EphB1 Associates with Grb7 and Regulates Cell Migration*

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EphB1 is a member of the Eph family of receptor tyrosine kinases that play important roles in diverse biological processes including nervous system development, angiogenesis, and neural synapsis formation and maturation. Grb7 is an adaptor molecule implicated in the regulation of cell migration. Here we report identification of an interaction between Grb7 and the cytoplasmic domain of EphB1 by using Grb7 as a "bait" in a yeast two-hybrid screening. Co-immunoprecipitation was used to confirm the interaction of Grb7 with the cytoplasmic domain of EphB1 as well as the full-length receptor in intact cells. This interaction is mediated by the SH2 domain of Grb7 and requires tyrosine autophosphorylation of EphB1. Furthermore, Tyr-928 of EphB1 was identified as the primary binding site for Grb7. Stimulation of endogenous EphB1 in embryonal carcinoma P19 cells with its ligand ephrinB1 increased its association with Grb7, which is consistent with a role for the autophosphorylation of EphB1. We also found that EphB1 could phosphorylate Grb7 and mutation of either Tyr-928 or Tyr-949 to Phe decreased this activity. Finally, we show that EphB1 could stimulate fibroblast motility on extracellular matrix in a kinase-dependent manner, which also correlated with its association with Grb7. Consistent with this, co-expression of Grb7 with EphB1 further enhanced cell motility, whereas co-expression of the Grb7 SH2 domain abolished EphB1-stimulated cell migration. Together, our results identified a novel interaction between EphB1 with the adaptor molecule Grb7 and suggested that this interaction may play a role in the regulation of cell migration by EphB1.

Grb7 is the founding member of a family of adaptor molecules that also include Grb10 and Grb14. The Grb7 family members share similar structural organizations, including an amino-terminal proline-rich region, a central segment termed the GM domain (for Grb and Mig) region, which includes a pleckstrin homology domain, and a carboxyl-terminal SH2 domain. Grb7 family members have been shown to interact with a variety of cell surface receptors and other signaling proteins (1, 2). Many of these interactions are mediated by the SH2 domain of a Grb7 family member and a phospho-tyrosine motif in the activated receptor/other signaling molecules. These interactions have been proposed to play a role in the regulation of mitogenic signaling pathways. A number of recent studies provided evidence to support a role of Grb10 in cell proliferation and cell survival (3, 4), although a similar role for the other family members Grb7 and Grb14 is not clear (2).

The central GM domain of Grb7 family adaptors share significant sequence homology with Mig-10, a Caenorhabditis elegans gene that has been implicated in neuronal migration in C. elegans embryonic development (5, 6). This suggests that Grb7 may play a role in the regulation of mammalian cell migration. Indeed, recent studies from our laboratory showed that Grb7 participates in signal transduction pathways in integrin-mediated cell migration (11, 12). We found that the SH2 domain of Grb7 could directly interact with focal adhesion kinase (FAK), which is a cytoplasmic tyrosine kinase known to mediate integrin signaling in cell migration (7–10). Grb7 interaction with FAK required autophosphorylation of FAK at Tyr-397, and this interaction is increased upon integrin-mediated cell adhesion, which stimulates FAK activation and autophosphorylation. Inducible over-expression of Grb7 in NIH3T3 cells enhanced cell migration toward fibronectin, whereas the SH2 domain inhibited cell migration. Association of Grb7 with FAK allowed phosphorylation by FAK, which was shown to be critical in the regulation of cell migration (11, 12). It is not clear, however, whether Grb7 interactions with other signaling molecules and/or cell surface receptors also play a role in the regulation of cell migration.

With 15 members by the last count, Eph kinases constitute the largest family of receptor protein tyrosine kinases. According to sequence homology and ligand-binding specificity, they are divided into two subfamilies. EphA kinases bind to ephrinA ligands that are anchored to cytoplasmic membrane through a glycosyl phosphatidylinositol linkage, whereas EphB kinases bind to ephrinB ligands that have a single transmembrane domain. Interestingly, receptor-ligand relationships between Eph kinases and ephrins are not distinct in that both Eph kinases and ephrins can transmit signals to the interior of juxtaposing cells (13–16). The Eph-ephrin interaction and ensuing bi-directional signaling have been implicated in diverse biological processes including nervous system development, angiogenesis, and neural synapsis formation and maturation (see Refs. 17–20 for recent reviews).

