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The Role of Janus Kinase 2 (JAK2) in the Pathologenesis of Myeloproliferative Disorders

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Abstract. The myeloproliferative disorders, polycythemia vera, essential thombocythemia, and primary myelofibrosis are clonal disorders of multipotent hematopoietic progenitors. The genetic cause of these diseases was not known until 2005, when several independent groups demonstrated that most patients with PV, ET and PMF acquired a single point mutation in the cytoplasmic tyrosine kinase, such as *JAK2 (JAK2 V617F)*. These discoveries have changed the landscape for diagnosis and classification of PV, ET and PMF, and have shown the ability of genomic technologies to identify new molecular targets in human malignancies with pathogenetic, diagnostic and therapeutic significance.

Keywords: Leukemia, Tyrosine kinase, Myeloproliferative disorders, JAK 2 mutations

Introduction

Myeloproliferative disorders (MPD) are clonal disorders of hematopoietic progenitors, and include the classical MPD chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), as well as chronic eosinophilic leukemia (CEL), chronic myelomonocytic leukemia (CMML), and systemic mastocytosis (SM) and others. The different myeloproliferative disorders (MPD) can be classified by the predominant

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terminally differentiated myeloid cell involved in the disorder, and for each terminally differentiated myeloid cell there is a clinically distinct MPD. Different approaches have been used to identify the activating alleles that cause these disorders, and in all cases these alleles result in constitutive tyrosine kinase signaling. HSC, hematopoietic stem cell; JAK2, Janus kinase 2; MPL, thrombopoietin receptor; PDGFR, platelet derived growth factor receptor (Fig. 1). Although, each of the MPD is recognized as a distinct clinicopathological entity, these disorders share cardinal features that distinguish the MPD from other myeloid malignancies[1], namely myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). In the past two decades, mutant alleles have been identified in CML, CMML, CEL and SM[2-5]. In each case the causative mutation results in constitutive activation of tyrosine kinase

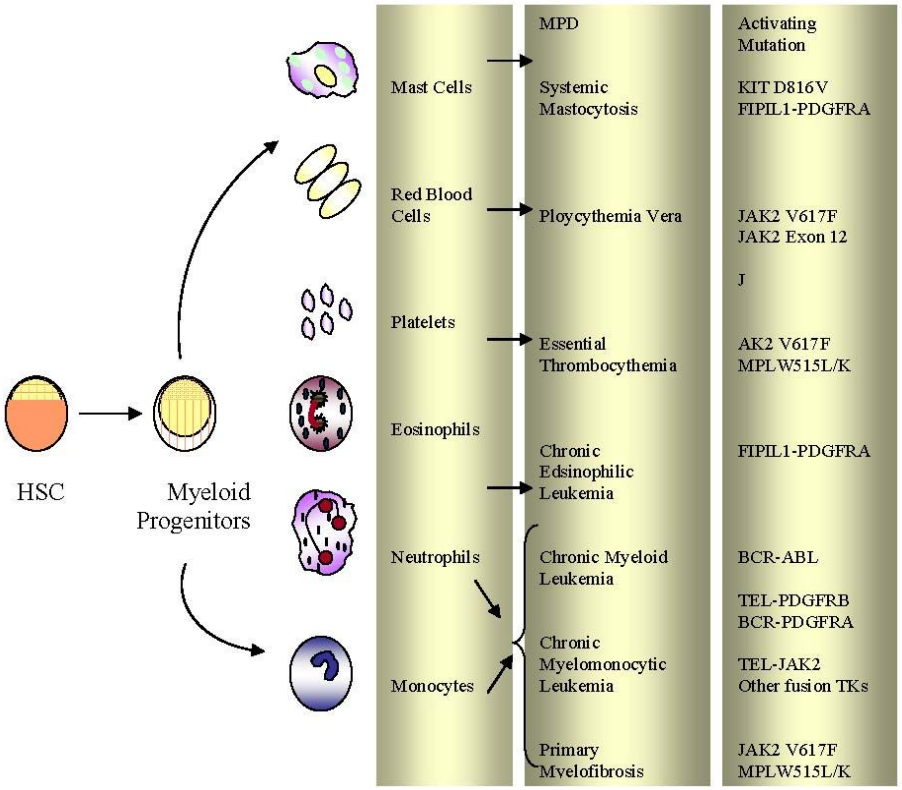

Fig.. 1. MPD classification and molecular pathogenesis.

