p38 Mitogen-Activated Protein Kinase Has Different Degrees of Activation in Myeloproliferative Disorders and Myelodysplastic Syndromes

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Key Words: p38 Mitogen-activated protein kinase; Myeloproliferative disorder; Myelodysplastic syndrome

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Abstract

The goal of the present study was to evaluate the activation patterns of p38 mitogen-activated protein kinase (MAPK) in myeloproliferative disorders (MPDs) and myelodysplastic syndromes (MDSs). Phosphorylated (activated) p38 MAPK was analyzed immunohistochemically in formalin-fixed decalcified bone marrow core biopsy specimens from 32 MPD, 33 MDS, and 11 control cases. Moderate p38 activation was commonly seen in MDS, whereas weak p38 activation was seen in all MPD cases and all control cases but 1 in the erythroid lineage. In myeloid and megakaryocytic lineages, strong p38 activation was more commonly observed in MDS compared with MPD (myeloid, 22/33 vs 2/32; P < .0001; megakaryocytic, 18/23 vs 5/32; P < .0001) and control (myeloid, 22/33 vs 211; P = .012; megakaryocytic, 18/23 vs 3/9; P = .035) cases. Furthermore, weak p38 activation was observed in myeloid and megakaryocytic lineages in MPD compared with control (myeloid, 15/32 vs 1/11; P = .033; megakaryocytic, 16/32 vs 0/9; P = .007) cases. Increased p38 MAPK activation may have a role in inhibiting hematopoiesis leading to cytopenias in MDS, and relatively decreased p38 activation in MPD might promote hematopoiesis, resulting in cytosis.

p38 mitogen-activated protein kinase (MAPK), a member of the MAPK family, is activated by stress stimuli, cytokines, and growth factors via phosphorylation of Thr180/Tyr182 sites. Activated p38 MAPK mediates important and diverse cellular functions, including activation of transcription factors and regulation of cytokine production, the cell cycle, cell differentiation, cell movement, apoptosis, and cell death.1-4

The function of p38 in normal human hematopoiesis is primarily inhibitory, mediating proapoptotic and growth inhibitory signals. For example, the p38 pathway is activated in response to treatment of human hematopoietic cell lines with inhibitory cytokines such as type I interferons (IFNs), IFN-α and IFN-β5,6 tumor necrosis factor α7 transforming growth factor β8 and type II IFNs.7 In addition, it has been suggested that most hematopoietic growth factors and myelosuppressive cytokines exert their effects in regulating and balancing normal hematopoiesis through activation of the MAPK pathways.7-11 Furthermore, p38 MAPK can further regulate the expression of cytokines that activate themselves, making it a complicated yet efficient regulation loop.12

Myelodysplastic syndromes (MDSs) are characterized by refractory cytopenias due to ineffective hematopoiesis in the bone marrow, leading to considerable morbidity and mortality.13-15 Recently, overactivation of p38 MAPK has been implicated in the pathophysiology of MDSs, most likely by inhibiting hematopoiesis in vitro. Katsoulidis et al16 showed that p38 MAPK inhibitor enhances hematopoietic colony formation from the bone marrow of patients with MDS. Navas et al17 reported similar findings in which pharmacologic inhibition of p38-α led to a dose-dependent increase in erythroid and myeloid colony formation in bone marrow from patients with MDS in vitro.
MATERIALS AND METHODS

Cases and Bone Marrow Biopsy Specimens

We studied 32 MPD and 33 MDS cases. All cases were retrospectively selected by reviewing our files from 1996 to 2005, and only cases with an adequate amount of biopsy tissue were included. The pathologic and clinical findings of these cases were reviewed, and the diagnoses were confirmed and classified according to the World Health Organization classification scheme. The MPD cases included 14 cases of CML; 4 of PV; 4 of ET; 5 of myelofibrosis; and 5 of MPD, unclassifiable. The MDS cases included 1 case of refractory anemia (RA); 4 of RA with ringed sideroblasts; 12 of RA with excess blasts; 8 of acute myelogenous leukemia transformed from MDS; 2 of refractory cytopenia with multilineage dysplasia; 2 of MDS associated with isolated del(5q) chromosome abnormality (5q– syndrome); 3 of therapy-related MDS; and 1 of MDS, unclassifiable. The control samples were from 11 cases with bone marrow core biopsy specimens free of MPD, MDS, leukemia, and other hematologic malignancies. The 11 patients were chemotherapy-naive with lymphoma whose bone marrow core biopsy specimens were obtained for staging purposes. Approval of the study was obtained from the institutional review board of the Methodist Hospital, Houston, TX.