The regulatory functions of Eph kinases have been attributed to repulsive guidance of axon and cell migration in some cases and establishment and remodeling of cell-cell interactions in other situations (17, 18, 21, 22). Recent studies suggested that several Eph receptors could regulate cell adhesion...
and migration on extracellular matrix (ECM) (23–30). EphB2 could down-regulate integrin activity, possibly through tyrosine phosphorylation and inactivation of R-Ras (23), a positive regulator of integrin function (24, 25). In PC-3 cells, ligand stimulation of endogenous EphA2 caused cell rounding due to integrin affinity down-regulation, concomitant with focal adhesion kinase dephosphorylation (26). Induction of cell de-adhesion was also observed following ligand activation of EphA3 transfectants into 293 cells (27, 28). The adhesion regulatory functions of Eph kinases appear to be cell type-specific; transfection of EphA8 into HEK 293 cell inhibited cell adhesion (29), whereas in NIH 3T3 cells, the similarly transfected EphA8 stimulated integrin-mediated cell adhesion (30). Recently, EphA4 and other EphA kinases were found to associate with ephexin, a guanine nucleotide exchange factor for Rho family small GTPases (31). Upon EphA kinase ligation, ephexin mediates activation of RhoA and suppression of Cdc42 and Rac1, consistent with the repulsive guidance function of Eph kinases.

To further explore the role and mechanisms of Grb7 in cell migration, we used yeast two-hybrid screening to identify novel cellular proteins that bind to Grb7. We report here the identification of the association of EphB1 with Grb7 in an activation-dependent manner and the effect of EphB1 expression on cell migration. These results suggest the potential role of Grb7 in mediating the signaling pathways triggered by the EphB1 receptor in cell migration.

MATERIALS AND METHODS

Reagents—Protein A-Sepharose 4B, glutathione-agarose beads, and human plasma fibronectins were purchased from Sigma. LipofectAMINE was purchased from Invitrogen. The following antibodies were purchased as indicated: mouse α-phosphotyrosine monoclonal antibody PY-20 from Transduction Laboratories (Lexington, KY); rabbit polyclonal α-Grb7, rabbit polyclonal α-HA, and α-myc monoclonal antibodies from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Ephrin B1-Fc was as described previously (26), and goat anti-human IgG, Fcγ fragment-specific, was purchased from Jackson ImmunoResearch.

Yeast Two-hybrid Screen—CDNA encoding full-length Grb7 or its Pro-GM segment was excised from pkH3-Grb7 or pkH3-Pro-GM (12) and inserted into the bait vector pGBT9 (Clontech) to generate pGTP-Grb7 or pGTP-Pro-GM, respectively. The H7F7 yeast strain was first transformed with pGTP-Grb7 and subsequently with a HeLa cell cDNA library fused to the GAL4 transcriptional activation domain (32) (generous gift of Dr. G. Hannon). Transformants were plated on agar selection medium lacking tryptophan (Trp−) (generous gift of Dr. G. Hannon). Transformants were picked and then ligated with pkH3 that had been digested with SalI followed by fill-in by T7 DNA polymerase and digested with BamH1, resulting in pHAN. Immunoprecipitation and Western Blotting—For most experiments, subconfluent cells were washed twice with ice-cold PBS and then lysed with 1% Nonidet P-40 (Sigma) as a template. The PCR product was digested by BamH1, purified from gel, and then ligated with pkH3 that had been digested with SalI and then inserted into the corresponding site in pkH3, which had been digested with SalI and EcoRI and then inserted into the cellus. The construct was then digested with BglII and EcoRI, and the segment encoding the cytoplasmic domain of EphB1 was replaced by the corresponding fragment from pkH3-EphB1cyto-βd, pkH3-EphB1cyto−Y594F, or pkH3-EphB1cyto−Y928F to generate pKCH-EphB1-βd, pKCH-EphB1-Y594F, or pKCH-EphB1-Y928F, respectively.
interaction of Grb7 with EphB1cyto, we tested potential interaction of EphB1cyto interaction with a Grb7 truncation mutant lacking the SH2 domain (designated Pro-GM) in a yeast two-hybrid system (Fig. 1). As expected, EphB1cyto interacted with the full-length Grb7 encoded by pGBT-Grb7 but not pGBT9 vector alone. Interestingly, EphB1cyto did not show any interaction with Pro-GM. These results suggested that EphB1cyto also interacted with Grb7 through its SH2 domain.

