Identification of a New Adapter Protein That May Link the Common β Subunit of the Receptor for Granulocyte/Macrophage Colony-stimulating Factor, Interleukin (IL)-3, and IL-5 to Phosphatidylinositol 3-Kinase*

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The high affinity receptor for human GM-CSF (hGM-CSF)1 is a heterodimer consisting of two subunits, termed α and β, which are transmembrane proteins of 75–85 and 120–135 kDa, respectively (1–3). The α subunit is specific for the hGM-CSF receptor (hGMR) and binds hGM-CSF with low affinity (1). The β subunit, which is shared by the human receptors for GM-CSF, interleukin (IL)-3 and IL-5, cannot bind hGM-CSF by itself but is required for high affinity binding of hGM-CSF (3–5). Expression of the normal hGMR α and β subunits in established murine cells can generate a potent oncogenic signal in the presence of hGM-CSF (6).

The β subunit of hGMR is important for signal transduction (7, 8). Although the β subunit itself does not contain consensus sequences characteristic of protein kinases or protein phosphatases, hGM-CSF induces rapid phosphorylation of cellular proteins on tyrosine residues (9–12), indicating a functional association with cytoplasmic protein-tyrosine kinases. Candidates for these non-receptor tyrosine kinases are p130 (13–14), p92 (15), p53/p56 (16), and p62 (17).

Activation of phosphatidylinositol-3 kinase (PI 3-kinase) is one of the immediate cellular responses to stimulation by growth factors and cytokines, including hGM-CSF (17–20). PI 3-kinase is a heterodimer consisting of two subunits: an 85-kDa protein containing SH2 and SH3 domains (p85) and a 110-kDa catalytic subunit (p110) (21). p85 functions as an adapter molecule that targets p110 to activated growth factor receptors (19, 20, 22–24). In most cases, this is mediated by binding of the SH2 domains of p85 to the pYXXM consensus motif in activated tyrosine kinase receptors (25) like the platelet-derived growth factor receptor (26). However, the hGMR does not have this consensus sequence for PI 3-kinase binding nor the recognition motif pYXYX that has been recently described for binding of PI 3-kinase to the hepatocyte growth factor/scatter factor receptor (27).

A variation of the direct association of PI 3-kinase with receptor molecules is its indirect binding to activated receptors through a tyrosine-phosphorylated receptor substrate like the insulin receptor substrate 1 (IRS-1). After insulin receptor stimulation and tyrosine phosphorylation of IRS-1, the p85 subunit of PI 3-kinase associates through its SH2 domains with tyrosine phosphorylation sites within pYXXM motifs on IRS-1 (28). Other proteins with SH2 domains like Grb2 (29), Nck (30), and SH-PTP-2 (31) also associate with IRS-1. Therefore, the role of IRS-1 is to function as a multiside docking protein to link the upstream insulin receptor to several downstream adapter molecules of different signaling pathways, including the PI 3-kinase. Another PI 3-kinase adapter protein is the IL-4 receptor substrate 4PS, which links the activated IL-4 receptor to PI 3-kinase (32).

Here, we describe the identification of a new PI 3-kinase adapter protein of 80 kDa (p80), which is the major tyrosine-phosphorylated substrate in hGM-CSF-stimulated cells. p80 coprecipitates with the common β subunit and binds directly to the p85 subunit of PI 3-kinase. Our data suggest that p80 is a new adapter protein, which may participate in linking the activated hGM-CSF receptor to the PI 3-kinase pathway.

MATERIALS AND METHODS

CD1s—The hGM-CSF-dependent human erythroleukemia cell line TF-1 (33) was obtained from Atsushi Miyajima (DNAX Research Insti-
tute). These cells were cultured in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum and 3 ng/ml recombinant hGM-CSF. Control and v-fes-transformed NIH 3T3 cells have been described (34). pC012-EGFR cells are NIH 3T3 cells overexpressing human EGF receptors (35). Human recombinant EGF has been described (34). pCO12-EGFR cells are NIH 3T3 cells overexpressed human recombinant EGF. These cells were maintained in a minimal essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum (Life Technologies, Inc.) and 36 ng of human recombinant CSF-1 (Chiron Corp., Emeryville, CA) per ml.

