Gβγ pathways in cell polarity and migration linked to oncogenic GPCR signaling: potential relevance in tumor microenvironment

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Abstract

Cancer cells and stroma cells in tumors secrete chemotactic agonists that exacerbate invasive behavior, promote tumor-induced angiogenesis, and recruit protumoral bone marrow-derived cells. In response to shallow gradients of chemotactic stimuli recognized by G protein coupled receptors (GPCRs), G\(\beta\gamma\)-dependent signaling cascades contribute to specify the spatiotemporal assembly of cytoskeletal structures that can dynamically alter cell morphology. This sophisticated process is intrinsically linked to the activation of Rho GTPases and their cytoskeletal-remodeling effectors. Thus, Rho guanine nucleotide exchange factors, the activators of these molecular switches, and their upstream signaling partners, are considered participants of tumor progression. Specifically, phosphoinositide-3 kinases (class-I PI3Ks, \(\beta\) and \(\gamma\)) and P-Rex1, a Rac-specific guanine nucleotide exchange factor, are fundamental G\(\beta\gamma\) effectors in the pathways controlling directionally persistent motility. In addition, GPCR-dependent chemotactic responses often involve endosomal trafficking of signaling proteins, and coincidentally, endosomes serve as signaling platforms for G\(\beta\gamma\). In preclinical murine models of cancer, inhibition of G\(\beta\gamma\) attenuates tumor growth, whereas in cancer patients, aberrant overexpression of chemotactic G\(\beta\gamma\)-effectors and recently-identified mutations in G\(\beta\) correlate with poor clinical outcome. Here we discuss emerging paradigms of G\(\beta\gamma\) signaling in cancer, which are essential for chemotactic cell migration and represent novel opportunities to develop pathway-specific pharmacological treatments.
Introduction

In tumor microenvironments, cells adjust their shape and move in response to multiple factors secreted by cancer cells and stromal cells (Friedl and Alexander, 2011; Roussos et al., 2011). Inherent intracellular forces are generated by actin polymerization and ATPase activity of myosin motors, and these mechanical responses are tightly controlled by receptor-dependent signaling mechanisms that define the precise spatiotemporal reorganization of the actin cytoskeleton (Alexander and Friedl, 2012; Devreotes and Horwitz, 2015). In particular, chemotactic G protein-coupled receptors (GPCRs) elicit G protein-dependent signaling pathways linked to metastatic dissemination of cancer cells, formation of new blood vessels, and recruitment of proangiogenic cells from the bone marrow, all of which are critical to maintain cancer progression (Figure 1) (Dorsam and Gutkind, 2007; Lappano and Maggiolini, 2011).

Current models of GPCR-dependent chemotactic cell migration have evolved from studies on fast-moving cells that migrate individually, such as Dictyostelium amoebae and neutrophils. These generic pathways mediating migration suggest the involvement of Gβγ-dependent effectors, particularly PI3Ks and phosphatidylinositol (3,4,5)-trisphosphate-dependent effectors, including guanine nucleotide exchange factors for Rho GTPases. Cytoskeletal reorganization is launched by actin nucleation sites generated by effectors of GTP-bound Rho GTPases; RhoA, Rac1 and Cdc42 are the best characterized members of this family of molecular switches (Figure 2). These Rho-GTPase-dependent downstream mechanisms will not be discussed here, but readers are encouraged to consult excellent reviews covering these topics (Burridge and Wennerberg, 2004; Charest and Firtel, 2007; Rickert et al., 2000; Ridley, 2015; Ridley et al., 2003; Van Haastert and Devreotes, 2004). General mechanisms of directional cell migration provide a basis to explore GPCR-dependent cell migration linked to cancer
progression. However, in cancer settings, this process is further complicated by the influence of a plethora of stimuli in the microenvironment. Moreover, oncogenic pathways participating in chemotactic migration are affected by mutational changes, overexpression and differential repertoire of signaling systems which, among many other factors, eventually are integrated to facilitate individual and collective migration of cancer and protumoral cells (Figure 1).

