Membrane localization is not required for Mpl function in normal hematopoietic cells


Cellular trafficking of growth factor receptors, including cross-talk among receptors at the cell surface, may be important for signal transduction in normal hematopoietic cells. To test this idea, the signaling domain of Mpl (the thrombopoietin receptor) was targeted to the plasma membrane, or to the cytoplasm of murine marrow cells, and the ability of the cells to proliferate and differentiate in response to Mpl dimerized at the plasma membrane or free in the cytoplasm was assessed. Constructs encoding the signaling domain of Mpl linked to an FK506 binding protein domain (to permit dimerization by the membrane-permeable ligand AP20187)

Introduction

Hematopoietic growth factor receptors are transmembrane glycoproteins located in the plasma membrane of the cell. Binding of growth factor causes receptor dimerization and recruitment and activation of signaling molecules, thus initiating signal transduction.1 After ligand binding, growth factor receptors cluster in clathrin-coated pits, become internalized, and are targeted for degradation or recycled to the cell surface. The cell surface location of hematopoietic growth factor receptors permits communication with the extracellular milieu. Other attributes of cell surface location of these receptors that may facilitate signal transduction include organization into specific microdomains on the cell surface,2-3 preassembled signaling complexes,6,7 cross-talk among heterologous cytokine receptors,8-10 and commencement of normal intracellular trafficking of the receptor-ligand complex.11-14

A stringent test of growth factor receptor function is the ability to support the proliferation and differentiation of normal hematopoietic cells. We hypothesized that the location of a hematopoietic growth factor receptor in the plasma membrane was important not only for communication with the extracellular milieu but also for expression of the full repertoire of receptor functions. The availability of membrane-permeable agents that can bind and dimerize FK506 binding protein 12 (FKBP12) domains provided a mechanism to test this hypothesis.15-17,49,50 We linked the 121-amino acid signaling domain of the murine thrombopoietin receptor Mpl to a modified FKBP12 and targeted the constructs to the plasma membrane or to the cytoplasm of normal murine marrow cells. We found that the activation of Mpl in the cell cytoplasm or at the plasma membrane supported long-term proliferation of the cells, generation of myeloid, erythroid, and megakaryocytic progenitor cells, and terminal maturation of the cells.

Interactions between growth factor receptors and signaling molecules are normally subject to precise spacial and temporal regulation within the cell,18 and there is considerable cross-talk among hematopoietic growth factor receptors. However, the current report documents that normal cellular localization and trafficking of Mpl are not required for proliferation or differentiation in primary hematopoietic cells.

Materials and methods

Plasmid construction

The construct encoding membrane-targeted Mpl, containing a myristylation sequence, a modified FKBP12 domain, the 121-amino acid signaling domain of murine Mpl, and a C-terminal hemagglutinin HA epitope tag, is shown in Figure 1.16,17 The modified FKBP12 domain (FKBP12 with a phenylalanine-to-valine substitution at amino acid 36) allows for binding of the dimerizer AP20187.16,17,36 Membrane targeting is achieved by incorporating

From the Department of Medicine, Division of Hematology, University of Washington, Seattle; and the Department of Biochemistry, Howard Hughes Medical Institute, St Jude Children’s Research Hospital, Memphis, TN.

Submitted January 2, 2001; accepted May 10, 2001.

Supported by National Institutes of Health grants R01DK52997, R01DK57525, P01HL53750, P01DK47754, and DK49855 and by an award from the Fanconi Anemia Research Fund.

Reprints: C. Anthony Blau, Department of Medicine, University of Washington, Box 357710, 1959 NE Pacific St, Seattle, WA 98195; e-mail: tblau@u.washington.edu.

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localized to the cytoplasm. Both constructs encode an FKBP12 domain harboring a cytoplasmic Mpl construct lacks a myristylation motif and is, therefore, predicted to be dimerized with a stoichiometry of 2:1. Red indicates myristylation peptide; hatched, FKBP12 domain; yellow, Mpl signaling domain; black, HA epitope tag.

