Specific Involvement of Tyrosine 764 of Human Granulocyte Colony-Stimulating Factor Receptor in Signal Transduction Mediated by p145/Shc/GRB2 or p90/GRB2 Complexes

By John P. de Koning, Anita M. Schelen, Fan Dong, Carin van Buitenen, Boudewijn M.T. Burgering, Johannes L. Bos, Bob Löwenberg, and Ivo P. Touw

Signaling transduction from the granulocyte colony-stimulating factor receptor (G-CSF-R) occurs via multiple pathways, one of which involves activation of p21ras and mitogen-activated protein kinase. The SH2 domain-containing proteins Shc and GRB2 have been implicated in this latter signaling route. We studied the role of these proteins in signal transduction from wild type (WT) G-CSF-R, C-termina/ deletion mutants, and tyrosine-to-phenylalanine substitution mutants in transfectants of the mouse pro-B cell line, BAFl3. G-CSF stimulation of BAFl3 cells expressing WT G-CSF-R induced tyrosine phosphorylation of Shc. Anti-Shc antibodies co-immunoprecipitated tyrosine-phosphorylated 145-kD proteins (p145), whereas GRB2 immunoprecipitates contained phosphorylated Shc, Syk, and proteins of 145 and 90 kD (p90). Neither of these complexes were detected after activation of a C-terminal deletion mutant of G-CSF-R that lacked all four conserved cytoplasmic tyrosine residues. G-CSF induced formation of SyP/GRB2 complexes in all the tyrosine-substitution mutants, suggesting that this association did not depend on the presence of single specific tyrosine residues in G-CSF-R. In contrast, tyrosine 764 of G-CSF-R appeared to be exclusively required for tyrosine phosphorylation of Shc and its association with p145 and GRB2. In addition, tyrosine 764 also specifically mediated binding of GRB2 to p90 without the involvement of Shc. These findings indicate that tyrosine 764 of G-CSF-R has a prominent role in G-CSF signal transduction.

© 1996 by The American Society of Hematology.
phosphorylation of Shc. Activation of Shc is accompanied by tyrosine phosphorylation and association of 145-kD proteins of unknown identity, resulting in the formation of p145/Shc/GRB2 complexes. Tyrosine 764 is also predominantly involved in the phosphorylation of 90-kD proteins that form complexes with GRB2. Finally, we demonstrate that the C-terminal domain of activated G-CSF-R mediates binding of GRB2 to tyrosine-phosphorylated Syp. In contrast to the formation of p145/Shc/GRB2 or p90/GRB2 complexes, Syp/GRB2 binding is not mainly accomplished via one tyrosine residue of G-CSF-R.

**MATERIALS AND METHODS**

**Site-directed mutagenesis.** Human G-CSF-R cDNA was cloned in the pBlueScript vector (pBS) and in the eukaryotic expression vector pLNCX, as described previously. PCR techniques were used to individually substitute the four tyrosine residues located in the cytoplasmic domain of G-CSF-R for phenylalanine residues (Fig 1), according to a recently described method for site-directed mutagenesis. The oligonucleotides 5' CAGACCTI'TGTGCTGTGAGAACCT-3' (Y704F, containing a silent PstI site), 5' GATCAA-AGCCCCAAAAGCTITGAGAACCT-3' (Y729F, containing a silent ScaI site), 5'-GAGGCACTTTCTCGGTGAC-3' (Y744F), and 5'-AGCCCCAAAAGCTITGAGAACCT-3' (Y764F, containing a silent HindIII site) were designed as mutagenic primers. The human G-CSF-R cDNA cloned in pBS was used as the template in the first PCR, with a mutagenic primer and with primer M13-20 (5'-GTAGATCTI'TAGTCATGGGC'ITATGG-3') and RV25.1 (5'-GTAGATCTTATGCATGGGACTTG-3') and HindIII (Y764F) or sequenced following cloning in pBS (Y744F). To detect G-CSF-R proteins on the cell surface, cells were treated for 30 minutes at 4°C with rabbit anti-human G-CSF-R antibody 1729 (1:100 dilution; raised against amino acids 17 to 344 in the extracellular domain of the receptor). After washing, cells were incubated for 30 minutes at 4°C with a 1:40 dilution of fluorescein-conjugated goat-anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands). Samples were analyzed by flow cytometry using a FACSCAN (Becton Dickinson, San Jose, CA).