We focus this review on the role of Gβγ signaling in cancer. Emphasis is given to upstream effectors leading to the activation of Rho GTPases in the pathways that control chemotactic cell migration. In this window of molecular interactions, the participation of class-I PI3Ks (β and γ) and P-Rex1, a Rac-specific guanine nucleotide exchange factor is considered critical. We highlight the properties of P-Rex1, known to be activated in concert by Gβγ and phosphatidylinositol (3,4,5)-trisphosphate (Welch, 2015; Welch et al., 2002), as a putative scaffold of chemotactic signaling. The proposed role of this multidomain effector is based on its reported interactions with sphingosine-1-phosphate receptors and the serine/threonine kinases mTOR and type I PKA (Chavez-Vargas et al., 2016; Hernandez-Negrete et al., 2007; Ledezma-Sanchez et al., 2010). Overall, the proven relevance of multiple chemokines in oncogenic settings has pointed to GPCRs and their signaling effectors as possible pharmacological targets in cancer research.

**Protumoral chemotactic agonists**

Multiple agonists play a driving role in cancer progression. Oncogenic chemokines attract and consequently diversify the repertoire of cells associated with proliferating tumoral cells. Once incorporated as cellular components of the tumor stroma, fibroblasts, endothelial and immune cells also contribute to increase the variety of secreted chemokines that not only
stimulate the surrounding tissues, but also have a systemic effect: attracting cells from the bone marrow (Figure 1) (Kojima et al., 2010; Orimo et al., 2005; Scherz-Shouval et al., 2014; Wels et al., 2008). Tumor-associated bone marrow derived cells (BMDC) further support cancer progression via their ability to secrete chemokines, cytokines, growth factors, and proteins of the extracellular matrix (Gao et al., 2008; Nolan et al., 2007; Sekiguchi et al., 2011). Among BMDC’s protumoral effects, preparing the ground where metastatic niches are eventually established is particularly significant (Gao et al., 2008; Kaplan et al., 2005; Lyden et al., 2001; Peinado et al., 2012; Psaila and Lyden, 2009; Qian et al., 2011).

Examples of protumoral GPCR agonists with chemotactic properties include CCL2/5/22/25, CXCL1/2/5/8(IL-8)/12(SDF-1), and CX3CL1 (Bronte and Bria, 2016; Roussos et al., 2011; Ugel et al., 2015). In addition, bioactive lipids such as sphingosine-1-phosphate (Liang et al., 2013) and LPA (Jonkers and Moolenaar, 2009; Liu et al., 2009) also stimulate protumoral migratory pathways. CXCL12 and CCL2 attract monocytes that promote tumor-induced angiogenesis and contribute to the maintenance of an immunosuppressive microenvironment, which allows transformed cells to evade anti-neoplastic immune responses (Kitamura et al., 2015; Qian and Pollard, 2010). Furthermore, CXCL12 recruits endothelial progenitors from the bone marrow (Orimo et al., 2005), promotes angiogenic responses mediated by Rho GTPases (Carretero-Ortega et al., 2010), and plays a positive role in tumor vascularization (Guleng et al., 2005). Multiple cancer cells overexpress CXCR4 receptors, putatively enabling them to metastasize in response to CXCL12. Examples include breast carcinoma (Muller et al., 2001; Yagi et al., 2011), gastric carcinoma (Yasumoto et al., 2006), pancreatic cancer (Marchesi et al., 2004; Xu et al., 2015), ovarian carcinoma (Barbolina et al., 2010) and oral cancers (Delilbasi et al., 2004; Uchida et al., 2007). Metastatic signaling
mechanisms elicited by CXCR4 involve heterotrimeric Gi and G13 proteins. In these cases, chemotactic GPCRs activate Rho GTPases that control cell migration into the stroma of primary tumors and metastatic tissues. Therefore signaling pathways leading to the activation of Rho GTPases are considered central players in cancer settings (Dillenburg-Pilla et al., 2015; Tan et al., 2006; Yagi et al., 2011) (Figure 1). As an indicator of the emerging clinical importance of these pathways, a search for "chemokine and cancer" listed 108 studies in ClinicalTrials.gov.

The role of GPCRs, heterotrimeric G proteins, and their target RhoGEFs in tumor growth and metastasis. Cancer cells often hijack G protein signaling pathways, and this exacerbates the effect of oncogenes. Expressed in cancer and stroma cells in tumors, GPCRs act in concert with growth factor receptors, extracellular matrix proteins, and diverse agonists at various stages of oncogenic progression (Dorsam and Gutkind, 2007; Roussos et al., 2011) (Figure 1). Interestingly, these seven-transmembrane receptors further enhance the oncogenic potential of transformed cells by potentiating their paracrine effects. For instance, in highly metastatic breast cancer cells, Calcium Sensing Receptor (CaSR) promotes secretion of CCL22/MDC among other chemokines and growth factors that, in vitro, activate a chemotactic and angiogenic response. The mechanism involves transactivation of Epidermal Growth Factor Receptor and a positive loop based on the constitutive secretion of IL-6 (Hernandez-Bedolla et al., 2015). This GPCR also attenuates signaling effects of TGF-β, an important tumor suppressor. In particular, CaSR interferes with Smad2 phosphorylation and promotes its degradation. This inhibitory effect involves trafficking of CaSR in Rab11-positive endosomes (Organista-Juarez et al., 2013).

