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[Review]

THE ROLE OF HMGA2 IN THE PROLIFERATION AND EXPANSION OF A HEMATOPOIETIC CELL IN MYELOPROLIFERATIVE NEOPLASMS

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Abstract : Philadelphia chromosome-negative myeloproliferative neoplasms (MPN), which include polycythemia vera, essential thrombocythemia, and primary myelofibrosis, are characterized by clonal proliferative hematopoiesis with increased blood cell count. Clonal expansion mechanisms in MPN and related disorders such as myelodysplastic syndromes (MDS) remain to be elucidated. Although mutations in the *JAK2* gene lead to a proliferative hematopoiesis in majority of MPN and some MDS, the mutation alone does not cause a clonal expansion. In addition to *JAK2* mutations, several genetic abnormalities, including *TET2* and polycomb group genes involving epigenetic regulation have been reported in patients with MPN. Moreover, overexpression of *HMGA2* due to removal of specific sites in its 3′ untranslated region for regulatory let-7 micro RNAs may contribute to the proliferative hematopoiesis with conferring a growth advantage at the level of a hematopoietic stem cell in some cases with MPN.

INTRODUCTION

Philadelphia (Ph) chromosome-negative myeloproliferative neoplasms (MPN), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are characterized by clonal proliferative hematopoiesis with at least one lineage of increase in blood cell count[1]. MPN are slowly progressive, but sometimes complicated by secondary myelodysplastic syndromes (MDS)/acute myeloid leukemia (AML) or myelofibrosis (MF) with poor outcome[2].

In 1951, the entity of myeloproliferative disorders, which include PV, ET, PMF, and chronic myelogenous leukemia (CML), was described on the basis of clinical similarities[3]. For CML, Ph chromosome was discovered in 1960[4] and identified as a translocation in 1973[5], the *ABL* gene was shown in 1983[6], and BCR-ABL tyrosine-kinase inhibitor was developed in early 1990's[7]. In contrast, a mutation in the *Janus kinase 2* (*JAK2*) gene, namely *JAK2*V617F, was reported in majority of PV, PMF, and ET in 2005[8-11], more than 50 years after the description of this disease entity.

Discovery of *JAK2*V617F provided the novel insight that the mutation causes constitutive activation of JAK-STAT signaling pathway, which leads to a proliferation of blood cells. However, it turned out that the *JAK2*V617F does not necessarily confer a clonal growth advantage. In fact, both *JAK2*V617F⁺ and *JAK2*V617F⁻ cells similarly have clonality in X-linked clonality assay and chromosomal analysis in blood cells from MPN patients[12,13]. In addition, secondary AML following MPN often derive from *JAK2*V617F⁻ cells rather than *JAK2*V617F⁺ cells[13-15]. Furthermore, although *JAK2*V617F mutation is sufficient to cause MPN, *JAK2*V617F mutant cells failed to repopulate in bone marrow transplantations (BMT)[16,17].

Based on these findings, additional molecular pathogenesis other than *JAK2*V617F have been explored in MPN, bringing a variety of new insights, such as mutations and/or expression changes of genes involving cytokine signaling cascade, transcription, and epigenetic regulation[18-20]. In addition, overexpression of the high mobility group

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AT-hook 2 (HMGA2), which plays important roles in cell proliferation and differentiation, has been reported in MPN[21-26]. Overexpression of HMGA2 is often a consequence of the chromosomal rearrangement that removes 3´ untranslated region (UTR) of HMGA2, containing specific sites for *let-7* micro RNAs, which negatively regulate expression of HMGA2[27,28]. In our recent study, transgenic mice overexpressing Hmga2mRNA without its 3´UTR, indeed revealed a proliferative hematopoiesis mimicking MPN and expansion of a hematopoietic cell at the level of a hematopoietic stem cell (HSC), suggesting that HMGA2 expression possibly contributes to some etiology of MPN[29]. Here, we review the molecular pathogenesis and the role of HMGA2 in MPN.