To verify the yeast two-hybrid results and to further investigate the potential interaction of EphB1 with the Grb7 SH2 domain, a mammalian expression vector encoding the EphB1 cytoplasmic domain (EphB1cyto) with a triple HA tag fused to its amino terminus was generated as described in the “Materials and Methods.” Point mutations were introduced into EphB1cyto to generate the kinase-defective (EphB1cyto-kin) domain, missense mutations (EphB1cyto-Y594F and EphB1cyto-Y928F) in the same expression vector. Tyr-594 and Tyr-928 have been shown to mediate EphB1 interactions with Nck and LMW-PTP, respectively. CHO cells were co-transfected with pKH3 vectors encoding EphB1cyto or its mutants along with pDHGST-Grb7 encoding Grb7 fused to GST at the amino terminus (11). Two days after transfection, cells were lysed and GST-Grb7 complexes were precipitated with glutathione-agarose beads. After washing, the bound proteins were analyzed by Western blotting with antibody against EphB1. Fig. 2 shows that the wild type EphB1cyto and EphB1cyto-Y594F mutant associated with Grb7 in CHO cells. In contrast, EphB1cyto-kin was not associated with Grb7, and the EphB1cyto-Y928F mutant showed a significantly reduced association with Grb7. Similar expression levels of EphB1cyto and its mutants were verified by blotting of whole cell lysates with anti-EphB1 antibodies. We noticed that EphB1cyto-kin exhibited an increased mobility compared with wild type EphB1cyto on the gel, suggesting that EphB1 autophosphorylation affected its mobility on SDS-PAGE. Together, these results demonstrate the interaction of EphB1 with Grb7 and suggest that autophosphorylation of EphB1 at Tyr-928 plays a major role in mediating its interaction with Grb7 through the SH2 domain.

We next examined the association of the full-length EphB1 with Grb7 and its various domains in intact cells. CHO cells were co-transfected with expression vector encoding HA-tagged full-length EphB1 and pDHGST encoding Grb7 or its different segments with GST fused at their amino terminus. Two days after transfection, the lysates were prepared and GST fusion proteins were pulled down using glutathione-agarose beads. The bound proteins were then analyzed by Western blotting with polyclonal anti-HA antibody to detect associated HA-tagged EphB1 (Fig. 3A) or with anti-GST to detect the amount of Grb7 or its segments in the complex (Fig. 3B). EphB1 bound to full-length Grb7 and the SH2 domain of Grb7 but not the GM domain, Pro-GM domains, or GST alone control. Although the amount of GST-GM is slightly lower than the Grb7 or SH2 domain alone, comparable amounts of GST-Pro-GM were precipitated. Western blotting of whole cell lysates with anti-HA confirmed similar expression levels of EphB1 in all samples (Fig. 3C). Together, these results indicated that the SH2 domain of Grb7 also interacted with EphB1 through its SH2 domain.

Together, these results demonstrate the interaction of EphB1 with Grb7 and suggest that autophosphorylation of EphB1 at Tyr-928 plays a major role in mediating its interaction with Grb7 through the SH2 domain.

We next examined the association of the full-length EphB1 with Grb7 and its various domains in intact cells. CHO cells were co-transfected with the plasmids encoding bait and prey fusion proteins as indicated. Growth on Trp-Leu-His triple dropout plates is indicated. All transformants grew efficiently on Trp-

| Fusion partners | pGBT-9 | pGBT-Grb7 | pGBT-Pro-GM |
|-----------------|--------|-----------|-------------|
| pGAD            | −      | −         | −           |
| pGAD-EphB1cyto  | −      | +         | −           |

FIG. 1. Interaction of Grb7 with EphB1 cytoplasmic domain in the yeast two-hybrid system. A, schematic diagram of Grb7 and its Pro-GM segment used in the yeast two-hybrid assays. B, yeasts were co-transformed with the plasmids encoding bait and prey fusion proteins as indicated. Growth on Trp-Leu-His triple dropout plates is indicated. All transformants grew efficiently on Trp-Leu-His double dropout plates.

Fig. 1. Interaction of Grb7 with EphB1 cytoplasmic domain in the yeast two-hybrid system. A, schematic diagram of Grb7 and its Pro-GM segment used in the yeast two-hybrid assays. B, yeasts were co-transformed with the plasmids encoding bait and prey fusion proteins as indicated. Growth on Trp-Leu-His triple dropout plates is indicated. All transformants grew efficiently on Trp-Leu-His double dropout plates.