The oncogenic power of GTPase-deficient mutant Ga proteins, originally reported using transfected cellular models, has been confirmed in various types of human cancer. Examples include mutations in GNAQ (encoding Gaq), detected in almost 50% of ocular melanomas (Van...
Raamsdonk et al., 2009; Van Raamsdonk et al., 2010), and GNAS (encoding Gαs protein), initially characterized in pituitary tumors, and also identified in colon, hepatic, and pancreatic cancers (among others) using deep sequencing analysis, (Landis et al., 1989; O’Hayre et al., 2013).

To understand the mechanisms by which chemotactic GPCRs elicit local assembly of actin nucleation sites, it is critical to focus on Rho guanine nucleotide exchange factors (RhoGEFs) as effectors of heterotrimeric G proteins. Essentially, a polarized response marks the origin of cell protrusions to follow a chemotactic gradient. This is coordinated by Rho GTPases activated downstream of chemotactic GPCRs (Goicoechea et al., 2014; Kedziora et al., 2016; Vaque et al., 2013; Yagi et al., 2011). In this context, chemotactic stimuli eventually produce cytoskeletal structures as lamelipodia, filopodia and stress fibers, which are needed for cell protrusion and contraction. These examples of crosslinked fibers of polymerized actin and their interacting regulators are controlled by distinct Rho GTPases, such as Rac1, Cdc42 and RhoA (Figure 2) (Kozma et al., 1995; Nobes and Hall, 1995; Ridley and Hall, 1992; Ridley et al., 1992). Therefore, an instrumental role is anticipated for GPCR-regulated RhoGEFs, such as RH-RhoGEFs, P-REX1, and their upstream regulators as central controllers of the activation of Rho GTPases via the integration of second messengers, protein interactions and posttranslational modifications (Bracho-Valdes et al., 2011; Cook et al., 2014; Dillon et al., 2015; Hernandez-Negrete et al., 2007; Kim et al., 2011).

Receptors coupled to Gi, G_{12/13} and G_{q/11} activate Rho GTPases, either via Gβγ, in the case of Gi, or GTP-bound Gα subunits in the other cases (Figure 3). A group of RhoGEFs with a domain homologous to RGS, characteristic of proteins known as regulators of G protein signaling, are directly activated by Gα_{12/13} (Aittaleb et al., 2010; Fukuhara et al., 2000; Fukuhara
et al., 2001; Fukuhara et al., 1999; Hart et al., 1998; Kozasa et al., 2011; Vazquez-Prado and Gutkind, 2011), whereas p63RhoGEF and Trio are activated by Gαq/11 (Lutz et al., 2005; Rojas et al., 2007). These G protein-regulated RhoGEFs are members of a superfamily of DH-RhoGEFs characterized by a catalytic domain with homology to Dbl, which is common among the 70 members of the biggest family of RhoGEFs (Cook et al., 2014; Rossman et al., 2005; Schmidt and Hall, 2002).