signaling. Perhaps, the most important from a clinical perspective, specific inhibition of these activated kinases results in dramatic clinical efficacy in the treatment of MPD[4-7]. Collectively, these data indicate that tyrosine kinase activation is a common pathogenetic mechanism in MPD, moreover these mutated kinases serve as validated targets for the design of molecularly targeted therapies. Although, these discoveries provided an important insight into the pathogenesis and treatment of certain MPD, the genetic causes of the most common MPD remained unknown; until the identification of mutations that activate Janus kinase 2 (JAK2) signaling in most patients with PV, ET or PMF[8-11]. In this mini review report, the discussion will include our understanding of the genetic basis of these disorders, in particular relating to the role of JAK2 activation in the pathogenesis of PV, ET and PMF.

JAK2 V617F Mutations in PV, ET and PMF

In 2005, several independent groups used different experimental approaches to identify a recurrent mutation in the *JAK2* tyrosine kinase in most patients with PV, ET or PMF[8-11]. *JAK2* is a member of the Janus family of cytoplasmic non-receptor tyrosine kinases, which also includes JAK1, JAK3 and TYK2. The mutation is a G-T substitution at nucleotide 1849, which results in substitution of V-F at amino acid position 617 of JAK2 (*JAK2 V617F*). The mutation is present in hematopoietic cells, but not germline DNA in patients with MPD[8-11], demonstrating that *JAK2 V617F* is a somatic mutation that is acquired in the hematopoietic compartment. In addition, the *JAK2 V617F* allele can occasionally be present in different hematopoietic compartments[12,13], including B and T lymphoid cells. These findings suggest that the mutation might occur in the pluripotent hematopoietic stem cell; indeed, the *JAK2 V617F* allele has recently been identified in the hematopoietic stem cell (HSC) compartment in patients with PV[14]. These data are in agreement with the hypothesis that the self-renewing properties of HSCs are necessary for the MPD phenotype, and that activated tyrosine kinases can transform HSCs, but not myeloid progenitors that lack the capacity for self-renewal[15].

In the initial reports of the *JAK2 V617F* allele in PV, ET and PMF, it was noted that, although, most patients with MPD are heterozygous for *JAK2 V617F*, a subset of patients, most commonly with

PV, are homozygous for the *JAK2 V617F* allele[8-11]. The mechanism of homozygosity for *JAK2 V617F* is not loss of the wild-type allele, as is observed for classical tumor-suppressor genes, but instead results from mitotic recombination and duplication of the mutant allele MPD[8-11], known as acquired uniparental disomy (UPD). UPD involving chromosomal locus 9p24, including *JAK2*, had previously been noted in PV[16], and Kralovics *et al*. identified the *JAK2 V617F* allele through analysis of the minimal region of UPD in PV[11]. Homozygous *JAK2 V617F* mutant erythroid colonies can be grown from almost all patients with PV[17]; suggesting that UPD at the *JAK2* locus resulting in *JAK2 V617F* homozygosity is an early event in the pathogenesis of PV. By contrast, homozygous *JAK2 V617F* mutations are rarely observed in ET[8], and hematopoietic colonies have grown from ET patients are most commonly wild type or heterozygous with respect to *JAK2*. These data suggested that there are important genetic differences between PV and ET, and that duplication of *JAK2 V617F* is the most important to the pathogenesis of PV.

