone must have expanded reached clonal dominance. The latter has to do with the fact that the ratio in the inactivation of the two X-chromosomes must substantially deviate from the expected 50:50 random distribution, a phenomenon also called after its author Mary F. Lyon "Lyonization" (4), in order to reach a minimal threshold for statistical significance. In most cases this cutoff is set at 75:25 or 80:20 (5). Thus, XCIP is relatively insensitive and clonal dominance must reach 75–80 % in order to be detectable. The methodology to detect XCIP has progressed and many polymorphisms in X-chromosomal genes have been described that allow finding informative markers in most female patients (6). Some differences exist between assays that measure methylation of female X-chromosomes at the DNA level and methods that rely on the expression of mRNA from genes located on the X-chromosome. The latter methodology is preferable and yields more reliable results (7). Using XCIP it has been demonstrated that PV and primary myelofibrosis (PMF) are invariantly clonal, while a subgroup of ET patients has been reported to display polyclonal hematopoiesis (8, 9).

Cytogenetic analysis provided additional tools for studying clonal hematopoiesis in MPN. However, only 10–15 % of PV patients have abnormal karyotype at diagnosis and the most common abnormalities include trisomies (+8, +9, +1), and deletions on chromosome 20q (del20q) (10–12). More recently, microarray analysis of single nucleotide polymorphisms (SNPs) and copy number variation have yielded deeper insights into the molecular pathogenesis of MPN and have helped identifying new genes mutated in MPN (13–15).

From today's perspective, the term "polyclonal ET", which was established based on XCIP studies can be deceptive and has caused considerable confusion in the field. It is important to realize that the absence of clonality by XCIP simply indicates that there is no clone that has expanded and reached 75–80 % of the cells that were analyzed. However, due to the low sensitivity of XCIP, the presence of clones that have not yet reached clonal dominance cannot be excluded. Indeed, today we have an increasing number of somatic mutations that can be used as markers to identify clones within a mixture of hematopoietic cells (16). The JAK2^{V617F} mutation activates the tyrosine kinase domain of JAK2

Figure 1. Stem cell hierarchy and disease initiation



and constitutes the phenotypic driver mutation in MPN (17–20). Due to its high prevalence, it also represents a very useful and sensitive clonality marker in MPN. Quantitative analysis of JAK2^{V617F} by real-time PCR has revealed that ET patients have a significantly lower mutant allele burden than PV or PMF patients and in many ET patients the JAK2^{V617F} allele burden is below 20 % (21). Thus, the statement that some ET patients display polyclonal hematopoiesis may be true from the perspective of using XCIP to detect clonality, but patients with "polyclonal ET" can nevertheless have a subset of up to 50 % of cells that are clonal that escape detection by XCIP. True polyclonal MPD would imply that increased hematopoiesis is secondary to stimulation by a growth factor or an infectious agent or is inherited through the germline. Clonal analyses suggest that JAK2^{V617F} could be both an early and late event in MPN disease (13, 22–26). Interestingly, patients that carry mutation in both JAK2^{V617F} and TET2 genes frequently displayed bi-clonal disease (25–27).

Today, it is well established that B cells are part of the MPN clone in some but not all MPN patients, but T cells are almost never part of the MPN clone (28–32). Since only a minority of patients with MPN display the JAK2^{V617F} mutation in B-cells (32), individual patients could theoretically have initiated MPN disease at different levels in the progenitor and stem cell hierarchy (Figure 1). Transformation at the level of committed progenitors that obtained stem cell properties, i. e. self-renewal capacity, by expressing "stemmness" genes has been demonstrated in acute myeloid leukemia (Figure 1) (33–35), but functional studies with HSCs or later progenitors that carry the JAK2V^{617F} mutation to determine which fraction is that are capable of initiating MPN have not been performed. However, JAK2^{V617F} has not been shown to induce unlimited self-renewal of hematopoietic progenitors and JAK2^{V617F} was also shown to be present in isolated purified HSCs from all V617F-positive MPN patients studied (36). These results are consistent with the conclusions reached from the early clonality studies on MPN that were based on finding XCIP skewing in B lymphocytes that MPN is initiated at the level of multipotent HSCs (3). Consistantly, granulocytes form patients with a low mutant JAK2^{V617F} allele burden showed clonality by XCIP or other clonal markers such as del20q, indicating that other clonal events can precede the acquisition of JAK2^{V617F} (22). The presence of the JAK2^{V617F} mutation appears to skew HSCs towards an erythroid cell fate (36). More recently, analysis of human primary bone marrow cells from MPN patients showed that JAK2 mutations do not alter hematopoietic stem and progenitor cell compartment size or in vitro behavior, but generate expansion of later myeloid differentiation compartments (37).

Mouse models of MPN have shown that JAK2^{V617F} can lead to MPN with ET, PV or PMF phenotypes (38, 39). The earliest models using retroviral transduction of mouse bone marrow cells followed by transplantation into lethally irradiated mice demonstrated that the expression of mouse JAK2^{V617F} is sufficient to induce a phenotype resembling PV (17, 40–43). These mice showed massive increase in hematocrit and hemoglobin concentration and a variable degree of neutrophilia. In contrast to patients with PV, the platelet numbers in these mice remained normal or were even decreased. The following years, mouse models using transgenic constructs strategies were generated (44–46). These mice have a more physiological expression of JAK2^{V617F}, as a contrast to the retroviral models, which are over-expressing JAK2^{V617F}. The transgenic models were able to reproduce all phenotypes present in patients: increased red cell values, platelets and neutrophils as well as splenomegaly and progression to myelofibrosis (44–46). The latest generation of mouse models used knock-in constructs of JAK2^{V617F} to have a more physiological expression and timing of oncogene activation (47–50). These mouse models went further into analyzing stem/progenitor effects of JAK2^{V617F}. While all these models observed an increase of erythroid progenitors, there was a discrepancy in the effects observed on stem/progenitor cells. Two reports were able to see an increase in early myeloid progenitors as well as an increase or trend towards an increase of LSK stem/progenitor cells (47, 48). In contrast, Li and colleagues observed a reduction in stem/progenitor cell numbers as well as a reduced competitive potential in competitive transplantations (50). The reasons between these discrepancies are at present unclear. These mouse models have showed that MPN can be initiated by JAK2^{V617F} without the presence of additional mutations. However all models to date are based on polyclonal disease. Thus, the question whether a single hematopoietic stem cell carrying JAK2^{V617F} as the sole genetic alteration is sufficient to initiate MPN has not yet been conclusively answered.

In order to understand the process disease initiation, we need to obtain a complete knowledge of the somatic mutations that are present at diagnosis of MPN. Although it seems possible that JAK2^{V617F} alone can initiate MPN in some patients, there is increasing evidence that mutations in other genes collaborate in the early stages of disease evolution and in many instances precede the acquisition of JAK2^{V617F}.

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