Western blotting and signaling studies
Ba/F3 cells expressing wild-type Mpl or the membrane-tethered or cytoplasmic Mpl constructs were cultured in RPMI containing 10% FCS, penicillin, streptomycin, and murine IL-3 until a cell density of 5 to 7 × 10^3/mL was achieved with viability greater than 98% assessed by trypan blue exclusion. Cells were washed twice in RPMI and resuspended in RPMI with 0.5% bovine serum albumin (BSA) at a concentration of 2 × 10^6 cells/mL. After 6 hours of starvation, the cells containing wild-type Mpl were stimulated with thrombopoietin (20 ng/mL) and those containing dimerizer motifs were exposed to AP20187 (100 nM) for 1 to 180 minutes. Cells were washed twice in ice-cold phosphate-buffered saline, and total cell lysates were generated as previously described. Protein concentrations were measured using a modified Lowry assay (Bio-Rad, Hercules, CA). For JAK2 analysis, 1 mg total protein was immunoprecipitated with JAK2 antisera (2 µL; Upstate Biotechnology, Lake Placid, NY), and immune complexes were collected with Protein A-Sepharose beads (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoprecipitated protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% acrylamide gels. After transfer to nitrocellulose membranes, the blots were probed with an antiphosphotyrosine antibody (4G10; Upstate Biotechnology) and visualized by chemiluminescence. For JAK2 analysis, 1 mg total protein was immunoprecipitated with JAK2 antisera (2 µL; Upstate Biotechnology), and immune complexes were collected with Protein A-Sepharose beads (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoprecipitated protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% acrylamide gels. After transfer to nitrocellulose membranes, the blots were probed with antibodies specific for phospho-JAK2 (generous gift from David Frank, Boston, MA) or doubly-phosphorylated (active) MAPK (Promega, Madison, WI). Blots were visualized by chemiluminescence using goat anti–rabbit immunoglobulin G (IgG) conjugated to HRP (Bio-Rad). Membranes were stripped and reprobed with a STAT5 antibody (Santa Cruz) and Erk1/2 antibody (Upstate Biotechnology) to demonstrate equivalent loading of lysates in each lane.

Retroviral transduction of Ba/F3 cells
Supernatant was collected from confluent retroviral producer cell clones packaging virus encoding either the membrane-targeted or the cytoplasmic form of Mpl. Retroviral supernatants were filtered through a 0.45 µm filter (Millipore), and 3 mL supernatant was added to a mixture of 2 × 10^5 Ba/F3 cells in 7 mL RPMI composed of 10% FCS and 5% conditioned media containing murine interleukin-3 (IL-3) (Stem Cell Technologies, Vancouver, BC, Canada). Polybrene was added to a final concentration of 8 µg/mL, and the cells were incubated for 48 hours at 37°C in a humidified incubator containing 5% CO2. After 48 hours of prestimulation with IL-3, IL-6, and stem cell factor, the cells were transferred onto irradiated (1500 cGy) retroviral producer cells and were cocultivated using identical growth conditions except for the addition of polybrene (8 µg/mL). Marrow cells were harvested after 48 hours of cocultivation and maintained in suspension culture. Transduction efficiency was assessed in colony assays (described below) using G418 (Gibco/BRL) at a concentration previously shown to prevent colony formation by nontransduced cells (800 µg/mL).

Suspension cultures
After retroviral transduction, marrow cells were cultured in Iscoves modified Dulbecco medium (IMDM) containing 10% FCS, 50 U/mL penicillin, and 50 µg/mL streptomycin either in the presence or absence of AP20187 (100 nM). AP20187 was a gift of ARIAD Pharmaceuticals (Cambridge, MA). Cell numbers were determined on the days indicated.

Hematopoietic colony assays
The numbers of erythroid (burst-forming units [BFU]-E), myeloid (CFU-granulocyte macrophage [GM]), and megakaryocytic (CFU-Meg) progenitor cells in the suspension cultures of the transduced marrow cells were quantitated in hematopoietic colony assays. Cells were washed free of AP20187 before plating. To quantitate BFU-E, the cells were plated in 1.4% methylcellulose (Dow Chemical, Midland, MI) in IMDM supplemented with 30% fetal bovine serum (FBS) and 10 ng/mL SCF.
with 25% FCS, 1% BSA, 5 × 10⁻⁵ M β-mercaptoethanol (Sigma Chemical, St Louis, MO), 1% penicillin–streptomycin–fungizone (Sigma), rat stem cell factor (100 ng/mL; a gift from Amsgen, Thousand Oaks, CA), and erythropoietin (2 U/mL). Plates were incubated at 37°C in a humidified incubator supplemented with 5% CO₂ and B/13-E-treated colonies were counted on day 8. Myeloid colonies (CFU-GM) were quantitated by plating the cells in 1.4% methylcellulose in IMDM supplemented with 25% FCS, 1% BSA, and murine IL-3 (100 U/mL; a gift from Dr Ken Kaushansky, University of Washington, Seattle). Colonies were counted on day 5. Megakaryocytic colonies (CFU-Meg) were quantitated by plating the cells in 0.28% agar (Difco, Detroit, MI) in IMDM supplemented with 15% horse serum (HyClone, Logan, UT), 5 × 10⁻⁵ M β-mercaptoethanol, murine thrombopoietin (10 ng/mL; a gift from Dr Ken Kaushansky), IL-3 (10 U/mL), and stem cell factor (10 ng/mL). Colonies were counted on day 5.