After the discovery of the *JAK2 V617F* allele, sensitive, allele-specific assays have been used to assess the frequency of *JAK2 V617F* mutations in different malignancies[10,17-19] (Table 1). Although, *JAK2 V617F* mutations are most common in PV, ET and PMF, they occur less commonly in other myeloid diseases; including CMML, MDS and AML[19-22], whereas acquisition of *JAK2 V617F* does not occur in lymphoid malignancies or in solid tumors. The predilection of *JAK2 V617F* mutations for myeloid malignancies is surprising, given that there is significant evidence that activation of Jak-Stat (signal transducer and activator of transcription) signaling occurs in a wide spectrum of human malignancies[23]. Existing genetic data suggested that there are desperate mechanisms for activation of Jak-Stat signaling in different malignancies; including *JAK2 V617F* mutations in myeloid malignancies, *JAK3* mutations in megakaryoblastic leukemia[24]. *JAK2* amplification and suppressor of cytokine signaling 1 (SOCS1) mutations in Hodgkin disease and mediastinal large B-cell lymphoma[25-28], and promoter hypermethylation of *SOCS1* in multiple myeloma[29]. As high-resolution genomic strategies improve, our understanding of the cancer genome novel genomic events that result in activation of Jak-Stat signaling are likely to be identified in hematopoietic and non-hematopoietic neoplasms.

Table 1. Frequency of the *JAK2 V617F* allele in myeloid disorders.

Disease	Frequency
Polycythaemia Vera	81-99%
Essential Thombocytosis	41-72%
Primary Myelofibrosis	39-57%
Chronic Myelomonocytic Leukemia	3-9%
Myelodysplasia*	3-5%
Acute Myeloid Leukemia‡	< 5%

**Most common in patients with refractory anemia with ringed sideroblasts and thombocytosis. A clinically distinct subtype of myelodysplastic syndromes. ‡Most common in patients with a previous history of polycythemia vera, essential thrombocytopenia and primary myelofibrosis.*

JAK2 V617F and Signal Transduction

The Jak Kinases normally function through their association with cytokine receptors that lack intrinsic kinase activity. Ligand binding to the appropriate cytokine receptor results in Jak kinase phosphorylation and activation, cytokine receptor phosphorylation, recruitment and phosphorylation of Stat proteins; and the activation of downstream signaling proteins. The specificity of different cytokine receptors for one or more different Jak kinases accounts in part for their differential effects on signal transduction. Genetic deletion of JAK2 results in embryonic lethality owing to a lack of definitive erythropoiesis, and JAK2-deficient hematopoietic progenitors do not respond to erythropoietin (EPO) stimulation; these data demonstrate JAK2 is the sole Jak kinase responsible for EPO receptor (EPOR) signaling[30].

The Jak kinases have seven homologous domains (JH1-7), which include the catalytic kinase domain (JH1) and a catalytically inactive pseudokinase domain (JH2). The *JAK2 V617F* point mutation results in a single amino acid substitution within the JH2 domain of JAK2. It has been suggested that the JH2 domain serves as an auto inhibitory function similar to the juxtamembrane domain of receptor tyrosine kinases[31], and that valine 617 has an important role in mediating JAK2 kinase autoinhibition[32]. The valine-to-phenylalanine substitution at codon 617 might abrogate autoinhibition and result in constitutive kinase activity[33], although, structural insight is needed to determine if this is the case. The *JAK2 V617F* protein has constitutive kinase activity[34], and when expressed *in vitro JAK2 V617F*, but not wild-type JAK2, is constitutively phosphorylated[8], which is consistent with the notion that *JAK2 V617F* is a gain-of-function mutation with respect to JAK2 kinase activity.

Expression of *JAK2 V617F* confers cytokine hypersensitivity and cytokine-independent growth to hematopoietic cells, which are characteristic features of hematopoietic colonies grown from patients with PV[35]. JAK2V617F-mediated transformation to cytokine-independent growth is most efficient in hematopoietic cells that co-express the EPOR, the thrombopoietin receptor (MPL), or the granulocyte colony-stimulating factor receptor (GCSFR)[36]. Unlike most cytokine receptors, EPOR, MPL and GCSFR are homodimeric type I cytokine receptors that are expressed on cells of the erythroid, megakaryocytic and granulocytic lineages, respectively. Although, these data do not exclude the possibility that *JAK2 V617F* interacts with non-homodimeric hematopoietic cytokine receptors; unlike other activated tyrosine kinases that have been identified in human malignancies, JAK2V617F-mediated hematopoietic cell transformation requires interaction with a cytokine receptor scaffold. In addition, the predilection of the *JAK2 V617F* allele for proliferative syndromes involving the erythroid, megakaryocytic and granulocytic lineages, might in part be explained by differential cytokine receptor expression during hematopoietic differentiation.

