Prevalence of MPL W515L/K Mutations in Taiwanese Patients With Philadelphia-negative Chronic Myeloproliferative Neoplasms

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Background: The discovery of Janus kinase 2 (JAK2)-V617F has provided important insight into the pathogenesis of Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs); however, the etiology of $JAK2^{V617F}$-negative Ph-negative MPN remains unidentified. MPL^{W515L} and MPL^{W515K} ($MPL^{W515L/K}$) are 2 gain-of-function mutations, which have been found in some Ph-negative MPN patients from Western countries. However, little is known about the incidence of these mutations in Taiwanese Ph-negative MPN patients.

Methods: We determined the MPL sequence of DNA samples from 105 patients, including 88 patients with Ph-negative MPNs and 17 with myelodysplastic syndrome, using polymerase chain reaction amplification of the cytokine receptor MPL exon 10 sequence.

Results: All the patients were normal at codon 515 regardless of their JAK2 status.

Conclusion: The MPL W515L/K mutations are rare in Taiwanese patients with Ph-negative MPNs. [*J Chin Med Assoc* 2010;73(10):530–532]

Key Words: essential thrombocythemia, idiopathic myelofibrosis, MPL mutation, myeloproliferative disorders, polycythemia vera

Introduction

The Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs) are classified by the World Health Organization into polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF), and chronic myeloid leukemia together with rarer subtypes such as chronic neutrophilic leukemia, hypereosinophilic syndrome and chronic eosinophilic leukemia.[1] These disorders share many features, such as hypercellularity of the marrow, unstimulated overproduction of 1 or more lineages of blood cells, increased risk of thrombosis and bleeding, and they may spontaneously convert to acute leukemia, and also to marrow fibrosis.[2] These clonal hematopoietic malignancies are considered to arise from transformation of pluripotent stem cells, and this leads to an increased production of erythrocytes, leukocytes and/or platelets.[3,4] The pathogenesis of Ph-negative MPNs has been investigated but has remained unclear. Several studies have demonstrated a breakthrough finding by detection of an activating mutation V617F in Janus kinase 2 (JAK2) in some Ph-negative MPN patients. This shed new light on the pathogenesis of Ph-negative MPNs.[5–8] This JAK2 V617F mutation was found in 70–90% of patients with PV, in 35–70% with ET, and in 30–50% with IMF. Molecular and clinical evidence has shown that the JAK2 V617F mutation has a direct causal role in the pathogenesis of Ph-negative MPNs.[9,10]

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The discovery of $JAK2^{V617F}$ has provided important insight into the pathogenesis of PV, ET and IMF, but the etiology of $JAK2^{V617F}$-negative Ph-negative MPN remains unknown. Recently, sequence analysis of the erythropoietin receptor, thrombopoietin receptor and granulocyte-colony stimulating factor receptor in JAK2-negative PV, ET and IMF led to the discovery of a somatic tryptophan-to-leucine substitution mutation at the transmembrane juxtamembrane junction of the thrombopoietin receptor MPL (MPL^{W515L}).[11] In addition, a small number of patients have an alternate mutation at codon 515, which results in a tryptophan-to-lysine substitution (MPL^{W515K}).[12] Expression of MPL^{W515L} in recipient mice results in a myeloproliferative disease with similarities to human IMF, including reticulin fibrosis, megakaryocytic hyperplasia, splenomegaly, and extramedullary hematopoiesis. Similar to $JAK2^{V617F}$, MPL^{W515L} is an acquired mutation that induces cytokine-independent growth and thrombopoietin hypersensitivity, and results in constitutive phosphorylation of JAK2, STAT3, STAT5, AKT, and ERK. The frequency of the gain-of-function mutation $MPL^{W515K/L}$ was reported to be 1% in ET and 5% in IMF in the USA.[12] Some patients may harbor coexisting $JAK2^{V617F}$ and $MPL^{W515K/L}$ mutations.[13,14] Although $MPL^{W515L/K}$ mutations are much less common than $JAK2^{V617F}$, the detection of MPL^{W515L} in MPN patients may provide valuable information. We have determined the frequency of the $JAK2^{V617F}$ mutation in Taiwanese patients,[15] but the frequency of MPL^{W515} mutations has not yet been determined. Therefore, we conducted this study to determine the prevalence of $MPL^{W515K/L}$ mutations in Taiwanese patients with Ph-negative MPN and myelodysplastic syndrome.

