Membrane Targeting of Grb2-associated Binder-1 (Gab1) Scaffolding Protein through Src Myristoylation Sequence Substitutes for Gab1 Pleckstrin Homology Domain and Switches an Epidermal Growth Factor Response to an Invasive Morphogenic Program

Christiane R. Maroun,* Monica A. Naujokas,* and Morag Park†‡§

Departments of *Medicine, †Biochemistry, and §Oncology, Molecular Oncology Group, McGill University Health Centre, McGill University, Montreal, Quebec, H3A 1A1, Canada

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The hepatocyte growth factor receptor tyrosine kinase Met promotes cell dissociation and the inherent morphogenic program of epithelial cells. In a search for substrates downstream from Met, we have previously identified the Grb2-associated binder-1 (Gab1) as critical for the morphogenic program. Gab1 is a scaffold protein that acts to diversify the signal downstream from the Met receptor through its ability to couple with multiple signal transduction pathways. Gab1 contains a pleckstrin homology (PH) domain with specificity for phosphatidylinositol 3,4,5-trisphosphate. The phospholipid binding capacity of the Gab1 PH domain is required for the localization of Gab1 at sites of cell-cell contact in colonies of epithelial cells and for epithelial morphogenesis, suggesting that PH domain-dependent subcellular localization of Gab1 is a prerequisite for function. We have investigated the requirement for membrane localization of Gab1 for biological activity. We show that substitution of the Gab1 PH domain with the myristoylation signal from the c-Src protein is sufficient to replace the Gab1 PH domain for epithelial morphogenesis. The membrane targeting of Gab1 enhances Rac activity in the absence of stimulation and switches a nonmorphogenic noninvasive response to epidermal growth factor to a morphogenic invasive program. These results suggest that the subcellular localization of Gab1 is a critical determinant for epithelial morphogenesis and invasiveness.

INTRODUCTION

The process of epithelial morphogenesis is crucial during embryonic development and in wound healing in the adult. This process requires the coordination of multiple cellular functions, including cellular proliferation, survival, migration, invasion, and differentiation, as well as remodeling of the extracellular matrix (reviewed in Gumbiner, 1992). A potent epithelial morphogen is the mesenchymally derived hepatocyte growth factor (HGF) (reviewed in Matsumoto and Nakamura, 1997). Through the activation of its receptor tyrosine kinase Met, expressed in epithelial and endothelial cells, HGF initiates the intrinsic morphogenic program of epithelial cells grown in three-dimensional matrix cultures (Montesano et al., 1991; Weidner et al., 1993; Soriano et al., 1995) and regulates multiple biological responses critical to the morphogenic process, including cell proliferation and survival, as well as the remodeling of epithelia (Gherardi et al., 1989; Nakamura, 1991; Weidner et al., 1993; Zhu et al., 1994). An in vivo role for HGF and the Met receptor has been demonstrated during the development of the liver, the development and innervation of skeletal muscle, and the growth of axonal cones (Yang and Park, 1993; Schmidt et al., 1995; Uehara et al., 1995; Ebens et al., 1996; Maina et al., 1997). Consequently, the involvement and biological activities of HGF and Met need be tightly controlled, because dysregulation of either has been associated with multiple neoplasias (reviewed in Vande Woude et al., 1997).

Although the ability of Met to regulate a morphogenic program has been extensively documented, the molecular mechanisms underlying this function remain ill defined. To dissect Met-dependent signals for a morphogenic program, we have established an epithelial model by using Madin-Darby canine kidney (MDCK) cells that express chimeric CSF-Met receptors, allowing structure/function analyses of
Met-dependent signals (Zhu et al., 1994; Fournier et al., 1996). These studies have revealed that two tyrosine residues in the carboxy terminus of the Met receptor (Y1349 and Y1356) are required for all biological activities of the receptor and provide docking sites for downstream signaling proteins (Weidner et al., 1993; Zhu et al., 1994). Tyrosine 1356 forms a docking site for the binding of the Grb2 and Shc adapter proteins, in addition to the indirect recruitment, via Grb2, of the Grb2-associated binder-I (Gab1) docking protein (Ponzetto et al., 1994; Fixman et al., 1996, 1997; Weidner et al., 1996; Nguyen et al., 1997; Peschard et al., 2001). Mutants of the Met receptor with decreased ability to recruit Grb2 fail to form branching tubules upon Met activation (Fournier et al., 1996). Importantly, the overexpression of Gab1 in these cells rescues the Met-dependent morphogenic program (Maroun et al., 1999a). This identifies Gab1 as a critical mediator of the epithelial morphogenic program and provides a biological model to perform structure/function analyses of Gab1-derived signals essential for this process.

Gab1 belongs to a family of docking proteins, including Gab2 and the recently identified Gab3 in mammals, as well as DOS and SOC-1 in Drosophila melanogaster and Caenorhabditis elegans, respectively (Holgado-Madruga et al., 1996; Raabe et al., 1996; Gu et al., 1998; Nishida et al., 1999; Zhao et al., 1999; Liu and Rohrschneider, 2002; Wolf et al., 2002). Despite low sequence homology, these proteins contain common features. All Gab proteins share greatest homology within their pleckstrin homology (PH) domain. In addition, Gab family proteins contain numerous tyrosine residues that, when phosphorylated, provide binding sites for the Src homology (SH)2 domains of multiple signaling proteins. Gab family proteins are phosphorylated after the activation of several families of receptor tyrosine kinase, cytokine and T- and B-cell antigen receptors, as well as nonreceptor tyrosine kinases, and act to diversify and potentiate signals downstream from these receptors (reviewed in Liu and Rohrschneider, 2002), yet the exact role for each Gab family member has not been elucidated.

Gab1 acts to diversify the signal downstream from the Met receptor. After the activation of the Met receptor, phosphorylation of Gab1 provides binding sites for the p85 subunit of the phosphatidylinositol-3-kinase (PI3K), phospholipase Cyl, the tyrosine specific phosphatase SHP-2, and the Crk adapter protein (Garcia-Guzman et al., 1999; Maroun et al., 1999a, 2000; Gual et al., 2000; Lamorte et al., 2000; Sakkab et al., 2000; Schaeper et al., 2000). Structure/function analyses of Gab1 have revealed that the integrity of its PH domain and association with the SHP-2 phosphatase or Crk are essential for Met-dependent epithelial morphogenesis (Maroun et al., 1999a,b, 2000; Lamorte et al., 2002b). Although the mechanisms through which Gab proteins are recruited to different receptors are still a subject of analysis, Gab1 contains an atypical proline-rich motif specific for the C-terminal SH3 domain of Grb2 (Lock et al., 2000). This allows the indirect recruitment of Gab1 via the Grb2 adapter protein to multiple receptors, including the epidermal growth factor (EGF) receptor (Lock et al., 2000; Rodrigues et al., 2000; Schaeper et al., 2000; Ong et al., 2001). In addition, Gab1 contains a unique proline-rich Met binding domain that interacts in a Grb2-independent manner with the Met receptor (Weidner et al., 1996; Lock et al., 2000, 2002; Schaeper et al., 2000).