Mutations in cytokine signaling-related genes

Proliferative hematopoietic features in MPN such as increased blood cell counts and cytokine-independent hematopoietic colony formation are caused by a constitutive activation of a certain signaling pathway[20]. Mutations in *JAK2*, including *JAK2*V617F, which are the most common genetic abnormality in MPN, lead to phosphorylation of JAK2 tyrosine kinase in hematopoietic cells even in the absence of cytokines[8-11]. Following phosphorylation of JAK2, downstream signaling is activated via transcription factors STAT3 and STAT5, MAP kinases, PI3K and AKT[30].

JAK2 kinase has seven homology domains, from JH1 to JH7 and plays a important role in the proliferation of myeloid cells by hematopoietic growth factor cytokines. *JAK2* mutations in MPN occur around the JH2 pseudokinase domain, which negatively regulates the kinase activity induced by JH1 domein[31]. The *JAK2*V617F derives from a substitution of thymine for guanine in exon 14 where JH2 domain locates. On the other hand, mutations in exon 12 of *JAK2* span a linker between the JH2 and SH2 domeins[32,33]. Both the *JAK2*V617F and mutations in exon 12 of *JAK2* modify the structure of JAK2 JH2 pseudokinase domain in a similar manner. However, whereas *JAK2*V617F mutation is found in more than 95% of PV, 50-70% of ET, 40-50% of PMF, and also up to 10% of MDS, mutations in *JAK2* exon 12 is detected only in the PV.

Mutations in *MPL* encoding the thrombopoietin receptor also cause a constitutive activation of JAK2 kinase and downstream signaling pathway[34-37]. The hot spot of the *MPL* mutations, amino acid 515 locates on next to the transmembrane domain in cytoplasm, around where 5 amino acids play a key role to prevent spontaneous activity of MPL as a receptor[38]. *MPL*515 mutations (W515K/L/A) have been observed in up to 15% of *JAK2*V617F-negative ET or PMF.

Although these "gain-of-function" mutations explain proliferation of blood cells, it remains uncertain how an MPN clone, such as a *JAK2*V617F$^+$ cell acquire a clonal growth advantage. *JAK2*V617F$^+$ hematopoietic cells fail to repopulate in competitive repopulation assays using some *JAK2*V617F knock-in model animals[16,17] and in a coincidental human hematopoietic stem cell transplantation from *JAK2*V617F$^+$ idiopathic portal hypertension patient to a patient with MDS[39]. Moreover, even without a mutation in *JAK2*, *MPL*, or other signaling-related genes, a signaling involving JAK-STAT pathway are generally activated in MPN hematopoiesis, in which the cause of signaling activation is largely unknown[30,40,41]. Indeed, erythropoietin-independent erythroid colony formation from progenitor cells with only wild-type *JAK2* has been shown[42]. These findings suggest that an unknown additional event might be required for an MPN cell to acquire a clonal growth advantage over a wild-type cell or lead to activation of JAK-STAT signaling pathway independent on *JAK2* mutations.

Mutations in epigenetic regulators

Significant advances in whole genome assays after the discovery of the *JAK2*V617F led to an increasing numbers of discoveries in mutations of MPNs. Many of these genes such as *Ten-Eleven-Translocation 2* (*TET2*)[43], *Additional sex combs like 1* (*ASXL1*)[44], and *Enhancer of zeste homolog 2* (*EZH2*)[45-47] involve in epigenetic regulations.

By hydroxylation of 5-methylcytosine (5-mC), TET2 generates 5-hydroxymethylcytosine (5hmC)[48], which may contribute to cytosine demethylation. Loss-of-function mutations in TET2 have been reported in wide range of myeloid malignancies, including MPN. Interestingly, *DNA-methyltransferase 3* (*DNMT3*), of which mutations was recently found in patients with AML[49,50], generates 5-mC by methylating cytosine[51], indicating a direct interaction between *TET2* and *DNMT3*, both of which may be important for the differentiation of HSCs[52,53]. More recently, mutations in *DNMT3* has been also reported in MPN[54,55].