Immunohistochemical Analysis for the Expression of p38 MAPK

p38 is activated by phosphorylation, and phosphorylated p38 MAPK was analyzed by immunohistochemical analysis using an antibody specific to phosphorylated p38 MAPK as a marker for activation (phosphorylated p38 MAPK, Thr180/ Tyr182 [12F8], Cell Signaling Technology, Danvers, MA). The specificity of this protein has been validated by using Western blot.20 Formalin-fixed, decalcified bone marrow core biopsy specimens from MPD, MDS, and control cases were obtained. Total p38 MAPK expression was also evaluated by immunohistochemical analysis using an antibody targeting p38 MAPK regardless of phosphorylation status (Cell Signaling Technology) in subsets of these cases.

The antigen-retrieval procedure consisted of steaming slides for 30 minutes in DakoCytomation Target Retrieval solution, pH 9.0, that was ready to use (DakoCytomation, Carpinteria, CA). All slides were immersed in 3% hydrogen peroxide for 10 minutes to block the endogenous peroxidase. Sections were then incubated at 4°C overnight with phosphorylated p38 antibody at a dilution of 1:25 and the total p38 antibody at a dilution of 1:10. After rinsing, sections were incubated with the secondary antibodies, DAKO EnVision+ Dual Link System HRP (DAKO, Carpinteria, CA), for 30 minutes. Sections were washed 3 times with phosphate-buffered saline, followed by application of the liquid Diaminobenzidine (DAB) Substrate Pack (DAKO) according to the manufacturer’s instructions. Appropriate positive and negative controls were used to ensure the accuracy of the staining.

Interpretation of Immunohistochemical Staining

Subsets of cases from MPD (CML, PV, ET, myelofibrosis, and MPD, unclassifiable) and control cases were evaluated for total p38 expression, and there were no observed differences in total p38 MAPK staining, suggesting that the total p38 MAPK protein was equally expressed in all cases. Therefore, to be cost- and effort-effective, further analysis was done only for phosphorylated p38 MAPK. Inactivated p38 has been shown to be located in the cytoplasm, but following its phosphorylation, the activated p38 is translocated to the nucleus for its transcriptional regulatory function.21-30

The degrees of p38 MAPK activation in erythroid, myeloid, or megakaryocytic lineages were systematically reviewed in a semiquantitative manner by 2 of us (M.S. and C.-C.C.), without knowledge of diagnosis, by scoring the percentage of cells showing nuclear and cytoplasmic staining of phosphorylated p38 MAPK in each lineage. We considered any cells with recognizable nuclear staining with a 20× objective lens as positive staining, regardless of staining intensity. In cases classified as acute myelogenous leukemia transformed from MDS, we analyzed only lineage-specific cells that could be readily identified based on morphologic features for the purpose of comparison with other cases.
Representative examples showing different degrees of phosphorylated p38 immunohistochemical staining in myeloproliferative disorder (MPD) and myelodysplastic syndrome (MDS) in each lineage of hematopoietic cells. Erythroid cells (arrows) showing weak to virtually absent staining in MPD (A, ×40) but strong staining in MDS (D, ×40); myeloid cells (arrows) with weak staining in MPD (B, ×40) but strong staining in MDS (E, ×40); and megakaryocytes (arrows) with moderate staining in MPD (C, ×40) but strong staining in MDS (F, ×40).
The entire marrow core biopsy sample of each case was evaluated by visual estimation and recorded in 10% increments. Any discrepancies were resolved by reviewing the slides with a multihedged microscope to achieve consensus. Although the recorded values were based on estimation done in 10% increments, they included evaluation of the entire bone marrow core and would include a minimum of 2,000 cells, including a minimum of 20 megakaryocytes, evaluated. “Weak,” or 1+, staining was defined as 0% to 20% of the cells staining with phosphorylated p38. “Moderate,” or 2+, and “strong,” or 3+, staining were defined as 21% to 50% and more than 50% of the cells staining with phosphorylated p38, respectively. Differences in proportions of degrees of p38 activation among MDS, MPD, and control cases in each lineage were tested by using the Fisher exact test.