Antibodies—Rat monoclonal antibody (mAb) CRS1, directed against the β subunit of hGMR, has been previously described (37) and was obtained from Atsushi Miyajima; a rabbit serum directed against the B55 unit of the PI-3 kinase was purchased from Transduction Laboratories (Lexington, KY); rabbit antisera directed against Shc and Jak2, and mAb directed against phosphotyrosine were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); mAb against Src (Mab327) was obtained from J an Brugge; mAb against Yes was obtained from Marius Sudol (38).

Stimulation of Cells—TF-1 cells were starved for 18 h in RPMI 1640 medium containing 1% (v/v) bovine serum albumin (fraction V; Sigma) at a cell density of 5 × 10⁵ cells/ml. Cells were then resuspended in fresh medium containing 0.1% (v/v) bovine serum albumin at a cell density of 1 × 10⁶ cells/ml, treated for 30 min with 50 μM sodium orthovanadate (Aldrich), and then were incubated for 2 min at 37°C in the absence or presence of 25 ng/ml hGM-CSF unless otherwise indicated. After incubation, cells were diluted into cold phosphate-buffered saline containing 50 μM sodium orthovanadate, and cell lysates were prepared as described below. The stimulation of BAC1.2F5 cells with 35 ng/ml CSF-1 and stimulation of pCO12-EGFR cells with 100 ng/ml EGF were carried out as described previously (39).

Preparation of Cell Lysates and Protein Analysis—Untreated or hGM-CSF-stimulated cells were lysed in Nonidet P-40 lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1% (v/v) Nonidet P-40, 2 mM EDTA, 10% (v/v) glycerol, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and 2% (v/v) Trasylol (FBA Pharmaceuticals, New York)). In some experiments, 10 mM CHAPS (Pierce) was used instead of Nonidet P-40, as indicated in the figure legends. Insoluble material was removed by centrifugation at 4°C for 10 min at 14,000 × g. Protein concentration was determined using the Bio-Rad protein assay according to the manufacturer (Bio-Rad), and cell lysates were stored in liquid nitrogen until use. Equal amounts of protein were used for electrophoretic analysis.

Cell lysates were immunoprecipitated with the indicated antibodies, and the immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis and Western blot analysis were carried out as described previously (39). Filters were developed with ECL (Amersham). For reprobing, bound antibodies were removed from the nitrocellulose membrane by incubation in stripping buffer (62.5 mM Tris, pH 6.7, 2% SDS, and 100 mM β-mercaptoethanol) for 30 min at 50°C. Stripped filters were washed five times for 10 min in TTBS before blocking and incubation with other antibodies. The same filter was used up to four times.

Purification of Bacterial Glutathione S-Transferase Fusion Proteins—pGEX-3X control and pGEX-fusion proteins were expressed with 0.1 mM isopropyl-β-D-thiogalactopyranoside, the induced bacteria were lysed by sonication in buffer containing 1% (v/v) Triton X-100, 50 mM HEPES (pH 7.4), and the induced proteins were adsorbed with glutathione-agarose (Sigma) as described previously (39).

PI-3 Kinase Assay—TF-1 cells were serum-starved for 18 h, stimulated for 2 min with 50 ng/ml human GM-CSF, lysed in Nonidet P-40 lysis buffer, and immunoprecipitated with anti-phosphotyrosine mAb PY20 (ICN, Costa Mesa, CA) or with anti-p85 mAb (UBI) as described above. Immunoprecipitates were washed three times in phosphate-buffered saline containing 1 mM sodium vanadate and 1% Nonidet P-40, two times in 50 mM Tris, pH 7.6, with 0.5 mM LiCl, once in TNE (10 mM Tris, pH 7.6, 100 mM NaCl, and 1 mM EDTA), and once with 20 mM HEPES, pH 7.6. PI-3 kinase activity in the immunoprecipitates was measured by its ability to phosphorylate phosphatidylinositol 3-phosphate as described previously (40). Phosphatidylinositol 3-phosphate was visualized by autoradiography for the times indicated in the figure legends.