FIG. 2. Association of Grb7 with EphB1 cytoplasmic domain and its mutants in mammalian cells. CHO cells were co-transfected with plasmid pDHGST-Grb7 and pKH3-EphB1cyto, pKH3-EphB1cytokd, pKH3-EphB1cyto-Y594F, or pKH3-EphB1cyto-Y928F, as indicated. Grb7 was pulled-down from the lysates using glutathione-coupled agarose beads. These were then analyzed by Western blotting with anti-EphB1 (left four lanes). Aliquots of the lysates (whole cell lysates) were also analyzed directly by Western blotting with anti-EphB1 to verify similar expression levels in all samples (right four lanes). Molecular mass positions (kDa) are shown on the left. The arrow on the right indicates the position of EphB1 cytoplasmic domain.

Fig. 2. Association of Grb7 with EphB1 cytoplasmic domain and its mutants in mammalian cells. CHO cells were co-transfected with plasmid pDHGST-Grb7 and pKH3-EphB1cyto, pKH3-EphB1cytokd, pKH3-EphB1cyto-Y594F, or pKH3-EphB1cyto-Y928F, as indicated. Grb7 was pulled-down from the lysates using glutathione-coupled agarose beads. These were then analyzed by Western blotting with anti-EphB1 (left four lanes). Aliquots of the lysates (whole cell lysates) were also analyzed directly by Western blotting with anti-EphB1 to verify similar expression levels in all samples (right four lanes). Molecular mass positions (kDa) are shown on the left. The arrow on the right indicates the position of EphB1 cytoplasmic domain.

FIG. 3. Binding of full-length EphB1 to Grb7 and its segments. CHO cells were co-transfected with pKH3-EphB1 encoding HA-tagged full-length EphB1 and pDHGST, pDHGST-Grb7, pDHGST-GM, pDHGST-Pro-GM, and pDHGST-SH2, as indicated. Two days after transfection, cells were lysed and Grb7 or its segments were pulled-down from the lysates using glutathione-coupled agarose beads. They were then analyzed by Western blotting with anti-HA (A) or anti-GST (B). Aliquots of the lysates (WCL) were also analyzed directly by Western blotting with anti-HA to verify similar expression levels EphB1 (C). Molecular mass positions (kDa) are shown on the left.
domain of Grb7 is sufficient for mediating specific binding of Grb7 to EphB1 in mammalian cells and that other domains are not involved in the interaction. In addition we found that Grb7 did not interact with EphB3, another member of the EphB family, in similar experiments (data not shown), providing further support for the specificity of Grb7 interaction with EphB1.

The above data show clearly that EphB1 could interact with Grb7 in a phosphorylation-dependent manner. However, the biological significance of this interaction can only be established if this interaction is regulated by activation of endogenous EphB1 by its ligand. To investigate this possibility, we employed P19 cells that express endogenous EphB1. The cells were transiently transfected with a mammalian expression vector pHAN-Grb7 encoding a His-tagged Grb7. Two days after transfection, the cells were treated with EphB1 ligand ephrin-B1-Fc or Fc control. Cell lysates were then prepared, and EphB1 complexes were precipitated with ephrin-B1-Fc-protein A/G beads. After washing, the precipitated complexes were resolved on SDS-PAGE and subjected to Western blotting using anti-Grb7 antibody. Fig. 4 shows that endogenous EphB1 associates with Grb7, and treatment of cells with the ligand ephrin B1 further enhanced EphB1/Grb7 association.

Our previous studies (12) showed that Grb7 could be phosphorylated by FAK, which plays a role in the regulation of cell migration by FAK. To further investigate the potential cellular function of EphB1 binding to Grb7, we examined the possibility of tyrosine phosphorylation of Grb7 by EphB1. We first analyzed the kinase activity of the EphB1 and its mutants because a number of reports suggested that mutation of the corresponding Tyr-594 residue in EphB2 attenuated its catalytic function (34, 35). CHO cells were transfected with expression vectors encoding HA-tagged EphB1 or its mutants. Lysates were prepared two days after transfection and analyzed for autophosphorylation by immunoprecipitation with anti-HA followed by Western blotting with anti-phosphotyrosine antibody PY-20. Fig. 5A shows that the wild type EphB1 exhibited strong autophosphorylation (top panel). Western blotting of the lysates from parallel samples with anti-HA verified similar expression levels of the constructs (bottom panel). These results suggested that mutation at Y594 also attenuated the kinase activity of EphB1, as in the case of EphB2 reported earlier (34, 35).