### Results

Different degrees of nuclear and cytoplasmic expression of phosphorylated p38 staining were observed in 3 cell lineages (erythroid, myeloid, and megakaryocytes) among MDS and MPD cases (Image 1). As shown in Table 1, the bone marrow control cases showed weak (10/11) to moderate (1/11) p38 MAPK activation in the erythroid lineage, mostly moderate (8/11) activation in the myeloid cell lineage, and moderate (6/11) to strong (3/11) activation in megakaryocytic cells. The majority of the MPD cases showed weak (32/32) p38 activation in the erythroid lineage, weak (15/32) to moderate (15/32) p38 MAPK activation in myeloid cells, and weak (16/32) to moderate (11/32) activation in megakaryocytic cells. On the other hand, as shown in Table 2, in MDS

### Table 1
**Degree of Phosphorylated p38 Activation in MPD (Including Subtypes) Compared With Control Cases**

<table>
<thead>
<tr>
<th>Cell Lineage/ Degree of Staining</th>
<th>MPD (n = 32)</th>
<th>Control Cases (n = 11)</th>
<th>CML (n = 14)</th>
<th>ET (n = 4)</th>
<th>MF (n = 5)</th>
<th>PV (n = 4)</th>
<th>MPD-U (n = 5)</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td><strong>Erythroid</strong></td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
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<td>0 (0)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<td></td>
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<tr>
<td>Weak (1+)</td>
<td>15 (47)</td>
<td>1 (10)</td>
<td>5 (36)</td>
<td>1 (25)</td>
<td>4 (80)</td>
<td>2 (60)</td>
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<td></td>
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<td>1 (7)</td>
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<td>1 (20)</td>
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<tr>
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<td>5 (16)</td>
<td>3 (27)</td>
<td>1 (7)</td>
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<td>0 (0)</td>
<td>1 (25)</td>
<td>2 (40)</td>
<td>.341</td>
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</table>

CML, chronic myelogenous leukemia; ET, essential thrombocythemia; MF, myelofibrosis; MPD, myeloproliferative disorders; MPD-U, MPD, unclassifiable; PV, polycythemia vera.

* Data are given as number (percentage).

† Fisher exact test.

### Table 2
**Degree of Phosphorylated p38 Activation in MDS (Including Subtypes) Compared With Control Cases**

<table>
<thead>
<tr>
<th>Cell Lineage/ Degree of Staining</th>
<th>MDS (n = 33)</th>
<th>Control Cases (n = 11)</th>
<th>RA (n = 1)</th>
<th>RARS (n = 4)</th>
<th>RAEB (n = 12)</th>
<th>MDS-AML (n = 8)</th>
<th>RCMD (n = 2)</th>
<th>5q− Syndrome (n = 2)</th>
<th>t-MDS (n = 3)</th>
<th>MDS-U (n = 1)</th>
<th>P*</th>
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<tr>
<td><strong>Erythroid</strong></td>
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<tr>
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<td>10 (91)</td>
<td>1 (100)</td>
<td>1 (25)</td>
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<td>0 (0)</td>
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<td><strong>Myeloid</strong></td>
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<tr>
<td>Weak (1+)</td>
<td>0 (0)</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<td>7 (58)</td>
<td>7 (88)</td>
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</table>

MDS, myelodysplastic syndrome; MDS-AML, acute myelogenous leukemia transformed from MDS; MDS-U, MDS, unclassifiable; NE, not evaluable; RA, refractory anemia; RAEB, RA with excess blasts; RARS, RA with ringed sideroblast; RCMD, refractory cytopenia with multilineage dysplasia; t-MDS, therapy-related MDS.

* Data are given as number (percentage). 5q− syndrome is an MDS associated with isolated del (5q) chromosome abnormality.

† Fisher exact test.
cases, p38 activation was weak (9/33) to moderate (24/33) in the erythroid lineage, moderate (11/33) to strong (22/33) in myeloid cells, and moderate (4/33) to strong (18/33) in the megakaryocytic lineage.

When we compared the staining patterns of MDS cases with those of MPD and with control cases, significant differences in p38 activation patterns were observed. In myeloid and megakaryocytic lineages, strong p38 activation was more commonly observed in MDS than in MPD cases (myeloid, 22/33 vs 2/32; P < .0001; megakaryocytic, 18/23 vs 5/32; P < .0001). In contrast, weak p38 activation was more commonly observed in myeloid and megakaryocytic lineages in MPD compared with control cases (myeloid, 15/32 vs 1/11; P = .033; megakaryocytic, 16/32 vs 0/9; P = .007) (Table 2). In contrast, weak p38 activation was more commonly observed in myeloid and megakaryocytic lineages in MPD compared with control cases (myeloid, 15/32 vs 1/11; P = .033; megakaryocytic, 16/32 vs 0/9; P = .007) (Table 2). In the erythroid cells, moderate p38 activation was seen in the vast majority of MDS cases compared with MPD (24/33 vs 0/32; P < .0001) and control (24/33 vs 1/11; P = .0003) cases. There were no significant differences in p38 MAPK activation in the erythroid lineage between MPD and control cases. We did not observe significant differences in p38 MAPK activation in any of the cell lineages among different subtypes of MPD or MDS, although the number of cases in each subtype was relatively small.

