JAK2 and TET2 Mutation in Polycythemia Vera

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Abstract

The Ten-Eleven Translocation-2 (TET2) gene, located on chromosome 4q24, has been implicated in hematological malignancies. The TET2 gene shows mutations in variable myeloid malignancies with the involvement of 15% of myeloproliferative neoplasms (MPNs). The inactivation of the TET2 gene in both mice and humans has shown a high degree of deregulation of the hematopoiesis process leading to hematological malignancies. Polycythemia vera (PV), an MPN characterized by increased red blood cell mass, has been associated with the TET2 gene. Furthermore, TET2 genes have been found to facilitate Janus kinase-2 and signal transducer activator of transcription 5, as well as modulate the epigenetic composition of genomic DNA. However, little is known about the role of TET2 mutations in patients with PV. Several studies have been conducted to further assess the significant role of TET2 gene function in various disease processes and prognoses to enhance the management and care of these patients.

Introduction And Background

Myeloproliferative neoplasms (MPNs) include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (MF), and chronic myeloid leukemia (CML) among several other disorders. CML is a Philadelphia chromosome-positive MPN, whereas others are negative for the Philadelphia chromosome. Several genes are implicated in myeloproliferative diseases including PV. In 2005, a recurrent point mutation in Janus kinase-2 (JAK2) exon 14 was identified in patients with MPNs. This mutation results in valine-to-phenylalanine substitution at position 617 in JAK homolog autoinhibitory domain. This substitution results in a gain-of-function of JAK2 which autonomously activates downstream signaling pathways, including Janus kinase/signal transducer and activator of transcription (JAK-STAT), phosphatidylinositol 3-kinase/protein kinase B, and extracellular signal-regulated kinases/microtubule-associated protein kinase [1,2]. Mutations in JAK2 exon 12 and MPL515 genes have also been implicated in MPNs which alter the JAK-STAT signaling pathway. However, the contribution of these genes has not been clearly identified in MPN phenotype. Myeloproliferative leukemia (MPL) and JAK2, although strongly associated with MPNs, do not necessarily specify clinicopathologic correlation. Growing evidence suggests the role of other genetic factors impacting the pathogenesis of MPNs. PV shows a higher expression of JAK2, but at the same time, JAK2 is not exclusive to PV in the MPN spectrum. Similar data have also been seen with the MPL mutant gene. This signifies the importance of the identification of more molecular alterations [1-5]. In recent studies, along with JAK2V617F, a coexisting Ten-Eleven Translocation-2 (TET2) gene, loss-of-function mutation has been found in the minimal loss of heterozygosity region of chromosome 4q24 in MPN patients [2]. TET2 has pleiotropic roles during hematopoiesis, including stem-cell self-renewal, lineage commitment, and terminal differentiation of monocytes [6]. TET2 is a known tumor suppressor gene.

PV is a monoclonal proliferative disorder of multipotential myeloid progenitor cells, increasing the cell count of all three lineages [7]. The disorder has a prevalence of 0.68-2.6 per 100,000 individuals [8]. It is characterized by increased red blood cell (RBC) mass and is associated with an increased risk of thrombotic events, leukemic transformation, and MF. JAK2V617F mutations are found in greater than 95% of PV patients. However, in patients with PV who were negative for JAK2V617F, other abnormalities were found in JAK2 exon 12 which induced activation of the JAK-STAT pathway at a greater level than the JAK2V617F allele [3]. This has been the basis for the World Health Organization’s definition of PV or other myeloproliferative disorders including ET which was revised in 2008. The diagnostic criteria include the detection of JAK2 mutation in exon 12 or 14. However, it is unclear how a single mutation in JAK2 can lead to different clinical phenotypes of MPN. JAK2 mutations do not explain the variable prognosis among patients with PV, which can perhaps be explained by the variable burden of this mutation in hematopoietic cells as well in genes other than JAK2. Furthermore, variations in the expression of alternative genes and epigenetic modifications may account for some of these disparities. Several other genes are implicated in PV including TET2 genes [4]. TET2 genes facilitate JAK2 or STAT5 signal transduction as well as modulate the epigenetic composition of genomic DNA, including DNA and histone methylation and acetylation [5].