**DNA synthesis assay.** DNA synthesis was assayed by [3H]-thymidine (3H-TdR) uptake, as described previously. Briefly, cells (1 x 10^6) were incubated in triplicate in 100 μL 10% fetal calf serum-RPM1 medium supplemented with titrated concentrations of human G-CSF or murine IL-3 in 96-well plates for 48 hours. Eight hours before cell harvesting, 0.1 μCi [3H-TdR (2 Ci/mmol/L; Amer- sham International, Amersham, UK) was added to each well. 3H- TdR incorporation was measured by liquid scintillation counting.

**Preparation of cell lysates, immunoprecipitation, and Western blotting.** Before stimulation, cells were deprived of serum and factors for 10 hours at 37°C in RPMI 1640 medium. One hour before addition of growth factors, 10 μmol/L sodium orthovanadate (Na3V04) was added. Cells (5 x 10^5/mL) were incubated in RPMI 1640 medium in the presence of human G-CSF (100 ng/mL) or murine IL-3 (1 μg/mL) for 1 to 30 minutes at 37°C or without factors (control). At the different time points, 10 vol ice-cold phosphate-buffered saline and 10 μmol/L Na3V04 were added. Subsequently, cells were centrifuged and lysed by incubation for 1 hour at 4°C in lysis buffer (20 mmol/L Tris hydrochloride, pH 8.0, 137 mmol/L NaCl, 10 mmol/L EDTA, 100 mmol/L NaF, 1% Nonidet P-40, 10% homogenized and centrifuged.

**Transfection of BAF3 cells was performed by electroporation.** Following gene transfer, cells were cultured in IL-3—containing medium for 24 hours and then selected in G418 (Gibco-BRL, Breda, The Netherlands) at a concentration of 1.5 mg/mL. Multiple clones were expanded for further analysis. Reverse transcriptase-PCR using the primers FRM1 (5'-CTGCTGTGTTAAACCTGCCT-3') and RV25.1 (5'-GTAGATCTTATGCATGGGACTTG-3') was performed to check the proper expression of WT and mutant G-CSF-R constructs. The products were either digested by PstI (Y704F), ScaI (Y729F), and HindIII (Y764F) or sequenced following cloning in pBS (Y744F). To detect G-CSF-R proteins on the cell surface, cells were treated for 30 minutes at 4°C with rabbit anti-human G-CSF-R antibody 1729 (1:100 dilution; raised against amino acids 17 to 344 in the extracellular domain of the receptor). After washing, cells were incubated for 30 minutes at 4°C with a 1:40 dilution of fluorescein-conjugated goat-anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands). Samples were analyzed by flow cytometry using a FACSCAN (Becton Dickinson, San Jose, CA).
Parental

WT

Y704F

Y729F

Y744F

Y764F

Fig 2. Flow cytometric analysis of G-CSF-R expression on parental BAF3 cells and BAF3 transfectants. Cells were stained either with rabbit anti-human G-CSF-R antibodies followed by fluorescein-conjugated goat-anti-rabbit IgG (---) or with fluorescein-conjugated goat-anti-rabbit IgG alone (-----). a.u., arbitrary units.

glycerol, 2 mmol/L Na$_2$VO$_4$, 1 mmol/L Pefabloc SC, 50 μg/mL aprotinin, 50 μg/mL leupeptin, 50 μg/mL bacitracin, and 50 μg/mL iodoacetamide. Insoluble materials were removed by centrifugation for 30 minutes at 10,000 x g at 4°C. Immunoprecipitations were performed essentially as previously described. In brief, supernatants of the clarified cell lysates were incubated overnight at 4°C with either anti-Shc or anti-GRB2 (Santa Cruz Biotechnology Inc, Santa Cruz, CA) antibodies. Protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) were then added for 1 hour at 4°C. After washing the beads with ice-cold lysis buffer five times, bound proteins were eluted by boiling for 5 minutes in sodium dodecyl sulfate (SDS) sample buffer. Following SDS–polyacrylamide gel electrophoresis (SDS-PAGE), proteins were electroblotted onto nitrocellulose (0.2 μm; Schleicher & Schuell, Dassel, Germany). Filters were blocked by incubation in 0.3% Tween 20 in Tris-buffered saline (TBS) for 1 hour at 37°C, washed in TBST (0.05% Tween 20 in TBS), and incubated with antibodies diluted in TBST. Antibodies used for Western blotting were antiphosphotyrosine antibody 44310 (Upstate Biotechnology Inc, Lake Placid, NY), anti-Shc, anti-GRB2, and anti-Syp (Santa Cruz). After washing with TBST, immune complexes were detected with horseradish peroxidase–conjugated species-specific antiserum (DAKO, Glostrup, Denmark), followed by enhanced chemiluminescence reaction (DuPont, Boston, MA). In some instances, membranes were stripped in 62.5 mmol/L Tris hydrochloride, pH 6.7, 2% SDS, and 100 mmol/L β-mercaptoethanol at 50°C for 30 minutes, reblocked, washed, and reprobed.

