

Regulation and Physiological Functions of G12/13-Mediated Signaling Pathways

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Key Words

Regulation of G12/13-mediated signaling pathways · Gα12/13-RH-RhoGEF-Rho pathway · Regulation of G12/13 · Regulation of effectors by G12 · Crosstalk between G12/13-mediated and other signaling pathways · Cell proliferation and transformation · Cell morphology and motility · The cardiovascular system · The immune system · The neuronal system

Abstract

Accumulating data indicate that G12 subfamily (Gα12/13)-mediated signaling pathways play pivotal roles in a variety of physiological processes, while aberrant regulation of this pathway has been identified in various human diseases. It has been demonstrated that Gα12/13-mediated signals form networks with other signaling proteins at various levels, from cell surface receptors to transcription factors, to regulate cellular responses. Gα12/13 have slow rates of nucleotide exchange and GTP hydrolysis, and specifically target RhoGEFs containing an amino-terminal RGS homology domain (RH-RhoGEFs), which uniquely function both as a GAP and an effector for Gα12/13. In this review, we will focus on the mechanisms regulating the Gα12/13 signaling system, particularly the Gα12/13-RH-RhoGEF-Rho pathway, which can regulate a wide variety of cellular functions from migration to transformation.

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Introduction

The α subunits of heterotrimeric G proteins are classified into four subfamilies based on the homology of their amino acid sequences: Gs, Gi, Gq, and G12 [1]. The G12 subfamily is composed of Gα12 and Gα13 [2]. Accumulating evidence indicates that G12/13-mediated signaling pathways are involved in a variety of physiological processes, including embryonic development, cell growth, cell polarity and migration, angiogenesis, platelet activation, the immune response, apoptosis, and neuronal responses [3–15]. In the nervous system, this pathway plays an important role in neuronal migration, axonal guidance, formation of cerebellar and cerebral cortices, and neurotransmitter release [11, 16–20]. In addition, abnormal regulation of this pathway has been found in disease conditions such as leukemia, cell transformation, tumor cell invasion and metastasis, hypertension, and ataxia [14, 18, 21–27]. However, significant differences between Gα12 and Gα13 signaling have been demonstrated from knockout mouse studies [28, 29]. Crosstalk between Gα12/13 signaling and other G protein signals, especially from Gαq, have also been reported [3, 11, 28, 30–36]. These results suggest that Gα12/13-mediated signaling pathways will play a critical role in many important biological responses. In this review, we will focus on the mechanisms by which the Gα12/13 signaling system regulates a wide variety of cellular functions.

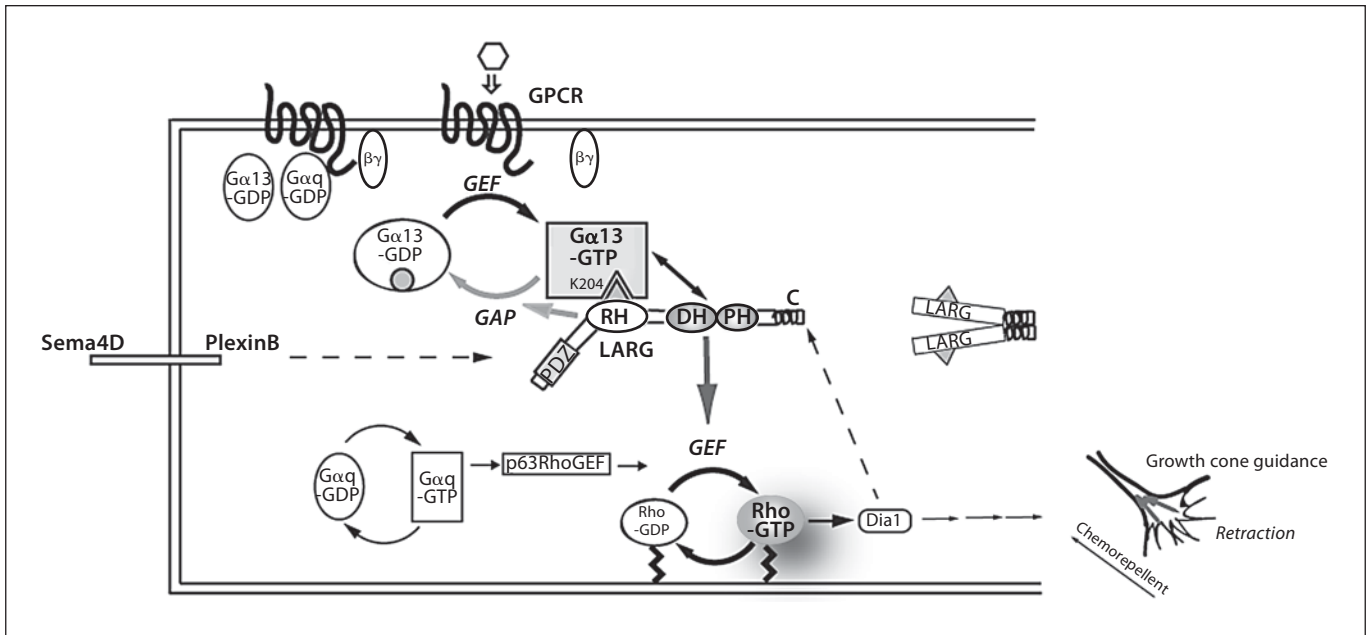


Fig. 1. The G12/13-mediated signaling pathway interacts with diverse proteins at various levels. Upon ligand binding, GPCRs catalyze the exchange of GDP for GTP on Gα13 subunits resulting in activation of the Gα subunit and release of Gβγ. Both Gα-GTP and free Gβγ interact with diverse downstream effectors. LARG is an effector for Gα13, which is a GEF for the monomeric GTPase RhoA. Upon binding of Gα13-GTP to LARG, the DH/PH domains activate RhoA by facilitating the exchange of GDP for GTP. LARG, in turn, stimulates the intrinsic GTPase activity of Gα13 through its N-terminal RH domain. Gα-GDP dissociates from

effectors and reassociates with Gβγ to terminate the signal. Thus, regulation of the G protein cycle of activation and deactivation profoundly affects the cellular response. The amplitude and duration of the signal response are determined by the amount of GTP-bound Gα subunit. A dotted line indicates evidence for direct activation of LARG without activated Gα13. Domains: RH = RGS (regulator of G protein signaling) homology; DH = Dbl homology; PH = pleckstrin homology; PDZ = PSD-95/SA90-Discs-large-ZO-1. See the text for details.

