mutations is inherent in the disease process. If so, it would occur stochastically such that individual risk could not be assessed a priori. Alternatively, patients could present with high risk mutations at diagnosis—these then simply need to be identified and considered when assessing therapeutic options.

One hypothesis for explaining the acquisition of mutations and the observed variability in disease progression is the presence of a “hypermutable state” in MPNs.\(^7,8\) In this model, individual patients are thought to acquire different additional mutations during disease progression, and the nature of these mutations directs the clinical phenotype. The data presented by Skoda’s group clearly argue against a hypermutable state in MPNs. The vast majority (95%) of all mutations detected were already present in the first sample analyzed. In addition, using 2 different methods, the authors calculate a mutation rate of 1 mutation in the genes analyzed in 45 to 66 patient-years. This calculation, however, raises 1 question. Of the 197 patients analyzed, 33% already carried at least 2 mutations. The average age at diagnosis in this patient cohort was 51 years for essential thrombocythemia (ET) and 58 or 61 years, respectively, for polycythemia vera (PV) and primary myelofibrosis (PMF) patients. Nonetheless, 27% of the ET patients had already acquired at least 2 mutations, in a time frame calculated to suffice only for 1 mutation. Prior to disease manifestation, therefore, some patients must have incurred a higher mutation rate.

Because transformation to acute leukemia, which is often highly refractory to treatment, is clinically the most challenging complication experienced by these patients, early predictors of leukemic risk are of utmost importance. Lundberg et al show that mutations in the tumor suppressor p53 are present at very low levels (so-called “subclonal levels,” where a very small percentage of the patient’s cells carry the aberration) in a small number of MPN patients. With 1 exception, 4 of the 5 patients carrying p53 mutations transformed to AML, with a latency of between 5 and 10 years. Because the p53 mutations were observed at such low levels, modern sequencing technologies (next-generation sequencing [NGS]) are required for their detection. Given their clinical importance, detection of p53 mutations by NGS should be considered in MPN patients, especially in light of 1 unexpected observation in this data set: of the 5 patients in which p53 mutations were identified, 3 were diagnosed with ET, generally considered to carry a significantly lower risk of leukemic transformation than PV or especially PMF. Moreover, ET is frequently diagnosed at a younger age, as also seen in the current cohort. ET patients with p53 mutations therefore present with an unanticipated high risk, one that is inapparent by clinical means, but may frequently be good candidates for bone marrow transplantation (BMT). BMT is the only curative approach to MPNs and one that may preempt leukemic transformation. A similar argument may apply to select patients with TET2 mutations, which are also shown to be associated with poor outcome in this study. However, the rate of leukemic transformation in TET2-mutated patients was only 30% compared with the 80% in p53-mutated patients (with the caveat of small number errors in both cohorts); hence, the decision for BMT must consider this.

The rapidly decreasing costs of NGS analysis may soon allow an economical use of this technology for the detection of clinical risk in MPN patients, a cohort that today appears undiscernibly heterogeneous in outcome. In this way, MPNs may follow the successful path forged by >10 years of molecularly guided therapeutic trials in AML, which have led to both improved molecular risk stratification and the development of targeted therapies for select molecularly defined groups of patients.\(^9\)

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Reference section


Red Cells, Iron, & Erythropoiesis

Comment on Garcia-Santos et al, page 2269

Toward unraveling heme regulation

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In this issue of Blood, Garcia-Santos et al advance the field of heme regulation, a highly complex process involving iron and globin metabolism. They focus on a key enzyme involved in heme catabolism, heme oxygenase 1 (HO-1), which, ironically, has been poorly investigated in erythroid cells, the largest pool of heme-containing cells.\(^1\)

They address for the first time the role of the inducible HO-1 during murine erythroid differentiation.

Hemoproteins are involved in a broad spectrum of crucial biological functions, including oxygen binding, oxygen metabolism, and electron transfer.\(^2\) Hemoglobin is the most abundant hemoprotein, and the highest amount is found in circulating red blood cells. A fine balance between heme synthesis and catabolism
is pivotal because non–protein-bound heme results in cell damage and tissue injury. Heme oxygenases are the initial and rate-limiting enzymes in the breakdown of heme.\(^3\)

HO-1 has been given much attention in the literature on nonerythroid cells and hepatocytes. Here, Garcia-Santos and colleagues first explore the expression of HO-1 in mouse erythroid cells isolated from bone marrow. Next, they address the role of HO-1 through a series of in vitro experimental investigations that explore both the effect of HO-1 overexpression and suppression in different murine cell models. Their final step is to challenge their results in vivo with an HO-1 knockout mouse model.

The authors first demonstrate that HO-1 is highly expressed in murine erythroid cells and upregulated during in vitro erythroid differentiation. Furthermore, they demonstrate in a cell model of erythroid differentiation that intracellular heme concentrations and globin production are negatively impacted by HO-1 overexpression. They additionally show that this decrease in globin production most likely results from less heme, because it was associated with the activation of the globin suppressor heme-regulated eIF2\(\alpha\) kinase, which is normally negatively regulated by heme. Overexpression of HO-1 also leads to a decrease in transferrin receptor (TfR), both at the messenger RNA and surface protein levels, and subsequently to a decreased transferrin-dependent iron uptake (see figure). This decrease in iron uptake is partially responsible for reduced heme levels because supplying the cells with a different source of iron that bypasses the TfR pathway leads to incorporation into heme and normal heme biosynthesis.

Conversely, in fetal liver cells derived from HO-1 knockout mice, the authors show a significant increase in globin expression during erythroid differentiation. This finding is associated with increased TfR messenger RNA and surface protein as well as globin.

Finally, the authors confirm the in vitro cellular model data by performing direct measurements in circulating reticulocytes from HO-1\(^{-/-}\) and HO-1\(^{+/+}\) mice. They show, in HO-1\(^{-/-}\) mice, higher expression of TfR at the cell surface, increased iron uptake and globin expression, and a decreased level of active heme-regulated eIF2\(\alpha\) kinase.

Altogether these experiments point to the role of HO-1 as a coregulator of globin production, suggesting that under physiological conditions, appropriate levels of HO-1 guarantee optimal globin production (see figure).

Garcia-Santos and colleagues suggest a potential consequence of clinical relevance in thalassemia syndromes, notably \(\beta\)-thalassemia, which is characterized by an imbalance of globin chains with an excess of unpaired \(\alpha\)-globin chains.\(^4\) It has previously been demonstrated that entrapment of purified \(\alpha\)-hemoglobin chains within normal human erythrocytes significantly enhanced oxidant stress and resulted in pathological changes characteristic of thalassemic cells in vivo.\(^5\) The degree of ineffective erythropoiesis also correlates with the degree of imbalanced globin chain synthesis.\(^6\) Garcia-Santos and colleagues propose that unpaired \(\alpha\)-globin chains may liberate their heme, thereby inducing HO-1. This abnormal and sustained HO-1 overexpression may further contribute to oxidative damage and to erythroid apoptosis, as evidenced by a significantly higher level of reactive oxygen species and elevated apoptosis found in differentiating MEL cells overexpressing HO-1 compared with control.

Future investigations of HO-1 effect in human erythroid cells and human thalassemic progenitors are warranted in order to extend these findings to human pathology.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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