Glutathione S-transferase/GRB2 capture. Cell lysates, prepared as described earlier, were incubated for 2 hours at 4°C with 2 μg glutathione S-transferase (GST)/GRB2 fusion protein bound to glutathione-Sepharose beads (Pharmacia). After washing the loaded beads in ice-cold lysis buffer five times, bound proteins were eluted by boiling for 5 minutes in SDS sample buffer, separated by SDS-PAGE, and analyzed by Western blotting, as described earlier.

RESULTS

Expression of and mitogenic signaling by different forms of G-CSF-R. To determine expression levels of mutated G-CSF-R proteins as compared with WT proteins in BAF3 transfectants, cells were stained with anti-human G-CSF-R antibodies and analyzed by flow cytometry. Examples of clones expressing WT or mutant G-CSF-R proteins are shown in Fig 2. The abilities of transfectants to proliferate in response to G-CSF were examined in ³H-TdR uptake assays. G-CSF responses of BAF3 cells expressing the tyrosine-to-phenylalanine substitution mutants were comparable to responses of BAF3 cells expressing WT G-CSF-R (Fig 3). Repeated analyses of at least three independent clones of each mutant gave identical results. G-CSF-R levels and G-CSF responses of BAF3 cells expressing deletion mutants M1 and DA have been described previously and were essentially similar to those of BAF3 cells expressing WT G-CSF-R.¹¹

G-CSF induces tyrosine phosphorylation of Shc. To investigate whether Shc is tyrosine-phosphorylated after G-CSF treatment, BAF3 cells expressing WT G-CSF-R were stimulated with G-CSF for 1 to 30 minutes. For comparison, cells were incubated in parallel without growth factor or with IL-
SHC- AND GRB2-MEDIATED SIGNALING BY G-CSF-R

135

100
ment (Fig 4A and B). Reprobing of the blot with anti-Shc antibodies confirmed equal loading of the samples (Fig 4C) and showed that the 145-kD proteins were not recognized on Western blots by anti-Shc antibodies, and thus co-precipitated by virtue of their association with Shc (data not shown).

Tyrosine 764 of G-CSF-R is exclusively involved in activation of Shc. To determine which of the tyrosine residues located in the cytoplasmic domain of G-CSF-R are involved in activation of Shc, BAF3 transfectants expressing C-terminal deletion or substitution mutants of G-CSF-R (Fig 1) were studied. G-CSF-stimulated tyrosine phosphorylation of Shc and its associated p145 was observed in cells expressing WT G-CSF-R and substitution mutants Y704F, Y729F, and Y744F (Fig 5A and B). In contrast, no activation of Shc and p145 was seen after G-CSF stimulation in BAF3 transfectants expressing substitution mutant Y764F and deletion mutants M1 and DA, despite the equal presence of Shc proteins as compared with WT G-CSF-R transfectants (Fig 5C). In parallel control incubations, IL-3 induced tyrosine phosphorylation of Shc and p145 in all of these transfectants. Repeated analyses of at least three independent clones of each mutant gave identical results.