Members of the Gab family of proteins all contain a conserved amino-terminal PH domain. Structural studies of PH domains have revealed common motifs that bind to phosphoinositides present in cellular membranes with different degrees of affinity and specificity (reviewed in Lemmon and Ferguson, 2000; Maffucci and Falasca, 2001; Lemmon et al., 2002). Although PH domains act to target proteins to phosphoinositide-rich membrane domains, some evidence supports a role for PH domains in protein–protein interactions (Burks et al., 1998; Farhang-Fallah et al., 2000). A full understanding of the physiological relevance of such interactions is still lacking and has not been addressed for Gab family members. The Gab1 PH domain binds phosphatidylinositol-3,4,5-trisphosphate (PIP3) (Isakoff et al., 1998; Maroun et al., 1999a,b; Rodrigues et al., 2000). In colonies of MDCK cells, Gab1 is predominantly localized at sites of cell-cell contacts. This localization is dependent on an intact PH domain, and cellular PI3K activity, indicating that PH domain–PIP3 interactions are critical for Gab1 subcellular localization (Maroun et al., 1999a,b). Moreover, Gab1 proteins with a deletion of the PH domain or point mutations that abrogate lipid binding, fail to promote epithelial morphogenesis in response to Met activation, although these mutant proteins become phosphorylated and associate with signaling proteins (Maroun et al., 1999a,b). Although these results indicate that the Gab1 PH domain is critical for Gab1 biological functions, the mechanism through which it mediates its functions is still unclear. It may act to target Gab1 to PIP3-rich membrane microdomains or be engaged in other unknown protein or lipid interactions.

In this article, we have directly tested the role of membrane localization of Gab1 in its ability to induce a morphogenic response, by substituting the myristoylation signal from the c-Src protein for the Gab1 PH domain. N-terminal myristoylation has been used successfully as a tool to study the effect of subcellular localization on the function of several signaling proteins, including protein kinase B/Akt and SOS (reviewed in Aronheim et al., 1994; Resh, 1999; Reuther et al., 2000). Herein, we show that replacement of the PH domain of Gab1 with the c-Src Myristoylation signal is sufficient for the recruitment of Gab1 to the plasma membrane and the rescue of the morphogenic program of Met receptor mutants. Moreover, the expression of Myr-Gab1 fusion proteins, but not wild-type Gab1, promotes a morphogenic program in response to EGF. These findings provide evidence that the subcellular localization of Gab1 has direct consequences on epithelial invasiveness and morphogenesis.

**MATERIALS AND METHODS**

**Cell Culture and DNA Transfections**

MDCK cells were maintained in DMEM containing 10% fetal bovine serum (FBS). MDCK cell lines expressing wild-type CSF-Met receptor and mutants thereof were generated by retroviral infection (Fournier et al., 1996), and stable cell lines expressing wild-type Gab1 have been described previously (Maroun et al., 1999a). Myristoylated-Gabl or Myr-ΔPH Gab1 in pcDNA3.1 were cotransfected with PLXSH, which confers resistance to hygromycin (300 μg/ml).

For transient transfection assays, 293T cells were seeded at 10^4/100-mm Petri dish and transfected 24 h later by calcium phosphate precipitation with plasmid DNA encoding wild-type or Myr-Gabl mutant proteins, or Rac1 proteins as indicated. Sixteen hours later,
cells were washed once in DMEM medium lacking FBS and cultured in media containing 10% FBS. After 48 h, cells were serum starved in 0.1% FBS for 3 h and then harvested for the respective assays.

**Antibodies and Reagents**

Anti-hemagglutinin (HA) (HA.11) was purchased from Babco (Richmond, CA), anti-AKT (sc-1618) from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-SHP-2 monoclonal antibody from Transduction Laboratories (Lexington, KY). Anti-phospho-ATK (Ser473) and anti-phospho-Erk1/2 (Thr202/Tyr204) were purchased from New England Biolabs (Beverly, MA) and total Erk (p42/p44) antibody was a gift from Dr. J. Blenis (Harvard Medical School, Boston, MA). Anti-β-catenin was obtained from Transduction Laboratories and ZO-1 from Zymed (South San Francisco, CA). Rac1 antibody was purchased from BD Transduction Laboratories (Mississauga, Ontario, Canada), anti-GST and anti-STAT5 from Santa Cruz Biotechnology, LY294002 from BIOMOL Research Laboratories (Plymouth Meeting, PA), Alexa 488-phalloidin from Molecular Probes (Eugene, OR), and Cy3-anti-mouse from Jackson Immunoresearch Laboratories (West Grove, PA). HGF was generously provided by Dr. George Vande Woude (Van Andel Research Institute, Grand Rapids, MI), rh-CSF-1 from Genetics Institute (Boston, MA), and EGF from Roche Diagnostics (Laval, Quebec, Canada). PRKSmac-Rac1 plasmin were kindly provided by Dr. Alan Hall (University College London, London, United Kingdom), GST-CRIB (University of Connecticut Health Center, Farmington, CT).

**Cloning of Myr-Gab1 Proteins**

A c-Src myristoylation signal-Gab1 chimeric protein was generated using the following primers: 5′-AGCTTATGGGGACGCAGCAGACGAAGCCGCAAGCAGGCCGGTGAGCTACGCTG-3′ and 5′-GATCCACGCAGTATCTGGAGACGTCTCAGTTCAATCGATACAGTGTTGATACAGGCAGCGCTACAATGTTCAACGATACATGGTCTTTG-3′. Which contain the c-Src myristoylation sequence from chicken c-Src, including the c-Src translation initiation codon and polybasic region as described in Kamikura et al. (2000). In addition, an HA tag was included (sequence underlined). Primers were phosphorylated using T4 polynucleotide kinase (New England Biolabs), annealed, and inserted as a HindIII-BamHI fragment into pKSII+ vectors described previously (Maroun et al., 1999a) as BamHI-EcoRI fragments and ligated in frame into the pKSII+ plasmid containing the myristoylation-HA sequence and then subcloned into pCDNA3.1 as KpnI-NotI fragments. The sequence of the Myr-HA and into the N terminus of Gab1 was confirmed by sequencing.