The group of RH-RhoGEFs (also been named RGS-RhoGEFs or RGL-RhoGEFs) includes p115RhoGEF/ARHGEF1 (Hart et al., 1998), PDZ-RhoGEF/ARHGEF11 (Fukuhara et al., 1999), and LARG/ARHGEF12 (Fukuhara et al., 2000) (Figure 3). The interaction between Gα13 and the RGS-like domain, involving the switch-regions of Gα13 and most of the GTPase domain (Vazquez-Prado et al., 2004), is reminiscent of the interaction between Gα subunits and RGS proteins. Thus, no second messengers are required as intermediates in the activation of RhoA by these G proteins, but membrane localization of Gα13 is essential (Vazquez-Prado et al., 2004). The RGS-like domains of PDZ-RhoGEF and p115RhoGEF interacting with Gα13 or a chimeric Gα13/i GTPase, respectively, have been analyzed at the atomic level (Chen et al., 2008; Chen et al., 2005). These structures, together with multiple biochemical assays, support a model implying that Gα13 putatively removes inhibitory intramolecular interactions and facilitates the establishment of stimulatory ones critical for exposing the catalytic DH domain. As a consequence, RH-RhoGEFs interact with RhoA, promoting exchange of GDP for GTP in this small GTPase (Aittaleb et al., 2010). In a murine model of breast cancer, an aberrant coupling of G13 to CXCR4 receptors has been linked to metastatic progression of tumors via RhoA-dependent cytoskeletal pathways regulating cell migration (Yagi et al., 2011).
An alternative mechanism of activation of Rho GTPases, relevant to chemotactic and adhesion GPCRs signaling (Hamoud et al., 2014; Park et al., 2007; Stevenson et al., 2014; Watanabe et al., 2014), involves a second phylogenetically-conserved family of GEFs. This group of nonconventional GEFs is specific for Rac and Cdc42 GTPases. It is constituted by 11 DOCK proteins, characterized by their catalytic DHR2 domain, and 3 ELMO scaffolds, known as functional interactors of DOCK 1 to 5 proteins (Gadea and Blangy, 2014; Laurin and Cote, 2014). Diverse chemoattractants stimulate ELMO-2 translocation to the plasma membrane. This relocalization is mediated by Gβγ, which directly interacts with the amino-terminal region of ELMO (Wang et al., 2016). In vitro, the CXCL12-dependent chemotactic response of highly metastatic breast cancer cells requires the expression of ELMO-1/2 proteins, which seems to exert a redundant role. Interestingly, in invasive ductal breast carcinoma, ELMO-1 is overexpressed, whereas in a murine model of metastatic breast cancer, ELMO-1 knockdown prevented invasion to the lungs (Li et al., 2013). Moreover, in breast cancer cells, ELMO phosphorylation by Axl resulted in increased invasion (Abu-Thuraia et al., 2015). Consistent with the intervention of Axl in chemotactic GPCR signaling, in colon cancer cells, CXCL12 stimulated Axl expression. Furthermore, the effect of this chemokine on cell invasion is attenuated by Axl knockdown, indicating the existence of an aberrant positive loop that exacerbates the CXCR4-dependent invasive behavior of colon cancer cells (Heckmann et al., 2014).

The mechanisms by which Gβγ activates Rho GTPases appears to be diverse, and involve Gβγ-regulated RhoGEFs and other Gβγ effectors such as PI3Ks which, through phosphorylated lipid second messengers generated in the surroundings of Gβγ, activate PIP3-dependent RhoGEFs (Dbouk et al., 2012b; Donald et al., 2004; Dupre et al., 2009; Guzman-Hernandez et
al., 2009; Rosenfeldt et al., 2004; Schwindinger and Robishaw, 2001; Vadas et al., 2013; Welch et al., 2002). As shown in Figure 3, different RhoGEFs interact with Gβγ and are likely activated by the versatile heterodimer, but follow-up investigations are necessary. Additional assays such as pulldowns of active-RhoGEFs using recombinant nucleotide-free mutant Rho GTPases (Garcia-Mata et al., 2006) might help to discern whether they are sine qua non Gβγ-effectors.

Intervention of various RhoGEFs in chemotactic signaling pathways is likely redundant. This is in part explained by their sensitivity to phosphoinositide-derived second messengers and, for certain groups, to the fact that those with equivalent conserved structures might be co-expressed. For instance, a recent systematic analysis assessing endothelial expression of 81 RhoGEFs (70 homologous to Dbl and 11 of the DOCK family) revealed that more than half, including RH-RhoGEFs and P-Rex1, are abundantly expressed in VEGF-stimulated cells as well as in tumor endothelial cells, supporting their potential participation in tumor angiogenesis (Hernandez-Garcia et al., 2015).

**Availability of Gβγ for chemotactic signaling**

A recent revolution in the field of GPCR structural analysis has consolidated a rather simplistic model to explain how multiple GPCRs transduce their signals through a small group of heterotrimeric G proteins (Tesmer, 2016); agonist-occupied seven transmembrane receptors are visualized as signaling funnels by which hundreds of ligands use common mechanisms of transduction. They expose an intracellular pocket that serves as a docking site occupied by the carboxyl-terminal tail of appropriate Goα subunits in complex with Gβγ. Following this interaction, Goα releases its bound GDP, incorporates GTP, and dissociates from Gβγ and the receptor, initiating signaling events at the plasma membrane (Tesmer, 2016). It is well accepted
that this model explains multiple complex cellular processes. The emerging concept of biased agonism implicates a previously unnoticed sophisticated level of complexity. Current models of active-receptor conformation go beyond the originally postulated concept that functional receptors only existed as one of two possible conformations: active or inactive. Multiple lines of evidence suggest that various conformations of the receptors are able to promote heterotrimeric G protein-dependent and independent pathways (Liu et al., 2015b; Luttrell et al., 2015; Pupo et al., 2016).