G-CSF induces association of GRB2 with tyrosine-phosphorylated Shc, Syp, 90-kD, and 145-kD proteins. Anti-GRB2 immunoprecipitates from unstimulated and G-CSF- or IL-3-stimulated BAF3 cells expressing WT G-CSF-R were probed with anti-PY antibodies (Fig 6A). Following incubation with G-CSF and IL-3, tyrosine-phosphorylated proteins of approximately 52, 70, 90, and 145 kD co-precipitated with GRB2. The kinetics of G-CSF-stimulated association of GRB2 with tyrosine-phosphorylated 52- and 145-kD proteins were comparable to the kinetics of tyrosine phosphorylation of Shc and its association with p145, as seen in anti-Shc immunoprecipitates (Fig 4A and B). The somewhat lower reactivity of p145 with anti-PY antibodies observed after 5 minutes of stimulation with G-CSF was not confirmed in repeated experiments. Reprobing of the blot with anti-Shc antibodies identified the 52-kD protein as Shc (Fig 6B). Upon G-CSF stimulation, tyrosine phosphorylation of 70-kD proteins occurred within 1 minute and persisted for at least 30 minutes. Reprobing the blot revealed that a subfraction of the 70-kD proteins reacted with anti-Syp

3. IL-3 has previously been shown to induce tyrosine phosphorylation of Shc.22,23 Phosphorylation of Shc was induced following stimulation with G-CSF and IL-3 (Fig 4A). Of three Shc isoforms, ie, p46shc, p52shc, and p66shc, that have been described,27 p52shc was predominantly phosphorylated. Only minimal activation of p46shc was seen, whereas p66shc is not present at detectable levels in BAF3 cells (Fig 4C). Upon stimulation with G-CSF, tyrosine phosphorylation of Shc was induced within 1 minute, reached a maximum at 5 minutes, and declined after 10 minutes. Tyrosine-phosphorylated proteins of approximately 145 kD (p145) co-immunoprecipitated with tyrosine-phosphorylated Shc after G-CSF and IL-3 treat-

For personal use only.on November 11, 2017. For personal use only.
antibodies (Fig 6C). Essentially similar results were obtained using GST/GRB2 fusion proteins (Fig 7).

**Mutation Y764F reduces G-CSF-induced association of GRB2 with tyrosine-phosphorylated Shc, 90-kD, and 145-kD proteins.** Since G-CSF-R mutant Y764F failed to induce tyrosine phosphorylation of Shc and since GRB2 only associates with Shc in its tyrosine-phosphorylated form, anti-GRB2 immunoprecipitates from G-CSF-stimulated BAF3 cells expressing mutant Y764F should not contain Shc. As shown in Fig 8A, G-CSF stimulation only induced association of GRB2 with tyrosine-phosphorylated 70-kD proteins that partly hybridized with anti-Syp antibodies after reprobing (data not shown). The kinetics correspond to the kinetics seen in BAF3 cells expressing WT G-CSF-R. G-CSF stimulation did not induce association of GRB2 with phosphorylated Shc or with proteins of 145 kD, whereas in parallel control incubations, IL-3 stimulation did (Fig 8A and B). Although in the exposure presented the 145-kD proteins are not clearly visible after IL-3 stimulation, they were visible in longer exposures of the same blot (data not shown). In contrast to WT G-CSF-R, activation of mutant Y764F only marginally induced association of GRB2 with phosphorylated 90-kD proteins.

**DISCUSSION**

Signaling from G-CSF-R involves at least two major routes, i.e., the Jak/STAT and p21^{ras}/MAP kinase path-
SHC- AND GRB2-MEDIATED SIGNALING BY G-CSF-R

Fig 7. Association of GST/GRB2 fusion proteins with tyrosine-phosphorylated proteins. Lysates from BAF3 cells expressing WT G-CSF-R, treated as indicated in Fig 4, were incubated for 2 hours at 4°C with GST/GRB2 fusion proteins immobilized on glutathione-Sepharose beads. Bound proteins were eluted and subjected to SDS-PAGE. (A) Phosphotyrosine-containing proteins identified by Western blotting with 4G10. (B) Blot reprobed with anti-Shc antibodies.

ways. The membrane-proximal cytoplasmic regions of G-CSF-R, EPO-R, and other members of the cytokine receptor family have been shown to be primarily responsible for activation of Jak kinases. For some receptors, eg, EPO-R and the β,-chain of IL-3-R and granulocyte-macrophage colony-stimulating factor (GM-CSF)-R, it was demonstrated that Jak proteins physically associate with the membrane-proximal region. In EPO-R, a single conserved tryptophan residue in this region is crucial for binding of Jak2. So far, only limited information is available as to which regions or single amino acid residues within G-CSF-R are important for activation of p21^Ras^ and MAP kinase and which mechanisms connect G-CSF-R to this signaling pathway. Shc and GRB2 proteins have been implicated in linking different types of cytokine receptors to p21^Ras^ activation.