RESULTS

A Protein of 80 kDa Is the Major hGM-CFSF/hIL-3-dependent Phosphorylated Protein in hGM-CSF-stimulated Cells—To identify the PI-3-like protein that is involved in hGM-CSF signaling we analyzed lysates of human GM-CSF-stimulated TF-1 cells by anti-phosphotyrosine immunoblotting. As shown in Fig. 1, A and B, treatment of TF-1 cells with hGM-CSF induced tyrosine phosphorylation of proteins of 51, 56, 68, 80, 89, 92, 125, 130, 140, and 150 kDa. Tyrosine phosphorylation occurred within 30 s, reached a maximum between 2 and 5 min, and declined rapidly thereafter (Fig. 1A). The induction of tyrosine phosphorylation by hGM-CSF was concentration dependent, with a maximal effect at 5 ng/ml (Fig. 1B). The major induced tyrosine-phosphorylated protein was a broad band of ~85 kDa, which we have designated p80 (Fig. 1A, lanes 2–4). p80 is also the major tyrosine-phosphorylated protein after stimulation of TF-1 cells with human IL-3 (Fig. 1C, lane 3). Both hIL-3 and hGM-CSF induced an indistinguishable pattern of tyrosine-phosphorylated proteins (Fig. 1C), consistent with the idea that the common β subunit, which is shared between the receptors for human IL-3, IL-5, and GM-CSF, is a major determinant of signaling events induced by these cytokines.

Association of Tyrosine-phosphorylated p80 with the β Subunit of hGMR—To determine whether phosphotyrosyl p80 binds to the common β subunit, we analyzed immunoprecipitates of the β subunit for the presence of p80. Anti-phosphotyrosine immunoblot analysis showed that after hGM-CSF stimulation, p80 coprecipitated with the β subunit (Fig. 2, lane 1), suggesting that p80 can directly or indirectly associate with the common β subunit.

Tyrosine-phosphorylated p80 Binds to the p85 Subunit of Phosphatidylinositol 3-Kinase through the N-terminal and C-terminal SH2 Domains of p85—Since p80 was in the molecular weight range of the p85 subunit of PI-3 kinase (Fig. 1, A–C) and PI-3 kinase activity is stimulated by hGM-CSF (17), we analyzed whether this phosphorylated protein was the p85 subunit...
of PI 3-kinase. Anti-p85 antibodies precipitated a phosphotyrosyl protein that comigrated with p80 (Fig. 3A, lanes 2 and 4). However, stripping of the filter shown in Fig. 3A, lanes 3 and 4, and reprobing with anti-p85 antibodies showed that p80 had a different electrophoretic mobility than the p85 subunit of PI 3-kinase (Fig. 3B, lanes 3 and 4). Thus, our data indicate that p80 is not the p85 subunit of PI 3-kinase but that p80 associates with p85 after hGM-CSF stimulation.

To characterize the interaction between p80 and p85, we examined whether this association was mediated through phosphotyrosine-SH2 interactions. To examine this question, the C-terminal and N-terminal SH2 domains of p85 were expressed as glutathione S-transferase fusion proteins in bacteria. Immobilized C-terminal (SH2-C) and N-terminal (SH2-N) SH2 domains of the p85 subunit bound phosphorylated p80, and this association was dependent on stimulation with hGM-CSF (Fig. 3A, lanes 5–8). The C-terminal SH2 domain had higher affinity for p80 than the N-terminal SH2 domain (Fig. 3A, lanes 6 and 8). In addition to p80, two other phosphotyrosyl proteins with apparent molecular masses of 110 and 130 kDa were bound to the C- and N-terminal SH2 domains, respectively (Fig. 3A, lanes 6 and 8, respectively). None of these proteins were recognized by a glutathione S-transferase vector protein (Fig. 3A, lane 9). The association of p85 with p80 followed the same time course as the tyrosine phosphorylation of p80 (Figs. 3C and 1A), suggesting that p80 associated with p85/PI 3-kinase primarily through phosphotyrosine-SH2 interactions. However, at the present time we cannot rule out the possibility that p80 was already associated with p85 before hGM-CSF stimulation through other interactions not involving phosphotyrosine. This will be clarified when antibodies to p80 become available.