In vitro studies, demonstrate that the expression of *JAK2 V617F* activates multiple downstream signaling pathways[8,36], including the Stat family of transcription factors, the mitogen activated protein kinase (MAPK) signaling pathway, and the phosphotidylinositol 3-kinase (PI3K)-Akt signaling pathway. Cytokine ligands normally bind cytokine receptors, which results in Janus kinase 2 (JAK2) phosphorylation, recruitment of signal transducer; an activator of transcription (Stat) signaling proteins and phosphorylation, An activation of downstream signaling pathways, including Stat transcription factors, mitogen activated protein kinase (MAPK) signaling proteins, and the phosphotidylinositol 3-kinase (PI3K)–Akt pathway (Fig. 2). Most activated tyrosine kinases that have been identified in human malignancies activate these same signaling cascades. The role and requirement of the Stat, MAPK and PI3K-Akt signaling pathways in JAK2 V617F-mediated transformation of hematopoietic cells has not been fully elucidated. However, several lines of evidence suggest that activation of the Stat family of transcription factors is important in JAK2 V617F-mediated transformation. First, expression of either constitutively

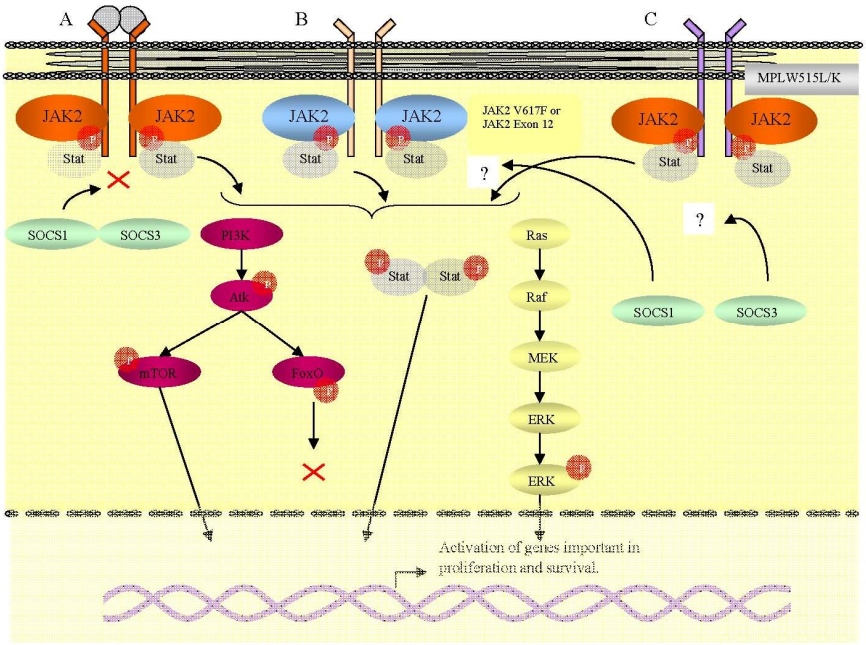

Fig. 2. The mechanism of activation of JAK2 kinase activity by mutations in the JAK2 signaling pathway. (A) Cytokine ligands normally bind cytokine receptors. (B) Mutant JAK2 (V617F and JAK2 exon 12 mutant kinases) lead to ligand-independent activation of downstream signaling pathways. (C) MPLW515L/K mutant thrombopoietin receptors are able to phosphorylate wild-type JAK2 in the absence of thrombopoietin.