We then analyzed possible phosphorylation of Grb7 by EphB1 (Fig. 5B). CHO cells were co-transfected with pHAN-Grb7 and pKCH vectors encoding HA-tagged EphB1, its mutants EphB1-kd, EphB1-Y594F, and EphB1-Y928F, or vector alone as a control. Two days after transfection, cell lysates were prepared and Grb7 was precipitated with Ni-beads and analyzed by Western blotting with PY-20. The top panel shows that co-expression of wild type EphB1, but not the kinase-defective EphB1, induced tyrosine phosphorylation of Grb7. Both EphB1-Y594F and EphB1-Y928F mutant also induced Grb7 phosphorylation, although to a lesser extent than the wild type EphB1. Western blotting of the precipitates by anti-Grb7 verified similar amounts of Grb7 in the samples (middle panel).

Because EphB1-Y594 mutant was still capable of binding to Grb7, we investigated the phosphorylation levels of EphB1 and its mutants that were associated with Grb7. Western blotting of Grb7 immunoprecipitates with PY20 revealed that wild type EphB1 was phosphorylated, whereas EphB1-kd was not. Interestingly, EphB1-Y594F was strongly phosphorylated (Fig. 5B, lower panel), correlating with its ability to bind Grb7 (Fig. 2). The strong phosphorylation of the co-precipitated EphB1-Y594F suggests that Grb7 may target the pool of the EphB1 mutant that was appropriately phosphorylated at sites that are targeted by the SH2 domain of Grb7, including Tyr-928. The reduced phosphorylation of Grb7 by the EphB1-Y594F

FIG. 4. Stimulation of Grb7 association with endogenous EphB1 upon ligand treatment. P19 cells were transfected with pHK3-Grb7 and then incubated with or without Ephrin-B1-Fc fusion protein as described under “Materials and Methods.” The cells were lysed and EphB1 complex was pull-downed with pre-clustered Ephrin-B1-Fc-Protein A/G beads. They were analyzed by Western blotting with anti-Grb7 (left panel). Aliquots of the lysates (WCL, right panel) were also analyzed directly with anti-Grb7 to show similar expression levels (right panel). Molecular mass positions (kDa) are shown on the left.

FIG. 5. Induction of tyrosine phosphorylation of Grb7 by EphB1. A, CHO cells were transfected with pKCH vector alone, pKCH-EphB1, pKCH-EphB1-kd, pKCH-EphB1-Y594F, or pKCH-EphB1-Y928F, as indicated. Two days after transfection, cells were lysed and the lysates were immunoprecipitated with anti-HA followed by Western blotting with PY-20 (top panel) or anti-HA (bottom panel). B, CHO cells were co-transfected with expression vector pHAN-Grb7 and pKCH vector alone, pKCH-EphB1, pKCH-EphB1-kd, pKCH-EphB1-Y594F, or pKCH-EphB1-Y928F, as indicated. Two days after transfection, cells were lysed and Grb7 was pulled-down from the lysates using Ni-beads. They were then analyzed by Western blotting with PY-20 to detect tyrosine phosphorylated Grb7 (top panel) and associated EphB1 or mutants (bottom panel) or anti-Grb7 to verify similar expression levels (middle panel). Molecular mass positions (kDa) are shown on the left.
mutant, relative to the wild type, could be accounted for by its reduced catalytic activity, which is consistent with previous reports for EphB2 mutation at similar site (34, 35).

Consistent with the lack of interaction between EphB1-Y928F and Grb7 (Fig. 2), only low levels of tyrosine-phosphorylated EphB1-Y928F were detected in Grb7 precipitates. The low level of Grb7 phosphorylation, despite the relatively intact catalytic activity of EphB1-Y928F, indicates that the physical interaction may be necessary for phosphorylation to occur.

To study whether EphB1 and its association with Grb7 could regulate cell migration, we tested the potential effects of EphB1 and its mutants on cell migration using a time-lapse imaging-based computerized motility analysis method called OMAware, as described previously (12). CHO cells were transiently transfected with expression vectors encoding EphB1 or its mutants along with pEGFP to mark the transfected cells. Two days after transfection, a fraction of cells were lysed and analyzed by Western blotting to verify similar expression levels (data not shown and Fig. 5). The remaining cells were used to evaluate the effects of these constructs on cell motility using OMAware. Fig. 6 shows that expression of wild type EphB1 stimulated CHO cell migration, whereas transfection of control pKH3 vector did not affect cell motility when compared with untransfected cells. Under similar conditions, the kinase-defective EphB1 did not stimulate cell migration. The Y594F mutant exhibits similar activity as the wild type EphB1, whereas the EphB1Y928F mutant shows reduced stimulation of cell migration (about 35% of that by the wild type EphB1).