In general terms, the canonical mechanism of heterotrimeric G protein signaling involves redistribution of GTP-bound G\(\alpha\) and G\(\beta\)\(\gamma\) at the internal surface of the plasma membrane (Hepler, 2014; Lambert, 2008). There, they interact with their effectors, which are either integral plasma membrane proteins or cytosolic signaling partners recruited via direct interactions. This stage of the process is traditionally limited to the plasma membrane, where the cycle is reinitiated when the actions of both G\(\alpha\) and G\(\beta\)\(\gamma\) are turned off.

The role of G\(\beta\)\(\gamma\) in the general model of GPCR signal transduction has been established after some initial controversy. Thirty years of signaling history have taken G\(\beta\)\(\gamma\) from an innocuous G\(\alpha\)-binding partner to a \textit{bona fide} signal transducer that targets multiple effectors. The report by Eva Neer and David Clapham recognizing G\(\beta\)\(\gamma\) as a powerful activator of ion channels (Logothetis et al., 1987) was initially received with some skepticism by the G-protein signaling community since the catalytic G\(\alpha\) subunits were in the spotlight of proposed transducing mechanisms. However, a key role for heterodimeric G\(\beta\)\(\gamma\) is now well accepted (see below), in addition to the influence of heterodimeric G\(\beta\)\(\gamma\) on G\(\alpha\) signaling apparent at various levels. G\(\beta\)\(\gamma\) serves as a GDI (guanine-nucleotide dissociation inhibitor) that keeps the switch
represented by $G\alpha$ in the off-conformation. $G\beta\gamma$ is needed to reinitiate a cycle of heterotrimeric G proteins by reconstituting new heterotrimers once GTP has been hydrolyzed by $G\alpha$, and it is essential for heterotrimeric G protein coupling to agonist-occupied receptors (Tang et al., 2006).

As transducers, $G\beta\gamma$ heterodimers are among the most ubiquitous signaling proteins; five different $G\beta$ and twelve $G\gamma$ are encoded in the human genome (Hurowitz et al., 2000). With the exception of $G\beta$5, which preferentially interacts with RGS 7 and 9 proteins, all $G\beta$ subunits interact with different $G\gamma$ subunits, although association preferences do exist (Mervine et al., 2006). Thus, multiple possible combinations, mainly limited by their expression in different tissues, exist. Therefore, $G\beta\gamma$ heterodimers exhibit an enormous signaling repertoire, perhaps comparable with that of dynamically modulated signaling lipids such as PIP3, a regular coactivator of $G\beta\gamma$ effectors. The existence of many different $G\beta\gamma$ heterodimers and their redundant biochemical effects are evidence of their important role in eukaryotic evolution (Krishnan et al., 2015).

Although $G\beta\gamma$ signaling is expected to be activated downstream of every heterotrimeric G protein, research indicates that some heterotrimers are better providers of signaling-ready $G\beta\gamma$ heterodimers than others. It is well accepted that GPCR-dependent chemotactic events are mediated by $G\beta\gamma$ released from Gi, but not from Gs or Gq (Neptune et al., 1999). Such singularity in $G\beta\gamma$ signaling might be explained by heterogeneous abundance of different heterotrimers, unique kinetics of GTP hydrolysis by $G\alpha$ subunits (influenced by different regulatory proteins) followed by recapture of free $G\beta\gamma$, or even differential degrees of dissociation between GTP-bound $G\alpha$ and $G\beta\gamma$ (Bondar and Lazar, 2014; Hepler, 2014).
Spatiotemporal asymmetry defined by Gβγ availability sets the basis of chemotactic signaling (Jin et al., 2000). Recent optogenetic and chemical biology approaches confirmed that local availability of Gβγ is sufficient to control polarized cell migration (O’Neill and Gautam, 2014; Surve et al., 2014). Gβγ recruits cytosolic signaling proteins to the plasma membrane as a consequence of its dissociation from Gαi-GTP, which is elicited by chemotactic GPCRs. These include PI3Ks and RhoGEFs (Figures 2 and 3), the most relevant Gβγ effectors involved in cell migration. Initial translocation of signaling proteins to the plasma membrane is frequently correlated to their activation. However, it should not be assumed that every signaling protein recruited by Gβγ will be fully activated by this interaction. In some cases, Gβγ might only contribute to the translocation of signaling proteins or it might act as a coactivator in concert with lipid second messengers or additional signaling proteins. For instance, PI3Kβ requires simultaneous input by Gβγ and growth factor receptors (Houslay et al., 2016). This early array of signaling interactions is subjected to regulatory circuits that amplify the initial inputs. In particular, PI3Kβ is sensitive to GTP-bound Rac and Cdc42 GTPases. Thus, PIP3 stimulates RhoGEFs that activate Rac, Cdc42, and, consequently, their downstream effectors. Among them, PI3Kβ putatively closes a circuit that produces more PIP3 (Fritsch et al., 2013). Overall, integration of these signaling inputs is finally manifested by polarized cytoskeletal arrangements that promote cell propulsion, which sustains directional migration (Figure 2).