Regulation of G12/13-Mediated Signaling Pathways

Heterotrimeric G proteins serve as key molecular switches to transduce signals from hormones, neurotransmitters, and other stimuli from cell surface receptors into cells by actively alternating their conformations between GDP-bound inactive and GTP-bound active forms (fig. 1). The transient protein-protein interactions induced by guanine nucleotide-dependent conformational changes of G proteins play central roles in these signaling pathways. In the current model, the ligand-activated G protein-coupled receptors (GPCRs) catalyze the exchange of GDP for GTP on Gα subunits [37]. Upon activation, three switch regions in the Gα subunit undergo significant conformational changes, followed by dissociation of the GTP-bound Gα subunit from the Gβγ subunits. Both Gα-GTP and free Gβγ interact with diverse downstream effectors, such as enzymes or ion channels, to transmit intracellular signals [38]. The Gα subunit hy-

drolyzes bound GTP to GDP by its intrinsic GTPase activity and this deactivation process is further accelerated by GTPase-activating proteins (GAPs) such as regulator of G protein signaling (RGS) proteins [39, 40]. Gα-GDP dissociates from effectors and reassociates with Gβγ to terminate the signal. Thus, regulation of G protein cycles of activation and deactivation profoundly affect the cellular responses and the amplitude and duration of the signal response are determined by the amount of GTP-bound Gα subunit.

Regulation of G12/13
Activation of G12/13 by GPCRs

More than 30 GPCRs are reported to couple to either G12, G13 or both, based on direct or indirect methods of evaluating G protein activation; see details in Riobo and Manning's review [41]. It is believed that the binding of a ligand changes the conformation of critical regions of the seven-transmembrane helix pocket of the GPCR, which

in turn causes conformational changes in the intracellular loops and COOH terminus. However, the precise activation mechanism of G proteins by agonist-activated GPCRs remains unknown.

In general, interaction between the C-termini of G α and GPCRs is considered to be responsible for G α activation. Although the exact interface between G α 12/13 and GPCRs remains unclear, antibodies against the C-termini of G α 12 or G α 13 subunits or peptides corresponding to 10–50 residues of the C-termini specifically attenuate the agonist-activated angiotensin (AT1), thrombin, and sphingosine-1-phosphate (S1P2 and S1P3) receptor signals in cells [25, 42, 43]. In addition, the N-terminal short sequences of α subunits of the G12 family, where G α 12 and G α 13 have low amino acid sequence homology, are reported to determine the selectivity of coupling to receptors [25, 42–44]. The use of chimeric G α 12 and G α 13 proteins, in which the N-terminal short sequences are replaced with each other, demonstrated that thrombin and lysophosphatidic acid (LPA) selectively activated G α 12 or G α 13 expressed in HEK 293 cells. From studies of other G α subfamilies, membrane-proximal regions of the second and third intracellular loops and the cytoplasmic tail of the receptor are generally believed to have an important role in G α activation [45, 46].

Many GPCRs are reported to simultaneously couple to and activate more than one G protein subfamily member [45]. Studies suggest that most receptors coupling to the G12 subfamily could couple to both G12 and G13, except for the 5-hydroxytryptamine 4 receptor, which only couples to G13 [41, 47]. Furthermore, most receptors coupling to G α 12 and/or G α 13 couple to other G proteins, especially to G α q [41, 45, 48]. These facts make evaluating the specificity of the signaling through G12 and G13 complicated. To analyze signaling through a specific receptor-G protein pair, Zhang et al. [48] evaluated G α activation directly and in 1:1 stoichiometry using thromboxane A₂ receptor (TP α)-G α 12 or -G α 13 fusion proteins in *Spodoptera frugiperda* (Sf9) cells. Interestingly, the results show that TP α -G α 12 responded to agonists with slow GTP γ S binding, whereas the TP α -G α 13 response was fast. These results contrast with the case of the purified G α proteins in vitro: G α 12 and G α 13 do not show any differences in GTP γ S binding kinetics. Ligand binding may induce a specific conformational change in TP α to influence the coupling efficiency to different G α subunits. In turn, it is also possible that binding with specific G α -GDP subunits may change the conformation of the receptor to define the affinity of the ligand for the receptor. Alternatively, the selectivity might be enhanced in

collaboration with associated proteins in the cell. Future structural analysis of a complex of a GPCR with heterotrimeric G α 12/13 proteins will provide information critical for answering these questions.

Deactivation of G α 12/13 by RGS Proteins

Like all other heterotrimeric G protein α subunits, G α 12/13 cycle between GDP- (inactive) and GTP-bound (active) states and possess an intrinsic ability to hydrolyze GTP to GDP. In vitro analysis has demonstrated that both recombinant G α 12 and G α 13 proteins have relatively slow rates of nucleotide exchange and GTP hydrolysis (G α 12: k_{on} , GTP γ S = 0.01 min⁻¹, k_{cat} = 0.1–0.2 min⁻¹, G α 13: k_{off} , GDP = 0.01 min⁻¹, k_{cat} = >0.2 min⁻¹) [49, 50]. This deactivation process is accelerated by the GAP activity of RGS proteins. p115RhoGEF and leukemia-associated RhoGEF (LARG) have been shown to act as specific GAPs for G α 12 and G α 13 in vitro [51–53]. p115RhoGEF, LARG, and PDZ-RhoGEF/GTRAP48 are the known members of the mammalian RhoGEF family, which contain an amino-terminal RGS homology (RH, also called rgRGS) domain (RH-RhoGEFs) that recognizes activated G α 12/13 [54–57] (fig. 2). In addition, RH-RhoGEFs contain central DH/PH (Dbl homology/pleckstrin homology) domains characteristic of GEFs for Rho family GTPases. In vitro, p115RhoGEF and LARG act as specific GAPs for G α 12 and G α 13, while the RGS domain of PDZ-RhoGEF lacks detectable GAP activity for these G α subunits [51, 52, 58]. At the same time, G α 12 and G α 13 subunits regulate the activity of Rho through RH-RhoGEFs [51–53, 55, 58]. RH-RhoGEFs directly link the activation of GPCRs by extracellular ligands to the regulation of Rho activity in cells. RH-RhoGEFs combine GAP and effector activity into a single molecule to regulate signaling from G α 12/13.

Although the sequence identity between the RGS domains from RH-RhoGEFs and other RGS family members is low (between 10 and 15%) [51], the high resolution crystal structures of the RGS domains from both PDZ-RhoGEF and p115RhoGEF have demonstrated that these domains share a similar tertiary fold composed of an all-alpha helical bundle [59, 60]. One structural divergence between these RGS domains and RGS4 is the extended C-terminus found in the RH-RhoGEFs, which forms α helices tightly associated with the core RGS domain through a large hydrophobic surface.