These results suggested that EphB1 could regulate cell migration in a kinase-dependent manner. In addition, they showed that the ability of EphB1 to stimulate cell migration correlated with its association with Grb7 (see Figs. 2 and 5), suggesting that EphB1 may regulate cell migration through its interaction with Grb7. To test this possibility, CHO cells were co-transfected with expression vector encoding EphB1 and pEGFP-Grb7 or pEGFP-Grb7SH2 encoding full-length Grb7 or the SH2 domain of Grb7, respectively. The cells were then subjected to motility assays using OMAware as described above. As shown in Fig. 6, expression of GFP-Grb7 alone stimulated cell migration as reported previously (11, 12). Co-expression of EphB1 with GFP-Grb7 further enhanced cell motility compared with expression of EphB1 with the GFP control or expression of GFP-Grb7 alone. Conversely, co-expression of EphB1 with the SH2 domain of Grb7 abolished EphB1-stimulated cell migration, presumably by disrupting EphB1 interaction with the endogenous Grb7. Together these results provide strong support for a role of EphB1 interaction with Grb7 in the regulation of cell migration.

**DISCUSSION**

Cell migration is critical for biological processes such as development, immune response, wound healing, and tumor metastasis. Initiation of cell migration involves detection of the gradients of soluble chemoattractants and/or immobilized environmental cues by cell surface receptors. The Eph receptor tyrosine kinases have been implicated to play a key role in the axon path-finding and migration of neurons as well as other cell types (17–19, 36). Increasing evidence suggests that, like other receptor tyrosine kinases, Eph receptors could associate with intracellular signaling molecules upon its activation (17, 37). However, the molecular mechanisms by which Eph receptors regulate cell migration is still poorly understood. In this report, we have identified the association of EphB1 with another signaling molecule Grb7. We showed that this interaction is dependent on EphB1 activation and requires Tyr-928 in the cytoplasmic domain of EphB1 and the SH2 domain of Grb7. Furthermore, we found that this interaction plays a role in the regulation of cell migration.

Grb7 was originally isolated as an epidermal growth factor receptor-binding adaptor protein (38), and soon after several other growth factor receptors and intracellular signaling molecules were reported to associate with Grb7 (1, 2). However, the functional significance of these specific interactions is still not completely understood. Recent studies from our laboratory and others (12, 39, 40) have suggested that Grb7 mediates stimulation of cell migration by integrin-FAK signaling via its interaction with FAK and phosphoinositides. These are the first experimental demonstrations of a specific cellular function for Grb7, which is consistent with previous hypothesis based on the homology of Grb7 with a C. elegans gene product Mig-10 involved in neuronal cell migration in embryonic development. Our findings in the current study indicated that Grb7 could also couple directly to receptor tyrosine kinases to mediate cell migration. This raises the interesting possibility that a general and specific cellular function of Grb7 is to mediate cell migration triggered by a variety of stimuli, which should be tested in future experiments.

Data presented here suggested that the interaction of Grb7 with EphB1 is mediated by the SH2 domain of Grb7 and the phosphorylated Tyr-928 of EphB1. Although it has been suggested to bind the pYXX motif, the SH2 domain of Grb7 has been shown to associate with phosphorylated tyrosines not present in this motif (11, 41, 42). Inspection of residues surrounding the Tyr-928 of EphB1 indicated the absence of the motif, therefore providing another exception regarding the preferred binding motifs for the SH2 domain of Grb7. Although it is not clear whether EphB1 could directly phosphorylate Grb7, EphB1 binding to Grb7 through Tyr-928 appears to play a role in its ability to induce Grb7 phosphorylation (Fig. 5) as well as its stimulation of cell migration (Fig. 6).