Structural analysis of heterodimeric Gβγ bound to different interactors assists in the understanding of how this non catalytic-signaling complex activates its effectors. For instance, in the case of GRK-2 (G protein-coupled receptor kinase-2), one of the few Gβγ effectors known at the structural level in complex with the heterodimer (Tesmer et al., 2005), GRK-2 is first
recruited to the membrane by $\text{G}_{\beta\gamma}$. Then, the spatial orientation of GRK-2 is fine-tuned by lipid second messengers that fix the PH-domain in a position that orients the catalytic domain in the proper conformation for access to its substrates and regulators (Yang et al., 2016). This general mechanism may also be effective for other cytosolic $\text{G}_{\beta\gamma}$-effectors that integrate PIP3 and $\text{G}_{\beta\gamma}$ signaling, such as P-Rex1. In contrast, the mechanism by which $\text{G}_{\beta\gamma}$ activates effectors already positioned in the plasma membrane, such as the GIRK channels, serves as a model for understanding effector activation independent of the cytosol to membrane translocation step, which is common for most $\text{G}_{\beta\gamma}$-effectors. According to the structural analysis of tetrameric GIRK-2 channels, together with four $\text{G}_{\beta\gamma}$ heterodimers, the orientation of $\text{G}_{\beta\gamma}$ is similar to that observed in the ternary complex of agonist-occupied $\beta_2$-adrenergic receptors coupled to nucleotide-free Gs (Rasmussen et al., 2011; Whorton and MacKinnon, 2013). In the channel, the intracellular domain of every one of its four subunits interacts with PIP2 and $\text{G}_{\beta\gamma}$, and this interaction fixes a pre-open channel conformation (Whorton and MacKinnon, 2013). It is interesting that the intracellular domains of the channel integrate phosphoinositides with $\text{G}_{\beta\gamma}$ signaling. Thus, in cellular settings, a common theme in the regulation of $\text{G}_{\beta\gamma}$-effectors appears to be the concerted participation of lipid second messengers.

The number of $\text{G}_{\beta\gamma}$-regulated signaling molecules is still expanding and seems to be related to multiple possible protein-protein interacting motifs at the surface of $\text{G}_{\beta\gamma}$. A diversity of complementary experimental approaches has revealed this broad array of $\text{G}_{\beta\gamma}$-docking sites. Peptides, identified by phage-display as interactors of different regions of $\text{G}_{\beta\gamma}$, interfere with the activation of some $\text{G}_{\beta\gamma}$-effectors, but not others (Scott et al., 2001). Correspondingly, point-mutations at different surface residues of $\text{G}_{\beta\gamma}$ affect a divergent pattern of $\text{G}_{\beta\gamma}$-regulated
effectors (Ford et al., 1998). Comparison of different structures in which Gβγ is part of a complex with GIRK-2, GRK-2, Go11, or Phosducin showed partial overlapping of the Gβγ-interacting surface, but specific differential areas of interaction were also recognized (Whorton and MacKinnon, 2013). Interestingly, a peptide corresponding to phosducin-like protein aminoterminal region competes with PI3Kγ for the interaction with Gβγ and attenuates the recruitment of this lipid kinase to the plasma membrane (Guzman-Hernandez et al., 2009). Moreover, this peptide inhibits AKT activation by sphingosine-1-phosphate in endothelial cells without affecting the simultaneous activation of ERK (Guzman-Hernandez et al., 2009). Furthermore, this peptide differentially inhibits chemotactic and angiogenic responses to sphingosine-1-phosphate without interfering with the effect of hepatocyte growth factor (Guzman-Hernandez et al., 2009). Altogether, these studies support the working hypothesis that differential inhibitors of Gβγ signaling, interfering with some effectors but not others, might reach clinical development with the final aim of interfering with aberrant Gβγ-dependent signaling pathways.