**Collagen Assays**

The ability of MDCK cells to form branching tubes was assayed as described previously (Maroun et al., 1999a, b). Briefly, 5 × 10^5 cells were resuspended in 500 μl of collagen solution (Vitrogen 100; Collagen Canada; Cohesion Technologies, Palo Alto, CA) prepared following the manufacturer’s instructions, and layered >350 μl of the collagen solution, in a 24-well plate. Cells were maintained in Liebowitz medium (Invitrogen, Carlsbad, CA) containing 5% FBS, and allowed to form cysts for 5–7 d. For stimulations, 15 U/ml HGF, 20 ng/ml rh-CSF1, or 100 ng/ml EGF was added to the Liebowitz medium containing 5% FBS. Tubs were apparent by light microscopy 5–10 d after addition of growth factors. The medium was changed every 5 d, and photographs taken at day 14 by using a Retiga 1300 digital camera (QIMAGING, Burnaby, British Columbia, Canada) and an Axiovert 135 microscope with a 10× objective objective.

**HGF Stimulation of MDCK Cell Lines Expressing Wild-Type and Myr-Gab1 Proteins**

Cells were seeded at 10^5/100-mm dish. Twenty-four hours later, cells were washed once with DMEM and starved overnight in 10 ml of DMEM containing 0.02% FBS. HGF or EGF was added at 100 U/ml and 100 ng/ml, respectively, in 2 ml for the indicated times. Cells were immediately lysed in 1 ml of lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml each of leupeptin and aprotinin, 1 mM Na3VO4).

**Immunoprecipitations and Western Blotting**

MDCK cell lysates (500 μg of total protein) were incubated with the indicated antibodies for 1 h at 4°C with gentle rotation. Twenty microliters of a 50% slurry of either protein A- or protein G-Sepharose was added for an additional hour to collect immune complexes. After three washes in lysis buffer, proteins were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were blocked for 1 h with 3% bovine serum albumin in TBST (10 mM Tris–HCl, pH 7.4, 2.5 mM EDTA, 150 mM NaCl, 0.1% Tween 20), and then with primary antibody (1:1000) for an additional hour. After five washes in TBST, proteins were revealed with secondary anti-mouse (Jackson Immunoresearch Laboratories) or protein A (Amersham Biosciences, Piscataway, NJ) conjugated to horseradish peroxidase. The proteins were visualized with an enhanced chemiluminescence detection system (Amersham Biosciences). For the determination of Erk and AKT phosphorylation, 50 μg of total cellular proteins was resolved on a 10% SDS-PAGE gel, transferred to a nitrocellulose membrane, and immunoblotted with an antibody specific for the activated form of Erk1 and Erk2, or AKT. Blots were stripped in 10 mM Tris, pH 2.3, containing 150 mM NaCl for 10 min. Blots were washed twice in TBST and then probed as indicated.

**Glutathione S-Transferase (GST) Association Assays**

Glutathione S-transferase (GST-Crk-SH2) fusion proteins were immobilized on glutathione-Sepharose beads, incubated with 500 μg of cell lysates from MDCK cells overexpressing wild-type Gab1 or Myr-Gab1 proteins, and stimulated or not with HGF (100 U/ml) or EGF (100 ng/ml). After 1 h on a rotator at 4°C, bound proteins were washed three times with lysis buffer, boiled in Laemmli buffer, and resolved by SDS-PAGE followed by Western blotting with anti-HA as indicated.

**Membrane Fractionation**

Cells (10^6) were cultured in 100-mm dishes (two plates per condition) for 48 h, serum starved for 24 h, and then stimulated or not as indicated with 100 U/ml HGF or 100 ng/ml EGF for 15 min. Cells were subsequently washed twice with cold phosphate-buffered saline (PBS) and scraped with a rubber policeman in a total of 0.5 ml of homogenization buffer (3 mM imidazole, pH 7.4, 8.5% sucrose). Homogenization was performed by passing cells through a 22-gauge needle 20 times. The homogenates were centrifuged at 800 × g for 10 min, and the postnuclear supernatant was subjected to ultracen-
trifugation at 100,000 x g for 30 min at 4°C. The pellet resulting from the ultracentrifugation contained total cellular membranes and the supernatant contained cytosolic proteins. The pellet was resuspended in 0.25 ml of homogenization buffer. Then 50 μl of lysates from either supernatant (S100) or pellet (P100) was resolved by SDS-PAGE on a 10% gel, transferred to nitrocellulose, and subjected to Western blotting by using anti-HA. Cellular compartments were confirmed using anti-Met and anti-STAT5 as membrane and cytoplasmic markers, respectively.

**Rac Activity Assays**

293T cells transiently transfected with WT or Myr-Gab1 and Rac1 plasmids, or MDCK cells expressing WT or Myr-WT Gab1 were grown in DMEM containing 10% FBS. Cells were serum starved for 3 h before the assay, in either 0.1% FBS (293T cells) or 0.02% FBS (MDCK cells). Cells were stimulated where indicated with either 100 U/ml HGF or 100 ng/ml EGF for the indicated times. Cells were collected at 3 h before the assay, in either 0.1% FBS (293T cells) or 0.02% FBS (MDCK cells). Cells were stimulated where indicated with either 100 U/ml HGF or 100 ng/ml EGF and lysed in Rac1 lysis buffer containing 25 mM HEPES, pH 7.5, 10 mM MgCl₂, 100 mM NaCl, and 1% NP-40, 5% glycerol, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium vanadate. GST-CRIB (the CRIB domain from PAK1 fused to GST) pull-down assays were performed as described previously (Royal et al., 2000). The relative Rac activity was obtained using the Scan Analysis program by quantitating the relative amount of Rac from the GST-CRIB pull-down assay in cells expressing Gab1 plasmids over that in the absence of exogenous Gab1. The values were then normalized to the relative levels of Rac expression obtained from each experimental group over that observed in cells expressing exogenous Rac, in the absence of Gab1 plasmids.