Methods

Genomic polymerase chain reaction (PCR)

This study was approved by the institutional ethics committee of Taipei Veterans General Hospital, Taiwan. Peripheral blood samples were collected from a total of 110 patients and informed consent was obtained from all patients.

Total DNA was isolated from leukocytes from patients' peripheral blood using a QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. The human MPL exon 10 sequence was amplified from 0.5–1.0 μg of genomic DNA by PCR using specific primers (forward primer: 5′ TGGGCCGAAGT CTGACCCTTT 3′; reverse primer: 5′ ACAGAGCGAA CCAAGAATGC-CTGT 3′).[10] The reaction was performed for 30 cycles with a standard protocol and an annealing temperature of 60°C. The products were resolved on 2.5% agarose gels and visualized by ethidium bromide staining. The specific band was purified and sequenced by an ABI PRISM 3100 Genetic Analyzer (ABI Applied Biosystems, Foster City, CA, USA).

Results

We extracted genomic DNA from the peripheral blood of 105 patients. Of these patients, 88 were Ph-negative MPN (Table 1) and 17 had myelodysplastic syndrome. Diagnosis of ET, PV and IMF followed the criteria of the Polycythemia Vera Study Group.[16,17]

After PCR amplification of the cytokine receptor MPL exon 10 and subsequent sequencing, we found

Table 1. Characteristics of 88 patients with Philadelphia-negative chronic myeloproliferative neoplasms

	Disease subtype		
	PV ($n=33$)	ET ($n=49$)	IMF ($n=6$)
Sex (M/F)	22/11	27/22	5/1
Age (yr)	68 ± 13	67 ± 14	72 ± 16
Median disease duration (mo)	55 (14–273)	35 (10–180)	124 (70–264)
WBC ($\times 10^9$/L)	12.1 ± 6.1	8.9 ± 4.8	8.9 ± 7.1
Hb (g/dL)	14.2 ± 2.1	12.6 ± 2.2	9.0 ± 1.8
Platelets ($\times 10^9$/L)	442 ± 338	583 ± 227	56 ± 32
Erythropoietin	7.7 ± 3.3	15.5 ± 11.6	18.4 ± 12
BM fibrosis (+)	37.5%	40%	100%
Splenomegaly	55%	53%	100%
CAD or HT (+)	54.5%	42.9%	33.3%
JAK2 V617F (+)	28/33 (85%)	29/49 (59%)	2/6 (33%)
Thrombosis/hemorrhage	7/1	13/1	0/1

PV = polycythemia vera; ET = essential thrombocythemia; IMF = idiopathic myelofibrosis; WBC = white blood cell count; Hb = hemoglobin; BM = bone marrow; CAD = coronary artery disease; HT = hypertension; JAK2 = Janus kinase 2.

that all the patients showed a normal codon at allele 515.

Discussion

Our data showed that 88 patients with Ph-negative MPN did not have $MPL^{W515L/K}$ mutations. Consistent with our results, Hsiao et al also reported that $MPL^{W515L/K}$ mutations could not be found in 53 Taiwanese patients with ET.[18] Pardanani et al suggested that MPL^{W515} mutations are disease-specific.[12] In the USA, they are prevalent in 5–9% of patients with IMF and in 1% of ET patients.[11,12] Similarly, Hu et al reported that 7% of American patients with IMF showed these 2 mutations.[19] However, we could not detect $MPL^{W515L/K}$ mutations in 6 patients with IMF.

It appears that the genetic origins of IMF represent the culmination of multiple genetic and possibly epigenetic events. A number of laboratories have suggested that additional genetic events might play a role in this process.[20–22] Comparative genomic hybridization studies have shown that gains of cytogenetic material occur in more than 50% of patients with IMF and most commonly involve gains of 9p, 2q, 3p, chromosome 4, 12q and 13q.[22] Furthermore, Dingli et al have identified an unbalanced translocation between chromosomes 1 and 6 with specific breakpoints (1,6) that they believe are highly specific to IMF.[20] These chromosomal sites may harbor additional genes that play a role in the origins of IMF. However, further studies with more patients are needed to determine the incidence and role of $MPL^{W515L/K}$ mutations in Taiwanese patients with IMF.

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