Previous studies (12, 39) suggested that FAK phosphorylation of Grb7 is important for its stimulation of cell migration. However, the Y594F mutant of EphB1 stimulated cell migration to a comparable level as the wild type EphB1 (Fig. 6),
although its induction of Grb7 phosphorylation is much lower due to its reduced catalytic activity (Fig. 5). Therefore EphB1 association with Grb7 through Tyr-928, but not the total level of Grb7 phosphorylation by EphB1, is critical for promotion of cell migration. These results, however, do not exclude the possibility that phosphorylation of particular Grb7 sites (e.g., those that are also phosphorylated by FAK that have been shown to correlate with stimulation of cell migration by FAK) by EphB1 and its Y924F mutant is important for promotion of cell migration by EphB1. Although Y924F showed reduced activity and autophosphorylation, it was able to bind Grb7, which may allow it to phosphorylate the “critical” Grb7 site. The wild type EphB1 may phosphorylate both the critical site and other sites of Grb7. The Y928F mutant, on the other hand, may phosphorylate only Grb7 sites other than the critical site (or at least reduced at the critical site) due to its inability to associate with Grb7. It is clear that additional studies to map the phosphorylation sites of Grb7 by EphB1 and FAK are important to further clarify the mechanisms by which Grb7 mediates stimulation of cell migration by EphB1 and FAK.

Previous studies (43, 44) have identified the interaction of EphB1 with several intracellular signaling molecules. Indeed, Tyr-928 of EphB1 has been mapped as the putative binding site for Grb10 and LMW-PTP (45, 46). Our previous data suggested that unlike Grb7, Grb10 appeared not to be involved in the regulation of cell migration despite their sequence homology (12). Although we could not exclude the possibility that the Y928F mutant failed to stimulate cell migration due to its lack of binding to LMW-PTP, our current data strongly support a role of EphB1 interaction with Grb7 (Fig. 3 and data not shown). Further investigations will be necessary to clarify the potential specific effects of different Eph receptors in various cells. Nevertheless, the current report identified a novel interaction between EphB1 with the adapter molecule Grb7 and the potential mechanisms of EphB1 regulation of cell migration.