**JAK2-deficient fetal liver cells**

Fetal liver cells were obtained from homozygous JAK2-deficient or wild-type littermate day 12.5 embryos. Similar studies were performed with fetal liver cells obtained from homozygous STAT5 A/B-deficient mice.28

**Detection of Mpl fusions in murine marrow cells by confocal microscopy and by flow cytometry**

Marrow cells transduced with either the cytoplasmic or the membrane-targeted Mpl construct were fixed in 0.5% paraformaldehyde and washed in saline. They were labeled with an anti-HA monoclonal antibody (10 μg/mL, BABBIO, Richmond, CA) for 20 minutes in the presence of 0.05% Triton X-100, then washed and labeled with a biotin-conjugated goat anti–mouse IgG followed by streptavidin–fluorescein isothiocyanate (FITC) (both from Jackson Immunoresearch, West Grove, PA). Nuclei were stained with propidium iodide (5 μg/mL) in 0.1% citrate, 0.05% Triton X-100, and 10 μg/mL DNase-free RNase (Boehringer Mannheim, Indianapolis, IN). Fluorescence was analyzed using a confocal microscope (Radiance 2000;Bio-Rad).

**Cellular fractionation and Mpl detection in COS-1 cells**

Cellular fractions were collected as reported by Koury et al.29 COS-1 cells were transfected with the membrane-targeted or the cytoplasmic Mpl constructs using the diethylaminoethyl dextran method,30 and 1 × 10⁷ cells were harvested using the sucrose step gradient, suspended in saline, and pelleted. Cells were incubated on ice in swelling buffer (including protease inhibitors) and homogenized with 50 strokes in a prechilled dounce homogenizer. Lysed cells were adjusted to 0.25 M sucrose, and the nuclei were pelleted. The supernatant was used for immunoprecipitations of cytoplasmic proteins with the anti-HA antibody, whereas the membrane pellet was floated on a sucrose enriched gradient (Bio-Rad) and detected with the primary HA antibody and secondary antibody goat anti–mouse HRP (Bio-Rad). Chemiluminescence detection (Amersham, Piscataway, NJ) was performed.

**Results**

**Dimerization of Mpl at the plasma membrane or in the cytoplasm stimulates proliferation in Ba/F3 cells**

G418-resistant Ba/F3 cell clones expressing either form of the Mpl fusion were isolated and tested for their ability to proliferate in response to the dimerizer AP20187. Proliferation assays were performed on individual clones. Mock-transduced Ba/F3 cells did not proliferate in response to AP20187 (data not shown). In contrast, the addition of AP20187 to cells expressing either form of Mpl stimulated a dose-dependent proliferative response (Figure 2A). Ba/F3 cell clones expressing the cytoplasmic or the membrane-targeted form of Mpl proliferated over a range of concentrations of AP20187. Cells expressing either form of Mpl proliferated at an AP20187 concentration as low as 1 nM. Previous work by our laboratory has shown that high concentrations of a monomeric antagonist of the receptor can competitively inhibit the proliferative response by inhibiting dimerization.21 These results suggest that dimerization of Mpl, in the absence of membrane localization, is sufficient to activate proliferative signaling in Ba/F3 cells.

**Signal transduction by membrane-tethered or cytoplasmic Mpl**

Signal transduction experiments were performed with Ba/F3 cell clones expressing either version of Mpl to look for activation of known signaling intermediaries of the thrombopoietin–Mpl system. No detectable phosphorylation of JAK2, STAT5, or ERK1/2 in either of the modified forms of Mpl was observed; this is in sharp contrast to thrombopoietin-stimulated Ba/F3 cells expressing the wild-type receptor that activated these molecules efficiently (Figure 2B).