Site-directed mutagenesis of both G α 13 and p115RhoGEF has provided important insights into the mechanism of the acceleration of GTPase activity by the RH domain of p115RhoGEF. In G α 13, lysine 204, located

in its switch I region, has been shown to be important for interaction with the RH domains of both p115RhoGEF and LARG [61, 62]. Residues 1–252 of p115RhoGEF stimulate the GTPase activity of $G\alpha_{13}$ similarly to the full-length protein [51, 53]. It has been demonstrated that residues at the N-terminus of the RH domain are required for RH-RhoGEFs to exert their GAP activity [53, 63]. In particular, residues 27–31 within a negatively charged cluster of residues are crucial for p115RhoGEF's GTPase activity. Mutations in this acidic patch reduce both binding to $G\alpha_{13}$ and stimulated GTP hydrolysis [63, 64]. Furthermore, a structural study of a complex between p115RhoGEF's RH domain and a $G\alpha_{13}/i1$ chimera has demonstrated that the interface is different from that of the RGS domain of RGS4 and $G\alpha_{i1}$ [54]. The N-terminal small element within the RH domain, which is required for GAP activity towards $G\alpha_{13}$, contacts the switch regions and the helical domain of $G\alpha_{13}/i1$. The core module of the RH domain binds to the switch II region and the $\alpha 3$ helix of $G\alpha_{13}/i1$ chimera, which is used for effector binding in other $G\alpha$ subunits. In spite of these structural studies, the molecular mechanism of GAP and GEF activation of RH-RhoGEFs upon $G\alpha_{12}/13$ binding has not yet been elucidated.

Post-Translational Modification of $G\alpha_{12}/13$

Lipid modifications affect the subcellular localization of $G\alpha$ subunits and the interactions of these subunits with other proteins. $G\alpha$ subunits are subjected to N-myristoylation, which is an irreversible, co-translational attachment of 14-carbon myristate to glycine at the N-terminus through an amide bond, and/or palmitoylation, which is a reversible, post-translational attachment of 16-carbon palmitate to cysteine residues near the N-terminus through thioester bonds. Both $G\alpha_{12}$ and 13 lack a glycine residue at the second site for myristoylation and thus are modified by only palmitoylation near their N-termini [65–67]. Although palmitoylation has no clear consensus sequence, one site (C12) in $G\alpha_{12}$ [65] and two sites (C14, C18) in $G\alpha_{13}$ [66] have been defined as sites of palmitate incorporation using mutants in which serine replaces these cysteine residues.

It has been demonstrated that palmitoylation of $G\alpha_{13}$ is critical for its association with the plasma membrane, translocation of its effector p115RhoGEF, and its ability to signal through RhoA [68]. It has also been reported that palmitoylation of $G\alpha_{12}$ but not $G\alpha_{13}$ is related to localization in lipid rafts [69]. A constitutively active mutant of $G\alpha_{12}$ which cannot be palmitoylated inhibits its transforming activity in NIH-3T3 cells [65]. It is interest-

ing to note that palmitoylation of another $G\alpha$ subunit, $G\alpha_z$, decreases its affinity for Gz GAP and its rate of GTP hydrolysis. However, this may not be the case with $G\alpha_{12}/13$ [70].

Phosphorylation of $G\alpha$ subunits is another important modification which regulates the amplitude and duration of G protein signals. $G\alpha_{12}$, as well as $G\alpha_z$, is a substrate for phosphorylation by protein kinase C (PKC) [71–74]. In vitro, $G\alpha_{12}$ is phosphorylated by PKC α , $-\delta$, $-\epsilon$, and $-\zeta$, and approximately 1 mol of phosphate was incorporated per mol subunit with PKC α [72]. $G\alpha_{12}$ stably expressed in NIH-3T3 cells is phosphorylated following treatment of the cells with PMA (phorbol 12-myristate 13-acetate) [72], and endogenous $G\alpha_{12}$ in human platelets is phosphorylated in response to PMA, thrombin, and the thromboxane A_2 receptor agonist U46619 [75]. Although the phosphorylation site has not been mapped, the N-terminal 50 amino acid residues comprise one possible region [72]. It is possible that Ser38 corresponding to Ser16 defined in $G\alpha_z$ may be the phosphorylation site. As to phosphorylation of $G\alpha_{13}$, there is a discrepancy between in vitro and cell-based experiments. In vitro experiments demonstrate purified $G\alpha_{13}$ is not a substrate for PKC α , $-\delta$, $-\epsilon$, and $-\zeta$ [72]. However, studies with intact cells show that $G\alpha_{13}$ in platelets is phosphorylated in response to PMA, and $G\alpha_{13}$ expressed in COS cells is effectively phosphorylated by PKC β , $-\delta$, and $-\epsilon$ [75]. This discrepancy might indicate that additional cellular factors are required for phosphorylation of $G\alpha_{13}$ by PKC.

It has been demonstrated that phosphorylation blocks the interaction of the $G\alpha_{12}$ subunit with $G\beta\gamma$, and $G\beta\gamma$ reciprocally blocks the phosphorylation of $G\alpha_{12}$ by PKC [72]. This appears to be consistent with the fact that $G\beta\gamma$ binds the N-terminal helix of $G\alpha$. $G\beta\gamma$ binding to the N-terminus of $G\alpha$ may hinder PKC from approaching sterically, or reciprocally, phosphorylation of the N-terminus may block $G\beta\gamma$ binding. Furthermore, Kozasa and Gilman [37] demonstrated that the rate of GTP γ S binding to phosphorylated $G\alpha_{12}$ is not inhibited by $G\beta\gamma$ whereas $G\beta\gamma$ inhibits GTP γ S binding to non-phosphorylated $G\alpha_{12}$. It is noteworthy that phosphorylation inhibits interaction of $G\alpha_z$, another substrate for PKC, with the RGS protein RGSZ1, while it is unknown if phosphorylation of $G\alpha_{12}$ produces a similar effect [76, 77]. Interestingly, a genetic screening using *Caenorhabditis elegans* suggested that the novel calcium-independent PKC θ/δ is a potential downstream target of $G\alpha_{12}$ [78]. Dhanasekaran et al. [79] have reported that Na^+/H^+ exchange activity stimulated by $G\alpha_{12}$ is lost after prolonged exposure of cells to PMA. Once activated upon binding of ligand to a

GPCR, $G\alpha_{12}$ is phosphorylated by PKC, which could be activated downstream of $G\alpha_{12}$ itself. The phosphorylated, activated, $G\alpha_{12}$ may have reduced interaction with $G\beta\gamma$, and be less susceptible to the GAP activity of RGS protein, prolonging the duration of signaling. The system would eventually desensitize because of a lack of reassociation between GDP-bound $G\alpha$ with $G\beta\gamma$ required for receptor-mediated reactivation. PKC likely attenuates the activity of $G\alpha_{12}$ in a negative feedback loop, while the mechanism of PKC activation by $G\alpha_{12}$ is unknown. Tyrosine phosphorylation of $G\alpha_{12}$ has not been reported.

Regulation of Effectors by G12

As mentioned above, $G\alpha_{12}$ and $G\alpha_{13}$ subunits directly activate RH-RhoGEFs to regulate the activity of the GTPase Rho [51–53, 55, 58]. p115RhoGEF was first identified as a direct downstream effector of the G12 subfamily 10 years ago. Since then, studies including the yeast two-hybrid system, have revealed more than 20 diverse proteins that directly interact with the G12 subfamily as well as RH-RhoGEFs; see recent reviews for further details [80, 81]. A well-established downstream effector of G12/13-mediated signaling is the monomeric GTPase RhoA, which is a regulator of a variety of intracellular processes including formation of actin stress fibers and assembly of focal adhesions, gene transcription, and control of cell growth [78, 82].