**Discussion**

In this study, we investigated the potential involvement of the p38 MAPK signaling pathway in the pathophysiology of MPD and MDS by examining the degrees of activation of all 3 hematopoietic cell lineages by using antibody specific to phosphorylated (activated) p38 MAPK. Our unique approach by evaluating p38 MAPK expression in all lineages based on the morphologic characteristics of each lineage has allowed us to support the findings of previous in vitro studies regarding the role of p38 MAPK in hematopoiesis. The results demonstrate that higher degrees of phosphorylated p38 MAPK expression are observed in MDS cases than in MPD and control cases in all lineages. These results agree with previous in vitro findings that increased p38 MAPK activation is observed in CD34+ cells from MDS cases and that inhibition of p38 MAPK restores the erythropoiesis and myelopoiesis in patients with MDS. Taken together, these results suggest that increased p38 MAPK activation may play a role in inhibiting erythropoiesis and myelopoiesis leading to anemia and neutropenia in patients with MDS.

Of importance, decreased p38 MAPK expression was observed in the majority of MPD cases as compared with control cases. We speculate that the decreased p38 MAPK activation in MPD may result in less inhibition of hematopoiesis and, thus, lead to the cytosis seen in MPD. In addition, our data suggest that p38 MAPK activation may also inhibit megakaryopoiesis because most MDS cases showed increased p38 MAPK activation in megakaryocytes while most MPD cases showed decreased p38 MAPK activation compared with control cases. Recent studies have demonstrated the p38 MAPK pathway as one of the intracellular signaling pathways involved in megakaryopoiesis, and in vitro pharmacologic inhibition of p38 has been shown to promote megakaryocytic differentiation of human leukemia cell lines. It would have been interesting to correlate the degrees of p38 expression in different lineages with the degrees of cytosis or cytopenias in different lineages to further support our hypothesis. However, this was not performed owing to the lack of CBC counts in a substantial portion of cases.

Of note, there were no significant differences in p38 MAPK expression status in the subtypes of MPD and cases of MDS in the present study. Although the number of cases in each subtype was relatively small, this finding suggests that the dysregulation of p38 MAPK activation may represent a common converging point for different mechanisms that may lead to inhibition or disinhibition (promotion) of hematopoiesis. This finding may have therapeutic implications because manipulating the p38 MAPK pathway may represent an effective and targeted method for treating all subtypes of MDS or MPD.

The mechanisms leading to this distinct difference in p38 MAPK activation in MPD and MDS remain to be fully elucidated. It is well established that various cytokines and growth factors that inhibit or promote hematopoiesis activate MAPK signaling pathways to generate their effects. We suspect that intracellular alterations in 1 or more of these cytokines or growth factors may be responsible for such a mechanism.
When interpreting the results of this study, some possible limitations should be considered. For the majority of MDS and MPD cases included in the study, we did not have data on possible therapeutic interventions that might have been introduced before bone marrow core biopsies were done. These interventions may include chemotherapeutic agents or hematopoietic growth factors that may directly or indirectly alter the p38 MAPK pathway, thereby modifying the degree of p38 expression in erythroid, myeloid, or megakaryocytic cell lineages. Consequently, the degree of p38 activation that was observed in our study may not reflect the true activation pattern expected in MDS or MPD. Also, bone marrow core biopsy specimens from patients with untreated lymphoma were used as control samples. Since the role of the p38 MAPK pathway in the pathogenesis and pathophysiology of lymphomas has not been well established, it is unclear whether the levels of p38 activation seen in the control cases were the result of an altered or dysregulated p38 signaling pathway. Of note, most of the MDS cases studied were high-grade MDS cases. Further studies with more cases of low-grade MDS are needed to confirm whether our findings are applicable to low-grade MDS as well. Similarly, the majority of the MPD cases in our study were CML. Future studies with more cases of ET and PV are needed to confirm our observations.

Our results indicate that phosphorylated p38 MAPK expression is significantly increased in MDS and decreased in MPD, suggesting that the p38 MAPK signaling pathway may have an important role in the pathogenesis of MDS and MPD. Future prospective studies with a larger sample are warranted to confirm our observations and to investigate the mechanisms leading to different p38 MAPK activation patterns in these diseases. Results from such studies may have implications in the search for possible therapeutic targets involving the p38 pathway in MDS and MPD.

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References


