| Title       | The role of HMGA2 in the proliferation and expansion of a hematopoietic cell in myeloproliferative neoplasms |
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| Citation    | Fukushima Journal of Medical Science. 58(2): 91-100                                                    |
| Issue Date  | 2012                                                                                                   |
| URL         | http://ir.fmu.ac.jp/dspace/handle/123456789/336                                                        |
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| DOI         | 10.5387/fms.58.91                                                                                      |
| Text Version| publisher                                                                                               |
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 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.

Discovery of JAK2V617F 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 JAK2V617F does not necessarily confer a clonal growth advantage. In fact, both JAK2V617F+ and JAK2V617F− cells similarly have clonality in X-linked clonality assay and chromosomal analysis in blood cells from MPN patients. In addition, secondary AML following MPN often derive from JAK2V617F− cells rather than JAK2V617F+ cells. Furthermore, although JAK2V617F mutation is sufficient to cause MPN, JAK2V617F mutant cells failed to repopulate in bone marrow transplantations (BMT). Based on these findings, additional molecular pathogenesis other than JAK2V617F 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. In addition, overexpression of the high mobility group
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 Hmg2mRNA 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\(^{30}\). Mutations in JAK2, including JAK2V617F, 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 an 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 domain\(^{11}\). The JAK2V617F 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 domains\(^{32,33}\). Both the JAK2V617F and mutations in exon 12 of JAK2 modify the structure of JAK2 JH2 pseudokinase domain in a similar manner. However, whereas JAK2V617F 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\(^{38}\). MPL515 mutations (W515K/L/A) have been observed in up to 15% of JAK2V617F-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 JAK2V617F\(^+\) cell acquire a clonal growth advantage. JAK2V617F\(^+\) hematopoietic cells fail to repopulate in competitive repopulation assays using some JAK2V617F knock-in model animals\(^{39,40}\) and in a coincidental human hematopoietic stem cell transplantation from JAK2V617F\(^+\) idiopathic portal hypertension patient to a patient with MDS\(^{41}\). 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\(^{40,41}\). 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 JAK2V617F 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 zest 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 HSC\(^{52,53}\). 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\(^{50}\). 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\(^{67,68}\). 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

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**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\(^{40}\). 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 (\(\Delta Hmga2\) mice)\(^{29}\), mimicking the truncation of **HMGA2** seen in the patients with MPN or PNH\(^{21-26,68}\). Hematopoiesis of \(\Delta Hmga2\) 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 \(\Delta Hmga2\) mice, increased expression of *Jak2* mRNA and cytokine-independent phosphorylations of STAT3 and AKT were observed in hematopoietic cells of \(\Delta Hmga2\) 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 \(\Delta Hmga2\) 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 \(\Delta Hmga2\) mice indicated that the **HMGA2** overexpression in the MPNs might be such an additional factor in MPN etiology (Fig. 2)\(^{29}\).

Hematopoietic cells of \(\Delta Hmga2\) 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 **JAK2**/V617F\(^-\) than **JAK2**/V617F\(^+\) 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\(^{28}\). Strikingly, they showed an evidence that Bmi1 directly repress expression of Hmga2 using chromatin immunoprecipitation assay for promoter area of Hmga2.

Serial BMT of \(\Delta Hmga2\) 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\(^{44,46}\). 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
clonal outgrowth of hematopoietic cells, which brought a long-term effect including continuous independence of blood transfusion in severe thalassemia\textsuperscript{70}.

Overexpression and/or truncation of \textit{HMGA2} has also been found in patients with PNH and MDS, in which bone marrow failure rather than proliferative hematopoiesis is a common feature\textsuperscript{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)-\(\alpha\) and interferon (IFN)-\(\gamma\)\textsuperscript{73 - 75}. It has also been suggested that an abnormal hematopoietic clone may preferentially survive the attack of IFN-\(\gamma\)-producing CTLs in these disorders\textsuperscript{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)\textsuperscript{77}. As well as PNH and MDS, it has been recently shown that TNF-\(\alpha\) may lead to a clonal selection of \textit{JAK2V617F}\textsuperscript{+} cells in MPN possibly due to a survival advantage against the TNF-\(\alpha\)\textsuperscript{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\textsuperscript{41}. It remains unknown if HMGA2 involve in such cytokine production, although immune response-related pathways were activated in HSCs of \(\Delta\text{HmgA2}\) mice in microarray analysis\textsuperscript{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\textsuperscript{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.
ACKNOWLEDGMENTS

We thank Professors M. Bessler and PJ. Mason for helpful discussions. This work was supported by the Research Grant of Aplastic Anemia & Myelodysplastic Syndromes International Foundation (3048-42179) and the Research Fellowship of Japan Society of Blood Transfusion and Cell Therapy to K. Ikeda. The NIH/NCI 2R01 CA105312 to Monica Bessler supported the generation of the ∆Hmg2 mice. K. Ikeda is a recipient of Fukushima Medical ∆Hmg2 Bessler supported the generation of the Ikeda. The NIH/NCI 2R01 CA105312 to Monica Society of Blood Transfusion and Cell Therapy to K. IKEDA et al. 96

REFERENCES

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood, 114 : 937-951, 2009.