Regulation of RH-RhoGEFs by $G\alpha_{12/13}$

Biochemical evidence using reconstitution systems with purified proteins has clearly demonstrated that the GEF activity of p115RhoGEF and LARG can be directly stimulated by $G\alpha_{13}$ [52, 58]. As mentioned above, RH-RhoGEFs combine GAP activity and effector activity into a single molecule. While it has been well demonstrated that p115RhoGEF and LARG serve as specific GAPs for $G\alpha_{12/13}$ through their RH domains, the molecular mechanism of RH-RhoGEF activation upon $G\alpha_{13}$ binding remains unclear. Some recent studies have provided information about the interface between $G\alpha_{13}$ and RH-RhoGEFs required for RH-RhoGEF activation.

A structural study using p115RhoGEF's RH domain and a $G\alpha_{13/i1}$ chimera has demonstrated that the core module of p115RhoGEF's RH domain binds to the region of $G\alpha_{13/i1}$ which is conventionally used for effector binding [54]. This result suggests roles for the RH domain in the stimulation of GEF activity by $G\alpha_{13}$ in addition to GAP activity. Several studies have indicated that regions outside of RH domain of RH-RhoGEFs, particularly the DH/PH domains, interact directly with activated $G\alpha_{13}$

[53, 58, 62]. Wells et al. [53] demonstrated that AlF_4^- -activated $G\alpha_{13}$ was able to bind directly to a deletion mutant of p115RhoGEF consisting of the DH and PH domains, although it was unable to stimulate the GEF activity of the fragment in vitro. Interaction through multiple interfaces, including the RH domains and DH/PH domains of RH-RhoGEFs, and $G\alpha_{13}$ might play an important role in stimulating GEF activity. Kinetic and thermodynamic analysis of the interaction between $G\alpha_{13}$ and LARG using surface plasmon resonance has demonstrated that the simultaneous binding of the RH domain and DH/PH domains with $G\alpha_{13}$ facilitates formation of the high affinity active $G\alpha_{13}$ -LARG complex [145].

In addition to analysis of the $G\alpha_{13}$ -binding surfaces on RH-RhoGEFs, the surfaces of $G\alpha_{13}$ necessary for binding to RH-RhoGEFs have also been characterized. One study, utilizing chimeras of $G\alpha_{13}$ and $G\alpha_{i2}$, revealed that not only the switch regions of $G\alpha_{13}$, but also a large portion of the Ras-like domain of $G\alpha_{13}$ are required for efficient Rho activation in cell-based assays [83]. Another study utilizing chimeras of $G\alpha_{12}$ and $G\alpha_{13}$ further identified that the C-terminal 100 amino acid residues of $G\alpha_{13}$ are required for activation of the GEF activity of p115RhoGEF and LARG, whereas the N-terminal α helical and switch regions of $G\alpha_{12}$ and $G\alpha_{13}$ are responsible for their differential GAP responses to the RH domain [84]. This result demonstrates that p115RhoGEF and LARG interact with distinct surfaces on $G\alpha_{13}$ for GAP activity or GEF activity regulation. Furthermore, we have demonstrated that the interaction of $G\alpha_{13}$ with LARG through the RH domain (a GAP interface) and the DH/PH domains (an effector interface) could coordinate together to stimulate the RhoGEF activity of LARG [145]. In summary, the spatial and kinetic connection of GAP and GEF activities within RH-RhoGEFs may help to regulate the amplification of G protein signaling, provide higher temporal resolution of the response, and increase the specificity of the signal output. Since $G\alpha_{12/13}$ have characteristically slow rates of nucleotide exchange and GTP hydrolysis, this mechanism could be a rational system for $G\alpha_{12/13}$ signaling to regulate multiple important cellular functions with fast responses as well as long-term processes.

On the other hand, binding of the activated $G\alpha_{12}$ does not activate the GEF activity of either p115RhoGEF or LARG in vitro. LARG activation requires not only binding to activated $G\alpha_{12}$ but also phosphorylation by a non-receptor tyrosine kinase [52, 58].

Phosphorylation

One mechanism regulating RH-RhoGEF activity is post-translational modification in the form of phosphorylation. Chikumi et al. [85] have demonstrated that the non-receptor tyrosine kinase FAK (focal adhesion kinase) phosphorylates both LARG and PDZ-RhoGEF, but not p115RhoGEF. By measuring the Rho activity of HEK293 cells overexpressing FAK and PDZ-RhoGEF, they have concluded that FAK enhances RhoGEF activity even in the absence of G α 12 or G α 13. They also observed that FAK can be activated by thrombin, G α 12, G α 13, and G α q through both Rho-dependent and -independent mechanisms, and proposed the existence of positive feedback regulation between Rho and FAK [85]. Another study using reconstitution and cell-based assays has demonstrated that G α 12 can stimulate the RhoGEF activity of tyrosine-phosphorylated LARG, but not non-phosphorylated LARG [52]. The direct phosphorylation of LARG by a non-receptor tyrosine kinase, Tec, *in vitro* greatly enhances the RhoGEF activity of LARG in response to G α 12, while it does not affect its basal RhoGEF activity. Although binding of Tec to G α 12 in cells was also demonstrated, the mechanism by which Tec is phosphorylated downstream of G α 12 remains unclear. It is interesting to note that thrombin, which can activate the G α 12/13 pathway, can also activate Tec [86] and that G α 12 promotes the kinase activity of Bruton's tyrosine kinase (BTK), another member of the Tec family [87]. Furthermore, overexpression of G α 12 and G α 13 stimulates auto- and transphosphorylation of Tec in NIH-3T3 cells [88]. One possibility is that activated G α 12 may recruit Tec to LARG and facilitate its phosphorylation.

The role of tyrosine kinases involved in the G α 12/13-Rho pathway is controversial. *In vitro*, the RhoGEF activity of non-phosphorylated LARG was stimulated by G α 13 but not G α 12, suggesting that the G α 13-LARG-Rho pathway does not require tyrosine kinases. However, in cell-based assays, the presence of Tec further potentiates the RhoGEF activity of LARG stimulated by G α 13 in NIH-3T3 or HeLa cells [52, 88]. In the context of cells, G α 13 and tyrosine kinases cooperatively and efficiently activate Rho. In contrast to the study by Suzuki et al., early studies of G α 12/13-mediated cytoskeletal reorganization suggested that tyrosine kinases might play a role in regulating Rho activation downstream of G α 13, but not G α 12 in PC12 cells or Swiss 3T3 cells [11, 89]. Tyrosine phosphorylation of PDZ-RhoGEF and LARG is an important post-translational modification regulating RhoGEF activity downstream of G α 12 and G α 13.