Stimulation of cells with other growth factors that also stimulate PI 3-kinase activity, namely CSF-1 and EGF did not induce tyrosine phosphorylation of p80 or its association with the p85 subunit of PI 3-kinase (Fig. 3D). Instead, p85 coprecipitated with an unknown tyrosine-phosphorylated protein of 110 kDa in CSF-1-stimulated BAC1.2F5 cells (Fig. 3D, lane 6), and, as previously shown (20), p85 coprecipitated with activated EGF receptor in EGF-stimulated pCO12-EGFR cells (Fig. 3D, lane 10).

We conclude that after hGM-CSF stimulation, p80 becomes rapidly phosphorylated on tyrosine and associates with the p85 subunit of PI 3-kinase by binding preferentially to the C-terminal SH2 domain of p85. Thus, p80 represents a novel signaling molecule that is capable of interacting with the p85 adapter protein in cells stimulated with hGM-CSF.

hGM-CSF Induces the Association of PI 3-Kinase Activity with Tyrosine-phosphorylated Proteins—Since hGM-CSF induced an association of the p85 subunit of PI 3-kinase with tyrosine-phosphorylated p80, we wanted to determine if this was accompanied by PI 3-kinase activation. Fig. 4 shows that hGM-CSF caused the appearance of PI 3-kinase activity in

![Fig. 2. Association of p80 with the common β subunit of hGM-R.](image)

![Fig. 3. p80 interacts with the p85 subunit of PI 3-kinase through the N- and C-terminal SH2 domains of p85.](image)
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anti-phosphotyrosine immunoprecipitates from stimulated TF-1 cells (Fig. 4, lanes 1 and 2) and that this was a fraction of the PI 3-kinase activity that was constitutively associated with p85 in the absence of hGM-CSF stimulation (Fig. 4, lanes 3 and 4). The correlation between appearance of PI 3-kinase activity in anti-phosphotyrosine immunoprecipitates and the binding of tyrosine-phosphorylated p80 to p85 and the hGMR β subunit further supports our model in which p80 links the hGMR to the PI 3-kinase pathway.

Association of p80 with Src Kinase Family Members—Several tyrosine kinases have been implicated in hGM-CSF-signaling. These include JAK2 (13, 14), Src family kinases (15, 16, 17), and Fps/Fes (15). To identify the tyrosine kinases that might be involved in phosphorylation of p80, we analyzed which tyrosine kinases were present in the p80-PI 3-kinase complex.

In preliminary experiments, we found that p60Src, p62(ts), and p53/p56Crk were expressed in TF-1 cells (data not shown). We then used an anti-Src antibody (SRC2) raised against residues 509–533 in the C-terminal region of human p60Src, which cross-reacts with p62(ts) and p53Crk, for immunoprecipitation and Western blot analysis. Immunoprecipitation of TF-1 lysates with anti-SRC2 antibodies resulted in precipitation of two proteins of 60 and 62 kDa (Fig. 5A), which were identified in separate gels as p60Src and p62(ts), respectively (data not shown). To analyze whether p80 and other tyrosine-phosphorylated proteins coprecipitated with p60Src and/or p62(ts), we reprobed the same filter with anti-phosphotyrosine antibodies. This analysis showed that after hGM-CSF stimulation, phosphotyrosyl proteins of 51, 56, 68, 76–85, 140, and 160 kDa were present in the anti-SRC2 immunoprecipitates (Fig. 5B). The broad band of 76–85 kDa in anti-SRC2 precipitates from hGM-CSF-stimulated cells (Fig. 5B) had the same electrophoretic mobility as p80, suggesting that p80 may be a substrate of p60Src and/or p62(ts). The 51-, 56-, and 68-kDa proteins (Fig. 5B) had the same electrophoretic mobility as the three isoforms of the adapter protein Shc, p46shc, p52shc, and p66shc (41). Stripping and reprobing of the filter with anti-Shc antibodies confirmed that p52shc (Fig. 5C) and p66shc (observed after long exposure) were present in anti-SRC2 immunoprecipitates from hGM-CSF-stimulated cells. Whether p46shc is also in the anti-SRC2 precipitate could not be ascertained because the IgG band interfered with detection of p46shc.