TET2 mutations often occur early during the development of human myeloid malignancies, including PV,
ET, MF, myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML). These mutations appear to target hematopoietic stem/progenitor cells [7,8]. However, studies have shown that TET2 gene mutations may also occur during later stages, which may explain the transformation of MPN to acute leukemia [6]. This implies that therapeutic targets may have to focus on hematopoietic stem cells or progenitor cells to eradicate these myeloproliferative conditions. Table 1 illustrates TET2 mutation prevalence in different myeloid malignancies [9], whereas Table 2 demonstrates the prevalence of TET2 mutation in MPNs [10].

| Myeloid malignancies                          | TET2 mutation prevalence |
|-----------------------------------------------|--------------------------|
| Acute myeloid leukemia                         | 12–24%                   |
| Chronic myelomonocytic leukemia                | 20–40%                   |
| Myelodysplastic syndromes                      | 19–26%                   |
| Myeloproliferative neoplasms                   | 7–13%                    |
| Systemic mastocytosis                          | 29%                      |

**TABLE 1: Prevalence of TET2 mutation in myeloid malignancies[9].**

| Myeloproliferative neoplasms                  | TET2 mutation prevalence |
|-----------------------------------------------|--------------------------|
| Polycythemia vera                              | 16.8%                    |
| Essential thrombocythemia                      | 9.8%                     |
| Myelofibrosis                                  | 15.7%                    |

**TABLE 2: Prevalence of TET2 mutation in myeloproliferative neoplasms[10].**

**Review**

**Overview of JAK2 and TET2 gene**

In 2009, the TET2 gene was described in myeloid malignancies along with its variants. The TET2 gene is located on chromosome 4q24. TET2 protein modulates DNA hydroxymethylation through the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, promoting DNA demethylation. The functional domain of TET2 is located at the C-terminus; it consists of a cysteine-rich domain as well as a double-stranded β-helix fold domain. Significant functions of TET2 include the hematopoiesis role, stem-cell self-renewal promotion, monocyte differentiation on the terminal stage, and lineage commitment. In addition, the TET2 gene is highly expressed in hematopoietic progenitor cells [11]. TET2 gene shows mutations in variable myeloid malignancies with the involvement of 15% of the MPNs. The inactivation of the TET2 gene in both mice and humans has shown a high degree of deregulation of the hematopoiesis process leading to hematological malignancies [12]. Moreover, the TET2 gene has been described as a tumor-suppressor gene with its homozygous and heterozygous mutations leading to hematopoietic malignancies in humans. Amino acid substitutions, frameshifting, in-frame deletions, and generated stop codons are all possible TET2 gene mutations. However, there is no precise pattern of genotypes with the associated hematological malignancies such as MDS, CMML, and AML [11]. Jung-Sook et al. reported that all patients carrying the TET2 mutation also carried the JAK2V617F mutation. There was no relation between the occurrence of the TET2 mutation and age, JAK2V617F allele burden, frequency of organomegaly, fibrosis of the marrow, hematologic indices, as well as thrombotic or hemorrhagic complications in all MPN patients [13]. Most TET2 mutations result in the loss of enzymatic function. The most common types of mutations are nonsense and frameshift ones, which occur before the C-domain. However, missense mutations and in-frame deletions also occur within the C-domain [14]. Figure 1 demonstrates the loss of function of TET2 along with mutation of JAK2V617F, leading to MPN and AML [15].
FIGURE 1: The progression of HSC to AML - TET2 and JAK2.

LOF: loss of function; HSC: hematopoietic stem cells; MPN: myeloproliferative neoplasm; JAK2V617F: Janus kinase 2; NPM1c: nucleophosmin; TET2: Ten-Eleven Translocation-2; AML: acute myeloid leukemia

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Perner F, Perner C, Ernst T, Heidel FH: Roles of JAK2 in aging, inflammation, hematopoiesis and malignant transformation. Cells. 2019, 8:854. 10.3390/cells8080854 [15].

Clinical implications of TET2 gene mutation and its association with polycythemia (in terms of diagnosis, treatment targets, and prognosis)

Patients with PV who are homozygous for JAK2V617F are prone to developing post-PV complications such as MF [16,17]. In addition, patients with JAK2V617F mutation who acquire BCR-ABL1 translocation are prone to developing CML. However, the role of TET2 in the progression or evolution of PV to MF, AML, or CML has not been established. Much controversy has been elicited regarding the prognosis of myeloid malignancies with TET2 mutations [18]. According to Tefferi et al., there were no clinically significant alterations in the prognosis or overall survival rate in patients diagnosed with MPNs based on findings from large cohort studies [19]. On the other hand, studies have demonstrated poorer outcomes in hematologic malignancies including CMML and AML associated with TET2 mutations with limited data on PV [20,21]. Moreover, TET2 mutations associated with malignancies have been shown to have a favorable response to hypomethylating agents in high-risk patients [11,22].