**Immunofluorescence**

MDCK cells (10⁴) overexpressing wild-type Gab1 or Myr-Gab1 mutants were plated on glass coverslips (Belco Glass, Vineland, NJ) in a 24-well dish (Nalge Nunc, Naperville, IL), for the indicated times in DMEM containing 10% FBS. Cells were stimulated or not with 50 U/ml HGF or 100 ng/ml EGF for the indicated times. Cells were fixed in 2% paraformaldehyde in PBS, for 30 min at room temperature, washed twice in PBS, and incubated for 10 min in PBS containing 50 mM ammonium chloride. After one additional wash in PBS, cells were treated with PBS containing 0.1% Triton X-100 and 5% FBS (buffer A) for 10 min at room temperature. Primary antibodies anti-HA, β-catenin, and ZO-1 were diluted (1:300) in buffer A and added for 10 min, and after three washes in the same buffer, Cy3-conjugated anti-mouse or Alexa 488-anti-rabbit (1:2000) was added for 10 min, followed by three washes in buffer A. Glass coverslips were mounted onto slides in Immunofluorescence medium (ICN Pharmaceuticals, Costa Mesa, CA), and pictures taken using a Retiga 1300 digital camera (QIMAGING) and an Axiovert 135 microscope (Carl Zeiss Canada) at ×63 magnification, or an LSM 410 confocal microscope (Carl Zeiss Canada) at a magnification of ×63, as indicated. For the modified indirect fluorescence assays with GST or GST-CRIB fusion proteins, cells were stimulated for 15 min, fixed, washed, and solubilized as described above. GST-fusion proteins were added at 100 μg/ml for 15 min, and after three washes, anti-GST antibody was added at 1:300, followed by the Cy3-anti-mouse.

**RESULTS**

**c-Src Myristoylation Signal Confers PH Domain-independent Membrane Targeting of Gab1**

To investigate whether membrane localization of Gab1 is essential for its function during the morphogenic program, we replaced the PH domain of Gab1 with the myristoylation signal found in the c-Src protein tyrosine kinase. This modification by the covalent attachment of myristate, results in protein association with the inner leaflet of the plasma membrane (Resh, 1999; Reuther et al., 2000). MDCK cell lines stably expressing an HA-epitope–tagged myristoylated ΔPH-Gab1 (Myr-ΔPH Gab1), or myristoylated WT-Gab1 (Myr-WT Gab1), were generated, and at least three cell lines with similar levels of protein expression were analyzed (Figure 1A shows two representative cell lines). As previously demonstrated, the deletion of the Gab1 PH domain resulted in the predominant localization of Gab1 in the cytoplasm in colonies of epithelial cells (Figure 1B; Maroun et al., 1999a). In contrast, Myr-ΔPH Gab1 was observed at sites of cell-cell contacts. All three cell lines tested showed similar subcellular localization of Myr-ΔPH Gab1, and one clone (Myr-ΔPH Gab1-A9) is shown (Figure 1). In addition, although the PH domain-dependent recruitment of WT-Gab1 to the membrane requires an intact inositolphospholipid binding site in the Gab1 PH domain and cellular PI3K activity (Figure 1B; Maroun et al., 1999), the inhibition of PI3K activity with LY294002 did not affect membrane localization of Myr-WT Gab1 or Myr-ΔPH Gab1 proteins (Figure 1). Further support that Myr-ΔPH Gab1 is localized in a membranous compartment is provided by biochemical fractionation of MDCK cells, expressing either a ΔPH Gab1 or Myr-ΔPH Gab1, into a soluble fraction representing the cytoplasmic compartment and a 100,000-g pellet containing the membrane fraction. Although the majority of ΔPH-Gab1 was expressed in the soluble cytoplasmic compartment, a significant portion of Myr-ΔPH Gab1 was localized in the membrane fraction (Figure 1C). The cytoplasmic and membrane compartments were confirmed using anti-STAT5 and anti-Met, respectively. Together, these results demonstrate that Gab1 can be targeted through the myristoylation signal of c-Src to a membranous compartment in a PH-domain and PIP3-independent manner.

**PH Domain-independent Membrane Targeting of Gab1 Rescues the Morphogenic Program in Cells Expressing Met Receptor Mutant Proteins**

To address whether membrane localization of Gab1 is critical for its function, or whether the PH domain of Gab1 exerts other functions, we assessed whether the Myr-ΔPH Gab1 protein can rescue the morphogenetic defect of CSF-Met receptor mutants (Fournier et al., 1996). MDCK cells expressing a chimeric CSF-Met receptor undergo a morphogenic program in response to CSF-1, whereas cells expressing a mutant CSF-Met (N1358H) that fails to recruit the Grb2 adapter protein and shows reduced association with Gab1 are unable to induce branching tubulogenesis in response to CSF-1 (Fournier et al., 1996). The overexpression of a WT-Gab1 protein in these cells rescues the morphogenic program in response to CSF-1, whereas a ΔPH-Gab1 protein fails to do so (Figure 2B; Maroun et al., 1999a). To test whether membrane targeting of Gab1 is sufficient for its ability to rescue the morphogenetic response, stable cell lines coexpressing the CSF-Met ΔGrb2 mutant and Myr-ΔPH Gab1 were generated. Three cell lines were analyzed, and cells expressing similar levels of Myr-ΔPH Gab1 and ΔPH-Gab1 were further characterized. As shown previously, cells expressing a ΔPH-Gab1 failed to rescue the tubulogenic defect (Figure 2B; Maroun et al., 1999a). Importantly, the overexpression of a Myr-ΔPH-Gab1 protein, to the same
level as a ΔPH-Gab1 protein rescued the morphogenic response (Figure 2, B and C). Hence, the c-Src myristoylation signal substitutes functionally for the Gab1 PH domain for epithelial morphogenesis. Because a Myr-ΔPH Gab1 protein was capable of rescuing the tubulogenic response of CSF-Met mutants, we determined whether constitutive membrane targeting of a wild-type Gab1 protein promoted a morphogenic response in the absence of Met activation. Despite membrane localization, cells expressing the Myr-WT Gab1 variant protein formed cysts comparable with those observed in cells expressing WT-Gab1 and failed to undergo a morphogenic program in the absence of stimulation. In addition, Myr-WT Gab1-expressing cells formed tubules in response to CSF-Met activation albeit to a lower extent, consistent with the lower level of expression of Myr-Gab1 proteins. Together, these results indicate that although membrane localization of Gab1 is necessary for the ability of Gab1 to rescue the tubulogenic defect of CSF-Met mutants, it is insufficient to promote a tubulogenic response in the absence of Met activation.