C-Terminal Regions and Oligomerization

Several studies have reported that RH-RhoGEFs oligomerize via their C-terminal regions [90–92]. It has been shown that RH-RhoGEF deletion mutants lacking their C-termini have an increased ability to stimulate Rho activation as compared to the full-length protein, and that the deletion of the C-terminal region alone is sufficient to increase the RhoGEF's transforming potential in cells [90, 93]. These results suggest that the RH-RhoGEF's activity may be negatively regulated *in vivo* through the C-terminus itself or by interaction of regulatory factors with this region. First, to identify regulatory proteins interacting with the C-terminus of RH-RhoGEFs, Eisenhaure et al. [91] utilized yeast two-hybrid screening with the C-terminus of the murine ortholog of p115RhoGEF, Lsc. Surprisingly, the only protein identified was a C-terminal fragment of Lsc itself, suggesting that it homo-oligomerizes via its C-terminal region. Using mutagenesis experiments, they further showed that homo-oligomerization and negative regulation of Lsc activity are distinct functions of its C-terminus. Disruption of a putative coiled-coil domain within the C-terminus impairs oligomerization, but does not result in enhanced Rho activation in cells. A subsequent study confirmed the homo-oligomerization of p115RhoGEF, LARG, and PDZ-RhoGEF via their C-terminal regions by co-immunoprecipitation using cells overexpressing the RH-RhoGEFs [90] (fig. 2). LARG and PDZ-RhoGEF have also been shown to possibly form hetero-oligomers with each other, but not with p115RhoGEF [90].

Interestingly, deletion of the C-terminus of PDZ-RhoGEF or p115RhoGEF does not affect or even reduces the RhoGEF activity *in vitro*, while it dramatically enhances the RhoGEF activity in cells [90, 93]. This discrepancy suggests that ancillary factors in cellular milieu might release the inhibition of the RH-RhoGEF's activity through the C-terminal region. A possible mechanism is that an activating protein releases the inhibition through the C-terminus to induce the active conformation of RH-RhoGEFs. One potential activating protein might be the active form of G α 13, since activation-dependent binding between the C-terminus of LARG and G α 13 has been observed [145]. Interestingly, the Rho effector Dia1 also binds to the C-terminus of LARG to potentiate its GEF activity, and its activation constitutes a positive feedback loop between LARG, RhoA, and Dia1 [94]. Another mechanism is post-translational modification at the C-terminal region. It is interesting to note that PDZ-RhoGEF is tyrosine-phosphorylated by FAK at its C-terminus [85].

A recent study has suggested that oligomerization of overexpressed LARG may regulate its intracellular localization. Oligomerization functions to prevent nucleocytoplasmic shuttling and to retain LARG in the cytoplasm, while the mechanism for regulation of LARG oligomerization and the function of LARG localized in the nucleus are unknown [92]. Further study is required to understand the physiological significance of oligomerization of endogenous RH-RhoGEFs.

Subcellular Localization

Translocation of RH-RhoGEFs to the plasma membrane where their target, Rho, is enriched seems to be another important mechanism for regulating RhoGEF activity. Intracellular localization of p115RhoGEF has been intensively studied. However, it should be noted that the distribution of endogenous and overexpressed RH-RhoGEFs might differ. Indeed, it has been demonstrated by subcellular fractionation of NIH-3T3 cells that endogenous p115RhoGEF is found mainly in the cytosolic fraction with approximately 10–20% of the protein localized to the membrane fraction, while overexpressed p115RhoGEF was distributed abundantly between both the membrane and cytosolic fractions [93]. Both immunocytochemical and cellular fractionation analyses have demonstrated that co-expression of the constitutively active form of G α 12 as well as G α 13 or stimulation of the thromboxane A₂ receptor, which couples with G α 12/13, induces redistribution of endogenous and overexpressed p115RhoGEF to the plasma membrane in HEK293 cells [68, 95]. Using overexpressed p115RhoGEF deletion mutants, these studies have identified the RH domain and the PH domain as essential for targeting of p115RhoGEF to the plasma membrane by G α 13. However, the RhoGEF activity of these mutants in the presence of G α 13 was not tested in cells. Furthermore, Bhattacharyya and Wedegaertner [64] tested the role of the acidic-rich region N-terminal to the RGS domain of p115RhoGEF in G α 13-dependent plasma membrane recruitment in cell-based assays, based on the finding that this region is important for interacting with G α 13 [54, 63]. Two point mutations in this region, Glu27Ala and Glu29Ala, impair the ability of p115RhoGEF to bind to G α 13 but did not affect its ability to localize to the plasma membrane [64].

Compared with p115RhoGEF, the mechanisms for regulating the subcellular distribution of PDZ-RhoGEF and LARG are less well understood. One immunohistochemistry study using polyclonal antibodies has shown that G α 12, G α 13, PDZ-RhoGEF and LARG proteins are distributed widely in the mouse nervous system, but lo-

calize to distinct morphological compartments within neurons. While LARG and G α 12 were mainly found in the somata of neurons, PDZ-RhoGEF and G α 13 were predominantly localized in the neuropil of central neurons [96]. An immunocytochemical analysis has demonstrated that endogenous PDZ-RhoGEF in Neuro2a cells is localized in the nucleus, cell body, and neurites, and upon stimulation of the LPA receptor, which is coupled to G α 12/13, PDZ-RhoGEF translocates to the tips of neurites, where Rho is enriched and cortical actin reorganization is induced [97]. Furthermore, analyses by Togashi et al. [97] revealed that a proline-rich motif C-terminally adjacent to DH/PH domains is essential for plasma membrane localization of PDZ-RhoGEF and cortical actin reorganization followed by cell rounding. However, the effects of mutating in this region on the interaction with G α 13 was not investigated. It has also been reported that PDZ-RhoGEF overexpressed in HEK293T, COS7, and Neuro2a cells is partially localized at or near the plasma membrane and co-localizes with cortical actin [98]. Immunoprecipitation and F-actin co-sedimentation assays demonstrated that PDZ-RhoGEF binds to actin. Mutants that fail to interact with the actin cytoskeleton display enhanced Rho activation compared with wild type PDZ-RhoGEF. Togashi et al. [97] also have reported that PDZ-RhoGEF appears to co-localize with microtubules. It has been proposed that not only G α 12/13 but also its downstream target myosin II may be involved in regulating PDZ-RhoGEF localization and activation, although the biochemical mechanism remains unknown [99]. These results imply that the interaction with actin, myosin, and microtubules might regulate PDZ-RhoGEF signaling.

In contrast to PDZ-RhoGEF, endogenous LARG in COS7 cells exhibits a predominantly cytoplasmic distribution [92]. In MDCKII cells, endogenous LARG was reported to be localized at the lateral membranes and slightly in the cytoplasm [100]. Overexpressed LARG seems to be distributed throughout the cytoplasm and does not co-localize with actin [98].