**Ability of membrane-tethered or cytoplasmic Mpl to support proliferation of normal hematopoietic cells**

Post-5-Fluorouracil murine marrow cells were cocultivated with retroviral producer cell lines for the membrane-targeted or the cytoplasmic versions of Mpl (Figure 1). Transduction efficiency rates (assayed by culturing the cells in the presence of G418) were 52% for membrane-tethered Mpl and 57% for cytoplasmic Mpl. To determine whether the Mpl signaling domain expressed freely in the cytoplasm could support the proliferation of
normal hematopoietic cells, these 2 populations of transduced cells were cultured in IMDM supplemented with 10% FCS and the dimerizer AP20187 (100 nM). Dimerization of cytoplasmic Mpl supported the proliferation of primary hematopoietic cells as effectively as dimerization of the membrane-tethered Mpl (Figure 3), resulting in more than a 10^7-fold increase in total cell numbers in a 50-day period in vitro.

To quantitate Mpl fusion protein expression, marrow cells expressing membrane-targeted Mpl, cytoplasmic Mpl, or a full-length form of Mpl with a C-terminal HA epitope tag were labeled with an anti-HA monoclonal antibody and a second antibody, as described above, and were analyzed by flow cytometry. Both the membrane-targeted Mpl fusion and the cytoplasmic Mpl fusion were expressed at approximately 50% of the level of the full-length Mpl. Murine marrow cells expressed approximately 1300 copies of full-length Mpl (assessed by Western blotting and compared with BaF3-Mpl cells, in which Mpl receptor display had been quantitated by Scatchard analysis of sodium iodide 125I-thrombopoietin binding). These results indicate that the expression levels of the membrane-targeted Mpl fusion and cytoplasmic Mpl fusion—fewer than 1000 copies per cell—are within the range of Mpl expression in normal megakaryocytes (12 000 Mpl receptors per cell) and normal platelets (25-200 receptors per platelet).

Growth rates of the cells expressing membrane-targeted or cytoplasmic forms of Mpl were identical. Sustained growth of both populations of cells was observed for more than 100 days (data not shown). Cell proliferation remained dimerizer dependent: when the cells were washed free of dimerizer and were resuspended in IMDM with 10% FCS, cell death ensued within 4 days. These results demonstrate that even with a low level of expression of the Mpl fusion proteins in normal hematopoietic cells (fewer than 1000 copies of the Mpl fusion protein per cell), dimerization of the Mpl signaling domain, engineered to be expressed free in the cytoplasm, can support long-term proliferation of normal hematopoietic cells.

Cellular localization of Mpl fusion proteins

Confocal microscopy was used to examine cellular localization of the 2 forms of Mpl in the transduced murine marrow cells (Figure 4). Cells that had been in suspension culture for approximately 100 days in the presence of AP20187 were labeled with an anti-HA monoclonal antibody, then by a biotinylated goat antimouse antibody followed by streptavidin conjugated to FITC, and then viewed under a confocal microscope. Distinct patterns of fluorescence are evident. Cells transduced with the membrane-targeted Mpl construct show intense fluorescence at the plasma membrane (Figure 4A), whereas cells transduced with the cytoplasmic Mpl construct show fluorescence in a punctate pattern throughout the cell cytoplasm, with fluorescence overlaying the nuclei and without fluorescence enhancement of the plasma membrane (Figure 4B).

Low levels of expression of the Mpl constructs in normal hematopoietic cells impeded attempts to use standard cell fractionation techniques to further investigate the cellular distribution of the membrane-targeted and cytoplasmic Mpl proteins. To circumvent this problem, both 2 Mpl constructs were transiently expressed in COS-1 cells, and a high level of expression of the proteins was achieved (Figure 5). Membrane-targeted and cytoplasmic versions of Mpl were expressed at similar levels in whole COS-1 lysates (Figure 5, lanes 1 and 2). The cytoplasmic version of Mpl was detected in the cytoplasmic fraction of the COS-1 cells (Figure 5, lane 4). However, though membrane-targeted Mpl was readily detected in the plasma membrane fraction of the COS-1 cells (Figure 5, lane 5), cytoplasmic Mpl was not detected (Figure 5, lane 6).