active STAT5 or its anti-apoptotic target gene BCL-X_L in human hematopoietic progenitors results in EPO-independent colony formation[37]; a hallmark of human PV. Moreover, STAT3 activation and BCL-X_L over expression are observed in most PV patient samples[38,39]. These data imply that Stat pathway activation is important in JAK2 V617F-mediated transformation, but do not indicate whether Stat pathway activation is necessary and/or sufficient for JAK2 V617F-mediated transformation. Murine bone marrow transplantation (BMT) assays using *Stat5a*; *Stat5b*-deficient mice have been used to show that STAT5 is required for hematopoietic transformation by the constitutively active TEL-JAK2 fusion tyrosine kinase[40], and future experiments will ultimately determine whether the same is true for *JAK2 V617F*. In

addition, the activation of signaling by the JAK2 V617F kinase might in part be due to escape from negative-feedback mechanisms important in attenuating JAK2 signal. JAK activity is negatively regulated by the Socs family of proteins, which normally bind to the Jak kinases and result in their degradation. In particular, SOCS1 and SOCS3 have been shown to bind to JAK2 and inhibit JAK2 catalytic activity[41,42]. Over expression of SOCS1 results in abrogation of *in vitro* and *in vivo* transformation by TEL-JAK2[43]. Although, expression of SOCS1 results in JAK2 and *JAK2 V617F* degradation and inhibition of kinase activity, the expression of SOCS3 paradoxically results in increased *JAK2 V617F* protein stability, increased SOCS3 phosphorylation and increased *JAK2 V617F* phosphorylation[44]. These data demonstrate that regulation of JAK2 kinase activity by SOCS3 is altered in the context of the V617F substitution, and suggest the possibility that therapeutic inhibition of SOCS3 might selectively attenuate *JAK2 V617F*, but not wild-type JAK2 signaling.

In vivo data from murine BMT experiments have provided important insights into the role of JAK2 activation in the pathogenesis of MPD. James *et al*. noted that the expression of *JAK2 V617F*, but not in wild-type JAK2, in a murine BMT assay results in significant erythrocytosis in recipient mice 28 days after transplantation[8,9]. Subsequent studies by several groups have confirmed and extended these findings[45-48] (Table 2). Several important observations can be made based on the data from these studies. First, although, expression of most activated tyrosine kinases in a murine BMT model results in a neutrophilic MPD most similar to human CML[47-50], the predominant phenotype that results from *in vivo* JAK2 V617F expression is erythrocytosis. By contrast, leukocytosis is observed in the Balb/C, but not the C57Bl/6 genetic background[45,47], suggesting that there are genetic modifiers which influence the phenotype of *JAK2 V617F*-positive hematopoietic progenitors. In addition, although thrombocytosis is commonly observed in PV and ET, expression of *JAK2 V617F* it does not induce thrombocytosis in recipient in mice. These data indicate that expression of *JAK2 V617F* by itself might result in human PV, but that additional genetic events are necessary for the development of ET and/or PMF.

Table 2. Murine models of MPD.

Mutant Allele	Strain	PV	Leukocytosis	Megakaryocytic Hyperplasia	Thrombocytosis	Myelofibrosis
JAK2 V617F[45,47,48]	Balb/C	Yes	Yes	Yes	No	Yes
JAK2 V617F[45-47]	C57 Bl/ 6	Yes	No	Yes	No*	Modest
JAK2 exon 12[53]	Balb/C	Yes	Yes	Yes	No	Yes
MPLW515L[55]	Balb/C	No	Yes	Yes	Yes	Yes

JAK2 V617F-Negative PV, ET and PMF

Although, *JAK2 V617F* mutations can be identified in many patients with PV, ET and PMF, a significant proportion of patients with ET, PMF and a small number of patients with PV are *JAK2 V617F* negative. Clonal hematopoiesis is observed in patients with *JAK2 V617F*-negative MPD[30], suggesting alternate alleles account for myeloproliferation in this setting. Moreover, serial assessment of *JAK2 V617F* status in *JAK2V617F*-negative MPD did not observe conversion to *JAK2 V617F*-positive MPD[51], indicating *JAK2 V617F*-negative MPD are pathogenetically distinct from *JAK2 V617F*-positive MPD.