The co-localization of PDZ-RhoGEF and LARG with receptors at the plasma membrane via their PDZ domains is clearly distinct from p115RhoGEF, which does not contain a PDZ domain (fig. 1, 2). Several studies using biochemical and cell-based analyses have demonstrated that plexin B1, which is a transmembrane receptor that mediates the repulsive cues of semaphorin 4D to initiate collapse of neurite growth cones in mammals, directly interacts through its C-terminus with the PDZ domains of PDZ-RhoGEF and LARG [101–104]. Taya et al. [100] have

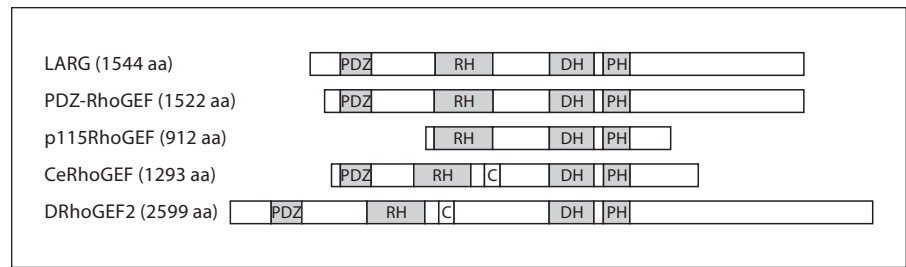


Fig. 2. A schematic representation of domain structures of RH-RhoGEFs. LARG, PDZ-RhoGEF and p115RhoGEF are the three known human RH-RhoGEFs. All RH-RhoGEFs contain RH domains followed by tandem DH/PH domains. The N-terminal region of PDZ-RhoGEF and LARG each contain a PDZ domain. For comparison, CeRhoGEF and DRhoGEF2, RGS-RhoGEFs from *C.*

elegans and *D. melanogaster*, respectively, are depicted as well. Unlike human RGS-RhoGEFs, CeRhoGEF and DRhoGEF2 each contain one C1 homology domain. Domains: RH = RGS homology domain; DH = Dbl homology; PH = pleckstrin homology; PDZ = PSD-95/SA90-Discs-large-ZO-1; C = C1 homology domain.

reported that the C-terminus of non-phosphorylated insulin-like growth factor-1 (IGF-1) receptor also directly interacts with LARG and that IGF-1 stimulation in MDCKII cells induces Rho activation.

Crosstalk between G12/13-Mediated Signaling and Other Signaling Pathways

The G12/13-mediated signaling pathway engages in crosstalk with other pathways at various levels such as GPCRs (described above), G proteins, and downstream effectors (fig. 1). At the G protein level, G α 12 and G α 13 interact directly and in an activation-dependent manner with the cytoplasmic tails of cadherins [105, 106]. Binding of activated G α 12 to the cytoplasmic tail of E-cadherin triggers the release of the transcriptional activator β -catenin attenuates the extracellular adhesive function of E-cadherin, and promotes cell migration [105, 106]. G α 12 may integrate the LARG-Rho signaling pathway controlling cytoskeletal rearrangement and the cadherin- β -catenin signaling pathway regulating cell-cell adhesion to govern cell migration in response to extracellular stimulation. It has also been reported that G α 12 interacts with and activates a member of the Tec family of non-receptor tyrosine kinases, BTK, while G α 13 binds and stimulates PYK2, another non-receptor tyrosine kinase [87, 107].

RH-RhoGEFs are also capable of interacting with various other cellular proteins. In particular, PDZ-RhoGEF and LARG are well known to interact with multiple cell surface receptors through their PDZ domain as previously described. One of the binding partners for the PDZ domain is plexin B1. Multiple studies have demonstrated that binding of the C-terminal PDZ-binding motif of

plexin B1 with both PDZ-RhoGEF and LARG activates RhoA and promotes growth cone collapse [101–104]. This pathway is also known to be involved in angiogenesis [108]. Additionally, the direct binding of the C-terminus of the IGF-1 receptor to LARG induces RhoA activation [100]. It is noteworthy that in these two cases, binding of plexin B or IGF-1 receptor activates the GEF activity of RH-RhoGEFs independently of G α 12/13 activation. Direct interaction between the PDZ domain of LARG and CD44 in human head and neck squamous carcinoma cells has also been reported [109]. This CD44-LARG complex interacts with the EGF receptor and activates the EGFR receptor kinase. The C-terminus of the LPA receptor also interacts with the PDZ domains of LARG and PDZ-RhoGEF [110]. p115RhoGEF has been reported to interact with the C-terminus of HIV-1's transmembrane protein gp41 [111]. This interaction inhibits the ability of p115RhoGEF to initiate Rho-dependent stress fiber formation and gene transcription. Interestingly, mutations in gp41 that block the interaction with p115RhoGEF inhibit the ability of the HIV-1 virus to produce infectious particles in certain cell types. This finding suggests that the interaction between gp41 and p115RhoGEF modulates the ability of the virus to replicate. As described above, the Rho effector Dial1 binds to the C-terminus of LARG, stimulates its GEF activity, and may constitute a positive feedback loop between LARG, RhoA, and Dial1 [94].

A deficiency of G α 13 in mice is embryonic lethal, while mice lacking G α 12 develop normally and do not exhibit any overt morphological or behavioral defects. This fact clearly shows that G α 13 and G α 12 mediate distinct signaling pathways. The studies of intercrosses of

G α 12-deficient mice, G α 13-deficient mice, and G α q-deficient mice indicate that the G α 12-mediated signaling pathway functionally interacts not only with the G α 13- but also with the G α q-mediated signaling systems [28]. Coordinated action from the two signals is known at the level of transcriptional factors [32] and protein kinases such as protein kinase D and PYK2 [36, 112]. The signals from G α 12/13 and G α q have been known to converge on Rho, which is a target for G α 12/13 [35, 113, 114]. Recently, p63RhoGEF, together with the related Dbl-family member Trio, has been identified as a direct effector of G α q and a guanine nucleotide exchange factor for RhoA, and the crystal structure of the G α q-p63RhoGEF-RhoA complex has also been determined [31, 33]. The crosstalk between G α 12/13- and G α q-mediated signals has been noted for smooth muscle contraction [30] and platelet activation [115].

Dissection of G12/13-Mediated Signaling Pathways Evoked by Specific G α -Effector Interactions

Accumulating evidence supports the idea that GPCR signals are amplified and integrated into the intracellular signaling network at the level of G proteins [116]. The G α subunit acts as the core of the signaling complex at the membrane, which is formed through transient protein-protein interactions between multiple signaling components.

Recently, some groups have tried to regulate the specific signal induced by the G α -effector interaction. Using substitution mutants of G α 12 at the specific interface for the G α 12-effector interaction, Meigs et al. [117] have successfully identified a variant of G α 12 that is selectively uncoupled from one signaling pathway while retaining signaling capacity through a separate pathway: it has impaired binding to RH-RhoGEFs and is unable to activate Rho, but retains coupling to the effector cadherin and the ability to trigger β -catenin release from the cytoplasmic domain of cadherin. A study using small interfering RNAs to eliminate specific RH-RhoGEF expression in kidney and prostate cells has demonstrated that specific G12-coupled receptors require specific RH-RhoGEFs for Rho activation [118]. Thrombin-mediated stimulation of Rho requires LARG, while the LPA-stimulated Rho response requires PDZ-RhoGEF. These efforts to evaluate the specific signals mediated by G12/13 could lead to development of specific modulators for biological responses induced by divergent effectors, and eventually drugs with fewer side effects.