**Gβγ-dependent activation of PI3K.** The PI3K/AKT/mTOR signaling pathway plays a central role in cell migration, among other fundamental physiological processes (Bracho-Valdes et al., 2011). In order to participate in a chemotactic response, heterodimeric Gβγ exhibits its signaling interface, covered in the basal non-stimulated condition by Gα, to directly interact with the catalytic subunit of PI3Kβ (via a low affinity site, stabilized with membrane lipids) or with adaptors of PI3Kγ (Dbouk et al., 2012a; Thorpe et al., 2015; Vadas et al., 2013).

Although molecular structures of Gβγ/PI3Ks signaling complexes are not yet available, the interaction between Gβγ and PI3Kβ has been mapped to a non-conserved region, including 24 amino acids between the C2 and helical domains of p110β catalytic subunit (Dbouk et al.,
Knock-in mice in which non-\(G\beta\gamma\)-sensitive PI3K\(\beta\) was expressed revealed that this lipid kinase integrates signaling inputs by \(G\beta\gamma\) and growth factor receptors (Houslay et al., 2016). The interaction of \(G\beta\gamma\) with p110\(\beta\) is essential for Gi-dependent AKT activation, chemotaxis and \textit{in vitro} transforming activity (Dbouk et al., 2012a). Interestingly, WDR26, an oncogenic scaffold overexpressed in breast cancer, facilitates PI3K\(\beta\) signaling by \(G\beta\gamma\) (Ye et al., 2016).

The interaction between \(G\beta\gamma\) and PI3K\(\gamma\) is mediated by the regulatory p101 and p87/p84 subunits, and the interaction supports the association of the lipid kinase to the membrane where \(G\beta\gamma\) then exerts a direct stimulatory effect on the catalytic subunit (Brazzatti et al., 2012; Shymanets et al., 2013). \(G\beta\gamma\) stabilizes the conformation of the catalytic p110\(\gamma\) subunit, specifically the linker region between RBD and C2 exposed by interaction with lipids. The existence of these molecular contacts has been revealed by hydrogen–deuterium exchange mass spectrometry approaches (Vadas et al., 2013).

In \(G\beta\gamma\), the interface of interaction with PI3Ks (\(\beta\) and \(\gamma\)) seems to be partially different for each isoform (Dbouk et al., 2012a). PI3K\(\beta\) interacts with \(G\beta\gamma\) in two regions. The first, located at the linker between the amino-terminal \(\alpha\)-helix of \(G\beta\) and its first blade, appears to be exclusive for this lipid kinase. The second region corresponds to an already mapped hotspot in \(G\beta\) important for the interaction with various effectors, including PI3K\(\gamma\), at the second blade (Dbouk et al., 2012a; Shymanets et al., 2012). As formerly demonstrated, inhibition of \(G\beta\gamma/\text{PI3K}\gamma\) interactions with a construct corresponding to the amino-terminal region of phosducin-like protein prevents Gi-dependent chemotactic and angiogenic responses elicited by sphingosine-1-receptors (Guzman-Hernandez et al., 2009). These data raise the possibility that the interface between \(G\beta\gamma\) and either PI3K\(\beta\) or PI3K\(\gamma\) is an attractive target for therapeutics.
P-Rex1 as a potential chemotactic scaffold

Chemotactic GPCRs stimulate polarized actin polymerization, and effectors of Rho GTPases are essential activators of actin nucleation, implying that GPCRs must activate Rho GTPase regulators at a specific time and location to remodel the cytoskeleton (Hall, 2012). The ability of Gβγ to activate PI3Ks, which generate PIP3, together with the widely accepted concept that PH domains interact with this lipid second messenger (Moravcevic et al., 2012), set the basis for a general model of RhoGEF activation. Accordingly, polarized production of PIP3 potentially stimulates multiple RhoGEFs, most of which contain PH domains (Hernandez-Garcia et al., 2015; Rossman et al., 2005). However, other elements that impact the timing and magnitude of RhoGEF activation are likely integrated at the plasma membrane if a mechanism that is able to support persistent directional migration is involved. These include integral plasma membrane proteins, such as activated GPCRs (Ledezma-Sanchez et al., 2010) and free Gβγ that establish direct interactions with RhoGEFs and may have a role as docking sites and allosteric activators, likely maintaining RhoGEFs recruited at the plasma membrane. Thus, interacting molecules might control the temporal nature and levels of activity of different RhoGEFs.