**Myr-Gab1 Proteins Convert EGF into a Morphogenic Invasive Response**

Stimulation of the epidermal growth factor receptor (EGFR) in MDCK epithelial cells, which express abundant levels of EGFR, does not lead to a morphogenic response (Maroun et al., 1999a). Moreover, the overexpression of Gab1 in these
cells does not alter this response (Maroun et al., 1999a). In contrast to indirect and direct modes of recruitment of Gab1 to Met, the recruitment of Gab1 to the EGFR is indirect and may promote a less stable association at the plasma membrane, which is insufficient to support a morphogenic response downstream from the EGFR. To test this, we have investigated whether the overexpression of membrane-targeted Gab1, Myr-WT Gab1, or Myr-ΔPH Gab1 could promote a morphogenic program in response to EGF. Cells overexpressing WT, ΔPH, Myr-WT, or Myr-ΔPH Gab1 were first assayed for their ability to form branching tubules in a collagen matrix. After 5 d of culture, all experimental groups formed cysts (Figure 3). Strikingly, stimulation with EGF resulted in the ability to form branching tubules in cells expressing Myr-WT Gab1 (Figure 3), but not in cells overexpressing WT-Gab1 (Figure 3; Maroun et al., 1999a). The PH domain of Gab1 was not required for this response because EGF stimulation of cells expressing Myr-ΔPH Gab1 formed branching tubules, although at a lower efficiency. Because overexpression of Myr-WT Gab1 or Myr-ΔPH Gab1 lead to a morphogenic program in response to EGF, we determined whether this correlated with an enhanced capacity to invade a three-dimensional matrix in a short-term assay. Cells were seeded in a collagen matrix and assayed 48 h later for their ability to invade into the neighboring collagen matrix. Although EGF was unable to mediate the invasion of cells.

Figure 2. PH domain-independent membrane recruitment of Gab1 rescues the morphogenic program in MDCK cells expressing Met receptor mutants. MDCK cell lines expressing a CSF-Met receptor mutant that fails to bind Gab2 were stably transduced with constructs encoding for ΔPH, Myr-ΔPH Gab1, WT or Myr-WT Gab1 proteins. (A) Level of expression of ΔPH, Myr-ΔPH Gab1, WT, or Myr-WT Gab1 in the different stable cell lines. (B) Cells expressing ΔPH (clone 1), Myr-ΔPH (clone C2) Gab1, WT or Myr-WT (clone B3) proteins were grown in collagen for 5 d, during which time they formed cysts. RhCSF-1 was added and renewed every 5 d. Fourteen days after the addition of rh-CSF-1 branching morphogenesis was visualized at a magnification of 10×. (C) Quantitation of the morphogenic response was performed as described in MATERIALS AND METHODS. The responses are plotted as the percentage of cysts that have undergone branching tubulogenesis. The values are derived from at least three experiments.
expressing wild-type Gab1, cells expressing either Myr-Gab1 or Myr-ΔPH Gab1 efficiently invaded the collagen matrix (Figure 4). The inability of EGF to promote an invasive program in cells expressing WT-Gab1 is not due to a defect in the intrinsic capacity of cells to invade a collagen matrix, because these cells invade in response to HGF (Figure 4). Thus, membrane targeting of Gab1 through the c-Src myristoylation signal switches the biological response to EGF from nontubulogenic, noninvasive into an invasive, morphogenic program, indicating that the localization of Gab1 provides an essential function for epithelial invasive-ness and tubulogenesis.

**Figure 3.** Myr-Gab1 converts a nonmorphogenic response downstream from the EGFR to a morphogenic response. (A) MDCK cells stably expressing WT, Myr-WT (clones O and Q), ΔPH, Myr-ΔPH (clones A9 and A11) Gab1 proteins were grown in a collagen matrix for 5 d after which 100 ng/ml EGF was added. The morphogenic response was evaluated 14 d later and quantitated as described in MATERIALS AND METHODS in B.

**Myr-WT Gab1 Induces Reorganization of Adhesion Junctions and Cell Dispersal**

The process of conversion of a polarized cyst of epithelial cells into a network of branched tubules in three-dimensional cultures involves remodeling of epithelial junctions, cell proliferation, and invasion (Pollack et al., 1998). In two-dimensional epithelial cultures, the remodeling of epithelial junctions in response to HGF occurs in a step-wise process, involving cell spreading and the loss of adhesion junctions, followed by the dissolution of tight junctions (Royal and Park, 1995; Potempa and Ridley,
In contrast, stimulation with EGF fails to promote the loss of adhesion and tight junctions and does not promote the dispersal of epithelial colonies (Khwaja et al., 1998). Because in cells expressing Myr-Gab1 proteins, the EGF signal resulted in an invasive response, we determined whether this coincided with changes in cellular junctions leading to cell dispersal. Cells expressing either WT or Myr-WT Gab1 proteins were stimulated with HGF or EGF for 3 h, 6 h, or overnight as indicated (Figure 5). The integrity of adherens and tight junctions was analyzed by fluorescent microscopy after labeling with anti-β-catenin and anti-ZO-1, respectively, and cell scatter was assessed by light microscopy (Figure 5). As shown previously for MDCK cells (Royal and Park, 1995), in cells expressing WT-Gab1, HGF induced cell spreading and loss of β-catenin and ZO-1 from cell-cell junctions, whereas EGF stimulation failed to induce loss of cell-cell junctions (Figure 5A and B) and to promote cell dispersal (Figure 5C). In contrast, both HGF and EGF stimulation of cells expressing Myr-WT Gab1

**Figure 4.** Myr-Gab1 expression promotes MDCK cell invasion of collagen, after EGF stimulation. (A) MDCK cells stably expressing WT, Myr-WT (clones Q), ΔPH, Myr-ΔPH (clones A11) proteins were cultured in collagen for 48 h, EGF (100 ng/ml) or HGF (10 U/ml) was added, and 3 d later pictures were taken using a Retiga 1300 digital camera and an AxiosVert 135 microscope (Carl Zeiss Canada) at a magnification of 10×. (B) Results were quantitated and plotted as the percentage of colonies that have invaded into the neighboring matrix.
induced the loss of β-catenin and ZO-1 from cell-cell junctions (Figure 5, A and B) and cell dispersal (Figure 5C). Interestingly, in the absence of stimulation with HGF or EGF, cells expressing Myr-Gab1 proteins showed enhanced spreading and decreased intensity of β-catenin and ZO-1 in adherens and tight junctions, respectively. Similar results were observed with cells expressing Myr-DPH Gab1 proteins (our unpublished data). These results suggest that membrane targeting of Gab1 through N-terminal myristoylation alone is sufficient to mediate changes in the actin cytoskeleton and the integrity of junctional complexes, and synergizes with EGF to promote the breakdown of cellular junctions in two-dimensional cultures.