Since p80 associates with the p85 subunit of PI 3-kinase (Fig. 5D), the filter shown in Fig. 5, A–C, was reprobed with anti-p85 antibodies to determine if p85 was also present in this complex. As shown in Fig. 5D, similar amounts of p85 were present in anti-SRC2 precipitates from unstimulated and stimulated TF-1 lysates. Thus, one or more Src kinases were associated with p85 before hGM-CSF stimulation. Recent reports that Src family kinases can associate with p85 in a phosphotyrosine-independent manner (42, 43) are consistent with the idea that p60Src and/or p62(ts) may be already complexed with p85 before hGM-CSF stimulation.

p80 also associated with p53/p56Crk in a hGM-CSF-dependent manner. As shown in Fig. 6, the time course of p80 association with Lyn was similar to the time course of tyrosine phosphorylation of p80 (Fig. 1A).

Since p53/p56Crk are not recognized by the anti-SRC2 antibody, these data indicate that at least two members of the Src family, i.e. p53/p56Crk and p60Src and/or p62(ts), associate with p80. Therefore, these kinases are very strong candidates for phosphorylation of p80 and other phosphotyrosyl substrates present in the p80-PI 3-kinase complex (e.g. Shc, p140, p160).

The Jak2 tyrosine kinase associated with the β subunit and has been implicated in the tyrosyl phosphorylation of the β subunit (14). Since p80 also associates with the β subunit, we analyzed the possibility that p80 is a substrate of Jak2. We confirmed that Jak2 becomes tyrosine phosphorylated after stimulation of TF-1 cells with hGM-CSF; however, we could not detect p80 in Jak2 immunoprecipitates, suggesting that p80 may not be a direct substrate of Jak2 (data not shown). Similarly, the tyrosine kinase Fps/Fes, which has been implicated in hGM-CSF signaling (15), was not present in the p80-PI 3-kinase complex (data not shown), suggesting that p80 was not a substrate of Fps/Fes either.

DISCUSSION

To analyze molecular events involved in hGM-CSF signaling that may also occur in vivo, we have used a human GM-CSF-dependent cell line that responds to physiological concentrations of the ligand. Our experiments resulted in the identification of a new phosphotyrosine protein (p80) that associates with the SH2 domains of the p85 subunit of PI 3-kinase and...
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Fig. 6. Association of Lyn with tyrosine-phosphorylated p80. Starved TF-1 cells were either not stimulated (lane 1) or stimulated with 25 ng/ml hGM-CSF (lanes 2–8) for various times (lane 2, 0.5 min; lane 3, 2 min; lane 4, 5 min; lane 5, 10 min; lane 6, 30 min; lane 7, 60 min; lane 8, 120 min). Nonidet P-40 lysates were immunoprecipitated (IP) with anti-Lyn antibodies and analyzed by anti-phosphotyrosine immunoblotting (P.Tyr). The positions of p80, p56<sup>lyn</sup>, p53<sup>ak2</sup>, and immunoglobulin heavy chain are indicated on the left. Molecular weight markers are indicated on the right.

with the common β subunit of the hGM-CSF/IL-3/IL-5 receptor. p80 Might Link the hGM-CSF Receptor to the PI 3-Kinase Pathway—Activation of PI 3-kinase is one of the immediate cellular responses to stimulation by growth factors and cytokines (18–20). As previously reported in other cells (17), hGM-CSF also caused an increase in PI 3-kinase activity in TF-1 cells, indicating that the PI 3-kinase pathway is involved in hGM-CSF signaling in these cells. PI 3-kinase is a heterodimer consisting of two subunits: an 85-kDa protein containing SH2 and SH3 domains (p85) and a 110-kDa catalytic subunit (p110) (21). p85 functions as an adapter molecule that targets p110 to activated growth factor receptors (19, 20, 22–24). In most cases, this is mediated by binding of the SH2 domains of p85 to the pYV<sup>IXM</sup> motif in activated receptors. The β subunit of the hGMR does not have this consensus sequence for PI 3-kinase binding (25) nor the recognition motif pYVXXV that has been recently described for binding of PI 3-kinase to the hepatocyte growth factor/scatter factor receptor (27). Therefore, p85 may bind indirectly to the hGMR via a tyrosine-phosphorylated adapter protein. This mechanism has been described for the insulin receptor whereupon ligand binding, p85 binds to IRS-1, a major phosphotyrosyl substrate of the insulin receptor (29, 44). Indirect binding of PI 3-kinase has also been reported for a cytokine receptor as well. p85 binds to IL-4 receptor via a tyrosine-phosphorylated adapter protein designated 4PS (32).