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References
1. Daly, R. J. (1998) Cell. Signal. 10, 613–618
2. Han, D. C., Shen, T. L., and Guan, J. L. (2001) Oncogene 20, 6315–6321
3. Minusone, A., Valentini, B., Reisnecker, M., Xu, S., and Baserga, R. (1997) J. Biol. Chem. 272, 26382–26387
4. Wang, J., Dai, H., Yousef, N., Moussaia, M., Deng, Y., Boufelliga, A., Swany, M., and Passalaqua, E. (1999) Mol. Cell. Biol. 19, 6217–6228
5. Manser, J., and Wood, W. B. (1990) Dev. Genet. 11, 49–64
6. Ooi, J., Tujikiv, I., Imanu, I., Morishita, M., Sugimachi, J., Buchberg, A. M., and Maroplos, B. (1995) Oncogene 10, 1623–1630
7. Parsons, J. T. (1996) Curr. Opin. Cell Biol. 8, 146–152
8. Hanks, S. K., and Polte, T. R. (1997) Bioessays 19, 137–145
9. Cary, L. A., and Guan, J. L. (1999) Front. Biosci. 4, D102–D113
10. Schlaeger, D. D., Hauck, C. R., and Sieg, D. J. (1999) Prog. Biophys. Mol. Biol. 71, 435–478
11. Han, D. C., and Guan, J. L. (1999) J. Biol. Chem. 274, 24425–24430
12. Han, D. C., Shen, T. L., and Guan, J. L. (2000) J. Biol. Chem. 275, 28911–28917
13. Holland, S. J., Gale, N. W., Bhamulu, G., Yancopoulos, G. D., Kenemeyer, M., and Paonson, T. (1996) Nature 383, 722–725
14. Bruckner, K., Pasquale, E. B., and Klein, R. (1997) Science 275, 1640–1643
15. Cowan, C. A., and Kenemeyer, M. (2001) Nature 413, 174–179
16. Lu, Q., Sui, E., Klein, R. S., and Plaxton, J. G. (1999) J. Cell. Biol. 156, 69–79
17. Wilkinson, D. G. (2001) Annu. Rev. Neurosci. 24, 155–182
18. Planagan, J. G., and Vanderhaegen, P. (1998) Annu. Rev. Neurosci. 21, 309–345
19. Adams, R. H., and Klein, R. (2000) Trends Cardiovasc. Med. 10, 183–188
20. Muri, K. K., and Pasquale, E. B. (2002) Neuron 33, 159–162
21. Gale, N. W., and Yancopoulos, G. D. (1997) Cell Tissue Res. 290, 227–241
22. Bao, B., and Drescher, U. H. (2000) Trends Cell Biol. 10, 145–149
23. Zou, J. X., Wang, B., Kato, M. S., Ziech, A. H., Pasquale, E. B., and Rusoslath, E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13813–13818
24. Zhang, Z., Nosito, K., Wang, H., Reed, J. C., and Rusoslath, E. (1996) Cell 85, 61–69
25. Wang, B., Zou, J. X., Ek-Rylander, B., and Rusoslath, E. (2000) J. Biol. Chem. 275, 5222–5227
26. Miao, H., Burnett, E., Kinch, M., Simon, E., and Wang, B. (2000) Nat. Cell. Biol. 2, 62–69
27. Boyd, A. W., and Lackmann, M. (2001) Sci. STKE 2001(112), RE20
28. Lawrence, I. D., Wimmer-Klein, S., Lock, P., Schwenklaeder, S. M., Down, M., Boyd, A. W., Aweof, P. F., and Lackmann, M. (2002) J. Cell. Sci. 115, 1059–1072
29. Choi, S., and Park, S. (1999) Oncogene 18, 5413–5422
30. Gu, C., and Park, S. (2001) Mol. Cell. Biol. 21, 4579–4597
31. Shama, S. M., Lin, M. Z., Goldberg, J. L., Estrach, S., Sahin, M., Hu, L., Bazzalakova, M., New, R., Cortes, G., Debant, A., and Greenberg, M. E. (2001) Cell 105, 233–244
32. Hannon, G. J., Demetrick, D., and Beach, D. (1993) Genes Dev. 7, 2378–2389
33. Cary, L. A., Chang, J. F., and Guan, J. L. (1996) J. Cell Sci. 109, 1787–1794
34. Binn, R. L., Taylor, P. P., Scherfer, F., Paonson, T., and Holland, S. J. (2000) Mol. Cell. Biol. 20, 4791–4805
35. Ziech, A. H., Pasquale, C., Freeman, A. L., Schneller, M., Hadman, M., Smith, J. W., Rusoslath, E., and Paonson, E. B. (2000) Oncogene 19, 177–187
36. Dodelet, V. C., and Pasquale, E. B. (2000) J. Biol. Chem. 275, 5413–5422
37. Defted, V. C., and Pasquale, E. B. (2000) Oncogene 19, 5614–5619
38. Holland, S. J., Polos, E. E., Wang, T., and Schlessinger, J. (1998) Curr. Opin. Neurobiol. 8, 117–127
39. Margolis, B., Silvennoinen, O., Comoglio, F., Ronprapunt, C., Skolnik, E., Ulrich, A., and Schlessinger, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8894–8898
40. Tanaka, T., Saito, K., Kawaguchi, H., Saeki, H., Ohno, S., and Wands, J. R. (2000) J. Cell. Physiol. 183, 411–415
41. Shen, T. L., Han, D. C., and Guan, J. L. (2002) J. Biol. Chem. 277, 29069–29077
42. Zhou, S., Margolis, B., Chaudhuri, M., Shoeison, S. E., and Cantley, L. C.
(95) J. Biol. Chem. 270, 14863–14866
42. Fiddes, R. J., Campbell, D. H., Janes, P. W., Sivertsen, S. P., Sasaki, H., Wallasch, C., and Daly, R. J. (1998) J. Biol. Chem. 273, 7717–7724
43. Stein, E., Huynh-Do, U., Lane, A. A., Cerretti, D. P., and Daniel, T. O. (1998) J. Biol. Chem. 273, 1303–1308
44. Yue, Y., Widmer, D. A., Halladay, A. K., Cerretti, D. P., Wagner, G. C., Dreyer, J. L., and Zhou, R. (1999) J. Neurosci. 19, 2090–2101
45. Stein, E., Cerretti, D. P., and Daniel, T. O. (1996) J. Biol. Chem. 271, 23588–23593
46. Stein, E., Lane, A. A., Cerretti, D. P., Schoecklmann, H. O., Schroff, A. D., Van Etten, R. L., and Daniel, T. O. (1998) Genes Dev. 12, 667–678
47. Hattori, M., Osterfield, M., and Flanagan, J. G. (2000) Science 289, 1360–1365
48. Lauffenburger, D. A., and Horwitz, A. F. (1996) Cell 84, 359–369
49. Huynh-Do, U., Stein, E., Lane, A. A., Liu, H., Cerretti, D. P., and Daniel, T. O. (1999) EMBO J. 18, 2165–2173
50. Bohme, B., VandenBos, T., Cerretti, D. P., Park, L. S., Holtrich, U., Rubsam-Waigmann, H., and Strebhardt, K. (1996) J. Biol. Chem. 271, 24747–24752
51. Holmberg, J., Clarke, D. L., and Frisen, J. (2000) Nature 408, 203–206
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