ASXL1 and *EZH2* belong to polycomb group genes (PcG), involving in histone methylation and chromatin modification. ASXL1 protein is a part of polycomb repressive deubiquitinase complex that regulates expressions of HOX-related genes and

deubiquitination of histone H2[56]. EZH2 protein is a member of the polycomb repressive complex 2, which play roles in proliferation, differentiation, identity maintenance, and plasticity of cells, and modifies chromatin structure[57]. EZH2 also methylates histone H3 at lysine 27[58]. Mutations in ASXL1 or EZH2 in MPN and other myeloid disorders generally result in a function loss, which raise another interesting aspect that these may be representative genes in the deletions of chromosomes 20q or 7q, respectively[44,45].

Overexpression and truncation of HMGA2

HMGA2 protein is a member of the HMGA family of nonhistone chromatin proteins, which also contains HMGA1a, HMGA1b, and HMGA1c[59]. DNA-binding AT-hook domains of HMGA2, which are encoded by first three exons of the *HMGA2* gene, can modulate transcription by affecting the DNA conformation of specific AT-rich regulatory elements promoting transcriptional activity[60,61]. The HMGA2 protein is important in a wide spectrum of biological processes, including cell proliferation, cell-cycle progression, apoptosis, and senescence[62,63]. HMGA2 is also thought to play a crucial role in self-renewal and control of differentiation of a variety of stem cells such as embryonic stem cells[64], neural stem cells[65], and cancer stem cells[66]. In particular, proliferation, cell-cycle progression, and differentiation control of tumor cells due to overexpression of *HMGA2* may lead to a growth advantage in several benign tumors and cancers [63,66].

HMGA2 exon 5 encodes the acidic C-terminal domain of the protein and contains the 3′UTR of the mRNA. The 3′UTR of HMGA2 contains specific sequences complementary to the *let-7*-family of miRNAs. Binding of the complementary sequences by let-7 miRNAs post-transcriptionally and negatively regulates HMGA2 mRNA and protein expression[27,28]. The Expression of HMGA2 protein is abundant during embryogenesis but very low in normal adult tissues, inversely correlating with that of *let-7* miRNAs[67]. Overexpression of HMGA2, however, are found in various benign and malignant tumors in adults and are thought to contribute to transformation in these tumors[62,63]. In most cases these tumors harbor a rearrangement of chromosome 12q13-15, the location of the HMGA2 gene, causing a truncation or deletion of the HMGA2 3′UTR, while sequences encoding the HMGA2 DNA binding domains are intact. Thus, chromosomal rearrangements within the HMGA2 locus deleting the let-7 binding sites may cause overexpression of HMGA2 protein with a preserved DNA binding capacity.
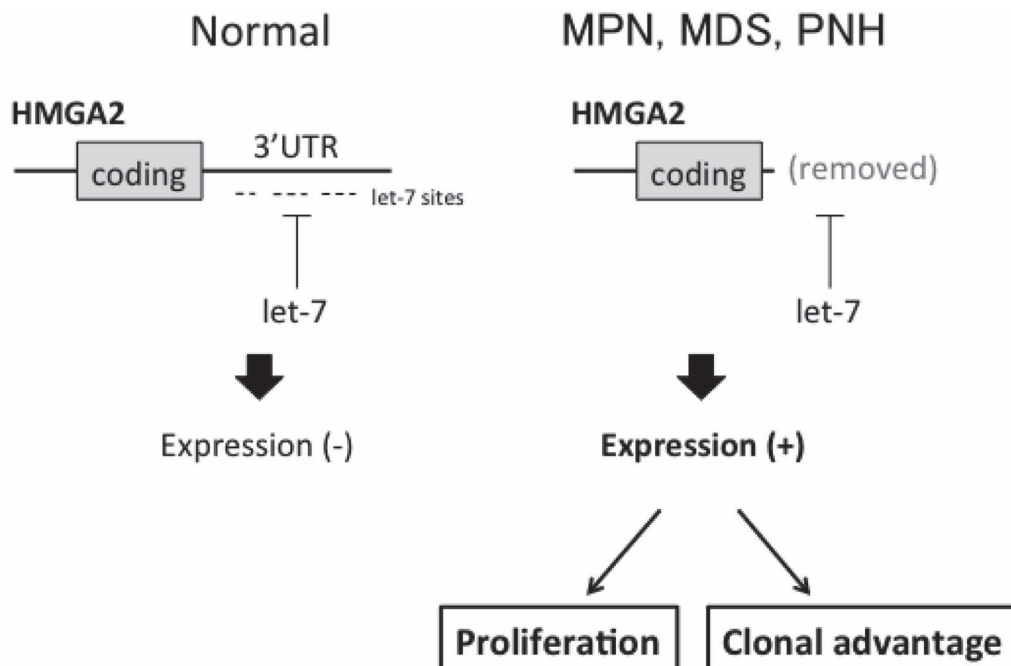
Overexpression and/or truncation of 3′UTR of