We postulate that p80 is a tyrosine-phosphorylated adapter protein that may link PI 3-kinase to the hGMR, either directly or indirectly. This is based on the observations that p80 was found in immunoprecipitates of the β subunit of hGMR and that phosphotyrosyl p80 was tightly bound to the p85 subunit of PI 3-kinase. Our experiments showed that the C-terminal and N-terminal SH2 domains of p85 recognized phosphorylated p80, strongly suggesting that this protein binds directly to p85 through SH2-phosphotyrosine interactions. The C-terminal SH2 domain of p85 had higher affinity for phosphorylated p80 than the N-terminal SH2 domain, as has been shown for the association between p85 and the activated platelet-derived growth factor receptor (45). Clarification of the nature of the association between p80 and the β subunit will require further analysis.

p80 Is Tightly Associated with Src Family Kinases—Phosphorylation of the multiple hGM-CSF-dependent targets probably requires the concerted action of several different protein-tyrosine kinases. Our data suggest that Src family kinases were responsible for phosphorylation of p80 and other phosphotyrosyl substrates in hGM-CSF-stimulated cells. In TF-1 cells, anti-Lyn immunoprecipitates from hGM-CSF-stimulated cells contained tyrosine-phosphorylated p80 (Fig. 6). This is of particular interest because p80 associates with the p85 subunit of PI 3-kinase, and a hGM-CSF-dependent increase in PI 3-kinase activity has been detected in anti-Lyn immunoprecipitates (17). In addition, we detected phosphorylated p80 in a complex with Src/Yes (Fig. 5B), and p80 was also phosphorylated in vitro when anti-Src/Yes immunoprecipitates were subjected to an in vitro kinase assay (data not shown). The Lyn and Yes tyrosine kinases have been proposed to be involved in hGM-CSF signaling on the basis of an increase in their in vitro kinase activity in hGM-CSF-stimulated hematopoietic cells (16, 17).

In addition to p80, other tyrosine-phosphorylated proteins were detected in anti-Src/Yes precipitates, i.e. Shc, p140, and p160, suggesting that these proteins might also be substrates of Src or Yes. p140 is a protein that associates with Shc after stimulation with several cytokines (46) including hGM-CSF through a new phosphotyrosine binding domain in the N terminus of Shc (47). p160 is an unknown protein. Interestingly, while the association of Src and/or Yes with tyrosine-phosphorylated p80, Shc, p140, and p160 was dependent on hGM-CSF, Src/Yes was already associated with the p85 subunit of PI 3-kinase in the absence of hGM-CSF stimulation. Since we did not detect tyrosine phosphorylation of the p85 subunit of PI 3-kinase in hGM-CSF-stimulated cells, the interaction between Src and/or Yes with p85 may not be dependent on SH2-phosphotyrosine interactions. This finding is consistent with recent reports that the SH3 domain of Src kinases binds directly to a proline-rich region of the p85 subunit of PI 3-kinase (42, 43). The presence of Src kinases in the PI 3-kinase complex before stimulation makes these kinases good candidates for phosphorylation of p80 or other proteins in this complex.

We also analyzed other tyrosine kinases for their association with p80. jak2 was found to be tyrosine phosphorylated after hGM-CSF treatment of TF-1 cells (13–14). However, no tyrosine-phosphorylated p80 was detected in anti-jak2 immuno precipitates (data not shown). Similarly, no phosphorylated p80 was found in immunoprecipitates of Hck or Fps/Fes from hGM-CSF-stimulated cells (data not shown). Therefore, we conclude that these kinases are unlikely to be involved in tyrosine phosphorylation of p80.

In summary, we have identified a new adapter protein, p80, that associates with the β subunit of the hGMR and with the p85 subunit of PI 3-kinase. p80 may link the hGMR to the PI 3-kinase signaling pathway. So far, tyrosine phosphorylation of p80 seems to be specific for signaling via the common β subunit of the hGM-CSF/IL-3/IL-5 receptors. We are now attempting to clone p80 to clarify its function and mechanism of action in hGM-CSF signaling.

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