In this study, we established that activation of G-CSF-R results in rapid tyrosine phosphorylation of Shc and association of Shc with phosphorylated proteins of approximately 145 kD. Similar observations have been made for other cytokine receptors, including those for IL-3, EPO, GM-CSF, macrophage colony-stimulating factor (M-CSF), and Steel factor. The identity of the 145-kD proteins is not yet known. Recently, it was shown that Shc contains a novel phosphotyrosine-binding (PTB) domain, distinct from SH2 domains, which is involved in the interaction of Shc with tyrosine-phosphorylated 145-kD proteins.

GRB2 immunoprecipitates from G-CSF–activated BAF3 cell transfectants expressing WT G-CSF-R contained tyrosine-phosphorylated proteins of 52 (identified as Shc), 70, 90, and 145 kD. The kinetics of G-CSF-induced GRB2 association with Shc were comparable to those of tyrosine phosphorylation of Shc. Complex formation of 145-kD proteins, Shc, and GRB2 did not occur in cells expressing G-CSF-R deletion mutants M1 or DA or substitution mutant Y764F, consistent with the notion that recruitment of these signaling molecules occurs exclusively via involvement of tyrosine 764 of G-CSF-R. Tyrosine 764 also determined G-CSF-induced association of GRB2 with tyrosine-phosphorylated 90-kD proteins, although some residual association of GRB2 with 90-kD proteins after activation of mutant Y764F was seen. This could be suggestive of an alternative less efficient mechanism of activation of 90-kD proteins. Notably, the 90-kD proteins were not found in Shc immunoprecipitates, suggesting that p90/GRB2 complexes are formed independently of Shc and p145. The signaling mole-
cule, Vav, with a molecular weight of 95 kD, has recently been shown to form complexes with GRB2.23 We therefore investigated whether the 90-kD proteins could be Vav. However, reprobing of blots with anti-Vav antibodies indicated that p90 is distinct from Vav.

The phosphotyrosine phosphatase (PTP) Syp (synonymous with PTP-1D, PTP-2C, SH-PTP2, or SH-PTP3) has been implicated in signal transduction mediated by platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, and the hematopoietic growth factors IL-3, GM-CSF, and IL-6.24,25,49,54,55 Syp binds to activated PDGF-R and EGF-R via SH2 domains and is then tyrosine-phosphorylated.24,25,56 Tyrosine phosphorylation of Syp enhances its catalytic activity.25 The spectrum of activities of Syp has not been fully elucidated. It has been suggested that Syp acts as a negative regulator of receptor signal transduction by dephosphorylation of receptors, similar to the structurally related hematopoietic cell phosphatase (HCP).57 However, in apparent contrast with this idea, Syp does not efficiently dephosphorylate EGF-R or PDGF-R.25 Alternatively, Syp could act as a positive regulator by dephosphorylating inhibitory phosphotyrosine sites. Recently, a novel function was attributed to Syp when it was discovered that tyrosine-phosphorylated Syp functions as an adapter between PDGF-R and GRB2,36,37 resulting in recruitment of the GRB2/Sos complex to the PDGF-R/Syp complex followed by activation of the p21Ras pathway.58 Our results suggest that Syp may have a similar role in G-CSF-mediated signal transduction.

G-CSF–induced Syp/GRB2 association was severely reduced in cells expressing C-terminal deletion mutant DA (containing only tyrosine 704) and was not detected in cells expressing deletion mutant M1 (lacking all cytoplasmic tyrosines). These findings established that the C-terminal region of G-CSF-R is required for association of GRB2 with activated Syp. However, in contrast to the formation of p145/She/GRB2 and p90/GRB2 complexes, G-CSF–induced Syp/GRB2 association did not predominantly depend on the presence of one particular tyrosine residue of G-CSF-R. This was evident from the observation that all tyrosine-to-phenylalanine substitution mutants of G-CSF-R were capable of inducing Syp/GRB2 complexes. Assuming that, in analogy with PDGF-R, Syp acts as an adapter between G-CSF-R and GRB2, the association is partly induced via tyrosine 704 and partly via other tyrosine(s). Because GRB2 immunoprecipitates of cells expressing mutant Y764F contained tyrosine-phosphorylated Syp but not the 90- and 145-kD proteins, the latter molecules are probably upstream of GRB2 in the signaling route, unless Syp would efficiently dephosphorylate the 90- and/or 145-kD proteins or interfere with their binding to GRB2.