Fig. 1. HMGA2 overexpression due to truncation of its 3′UTR in hematologic disorders. HMGA2 is overexpressed due to chromosomal rearrangement, which removes its 3′UTR containing specific sites for let-7 micro RNAs in MPN, MDS, and PNH, because let-7 negatively regulates expression of HMGA2. HMGA2 overexpression may lead to both proliferation and clonal advantage of hematopoietic cells.

HMGA2 have been found in patients with MPN, MDS and MDS/MPN[21-26]. Interestingly, it has been reported that *HMGA2* mRNA expression was significantly higher in PMF patients with *JAK2*V617F mutation than patients without the mutation[25]. Moreover, in two patients with paroxysmal nocturnal hemoglobinuria (PNH), a chromosomal rearrangement causing a truncation of the 3´UTR of the HMGA2 gene have been also found particularly in abnormal clone without cell surface glycosyl phosphatidylinositol proteins (PNH clone), leading to the overexpression of HMGA2 in PNH clones[68]. These findings suggested the hypothesis that overexpression of HMGA2 may confer a clonal growth advantage to an abnormal progenitor cell, thus contributing to pathogenesis in MPN or other clonal hematologic disorders (Fig. 1).

Recently, to study the consequence of overexpression of *HMGA2* in hematopoiesis, we generated a transgenic mouse line expressing a murine *Hmga2* cDNA with a truncation of its 3´UTR (Δ*Hmga2* mouse)[29], mimicking the truncation of HMGA2 seen in the patients with MPN or PNH[21-26,68]. Hematopoiesis of Δ*Hmga2* transgenic mice resembled MPN, characterized by increased peripheral blood cell counts in all blood cell lineages, hypercellular bone marrow, splenomegaly, increased colony formations and erythropoietin-independent erythroid colony growth. When we explored cause of the proliferative hematopoiesis of Δ*Hmga2* mice, increased expression of *Jak2* mRNA and cytokine-independent phosphorylations of STAT3 and AKT were observed in hematopoietic cells of Δ*Hmga2* mice. Therefore, activation of a pathway involving JAK-STAT and AKT may play a role in proliferative hematopoiesis due to overexpression of HMGA2. In addition, hematopoietic cells of Δ*Hmga2* mice showed an extreme growth advantage over wild-type cells in competitive repopulation assays and in serial BMT, indicating that overexpression of *HMGA2* leads to a proliferative growth advantage in hematopoietic cells at the level of HSC. Therefore, HMGA2 overexpression may explain both the proliferative hematopoiesis and clonal growth advantage of abnormal hematopoietic cells in some patients with MPN or PNH (Fig. 1), although the frequency of *HMGA2* dysregulation in these disorders, by gene rearrangement or other means, has yet to be determined.