**WT and Myr-WT Gab1 Proteins Are Phosphorylated with Similar Kinetics**

We have previously demonstrated that sustained phosphorylation of Gab1 correlates with a morphogenic program in response to HGF, whereas phosphorylation of Gab1 downstream from EGF is transient (Maroun et al., 1999a). Because expression of Myr-Gab1 proteins alters the biological responses downstream from the EGF receptor to an invasive program, we determined whether tyrosine-specific phosphorylation of Myr-Gab1 proteins was altered after EGFR activation. Cells expressing either WT or Myr-WT Gab1 proteins were stimulated with EGF or HGF for the indicated time. Gab1 was immunoprecipitated and tyrosine-specific...
phosphorylation was determined after Western blotting with an anti-phosphotyrosine antibody. Although HGF resulted in sustained phosphorylation of Gab1, the phosphorylation of Gab1 after EGF receptor activation was transient as demonstrated previously (Figure 6A; Maroun et al., 1999). Thus, the duration of phosphorylation was not altered in cells expressing the Myr-Gab1 protein, and the association of Myr-Gab1 with the phosphatase SHP-2 (Figure 6A), or the p85 subunit of PI3K (our unpublished data), was similar to that observed in cells expressing WT Gab1.

Stimulation of the Met receptor leads to sustained Erk activation, whereas EGF promotes a transient activation of Erk (Khwaja et al., 1998). Moreover, the inhibition of mitogen-activated protein kinase kinase (MEK), an upstream activator of Erk, by using synthetic inhibitors results in the abrogation both of cell dissociation and a morphogenic response supporting a requirement for sustained Erk activation in this process (Potempa and Ridley, 1998). We therefore investigated whether changes in the kinetics of Erk activation correlated with the ability of EGF to induce tubulogenesis and cell scatter in cells overexpressing the Myr-Gab1 proteins by using an antibody that recognizes phospho-Thr202 and Tyr204 (pErk) in the activation loop. Cells were stimulated for 5 and 180 min, and total cellular proteins were separated by SDS-PAGE and subjected to Western blotting by using anti-pErk. Both HGF and EGF induced Erk activation in cells expressing Myr-WT Gab1 in a manner similar to that observed in cells expressing WT Gab1 (Figure 6B). Consistent with previous data, HGF induced a sustained activation of Erk, whereas EGF induced a transient activation.

In addition to MEK, PI3K activity is critical for breakdown of adherens junctions and cell dispersal, and PI3K is recruited to Gab1 (Royal and Park, 1995; Khwaja et al., 1998; Potempa and Ridley, 1998; Maroun et al., 1999a; Rodrigues et al., 2000). As a readout for PI3K activity, we determined whether the phosphorylation of PKB/Akt was altered in cells overexpressing Myr-WT Gab1. Using a phospho-Ser473–specific antibody that recognizes active PKB/Akt, we show that the kinetics of phosphorylation of this protein is unchanged by overexpression of Myr-WT Gab1 compared with cells expressing WT Gab1 (Figure 6C). Together, these results suggest that although the expression of Myr-WT Gab1 dramatically changed the biological outcome after EGF receptor activation, this neither correlates with changes in the kinetics of tyrosine specific phosphorylation of Gab1 nor with its association with SHP-2, a critical signaling protein for the morphogenic response (Maroun et al., 1999a).

Furthermore, the activation of the Erk or the Akt pathways was not detectably modified in these cells compared with cells expressing WT Gab1.

**Myristoyl-tagged Gab1 Promotes Activation of Rac, Cell Spreading, and Actin Reorganization**

Although the kinetics of phosphorylation of Gab1 was not detectably altered, the subcellular distribution of Gab1–dependent signaling complexes may affect biological outcome. As described previously (Maroun et al., 1999), in low cell density cell cultures, WT-Gab1 is localized in the cytoplasm and recruited to lamellipodia on cells on the edge of the colony by 15 min after Met stimulation. In contrast, EGF stimulation did not demonstrate similar membrane recruitment of Gab1 (Figure 7A) and induced overall less membrane ruffling activity and lamellipodia formation. In analogous culture conditions, Myr-WT Gab1 was membrane localized before stimulation as shown by immunofluorescence and biochemical fractionation, which revealed that the majority of WT-Gab1 was expressed in the soluble, cytoplasmic compartment (Figure 7B). It is noteworthy that stimulation of WT-Gab1–expressing cells with HGF did not induce detectable membrane partitioning of Gab1 proteins, possibly due to the transient or unstable nature of the interaction of Gab1 with membrane inositolphospholipids, or with the Met receptor under serum-starved conditions.

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**Figure 6.** Myr-Gab1 expression does not alter either the kinetics of phosphorylation of Gab1 after HGF or EGF stimulation, or the kinetics of Erk and Akt/PKB activation. MDCK cells stably expressing WT or Myr-WT Gab1 proteins (10⁶ cells) were cultured for 24 h in DMEM containing 10% FBS. Cells were serum starved in medium containing 0.02% FBS for another 18 h. Stimulation with HGF (100 ng/ml) or EGF (100 U/ml) or EGF (100 ng/ml) was performed for the indicated time.

(A) Cells were lysed and subjected to immunoprecipitation assays with anti-HA followed by Western blotting either with anti-PY or anti-SHP2, as described in MATERIALS AND METHODS. Fifty micrograms of total cell lysates was probed with anti-HA. (B) Fifty micrograms of total cell lysates from cells stimulated for the indicated times was resolved by SDS-PAGE on a 10% gel, transferred to nitrocellulose, and probed for pErk. Blots were stripped and reprobed with an anti-total Erk antibody. (C) Cells stimulated as indicated were lysed, and 50 μg of total cell lysates was resolved by SDS-PAGE on a 10% gel. After transfer to nitrocellulose Western blotting with anti-pPKB/Akt was performed. Blots were stripped and reprobed for total Akt.

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portantly, significantly more Myr-Gab1 was localized to the membrane compartment in these conditions (Figure 7B).

Myristoylation-mediated targeting of Gab1 proteins to the membranous fraction would thus enhance a Gab1-dependent signal in the membrane, in response to EGF. In support of this, cells expressing Myr-WT Gab1 show actin reorganization and membrane ruffling in response to EGF compared with cells expressing WT-Gab1 (Figure 7A). However, the expression of Myr-Gab1 proteins alone, in the absence of stimulation, promotes some of the early morphological changes observed in response to HGF. Cells expressing Myr-WT-Gab1 were spread compared with cells expressing WT Gab1 (Figures 5, A–C, and 7A). This was not due to clonal differences, because at least three independent stable cell clones analyzed yielded similar results (our unpublished data). Consistent with changes in cell morphology, cells expressing Myr-WT Gab1 proteins showed actin remodeling, particularly in the formation of membrane extensions/lamellipodia (Figure 7A, arrowheads), in addition to a decrease in the integrity of adherens and tight junctions in cells expressing Myr-WT Gab1 as visualized by a decrease in β-catenin and ZO-1 localization at cell-cell junctions (Figure 5).