JAK2 Exon 12 Mutations in V617F-Negative PV

Although, most patients with PV are *JAK2 V617F* are positive when assessed using appropriately sensitive allele-specific assays, a small proportion of patients with PV are negative for the *JAK2 V617F* allele[10,19,20,52]. In order to search for alternate alleles that might result in the activation of Jak-Stat signaling, Scott *et al*. analyzed patients with *JAK2 V617F*-negative PV for somatic mutations in all exons of *JAK1*, *JAK2*, *JAK3*, *TYK2*, *STAT5A* and *STAT5B*[53]. Genomic analysis identified four novel somatic mutations in exon 12 of *JAK2*; one novel allele was a point mutation that results in the substitution of lysine for leucine at codon 539 (K539L), and three additional alleles were small deletions or insertions involving codons 538 to 543. *In vitro* colony assays showed that *JAK2* exon 12 mutations were present in all EPO-independent colonies have grown from these patients. Expression of these novel JAK2 mutant kinases in Ba/F3 cells co-expressing EPOR, resulted in transformation to factor-independent growth, and in activation

of downstream signaling pathways in an analogous fashion to the canonical *JAK2 V617F* allele. In addition, expression of the *JAK2* exon 12 mutations in a murine BMT assay recapitulated the phenotype of JAK2 V617F, with recipient mice developing; polycythemia, splenomegaly, and erythroid expansion. Unlike *JAK2 V617F*, *JAK2* exon 12 mutations are only observed in *JAK2 V617F*-negative PV, and are specific to patients who present with isolated erythrocytosis without concomitant leukocytosis or thrombocytosis. These data indicate that *JAK2* exon 12 mutations contribute to the pathogenesis of *JAK2 V617F*-negative PV, plus different activating JAK2 alleles are associated with different clinical phenotypes.

Additional Inherited and Acquired Alleles in MPD

Although, existing data indicates that acquisition of *JAK2 V617F* mutations contributes to the pathogenesis of PV, ET and PMF, there are probably additional genetic events that contribute to the development of these MPD. Given that the identical point mutation occurs in three related, but clinically distinct disorders, additional genetic factors must cooperate with the *JAK2 V617F* kinase to determine the phenotype of *JAK2 V617F*-positive MPD. Genetic data from families with a predisposition to develop MPD support this hypothesis. Several groups have identified families with more than one member with a diagnosis of PV, ET or PMF[60,61]. Gain-of-function mutations in KRAS[62] and RET[63] have been identified in familial cancer predisposition syndromes; however, analysis of *JAK2 V617F* mutational status in familial MPD has not identified the germline *JAK2 V617F* mutations[64,65]. By contrast, somatic *JAK2 V617F* mutations are identified in some, but not all, affected members in these kindreds. These data are consistent with the notion that there are heritable alleles that predispose to the acquisition of *JAK2 V617F* mutations and to the development of PV, ET and PMF. Although, the identity of these predisposition alleles is not known, it is attractive to hypothesize that these MPD predisposition alleles modulate JAK2 signaling and increase the selective advantage of cells that acquire the *JAK2 V617F* allele.

In addition, several lines of evidence suggest there might be a 'pre-*JAK2 V617F*' transformed hematopoietic progenitor. Patients with PV, ET or PMF are at an increased risk for the development of AML, and although *JAK2 V617F* mutations are relatively uncommon in *de novo*