2. Levine RL, Gilliland DG. Myeloproliferative disorders. Blood, 112 : 2190-2198, 2008.

3. Dameshek W. Some speculations on the myelo- dysisplasia with myelofibrosis. Cancer Cell, 110 : 1054-1061, 2006.

4. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst, 25 : 85-109, 1960.

5. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature, 243 : 290-293, 1973.

6. Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts van Kessel A, Bootsma D, Stone M, Heisterkamp N, Stephenson JR, Grosveld G, Ferguson-Smith MA, Davies T, Green AR. Mutation of JAK2 in the myelo- proliferative syndromes. Blood, 6 : 372-375, 1951.

7. Druker BJ, Tamura S, Buchduner E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med, 2 : 561-566, 1996.

8. James C, Ugo V, Le Couédic J-P, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslova H, Roland B, Bennacere-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythæmia vera. Nature, 343 : 1144-1148, 2005.

9. Kralovics R, Passamonti F, Buser AS, Teo S-siong, Tiedt R, Passweg JR, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med, 352 : 1779-1790, 2005.

10. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggan TJ, Wlodarska I, Clark JJ, Moore S, Adelsperger J, Koo S, Lee JC, Gabriel S, Mercher T, D’Andrea A, Fröhling S, Döhner K, Marynen P, Vandenberghë P, Mesa RA, Tefferi A, Griffin JD, Eck MJ, Sellers WR, Meyerson M, Golub TR, Lee SJ, Gilliland DG. Activating mutation in the tyrosine kinase JAK2 in polycythæmia vera, essential thrombocyæmia, and myeloid metaplasia with myelofibrosis. Cancer Cell, 7 : 387-397, 2005.

11. Baxter EJ, Scott LM, Campbell PJ, East C, Fouroulas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, Green AR. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative dis- orders. Lancet, 365 : 1054-1061, 2005.

12. Levine RL, Belisle C, Wadleigh M, Zahrich D, Lee S, Chagnon P, Gilliland DG, Busque L. X-inactivation-based clonality analysis and quanti- tative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. Blood, 107 : 4139-4141, 2006.

13. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, Erber WN, Kusec R, Larsen TS, Giraudier S, Le Bousse-Kerdilès MC, Griesshammer M, Reilly JT, Cheung BY, Harrison CN, Green AR. Mutation of JAK2 in the myelo- proliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. Blood, 108 : 3548-3555, 2006.

14. Theocharides A, Boissinot M, Girodon F, Garand R, Teo S-siong, Lippert E, Talman P, Tichelli A, Hermouet S, Skoda RC. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. Blood, 110 : 375-379, 2007.

15. Abdell-Wahab O, Manshouri T, Patel J, Harris K, Yao JJ, Hedvat C, Heguy A, Bueso-Ramos C, Kantarjian H, Levine RL, Verstovsek S. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukae- mias. Cancer Res, 70 : 447-452, 2010.

16. Mullanley A, Lane SW, Ball B, Meenderichian C, Okabe R, Al-Shahrour F, Paktimat N, Haydu JE, Housman E, Lord AM, Wernig G, Kharas MG, Mercher T, Kutz JL, Gilliland DG, Ebert
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24. Andrieux J, Demory J-L, Dupriez B, Quief S, Guglielmelli P, Zini R, Bogani C, Salati S, Pancrazzi 19. Tefferi A. Novel mutations and their functional 20. Vainchenker W, Delhommeau F, Constantinescu 23. Etienne A, Carbuccia N, Adélaïde J, Bekhouche I, 22. Storlazzi CT, Albano F, Locunsolo C, Lonace A, Cirmena G, Garuti A, Fugazza G, Rocchi M. HMGA2 overexpression in polycytemia vera with t(12;21)(q14;q22). Cancer Genet Cytogenet, 24: 115-118, 2007.