Lamellipodia formation and cell spreading in response to HGF are dependent on the small GTPase Rac1 (Royal et al., 2000). To investigate the possibility that Rac activity is elevated in cells expressing Myr-WT Gab1, increasing concentrations of WT or Myr-WT Gab1 were transiently cotrans-
fected into 293T cells with a plasmid encoding for wild-type Rac1. Forty-eight hours after transfection, cells were serum starved for 3 h before harvest in DMEM containing 0.1% FBS. Then 250 µg of cell lysates was subjected to a pull-down assay using a GST-CRIB fusion protein. Associated proteins were resolved by SDS-PAGE on a 12% gel, transferred to nitrocellulose, and probed with an anti-Rac antibody. Fifty micrograms of total cell lysates was resolved by SDS-PAGE on a 12% gel, the top part of the gel was probed with anti-HA, and the bottom with anti-Rac. The relative Rac activity was determined as described in MATERIALS AND METHODS by using the Scan Analysis program. (A) Representative of three experiments. (B) Relative Rac activity was obtained from three independent experiments performed as in A. (C) MDCK cells stably expressing WT or Myr-WT Gab1 proteins were stimulated as described in MATERIALS AND METHODS for 15 min. Cells were lysed and 500 µg of proteins was subjected to a pull-down assay using GST-CRIB fusion proteins. Associated proteins were resolved by SDS-PAGE on a 12% gel, transferred to nitrocellulose, and probed with an anti-Rac antibody. Fifty micrograms of total cell lysates was resolved by SDS-PAGE on a 12% gel, the top part of the gel was probed with anti-HA, and the bottom with anti-Rac. (D) MDCK cells stably expressing WT or Myr-WT Gab1 proteins were stimulated with HGF (100 U/ml) or EGF (100 ng/ml) for 15 min. Cells were lysed and 500 µg of proteins was subjected to a pull-down assay by using GST-Crk-SH2 domain fusion proteins, associated proteins were resolved by SDS-PAGE on an 8% gel, transferred to nitrocellulose, and probed with anti-HA. Levels of phosphorylation of Gab1 in these lysates as well as total levels of Gab1 are indicated.
stimulation either with HGF or EGF resulted in an increase in the association of Crk-SH2 domain with Gab1, but this interaction was not detectably altered after membrane targeting of Gab1 with c-Src-myristoylation signal (Figure 8D).

In MDCK and WT-Gab1–expressing cells, Rac is localized at sites of cell-cell junctions and relocalizes to the lamellipodia upon stimulation with HGF (Figure 9; Royal et al., 2000). However, stimulation with EGF does not lead to a similar subcellular distribution of Rac to lamellipodia (Figure 9).Because an elevated level of Rac activity was observed in Myr-WT Gab1–expressing cells, we investigated whether Rac could be localized in observed lamellipodia. In Myr-WT Gab1-expressing cells, elevated levels of Rac were present in membrane extensions in the absence of stimulation (Figure 9). Because an elevated level of Rac activity was observed in Myr-WT Gab1–expressing cells, we investigated whether Rac could be localized in observed lamellipodia. In Myr-WT Gab1-expressing cells, elevated levels of Rac were present in membrane extensions in the absence of stimulation (Figure 9). Moreover, to determine whether localization of Rac in lamellipodia correlated with Rac activity, we used a GST-CRIB fusion protein in a modified indirect fluorescence assay, in fixed, permeabilized cells. After an initial incubation in the presence of GST-CRIB, mouse anti-GST antibodies, and subsequently, Cy3-conjugated anti-mouse antibodies were used. These assays revealed that in WT-Gab1–expressing cells stimulation with HGF but not EGF resulted in an increase in the binding of GST-CRIB in lamellipodia (Figure 10). In contrast, the expression of Myr-WT-Gab1 enhanced the binding of GST-CRIB to membrane extensions even in the absence of stimulation (Figure 10). Together, these results indicate that the expression of Myr-Gab1 leads to the activation of pathways involved in elevating Rac activity and moreover, promotes the localization of active Rac to sites of lamellipodia extensions.

DISCUSSION

Our studies provide further insight into the mechanisms regulating epithelial morphogenesis. The molecular mechanism through which the Met receptor tyrosine kinase mediates its morphogenic activity involves the Gab1 scaffolding protein, which acts to diversify the signal downstream from the Met receptor (Maroun et al., 1999a). The ability of Gab1 to rescue the morphogenesis defect of Met receptor mutants depends on the integrity of its N-terminally located PH domain (Maroun et al., 1999a,b). This domain is required for the recruitment of Gab1 to the membrane and shows spec-
licity for PIP3 (Isakoff et al., 1998; Maroun et al., 1999b; Rodrigues et al., 2000). However, discrimination between a requirement for the Gab1 PH domain for biological activity and membrane localization had not been established. In this article, we show for the first time that membrane recruitment of Gab1, via a c-Src myristoylation signal, substitutes for the Gab1 PH domain for the morphogenic program downstream from the Met receptor. Furthermore, we show that enhanced membrane association of Gab1 synergizes with EGF and promotes a switch to an invasive, morphogenic response (Figures 3 and 4) may reflect an altered Gab1-dependent signal in response to EGF or alternatively an altered subcellular localization of the signal in response to EGF. Gab1 is tyrosine phosphorylated after stimulation of MDCK cells with HGF or EGF (Holgado-Madruga et al., 1996; Maroun et al., 1999a; Rodrigues et al., 2000). However, we have previously demonstrated that the kinetics of phosphorylation was distinct in response to HGF or EGF (Maroun et al., 1999a). Although HGF induced a sustained phosphorylation of Gab1, the phosphorylation of Gab1 in response to EGF was transient.