Generation of hematopoietic progenitor cells in the presence of membrane-targeted or cytoplasmic Mpl

To determine whether membrane-targeted and cytoplasmic Mpl constructs can support the generation of hematopoietic progenitor cells, aliquots of cells were removed from the liquid cultures at various time points, washed free of AP20187, and cultured in vitro in semisolid media in the presence of hematopoietic growth factors to detect erythroid (BFU-E), myeloid (CFU-GM), and megakaryocytic (CFU-Meg) progenitor cells. Both membrane-targeted and cytoplasmic Mpl supported the production of all 3 types of progenitor cells in a 1-month period in vitro (Table 1). There was...
no difference in the proportion of erythroid, myeloid, and megakaryocytic progenitor cells in the 2 suspension cultures. Dimerization of either the membrane-targeted Mpl or the cytoplasmic Mpl resulted in a 10 000-fold increase in the absolute number of hematopoietic progenitor cells in a 1-month period in vitro compared with the input number of progenitor cells.

**Terminal maturation of hematopoietic progenitor cells**

During the first 3 weeks of culture, neutrophils, macrophages, erythroid cells, and megakaryocytes were identified in the suspension cultures of cells transduced with either membrane-targeted Mpl or cytoplasmic Mpl (data not shown). At a later time point (102 days), mononuclear cells and megakaryocytes predominated (Figure 6). These results are similar to those we have reported previously using the membrane targeted mpl and indicate that cytoplasmic Mpl can also support the terminal maturation of committed progenitor cells.

**Requirement for JAK2 but not STAT5 signaling**

Day 12.5 fetal liver cells obtained from homozygous JAK2-deficient mice were used to determine whether membrane-targeted Mpl or cytoplasmic Mpl requires JAK2 (Table 2). JAK2+/+ cells, but not JAK2−/− cells, formed CFU-Mix in response to IL-3, and the reintroduction of JAK2 enabled the JAK2−/− cells to produce CFU-Mix. Transduction of the JAK2 +/+ cells, but not the JAK2−/− cells, with either form of Mpl resulted in colony growth in the presence of AP20187 (Table 2). These results demonstrate that both membrane-targeted Mpl and cytoplasmic Mpl require JAK2 for signal transduction in primary hematopoietic cells. In contrast to the results with JAK2−/− fetal liver cells, both the membrane-targeted Mpl and cytoplasmic Mpl could support AP20187-dependent colony growth in STAT5 A/B-deficient fetal liver cells, hematopoietic colonies were found in the presence of AP20187 (data not shown). These results indicate that neither membrane-targeted nor cytoplasmic Mpl requires STAT5 A/B for signal transduction in normal hematopoietic cells.

**Discussion**

The ability to dissociate Mpl function from its normal plasma membrane location demonstrates that membrane localization is not required for many aspects of Mpl function in normal hematopoietic cells. Remarkably, dimerization of the cytoplasmic version of Mpl in murine marrow cells resulted in the generation of myeloid, erythroid, and megakaryocytic progenitor cells in a 1-month period in vitro (Table 1) and in the emergence of terminally differentiated mature blood cells (Figure 6). Cytoplasmic Mpl lacks one attribute of wild-type Mpl: the ability to modulate circulating levels of thrombopoietin.

Numerous reports indicate that cross-talk occurs among hematopoietic growth factor receptors embedded in the plasma membrane, including physical association and cross-phosphorylation of heterologous receptors. Generalization of this concept has recently been challenged by studies of mice engineered to lack both common β chain shared by the IL-3, GM-CSF, and IL-5 receptors and the specific β chain of the IL-3 receptor. Marrow cells from these mice exhibit a normal proliferative response to G-CSF, erythropoietin (EPO) and stem cell factor, arguing that cross-talk involving the IL-3 receptor is not required by these cytokines. The current results suggest that interaction of Mpl with heterologous receptors within the plasma membrane also is not essential.

Cell surface proteins are not randomly distributed on the plasma membrane; rather, they are organized into microdomains that may be functionally important, including partitioning into or out of lipid-rich rafts, and caveolae, and other cell surface zones. Ligand binding to transmembrane hematopoietic growth factor receptors alters the distribution of receptors on the cell surface, perhaps facilitating signal transduction. Moreover, certain hematopoietic

**Table 1. Membrane-tethered or cytoplasmic Mpl can support the generation of progenitor cells**

<table>
<thead>
<tr>
<th>Cellular compartment</th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-Meg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mpl (membrane)</td>
<td>2590</td>
<td>173 ± 7</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Mpl (cytoplasmic)</td>
<td>1867</td>
<td>105 ± 10</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

Marrow cells transduced with either membrane-targeted Mpl or cytoplasmic Mpl were cultured in suspension in the presence of AP20187 (100 nM), and on day 33 progenitor cell assays were performed. Data show the average of duplicate plates (CFU-GM) or the mean ± SEM of triplicate plates (BFU-E, CFU-Meg). Colony assays from a second independent transduction of marrow cells produced similar results.