Uncertain prognosis in patients with TET2 mutations is largely due to studies lacking evidence of possible underlying and other associated mutations for the prognosis of TET2 mutations. In other words, there is a lack of evidence regarding whether TET2 mutations primarily dysregulate well-known pathways associated with hematopoietic transformation or constitute a novel, poorly discovered pathway toward malignancy pathogenesis [21]. To further elaborate, TET2 mutations may set the stage toward the pathogenesis of different hematologic malignancies and act jointly with other gene mutations at early stages. For example, TET2 mutations when combined with JAK2 and ASXL1 mutations give rise to PV and MF [23]. Overall, different combinations of gene mutations along with TET2 mutations markedly reflect on the prognosis. Of note, Świerczek et al. have suggested that TET2 mutations are not the PV initiating cascade, rather they occur after JAK2 mutations. It has also been suggested that combined JAK2 and TET2 mutations allow for dramatic proliferation over TET2-negative PV subclones, emphasizing the hypothesis that TET2 mutations increase the aggressive nature of JAK2 mutation-positive PV [24]. Interestingly, Ortmann et al. found that the order in which JAK2/TET2 mutations are acquired reflects on the clinical prognosis of patients diagnosed with PV. Patients with initial JAK2 mutation demonstrated a higher risk of thrombosis compared to a more indolent course in patients with initial PV-associated TET2 mutation [25].

With regard to the treatment, studies have shown that management with peginterferon alfa-2a can reduce JAK2V617F clones but not the TET2 mutant ones. As mentioned earlier, TET2 mutation leads to persistent clonal hematopoiesis [26]. Another study has shown that peginterferon alfa-2a-treated patients with both JAK2 and TET2 mutations had a less significant reduction in the burden of JAK2V617F compared to those with JAK2 but without TET2 mutations. The former group possessed a higher burden of JAK2V617F
mutation at the beginning of the therapy. Furthermore, the same study revealed that patients without complete remission were more likely to have additional mutations apart from JAK2 [27].

Conclusions
Several studies suggest that TET2 mutations are an early event in the development of myeloid malignancies, yet their function in normal cells and pathologic conditions remains to be elucidated. It is unclear what triggers these mutations and at what point in time. JAK2 mutation is associated with most MPNs including PV. However, a single mutation in JAK2 does not explain the variable prognoses among patients with PV. Several other genes including mutations in TET2 have been implicated. TET2 genes facilitate JAK2 or STAT5 signal transduction and allow the accelerated proliferation of cells in patients with JAK2-positive PV. Further, studies have shown that patients with initial JAK2 mutation demonstrated a higher risk of thrombosis compared to a more indolent course in patients with initial PV-associated TET2 mutation. However, these are early days and much remains unknown about TET2 mutations. As newer studies continue to shed light on this subject, whether TET2 genes may be targeted as a part of disease management or prognostication remains to be seen. Targeted disruption of genes in animal models, as well as perturbation of TET2 levels in normal and malignant cell types in vitro and in vivo, may offer clues to the understanding of the function of TET2.