DISCUSSION

An acquired somatic point mutation, *JAK2*V617F has been identified in majority of patients with MPN, but the precise etiology of MPN has not been determined. Mutations in other genes, notably a variety of epigenetic regulators including *TET2*, *ASXL1*, and *EZH2* have been found in some patients with MPN. In some cases more than one mutation is found in the same clone and in others leukemic transformation takes place in a cell not harboring the mutation[15], suggesting a more constitutive unknown condition may underlie in MPN. Our study of Δ*Hmga2* mice indicated that the *HMGA2* overexpression in the MPNs might be such an additional factor in MPN etiology (Fig. 2)[29].

Hematopoietic cells of Δ*Hmga2* mice also showed high expressions of *Jak2* mRNA and phosphorylated Stat3 or Akt[29], suggesting that constitutive JAK2-STAT3 and AKT activations induced by overexpression of HMGA2 may play a role in proliferative hematopoiesis. Although it should be investigated in the future how the expression of HMGA2 correlates with these pathways, it might contribute to activation of some signaling pathways shown in hematopoietic cells even without *JAK2* mutation of some MPN patients[30]. Interestingly, it has been reported that HMGA2 upregulation was more apparent in JAK2V617F[+] than JAK2V617F[-] cases in PMF[25]. On the other hand, HMGA2 contributes to chromatin extension and histone modification by directly binding to the DNA in various types of cells, suggesting that HMGA2 may also play some roles in epigenetic regulation in hematopoietic cells. Recently, Oguro *et al.* clarified that deletion of PcG-related Bmi1 causes MF after proliferation of megakaryocytes in part by derepression of Hmga2[69]. Strikingly, they showed an evidence that Bmi1 directly repress expression of Hmga2 using chromatin immunoprecipitation assay for promoter area of Hmga2.

Serial BMT of Δ*Hmga2* mice indicated that robust expression of HMGA2 may contribute to clonal growth advantage of an MPN clone by enhancing self-renewal capacity and function of HSCs[29], as well as other types of stem cells[64-66]. Interestingly, enhancement of HSC due to HMGA2 expression is observed not only in hematologic disorders but also in several human gene therapy trials using lenti- or retro-viral transduction of human genes[70,71]. In these studies, the virus was relatively often inserted into the HMGA2 locus, leading to removal of binding sites for *let-7* miRNA and
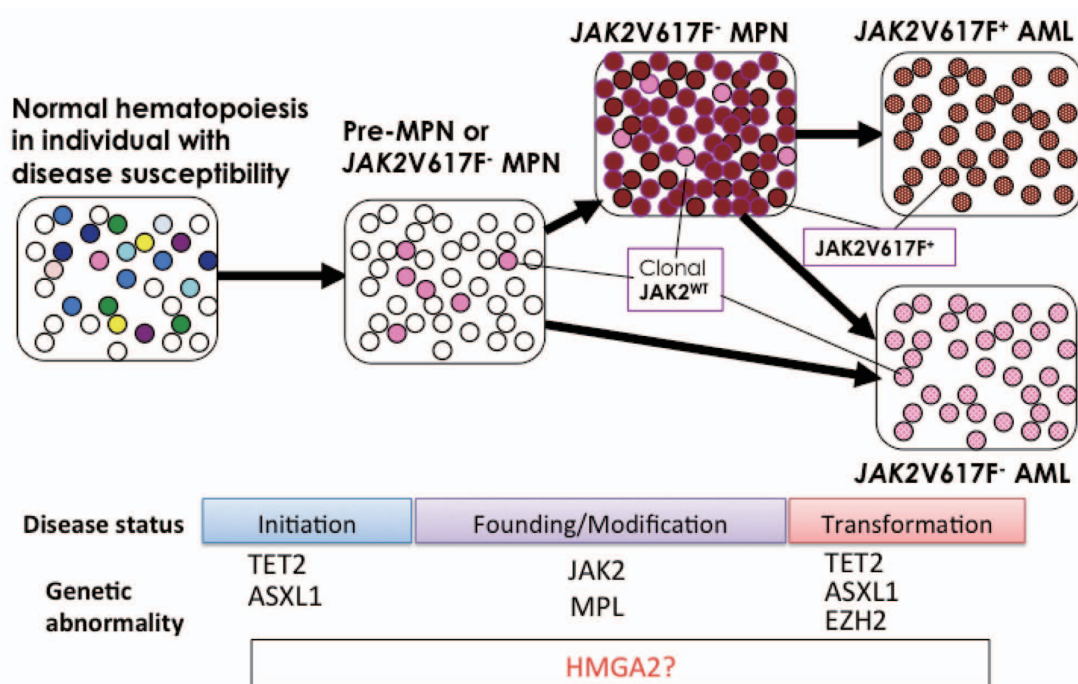
Fig. 2. Possible role of HMGA2 in disease progression of MPN. Detailed mechanism of disease progression remains unknown in MPN because *JAK2*V617F mutation may not provide a clonal growth advantage. *JAK2*V617F$^-$ cells as well as *JAK2*V617F$^+$ cells have clonality in blood cells from MPN patients, and secondary AML often arises from MPN *JAK2*V617F$^-$ cells, suggesting an underlying etiology in MPN such as HMGA2 overexpression, which may contribute to both cell proliferation and clonal growth advantage, in addition to mutations in *TET2*, *ASXL1*, and *EZH2*.