Physiological Function and Pathophysiological Significance of G12/13-Mediated Signaling Pathways

Advances in animal studies such as conditional knockout mice and genetic analysis in *Caenorhabditis elegans* and *Drosophila melanogaster* have imparted new biological significance to the G12 subfamily. Conservation of a G α 12/13-RH-RhoGEF-Rho signaling pathway through the course of evolution from the model organisms *C. elegans* and *D. melanogaster* to mammals is supported by genetic evidence. In a mutational screen to identify Rho signaling pathway components in *Drosophila*, Barrett et al. [9] identified the *DRhoGEF2* gene, which encodes a protein containing the tandem DH/PH domains characteristic of RhoGEFs, as an upstream Rho1 activator. Embryos lacking functional DRhoGEF2 show similar defects in the cell shape changes associated with gastrulation to embryos without functional Concertina, the single *Drosophila* G α 12/13 ortholog, suggesting that Concertina may propagate signals from an upstream ligand to Rho1 via DRhoGEF2 [9, 10]. Subsequently, the N-terminus of DRhoGEF2 was shown to have sequence homology with other putative RhoGEFs containing RH domains in their N-terminus [51]. Another study has demonstrated that in *C. elegans*, GPA-12, the ortholog of G α 12, and a RH-RhoGEF, CeRhoGEF, can interact in an activation-dependent manner and are co-expressed in some ventral cord motor neurons [119]. In the same study, silencing of either GPA-12 or CeRhoGEF using RNA interference (RNAi) results in a similar phenotype, namely defects in egg laying and embryonic lethality, further suggesting these two proteins function in the same pathway. Additional studies in *C. elegans* support the notion that one of the pathways acting upstream of Rho1 in acetylcholine-releasing motor neurons, where Rho1 stimulates the release of acetylcholine [120], depends on GPA-12, which acts via the single RH-RhoGEF ortholog, RHGF-1 [16]. It has been reported that Gq also acts via the UNC-73 RhoGEF, an ortholog of mammalian Trio, to increase Rho activity in neurons [121].

The first identified G α 12/13 function was the ability to induce oncogenic transformation [12–15]. Since then, many studies of the biological functions of G12/13 have concentrated on their roles in cell proliferation, cell migration, and morphological changes. Accumulating evidence indicates that the G12/13-mediated signaling pathway is involved in a variety of physiological and pathophysiological processes as mentioned in the introduction. Furthermore, some evidence indicates that G α 12 and

G α 13 have partially overlapping but distinct cellular and biological functions [28, 29], and that G12 or G13-mediated signals crosstalk with those from other G proteins, especially Gq to produce a biological response [3, 11, 28, 30–36].

Cell Proliferation and Transformation – Neoplastic Disorders

Notably, G α 12 and G α 13 are the only heterotrimeric G α subunits that have potent transforming capabilities when overexpressed as the wild-type forms. These tumorigenic and cell-proliferating effects of G12/13 seem to be mainly mediated by RhoA activation. Martin et al. [27] have demonstrated that overexpression of the thrombin protease-activated receptor-1 (PAR-1) promotes transformation and growth in NIH-3T3 cells through G α 12/13. PAR-1 stimulates the activity of the serum response factor and NF- κ B transcription factors, which are also effectors of RhoA. Furthermore, PAR-1 transforming activity is partially blocked by co-expression of dominant negative RhoA. Other studies also suggest that GPCRs upstream of G α 12 and G α 13 may promote tumorigenesis and tumor cell growth [25, 32, 122, 123]. Indeed, recent studies demonstrated that G α 12 protein levels are upregulated in human breast or prostate adenocarcinoma tissues [22, 23]. Interestingly, in contrast to the previous studies using non-transformed cells [12–15], G α 12 and G α 13 did not promote, and in some cases even inhibited, in vitro or in vivo proliferation of human breast or prostate cancer cell lines [22, 23]. Therefore, G α 12 and G α 13 may be important for promoting normal cell proliferation. In addition, it has been reported that G α 13-induced transformation uses a Rho-independent pathway via radixin, a member of the ERM family of proteins [124, 125].

It is noteworthy that unlike Ras, activating mutations in Rho have not been found in human cancers [126–128]. On the other hand, the regulators of Rho activation, Rho-GEFs have been isolated in screens for transforming genes. LARG was one of the very few RhoGEFs that have been found mutated in human cancers. LARG was originally identified as a novel protein fused to the MLL (mixed lineage leukemia) gene in a patient with acute myeloid leukemia [21]. It is interesting that in the MLL-LARG fusion, not only the DH/PH domains responsible for Rho activation but also the RH domain and a nuclear localization signal are retained. The MLL-LARG rearrangement is expressed as an in-frame fusion from the MLL promoter. A study has indeed shown that LARG is abundant in mouse hematopoietic stem cells [129]. Fur-

thermore, a recent analysis of bone marrow samples from patients with the preleukemic disorder Shwachman-Diamond syndrome demonstrated that in these patients LARG expression is dramatically increased [130]. However, further studies will be necessary to confirm the roles of LARG and MLL-LARG fusion protein in leukemic disease.

Cell Morphology and Motility – Failure in Gastrulation and Tumor Cell Invasion

Some aspects of the biological effects mediated by the G α 12/13 signaling pathway are contributed by the capability of their downstream effector, RhoA, to regulate cell morphology and motility. RhoA induces the assembly of contractile actin and myosin filaments (stress fibers), and is involved in cell contraction as well as in moving the body and tail of the cell behind the leading edge [128, 131, 132]. Cadherin, acting downstream of G α 12, also affects cell migration independently of Rho activation [105]. Gastrulation, which is regulated by the G α 12/13 ortholog and a RH-RhoGEF in *D. melanogaster* [9, 10], and tumor cell invasion, are related to these shape changes and movement.

Tumor Cell Invasion

Although G α 12 and G α 13 did not promote proliferation of human breast or prostate cancer cell lines, they promoted invasion by both types of cancer cells in vitro [22, 23]. Mouse mammary carcinoma cells implanted in the mammary fat pad of a mouse, which grow and metastasize in a manner similar to that of human breast cancer, has demonstrated that inhibition of G α 12/13 signaling by stable expression of the RH domain of p115RhoGEF in carcinoma cells reduced the rate of metastatic dissemination [22]. These studies suggest that Rho activation through G α 12/13 signaling is critical for promoting invasion. However, when seeded directly into the bloodstream, inhibition of G12/13 signaling by the RH domain had no effect on the ability of the cells to metastasize. Signaling via the G12-Rho pathway may coordinate with signals from cadherin, which influences cell-cell contacts, to promote invasion away from the primary tumor. However, it is possible that invasion stimulated by G α 12 and G α 13 may be cell type-specific [80].