**Table 2. Membrane-targeted Mpl and cytoplasmic Mpl require JAK2 for signaling in fetal liver cells**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Culture conditions</th>
<th>Colonies/10^5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>IL-3</td>
<td>198 ± 12</td>
</tr>
<tr>
<td>JAK2†</td>
<td>IL-3 plus EPO</td>
<td>92, 105</td>
</tr>
<tr>
<td>Mpl (membrane)‡</td>
<td>AP20187</td>
<td>9, 11</td>
</tr>
<tr>
<td>Mpl (cytoplasm)‡</td>
<td>AP20187</td>
<td>8, 9</td>
</tr>
</tbody>
</table>

†Fetal liver cells from either wild-type or JAK2-deficient day 12.5 embryos were cultured overnight with a JAK2 expressing retrovirus, then placed in colony assays. Results indicate the number of colonies per 10^5 cells plated from 2 animals.

‡Fetal liver cells from either wild-type or JAK2-deficient day 12.5 embryos were cultured overnight with a retrovirus encoding either membrane-targeted Mpl or cytoplasmic Mpl, then placed in colony assays. Results indicate the number of colonies per 10^5 cells plated from 2 animals.
growth factor receptors, including the EPO receptor, which is highly homologous to Mpl, are organized in preformed dimers or signaling complexes on the cell surface, which may serve to increase the effective concentration of receptors. EPO binding subtly alters the conformation of the preassociated receptors, triggering signal transduction. Thus, under normal circumstances, growth factor receptor display on the cell surface is subject to considerable organization. Despite these observations, the current report demonstrates that dimerization of the signaling domain of Mpl in the cell cytoplasm, independent of the topography of the plasma membrane, can support production of the full range of normal hematopoietic progenitor cells and their terminal maturation.

Like other cell surface hematopoietic growth factor receptors, Mpl is internalized after thrombopoietin binding. Normal cellular trafficking of receptors may be necessary for activation of the full complement of signaling pathways. For example, impairment of endocytically growth factor (EGF) receptor internalization through overexpression of a dominant-negative dynamin suppresses activation of the mitogen-activated protein kinases (MAPks) ERK1 and ERK2. Similarly, the intracellular routing of ErbB receptors affects signaling. Although a cytoplasmic PDGF receptor derivative became phosphorylated after dimerization, activation of p70 S6 kinase and MAPK failed to occur in the absence of membrane attachment. Similarly, the cellular compartment in which the epithelial growth factor (EGF) receptor is activated has important implications for signaling.

Deletion of the membrane-anchoring region of EGF resulted in intracellular activation of the EGF receptor and altered the organotypic growth pattern of an epithelial cell line. Furthermore, in another study, the addition of a KDEL endoplasmic reticulum retention motif to IL-3 resulted in intracellular activation of IL-3 receptor and autocrine proliferation of a factor-dependent cell line. However, this study did not examine the ability of intracellularly activated IL-3 receptor to support proliferation and differentiation of normal hematopoietic cells.

JAK2 is essential for signal transduction by wild-type Mpl. The current report demonstrates that both membrane-targeted Mpl and cytoplasmic Mpl mimic wild-type Mpl in their JAK2 dependence in primary hematopoietic cells. Like their wild-type counterparts, neither membrane-targeted Mpl nor cytoplasmic Mpl requires STAT5 to induce cell growth. It is possible that cytoplasmic JAK2 activation alone is sufficient to induce self-renewal among primitive multipotent hematopoietic cells. Signaling studies in Ba/F3 cells expressing the membrane-tethered or the cytoplasmic Mpl fusion protein did not show activation of JAK2. We interpret these results to mean that the Mpl fusion proteins activate JAK2 below the limit of detection by Western blot analysis.

In summary, these results show that plasma membrane localization is not required for expression of the full range of Mpl functions in normal hematopoietic cells, and they suggest that the major purpose of receptor display on the plasma membrane is to enable communication with the extracellular milieu.

Acknowledgments

We thank T. Clackson (ARIA Pharmaceuticals, www.ariad.com) for supplying AP20187, and James Yan and Hui Zeng for expert technical support.

References


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