25. Guglielmelli P, Zini R, Bogani C, Salati S, Pancrazzi 18. Delhommeau F, Jeziorowska D, Marzac C, Aliano S, Cirmena G, Garuti A, Fugazza G, Rocchi M. (WT1). Stem Cells, 34: 3´UTR - truncated 33. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Galinsky I, DeAngelo DJ, Clark JJ, Lee SJ, Golub Theocharides A, Ferrari M, Gisslinger H, Kralovics R, Cremonesi L, Skoda R, Cazzola M. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood, 111: 1686-1689, 2008.

26. Odero MD, Grand FH, Iqbal S, Ross F, Roman JP, Vijmanos JL, Andrieux J, Lai JL, Calasanz MJ, Cross NC. Dysregulation and overexpression of HMGA2 as a consequence of diverse chromosomal translocations in myeloid malignancies. Leukemia, 19: 245-252, 2005.

27. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science, 315: 1576-1579, 2007.

28. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMG2 oncogene. Dev, 21: 1025-1030, 2007.

29. Ikeda K, Mason PJ, Bessler M. 3´UTR-truncated Hmga2 cDNA causes MPN-like hematopoiesis by conferring a clonal growth advantage at the level of HSC in mice. Blood, 117: 5860-5869, 2011.

30. Kato J, Caceres N, Constantinescu SN. Aberrant signal transduction pathways in myeloproliferative neoplasms. Leukemia, 22: 1828-1840, 2008.

31. Saharinen P, Silvennoinen O. The Pseudokinase Domain Is Required for Suppression of Basal Activity of Jak2 and Jak3 Tyrosine Kinases and for Cytokine-inducible Activation of Signal Transduction. J Biol Chem, 277: 47954-47963, 2002.

32. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, Futreal PA, Erber WN, McMullin MF, Harrison CN, Warren AJ, Gilliland DG, Lodish HF, Green AR. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med, 356: 459-468, 2007.

33. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, Ferrari M, Gisslinger H, Kralovics R, Cremonesi L, Skoda R, Cazzola M. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood, 111: 1686-1689, 2008.

34. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I, DeAngelo DJ, Clark JJ, Lee SJ, Golub TR, Wadleigh M, Gilliland DG, Levine RL. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med, 3 : e270, 2006.

35. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford MR, Wilkins BS, Reilly JT, Hasselbalch HC, Bowman R, Wheatley K, Buck G, Harrison CN, Green AR. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood, 112 : 141-149, 2008.

36. Chaligné R, Tonetti C, Besancenot R, Roy L, Marty C, Mossuz P, Kiladjian JJ, Socie G, Bordessoule D, Le Bousse-Kerdiles MC, Vainchenker W, Giraudier S. New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. Leukemia, 22 : 1557-
rnout J, Beer P, Scott MA, Bareford D, Green AR, Huntly B, Erber WN. Clinical utility of routine MPL exon 10 analysis in the diagnosis of essential thrombocythaemia and primary myelofibrosis. Br J Haematol, 149: 250-257, 2010.

38. Staerk J, Lacout C, Sato T, Smith SO, Vainchenker W, Constantinescu SN. An amphipathic motif at the transmembrane–cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor. Blood, 107: 1864-1871, 2006.

39. Van Pelt K, Nollet F, Selleslag D, Knoops L, Constantinescu SN, Criel A, Billiet J. The JAK2V617F mutation can occur in a hematopoietic stem cell that exhibits no proliferative advantage: a case of human allogeneic transplantation. Blood, 112: 921-922, 2008.

40. Teofili L, Martini M, Cenci T, Petrucci G, Torti L, Storti S, Guidi F, Leone G, Larocca LM. Different STAT-3 and STAT-5 phosphorylation discriminates among Ph-negative chronic myeloproliferative diseases and is independent of the V617F JAK-2 mutation. Blood, 110: 354-359, 2007.

41. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, Estrov Z, Fridman JS, Bradley EC, Erickson-Vitanen S, Vadii K, Levy R, Tefferi A. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med, 363: 1117-1127, 2010.

42. Nussenzveig RH, Swierzczek SI, Jelinek J, Gaikwad A, Liu E, Verstovsek S, Prchal JF, Prchal JT. Polycythemia vera is not initiated by JAK2V617F mutation. Exp Hematol, 35: 32-38, 2007.

43. Delhommeau F, Dupont S, Della Valle Y, James C, Trannoy S, Massé A, Kosmider O, Le Couedic JP, Robert F, Alberdi T, Lécluse Y, Plo I, Dreyfus FJ, Marzac C, Casadevall N, Lacombe C, Romana SP, Dessen P, Soulier J, Viguier F, Fontenay M, Vainchenker W, Bernard OA. Mutation in TET2 in myeloid cancers. N Engl J Med, 360: 2289-2301, 2009.