The Cardiovascular System – Heart and Vascular Disease and Angiogenesis

A deficiency of G α 13 in mice impairs the development of the vascular system and is embryonic lethal [6]. Embryonic fibroblasts cultured from these mice show im-

paired motility in response to thrombin and LPA. Although vasculogenic blood vessel formation through the differentiation of progenitor cells into endothelial cells was not affected, angiogenesis, which includes sprouting, growth, migration and remodeling of existing endothelial cells, was severely disturbed [6, 29]. Endothelial-specific $G\alpha 13$ knockout embryos also showed a similar phenotype to that of $G\alpha 13$ null animals. However, restoration of $G\alpha 13$ expression in endothelial cells in $G\alpha 13$ conventional knockouts fails to completely rescue the phenotype, suggesting that $G\alpha 13$ expression in other cell types is necessary during embryonic development [7]. In contrast to the phenotype observed in $G\alpha 13$ null mice, mice lacking $G\alpha 12$ develop normally and do not exhibit any obvious morphological or behavioral defects. A double knockout of $G\alpha 12$ and $G\alpha 13$ produces developmental defects in the headfold, somites, and neural tube and these embryos arrest earlier than $G\alpha 13$ null embryos, suggesting that the function of $G\alpha 12$ is not completely redundant to that of $G\alpha 13$ during embryonic development [28]. In addition, a study suggests that semaphorin 4D/plexin B1-mediated angiogenic responses require Rho-mediated signaling via PDZ-RhoGEF or LARG [108]. As described above, binding of the C-terminal PDZ-binding motif of plexin B with both PDZ-RhoGEF and LARG activates RhoA.

Platelets

Studies using mice lacking $G\alpha 13$ in platelets revealed that this α subunit is involved in normal hemostasis and thrombosis. Platelets lacking $G\alpha 13$, but not $G\alpha 12$, have impaired shape changes and aggregation in response to multiple platelet activators in vitro, and fail to form stable thrombi ex vivo. The mice exhibit a large increase in tail-bleeding times [5].

Smooth Muscle

It is believed that upon binding with vasoconstrictors, receptors coupling to both Gq/11 and G12/13 stimulate phosphorylation of myosin light chain (MLC) via the Ca^{2+} /MLC kinase- and Rho/Rho kinase-mediated signaling pathways, respectively, to regulate vascular smooth muscle tone [30]. Recently, mice with conditional $G\alpha 12/13$ double deficiencies in smooth muscle cells have been developed [24]. Aortic segments from these mice show impaired contractile responses to the vasoconstrictors angiotensin II, thromboxane A_2 , and endothelin I. Furthermore, these mice are almost completely protected from salt-induced hypertension, while their basal blood pressure is unaffected. Similar phenotypes were observed in

mice lacking LARG in smooth muscle cells. These findings suggest that the $G\alpha 12/13$ -LARG pathway is a key regulator of vascular smooth muscle tone in the context of hypertension.

Heart

In cardiomyocytes, c-Jun NH₂-terminal kinase (JNK) activation triggers hypertrophic responses [133]. It has been reported that α_1 -adrenergic receptor-induced hypertrophic responses are mediated in part by a $G\alpha 12/13$ -Rho-JNK pathway, and in part by a Gq/11-JNK pathway that is Rho independent [134].

The Immune System

Several studies have suggested that $G\alpha 12/13$ signaling pathways play a pivotal role in regulating chemotaxis. Defects in Lsc (p115RhoGEF) null mice are primarily found in the immune system, which is reasonable as p115RhoGEF/Lsc is strongly expressed in hematopoietic tissue [4, 135–138]. These mice have reduced T-cell populations in their spleen and lymph nodes, as well as reduced numbers of marginal zone B cells in the spleen. Lsc null mice also display defects in lymphocyte migration, pseudopod formation, integrin-mediated adhesion and immune responses, suggesting that it is required for normal B- and T-lymphocyte function [4, 139].

The Neuronal System – Neurologic Disorders

The Rho family, which includes Rho, Rac, and Cdc42, has an important role in regulating actin cytoskeletal dynamics and has been implicated in growth cone guidance [140]. Rac and Cdc42 activation promote the formation of cell protrusions and adhesions at the leading edge, whereas Rho activation is thought to induce retraction through actinomyosin contraction at the trailing edge. It has been demonstrated that in neuronal cell lines, $G\alpha 12$ and $G\alpha 13$ specifically activate RhoA and cause Rho-dependent neurite retraction [11, 17]. Katoh et al. also have shown that $G\alpha q$ collaborates with $G\alpha 12$ and $G\alpha 13$ to regulate neurite retraction in a Rho activation-dependent manner. Furthermore, LPA and S1P have been shown to induce neurite retraction through GPCR and RhoA activation [17, 141].

Recently, Moers et al. [18] have developed mice with conditional ablation of the genes encoding both $G\alpha 12$ and $G\alpha 13$ in the nervous system. They crossed $G\alpha 12$ -deficient mice which were homozygous for a floxed $G\alpha 13$ allele with a transgenic mouse line expressing Cre under the control of the neuron-specific enhancer of the nestin promoter and the NEX promoter, which restricts recom-

ination to neuronal and glial precursor cells starting at E10.5 and to principal neurons of the forebrain excluding glial cells and interneurons, respectively. The mice showed neuronal ectopia of cerebral and cerebellar cortices due to overmigration of cortical plate neurons and cerebellar Purkinje cells, respectively. Embryonic cortical neurons and Purkinje cells isolated from these mice were unable to retract their neurites in response to LPA and SIP. This result indicates that the G α 12/G α 13 signaling pathway is involved in the proper positioning of migrating cortical neurons.

The semaphorin4/plexin B signaling pathway also may affect axon guidance through the G12/13-RH-Rho-GEF-RhoA signaling pathway. Sema4D stimulates RhoA activation through PDZRhoGEF and LARG as described above, while plexin B may suppress Rac function by competing with other Rac downstream targets, such as p21-activated kinase (PAK) [142]. A study suggests that the G12/13-RH-RhoGEF-RhoA signaling pathway is involved in Sonic hedgehog/Smoothed-mediated cellular responses, including stimulation of target gene promoters and inhibition of neurite outgrowth in neuroblastoma cells [143]. In addition, a study has demonstrated that morphological changes can also occur in glial cells in addition to neurons in response to G α 12/G α 13 activa-

tion [144]. This study also suggests that stimulation of the thromboxane A₂ receptor causes astrocyte proliferation mainly through a G α 12/G α 13 signaling pathway.

Future Directions

In this review, we have focused on studies highlighting the physiological significance of G12/13-mediated signaling and the regulatory mechanisms controlling this pathway. As focus on G12/13-mediated signaling has increased, it is becoming clear that these pathways participate in a variety of disease processes. In order to develop drugs to specifically regulate biological functions induced by G12/13-mediated signaling, it is essential to define the interfaces for protein-protein interactions in this signaling system. In the coming years, it will be important to analyze the molecular dynamics of these protein-protein interactions in conjunction with determining the structures of these complexes using high resolution X-ray crystallography.

Acknowledgement

This work was supported by a grant for Translational Systems Biology and Medicine Initiative (TSBMI) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and NIH grant GM61454.

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