clonal outgrowth of hematopoietic cells, which brought a long-term effect including continuous independence of blood transfusion in severe thalassemia[70].

Overexpression and/or truncation of *HMGA2* has also been found in patients with PNH and MDS, in which bone marrow failure rather than proliferative hematopoiesis is a common feature[26,68,72]. Bone marrow failure in these disorders is partly due to immunologic HSC injury by autoreactive cytotoxic T lymphocytes (CTLs) that produce tumor necrosis factor (TNF)-α and interferon (IFN)-γ[73-75]. It has been also suggested that an abnormal hematopoietic clone may preferentially survive the attack of IFN-γ-producing CTLs in these disorders[76], leading to the hypothesis that a second-hit genetic event beside the disease-initiating event involving HSC injury is necessary for an abnormal hematopoietic cell to acquire a clonal growth advantage and actually expand (two-hit-hypothesis)[77]. As well as PNH and MDS, it has been recently shown that TNF-α may lead to a clonal selection of *JAK2*V617F$^+$ cells in MPN possibly due to a survival advantage against the TNF-α[78]. This finding indicates that not only genetic abnormalities but also some sort of immunologic mechanisms or humoral factors may contribute to pathogenesis of MPN. In fact, high cytokine concentrations are significantly correlated with the poor prognosis of PMF, and recent JAK2 inhibitors benefit PMF patients in spleen size reduction and improving quality of life in part by reducing cytokine concentrations[41]. It remains unknown if HMGA2 involve in such cytokine production, although immune response-related pathways were activated in HSCs of Δ*Hmga2* mice in microarray analysis[29].

Unlike CML in which the BCR–ABL has been already targeted, pathogenesis of MPN is likely more complicated. In fact, in striking contrast of BCR–ABL tyrosine kinase inhibitors for CML, JAK2 inhibitors are facing the limitation in the effect on MPN[79-81], suggesting that further studies should focus on identifying a crucial therapeutic target among various factors in MPN. HMGA2 would be such a candidate therapeutic target because it may involve the pathogenesis of MPN in several ways including regulations of gene expressions, proliferative hematopoiesis, and clonal expansion.

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