Figure 10. Membrane localization of active Rac in cells expressing Myr-WT Gab1 proteins. WT or Myr-WT (clone Q) Gab1-expressing cells (10^6 cells) were grown overnight on glass coverslips in DMEM containing 10% FBS. Cells were stimulated with HGF (50 U/ml) or EGF (100 ng/ml) for 15 min, and then fixed in 2% paraformaldehyde. After permeabilization in PBS containing 0.1% Triton X-100 and 5% FBS, cells were incubated with GST-CRIB fusion proteins for 15 min. After three washes, anti-GST antibody was added followed by a CY3-conjugated anti-mouse antibody, and Alexa 488-phalloidin. Pictures were taken at a 63× magnification.
Given that in Myr-WT Gab1expressing cells, EGF induced a morphogenic response, membrane targeting of Gab1 may have allowed a sustained phosphorylation of Gab1 in response to EGF. However, compared with WT-Gab1 no detectable differences were observed in the kinetics of tyrosine phosphorylation of Myr-Gab1 in response to EGF (Figure 6). Furthermore, no significant changes were observed in Myr-Gab1–expressing cells compared with cells expressing WT-Gab1 in the kinetics of activation of Erk1/2 or Akt, by using phosphospecific antibodies (Figure 6). Importantly, the subcellular localization of Myr-Gab1 was distinct compared with WT-Gab1. Whereas in low cell density cultures WT-Gab1 was localized mainly in the cytoplasm and was recruited to the membrane and localized to lamellipodia upon stimulation with HGF, EGF neither induced lamellipodia formation nor the localization of Gab1 to the membrane at the leading edge of cells, on the periphery of the colony (Figure 7). In contrast, Myr-WT Gab1 or Myr-ΔPH Gab1 is localized at the cell membrane, as determined by indirect immunofluorescence and biochemical fractionation, in the absence of stimulation, and shows enhanced recruitment to membrane ruffles after stimulation with EGF (Figure 7).

The process of tubulogenesis and cell dispersal requires the remodeling of cell-cell junctions, and cell proliferation as well as invasion. In colonies of MDCK cells, HGF induces the loss of adherens junctions as well as tight junctions (Royal and Park, 1995). In contrast, although EGF stimulated spreading of MDCK cell colonies, it failed to promote the breakdown of adherens or tight junctions (Figure 5). However, even in the absence of stimulation, cells expressing Myr-Gab1 showed an altered cell morphology with increased cell spreading, remodeling of the actin cytoskeleton, and a decrease in staining of β-catenin and ZO-1 at cell-cell junctions (Figure 5).

Loss of adherens junctions in response to HGF requires the activation of the small GTP-binding protein Rac1 (Royal et al., 2000). Furthermore, Rac1 has been implicated in cell invasion in multiple cell types, where activated Rac1 promoted invasion by carcinoma cell lines, and dominant negative forms of Rac1 inhibited leptin-mediated invasion of a collagen matrix (Keely et al., 1997; Attoub et al., 2000; Banyard et al., 2000; Zhuge and Xu, 2001). Rac1 activity was elevated in cells expressing Myr-Gab1 compared with cells expressing WT-Gab1, and in transient assays Myr-WT and Myr-ΔPH Gab1, but not WT-Gab1, promoted Rac1 activation in the absence of HGF stimulation (Figure 8). Consistent with a role for Rac1 in junctional reorganization and cell spreading in multiple cell types, the presence of elevated Rac activity in cells expressing Myr-Gab1 may contribute to cell spreading as observed in cells expressing Myr-Gab1, and the biological switch observed after the activation of the EGFR. Hence, membrane targeting of Gab1 promotes some of the early responses to HGF, including remodeling of the actin cytoskeleton and junctional complexes. This alone is insufficient for morphogenesis, but can synergize with EGF-dependent signals to mediate cell invasion and morphogenesis.

The mechanism through which Myr-Gab1 promotes elevated basal Rac1 activity is currently unknown. After phosphorylation, Gab1 associates with the p85 subunit of PI3K and associated PI3K activity, as well as the Crk adapter protein. PI3K activity is required for the breakdown of adherens junctions and for activation of Rac in response to HGF, possibly through the targeting and activation of PH domain containing Rac exchange factors to PI3P-rich membrane domains. In addition, the Crk adapter protein interacts with a Rac exchange factor DOCK180 (Kiyokawa et al., 1998; Nolan et al., 1998). A conserved domain among DOCK180 family members directly binds to nucleotide-free Rac and can activate Rac in vitro. Moreover, a complex between Dock180, ELMO, and Rac has been implicated in Rac activation in vivo (Brugnera et al., 2002; Côte and Vuori, 2002). In support of this, the overexpression of Crk in MDCK cells elevates basal Rac activity and destabilizes adherens junctions (Lamorte et al., 2002a,b). Importantly, in the absence of detectable changes in the interaction of Gab1 with Crk and the p85 subunit of PI3K (Figure 8D; our unpublished data), the increased recruitment of Myr-Gab1 proteins to the membrane, under conditions where Gab1 shows basal levels of phosphorylation, may be sufficient to elevate the concentration of proteins, at the plasma membrane, involved in Rac activation, such as Crk or PI3K.

This is similar to a recent report, where the membrane targeting of insulin receptor substrate-1 (IRS-1) by using a CAAX motif from Ras resulted in enhanced recruitment of IRS-1 to the membrane. Although in contrast to our data, membrane targeting of IRS-1 led to enhanced activation of downstream pathways involving Akt and Erk in response to insulin (Kriauciuinas et al., 2000). Although these results apparently differ from ours, they have in common an increase in the local concentration of signaling proteins in the membrane. Thus, an increase in the level of activated Rac1 in the membrane observed in cells expressing Myr-Gab1, but not in cells expressing WT-Gab1, could contribute both to the breakdown of cell-cell junctions, possibly through the modulation of IQGAP1 (Kuroda et al., 1998), in addition to enhancing cell spreading and motility through the action of Rac on lamellipodia formation and cell migration (Van Aelst and D’Souza-Schorey, 1997; Hall, 1998).

The localization of Gab1 at the membrane is dependent on PI3P, whose levels and consequently Gab1 localization are positively regulated by PI3K and negatively regulated by the lipid phosphatase PTEN (Maroun et al., 1999a; Rodrigues et al., 2000). Amplification of PI3K or loss of PTEN is associated with tumor progression and invasion in multiple tumor types (Whang et al., 1998; Shayesteh et al., 1999; Kotelevets et al., 2001). The results presented here demonstrate for the first time that a membrane targeting signal can substitute for the Gab1 PH domain and that a membrane-targeted Gab1 leads to a switch to an invasive morphogenic program in response to EGF. This has important implications in human cancers where elevated PI3P levels through increases in PI3K or through a decrease in PTEN levels would enhance the localization of Gab1 at the membrane where we have shown it can promote an invasive response.

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