Additional Information
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References
1. Moran-Crusio K, Reavie L, Shih A, et al.: Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell. 2011, 20:11-24. 10.1016/j.ccr.2011.06.001
2. Quiviron C, Couronne L, Della Valle V, et al.: TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer Cell. 2011, 20:25-38. 10.1016/j.ccr.2011.06.005
3. Vannacci AM: Insights into the pathogenesis and management of thrombosis in polycythemia vera and essential thrombocythemia. Intern Emerg Med. 2010, 5:177-84. 10.1007/s11759-009-0519-3
4. Spivak JL, Considine M, Williams DM, et al.: Two clinical phenotypes in polycythemia vera. N Engl J Med. 2014, 371:808-17. 10.1056/NEJMoa1403141
5. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA: New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011, 118:1723-35. 10.1182/blood-2011-02-292102
6. Schaaf FX, Looser R, Li S, Hao-Shen H, Lehmann T, Tichelli A, Skoda RC: Clonal analysis of TET2 and JAK2 mutants suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. Blood. 2010, 115:2005-7. 10.1182/blood-2009-09-245581
7. Lundberg P, Karow A, Nienhoidl R, et al.: Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014, 125:2220-8. 10.1182/blood-2013-11-537167
8. Delhommeau F, Dupont S, Della Valle V, et al.: Mutation in TET2 in myeloid cancers. N Engl J Med. 2009, 360:2289-91. 10.1056/NEJMoa0810069
9. Patriarca A, Colaiazzo D, Tiscia G, et al.: TET2 mutations in Ph-negative myeloproliferative neoplasms: identification of three novel mutations and relationship with clinical and laboratory findings. Biomed Res Int. 2015, 2015:929840. 10.1155/2015/929840
10. Chia YC, Islam MA, Hider P, Woon PV, Johan MF, Hassan R, Ramlall T: The prevalence of TET2 gene mutations in patients with BCR-ABL-negative myeloproliferative neoplasms (MPNs): a systematic review and meta-analysis. Cancers (Basel). 2021, 13:53123078. 10.3390/cancers13133078
11. Feng Y, Li X, Cassady K, Zou Z, Zhang X: TET2 function in hematopoietic malignancies, immune regulation, and DNA repair. Front Oncol. 2019, 9:210. 10.3389/fonc.2019.00210
12. Pronier E, Delhommeau F: Role of TET2 mutations in myeloproliferative neoplasms. Curr Hematol Malig Rep. 2012, 7:57-64. 10.1007/s11899-011-0108-8
13. Ha JS, Jeon DS, Kim HR, Ryoo NH, Sub JS: Analysis of the ten-eleven translocation 2 (TET2) gene mutation in myeloproliferative neoplasms. Ann Clin Lab Sci. 2014, 44:173-9.
14. Ferrone CK, Ilyut-Hamron M, Rauh MI: Age-associated TET2 mutations: common drivers of myeloid dysfunction, cancer and cardiovascular disease. Int J Mol Sci. 2020, 21:626. 10.3390/ijms21020626
15. Perera F, Perner C, Ernst T, Heidel FH: Roles of JAK2 in aging, inflammation, hematopoiesis and malignant transformation. Cells. 2019, 8:854. 10.3390/cells8080854
16. Tefferi A, Lasho TL, Schwager SM, et al.: The clinical phenotype of wild-type, heterozygous, and homozygous JAK2(V617F) in polycythemia vera. Cancer. 2006, 106:631-5. 10.1002/cncr.21649
17. Koren-Mitchowits M, Landman J, Cohen Y, et al.: JAK2(V617F) allele burden is associated with transformation to myelofibrosis. Leuk Lymphoma. 2012, 53:2210-5. 10.3109/10428194.2012.682308
18. Cimmino L, Abdel-Wahab O, Levine RL, Aifantis I: TET family proteins and their role in stem cell differentiation and transformation. Cell Stem Cell. 2011, 9:193-204. 10.1016/j.stem.2011.08.007
19. Tefferi A, Pardanani A, Lim KH, et al.: TET2 mutations and their clinical correlates in polycythemia vera,
essential thrombocythemia and myelofibrosis. Leukemia. 2009, 23:905-11. 10.1038/leu.2009.47
20. Kosmider O, Gelsi-Boyer V, Ciudad M, et al.: TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009, 94:1676-81. 10.3324/haematol.2009.011205
21. Abdel-Wahab O, Mullally A, Hedvat C, et al.: Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009, 114:144--7. 10.1182/blood-2009-05-210039
22. Flach J, Dicker F, Schnittger S, Kohlmann A, Haferlach T, Haferlach C: Mutations of JAK2 and TET2, but not CBL are detectable in a high portion of patients with refractory anemia with ring sideroblasts and thrombocytosis. Haematologica. 2010, 95:518-9. 10.3324/haematol.2009.015631
23. Verstovsek S, Odenike O, Singer J, Gramant T, Al-Fayoumi S, Deeg HJ: Phase 1/2 study of pacritinib, a next generation JAK2/FLT3 inhibitor, in myelofibrosis or other myeloid malignancies. J Hematol Oncol. 2016, 9:137. 10.1186/s13045-016-0367-x
24. Swierczek SI, Yoon D, Bellanné-Chantelot C, et al.: Extent of hematopoietic involvement by TET2 mutations in JAK2V617F polycythemia vera. Haematologica. 2011, 96:775-8. 10.3324/haematol.2010.029678
25. Ortmann CA, Kent DG, Nangalia J, et al.: Effect of mutation order on myeloproliferative neoplasms. N Engl J Med. 2015, 372:601--12. 10.1056/NEJMoa1412098
26. Kiladjian JJ, Massé A, Cassinut B, et al.: Clonal analysis of erythroid progenitors suggests that pegylated interferon alpha-2a treatment targets JAK2V617F clones without affecting TET2 mutant cells. Leukemia. 2010, 24:1519-25. 10.1038/leu.2010.120
27. Quintás-Cardama A, Abdel-Wahab O, Manshouri T, et al.: Molecular analysis of patients with polycythemia vera or essential thrombocythemia receiving pegylated interferon α-2a. Blood. 2013, 122:895-901. 10.1182/blood-2012-07-442012