Collectively, our data could fit into a model in which recruitment of GRB2 to G-CSF-R occurs via multiple mechanisms, two of which depend on the presence of tyrosine 764 of G-CSF-R. Signaling via phosphotyrosine 764 of G-CSF-R is achieved through phosphorylation of either 145-kD or 90-kD proteins. In the first mechanism, phosphorylation of 145-kD proteins provides a binding site for the SH2 or PTB domain of She. She is subsequently tyrosine-phosphorylated and becomes associated with GRB2. In the second mechanism, phosphorylated 90-kD proteins directly bind to GRB2 without the involvement of She. In the third mechanism, cytoplasmic phosphotyrosines of G-CSF-R bind Syp, which subsequently becomes tyrosine-phosphorylated, thereby providing a binding site for GRB2. Finally, a fourth possibility that still needs to be experimentally addressed is that GRB2 may bind directly to tyrosine residues of G-CSF-R.

The physiologic significance of the different signaling mechanisms of G-CSF-CSF-R involving GRB2 remains to be established. It is anticipated that activation of the p21Ras pathway may occur via all these different mechanisms, involving multiple tyrosines of G-CSF-R. This would fit with the observation that a C-terminal truncation mutant of G-CSF-R, lacking tyrosine 764, is still capable of activation of p21Ras and MAP kinase.44 Activation of the p21Ras/MAP kinase pathway by IL-3/GM-CSF receptors has recently been shown to be essential for supporting the survival of cells by preventing apoptosis.59 It is likely that activation of this route through G-CSF-R serves a similar goal. Full understanding of the specific role of tyrosine 764 of G-CSF-R in signaling awaits elucidation of the identity and function of p90 and p145 proteins.

ACKNOWLEDGMENT

We thank Valeria Santini and Ruud Delwel for helpful discussions and Hans Vuijk and Arié Kievit for assistance with preparation of figures.

REFERENCES

4. Fukunaga R, Seto Y, Mizushima S, Nagata S: Three different
mRNAs encoding human granulocyte colony-stimulating factor receptor. Proc Natl Acad Sci USA 87:8702, 1990


38. Bennett AM, Tang TL, Sugimoto S, Walsh CT, Neel BG:
Protein-tyrosine-phosphatase SHFTP2 couples platelet-derived growth factor receptor \( \beta \) to Ras. Proc Natl Acad Sci USA 91:7335, 1994


41. Palacios R, Steinmetz M: IL-3-dependent mouse clones that express B-220 surface antigen, contain Ig genes in germ-line configuration, and generate B lymphocytes in vivo. Cell 41:727, 1985


45. Wittthuhn B, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O, Ihle JN: Jak2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following EPO stimulation. Cell 74:2227, 1993


56. Kazlauskas A, Feng G-S, Pawson T, Valius M: The 64-kDa protein that associates with the platelet-derived growth factor receptor \( \beta \) subunit via Tyr-1009 is the SH2-containing phosphotyrosine phosphatase Syk. Proc Natl Acad Sci USA 90:6939, 1993

57. Yi T, Mui AL-F, Krystal G, Ihle JN: Hematopoietic cell phosphatase associates with the interleukin-3 (IL-3) receptor \( \beta \) chain and down-regulates IL-3-induced tyrosine phosphorylation and mitogenesis. Mol Cell Biol 13:7577, 1993

58. Valius M, Kazlauskas A: Phospholipase C-\( \gamma \)1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor’s mitogenic signal. Cell 73:321, 1993

Specific involvement of tyrosine 764 of human granulocyte colony-stimulating factor receptor in signal transduction mediated by p145/Shc/GRB2 or p90/GRB2 complexes

JP de Koning, AM Schelen, F Dong, C van Buitenen, BM Burgering, JL Bos, B Lowenberg and IP Touw