AML they are present in many patients with AML secondary to a MPD[18]. However, two recent studies have demonstrated that a significant proportion of patients with a history of *JAK2 V617F*-positive MPD develop a *JAK2 V617F*-negative AML[51,66]. Cytogenetic and clonality analyses in a small number of cases suggest; the *JAK2 V617F*-positive MPD and *JAK2 V617F*-negative AML arise from the same clone[66]. Cytogenetic abnormalities occur in a subset of patients with PV, ET or PMF, including deletions on the long arm of chromosome 20[67]. In a small number of MPD patients, cytogenetic analyses show that all hematopoietic cells possess 20q deletions, but only a subset of these cells carry the *JAK2 V617F* allele[68,69]. These data indicate additional mutations can precede the acquisition of *JAK2 V617F* mutations; whether these mutations are distinct from the alleles that contribute to familial MPD is not known. Given that *JAK2 V617F*-negative MPD patients do not become *JAK2 V617F* positive during the course of their disease It is likely that the pre-*JAK2* clone does not manifest as a clinically apparent MPD, and the mutations that precede the acquisition of *JAK2 V617F* are distinct from those that activate signaling in the absence of *JAK2 V617F*. Given that murine BMT experiments suggest that *JAK2 V617F* is sufficient to induce a PV phenotype, cooperating alleles might not be present in all patients with *JAK2 V617F*-positive MPD.

Future Directions

Although, our understanding of the pathogenesis of PV, ET and PMF has greatly improved by the discovery of the *JAK2 V617F* allele, future studies will allow us to better understand the molecular pathogenesis of these MPD; to create more accurate genetic models of MPD and to develop molecularly targeted therapies for patients with these disorders. Current and future research into the genetic basis of MPD will include screens of JAK signaling molecules to identify mutant alleles in *JAK2*- and *MPL*-negative MPD, as well as genome-wide studies to identify inherited and/or acquired events that cooperate with *JAK2 V617F*. Moreover, detailed investigation of signal-transduction cascades activated by *JAK2 V617F* will delineate the role and requirement for different signaling pathways in hematopoietic transformation. Most importantly, the development of specific, potent inhibitors of JAK2 will allow assessing whether targeted therapy against

JAK2 results in significant clinical efficacy. Given that JAK pathway activation is commonly observed in many different human malignancies[24], it would predict that genomic screens will identify additional mutations that activate this signaling pathway in hematopoietic and epithelial tumors, The pharmacological inhibition of JAK kinase signaling will be of value in the treatment of human malignancies.

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دور التايروسين كاينيز في تطور سرطان الدم المزمن

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قسم تقنية المختبرات الطبية ، كلية العلوم الطبية التطبيقية ، مركز التميز لبحوث الجينوم الطبي ، جامعة الملك عبد العزيز جدة – المملكة العربية السعودية

المستخلص. ابيضاض الدم (اللوكيميا) يعرف بأنه خلايا الدم البيضاء السرطانية الغير ناضجة في نخاع العظام. ويمكن تقسيم هذا المرض إلى نوعين رئيسين، وهما مرض اللوكيميا الحاد في الخلايا المحببة (ميولويد لوكيميا)، واللوكيميا المزمن في الخلايا المحببة. الميولويد لوكيميا المزمن (chronic myeloproliferative disorders)، ينقسم إلى خمس مجموعات. وهذا النوع هو من الأمراض السرطانية الذي يمكن تشخيصه بسهولة حسب وجود الخلايا الغير ناضجة في نخاع العظام ومن ثم في الدم.

توجد بعض البحوث التي تؤكد أن الطفرات الجينية – التي تحدث في بعض المستقبلات الخلوية الموجودة في الخلايا الجذعية في نخاع العظام، لمرضى سرطان الدم – لها دورًا مهمًا في تطور المرض. ومن ضمن هذه المستقبلات الخلوية، وغير المستقبلات للتايروسين كاينيز (Non receptor tyrosine kinases and Receptor Tyrosine kinases) Jak2 وهو عبارة عن غير مستقبل للتايروسين كاينيز (non Receptor Tyrosine kinases)، وهي تعتبر من البروتينات السابحة في سيتوبلازم الخلية لتلك المستقبلات. وهذه الفئة من المستقبلات لها دور هام في نمو وتكاثر الخلايا الجذعية في نخاع العظام، وعلى هذا الأساس، أي طفرات جينية تحدث في الجين المسؤول عن Jak2 قد تؤدي إلى حدوث خلل في نمو تلك الخلايا الجذعية، مما يؤدي إلى حدوث تطورات في مرضى سرطان الدم.