44. Carbuccia N, Murati A, Trouplin V, Brecqueville M, Adélaïde J, Rey J, Vainchenker W, Bernard OA, Chaffanet M, Vey N, Birnbaum D, Moziozziacci MJ. Mutations of ASXL1 gene in myeloproliferative neoplasms. Leukemia, 23: 2183-2186, 2009.

45. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A, Hochhaus A, Drexlter HG, Duncombe A, Cervantes F, Oscier D, Boulwood J, Grand FH, Cross NC. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet, 42: 722-726, 2010.
Levine RL, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. Leukemia, 25 : 1219–1220, 2011.

56. Scheuermann JC, deAyala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, Wilm M, Muir TW, Müller J. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. Nature, 465 : 243-247, 2010.

57. Sauvageau M, Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. Cell Stem Cell, 7 : 299–313, 2010.

58. Zhou X, Benson KF, Przybysz K, Liu J, Hou Y, Zhou Z, Babcock W, Frei-Lahr D, Parker CJ, Kinoshita N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P. Transfusion independence and HMG2 activation after gene therapy of human β-thalassaemia. Nature, 467 : 318-322, 2010.

59. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenicity of breast cancer stem cells. FEBS Lett, 581 : 3533-3537, 2007.

60. Shichishima T, Noji H, Akutsu K, Osumi K, Shichishima A, Noji H, Maeda Y, Nishimura J, Kanakura Y, Kinoshita N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P. Transfusion independence and HMG2 activation after gene therapy of human β-thalassaemia. Nature, 467 : 318-322, 2010.

61. Chen B, Young J, Leng F. DNA bending by the mammalian high-mobility group protein AT hook. Biochemistry, 49 : 1590-1595, 1996.

62. Fusco A, Fedele M. Roles of HMGA proteins in cancer. Nat Rev Cancer, 7 : 899-910, 2007.

63. Young AR, Narita M. Oncogenic HMGA2: short or small? Genes Dev, 21 : 1005–1009, 2007.

64. Li O, Li J, Dröge P. DNA architectural factor and proto-oncogene HMGA2 regulates key developmental genes in pluripotent human embryonic stem cells. FEBS Lett, 581 : 3533-3537, 2007.

65. Nishino J, Kim I, Chada K, Morrison SJ. Hmg2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf expression. Cell, 135 : 227-238, 2008.

66. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenicity of breast cancer stem cells. Cell, 131 : 1109-1123, 2007.

67. Tzatsos A, Bardeesy N. Inka4/Arf regulation by let-7b and Hmg2a: a genetic pathway governing stem cell aging. Cell Stem Cell, 3 : 469-470, 2008.

68. Inoue N, Izui-Sarumaru T, Murakami Y, Endo Y, Nishimura J, Kurokawa K, Kuwayama M, Shime H, Machii T, Kanakura Y, Meyers G, Wittwer C, Chen Z, Babcock W, Frei-Lahr D, Parker CJ, Kinoshita T. Molecular basis of clonal expansion of hematopoiesis in 2 patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood, 108 : 4232-4236, 2006.

69. Oguro H, Yuan J, Tanaka S, Miyagi S, Mochizuki-Kashio M, Ichikawa H, Yamazaki S, Koseki H, Nakauchi H, Iwama A. Lethal myelofibrosis induced by Bmi1-deficient hematopoietic cells unveils a tumor suppressor function of the polycomb group genes. J Exp Med, 209 : 445-454, 2012.
78. Fleischman AG, Aichberger KJ, Luty SB, Bumm TG, Petersen CL, Doratotaj S, Vasudevan KB, LaTocha DH, Yang F, Press RD, Loriaux MM, Pahl HL, Silver RT, Agarwal A, O’Hare T, Druker BJ, Bagby GC, Deininger MW. TNFα facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms. Blood, 118: 6392-6398, 2011.

79. Pardanani A, Vannucchi AM, Passamonti F, Cervantes F, Barbui T, Tefferi A. JAK inhibitor therapy for myelofibrosis: critical assessment of value and limitations. Leukemia, 25: 218-225, 2011.

80. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner E, Lyons RM, Paquette R, Raza A, Vaddi K, Erickson-Viitanen S, Koumenis IL, Sun W, Sandor V, Kantarjian HM. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med, 366: 799-807, 2012.

81. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, McQuitty M, Hunter DS, Levy R, Knoops L, Cervantes F, Vannucchi AM, Barbui T, Barosi G. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med, 366: 787-798, 2012.