During cell migration, chemotactic Gi-coupled receptors promote lamellipodial protrusions. These structures, dynamically formed at the leading edge of advancing polarized cells, are formed by effectors of Rac GTPases. Of particular interest, the P-Rex1 family of guanine nucleotide exchange factors, specific for Rac, is synergistically activated by Gβγ and PIP3 (Donald et al., 2004; Rosenfeldt et al., 2004; Welch, 2015; Welch et al., 2002). Structurally, this group is characterized by a multidomain architecture. This group of GEFs, including P-Rex1 and P-Rex2 (with two isoforms), represents the most common set of effectors by which Gβγ...
activates Rho GTPases, particularly Rac. However, other RhoGEFs have been reported as interactors and effectors of Gβγ (Figure 3).

The molecular characteristics expected for a chemotactic scaffold are present in the P-Rex family of GEFs. They contain six well defined domains, which in P-Rex1 are known to participate in different interactions with plasma membrane proteins, phosphoinositides, and serine/threonine kinases (Figure 4). In particular, P-Rex1 interacts with the carboxyl-terminal region of GPCRs such as S1P1 receptors. In endothelial cells expressing P-Rex1 PDZ domains, these receptors exhibit a sustained chemotactic response to sphingosine-1-phosphate. Due to their interaction with these domains, S1P1 receptors maintain their functionality at the plasma membrane, in contrast to those that are internalized in the absence of P-Rex1 PDZ domains (Ledeza-Sanchez et al., 2010). Furthermore, P-Rex1 is recognized and activated by mTORC2 (Hernandez-Negrete et al., 2007), a multimeric serine-threonine kinase that can directly be activated by PIP3 (Liu et al., 2015a). Moreover, mTORC2 is also recognized by Gβγ, which positively regulates its participation in GPCR-dependent activation of AKT (Robles-Molina et al., 2014). Although it is not yet known whether all of the P-Rex1 interactors mentioned above are simultaneous partners of this GEF, these proteins might be part of dynamic signaling complexes, where P-Rex1 is a central platform that putatively maintains directional cell migration.

P-Rex1 is inhibited by PKA (Chavez-Vargas et al., 2016; Mayeenuddin and Garrison, 2006; Urano et al., 2008), and accumulating evidence indicates that regulation of P-Rex1 by PKA occurs via a complex mechanism involving phosphorylation-dependent inhibitory interactions (Chavez-Vargas et al., 2016). Interestingly, the type I PKA regulatory subunit directly interacts with P-Rex1 PDZ domains. In addition, PKA catalytic subunit phosphorylates
P-Rex1 at Ser-436 within the first DEP domain (Chavez-Vargas et al., 2016), whereas the carboxyl-terminal region of P-Rex1 is an indirect target of this kinase (Figure 4). As a consequence, P-Rex1 is inhibited by intramolecular interactions established between the phosphorylated domains and the catalytic DH-PH cassette (Chavez-Vargas et al., 2016).

In support of a spatiotemporal mechanism of reciprocal regulation between PKA and P-Rex1, experiments with fluorescently–tagged type I PKA regulatory subunit and P-Rex1 demonstrated that they translocate to the plasma membrane in response to CXCL12 (Chavez-Vargas et al., 2016). These results, together with the inhibitory effect of PKA-dependent P-Rex1 phosphorylation, indicate that type I PKA is a fine-tune control of P-Rex1 ready to modulate this GEF. In addition, the effect of cAMP on PKA dynamics suggests that the regulatory subunits of this kinase might also regulate P-Rex1 activity once the catalytic subunits are dissociated. Current efforts are addressing several intriguing hypothesis based on potential regulatory effects that go beyond P-Rex1 phosphorylation by PKA.

**Gβγ/PI3K/mTORC2/P-Rex1 axis in oncogenesis**

The fundamental role of Gβγ in GPCR-dependent chemotactic cell migration suggests that it plays a role in metastatic cancer; this is supported by preclinical evidence and cancer-related mutations in Gβ (Dbouk et al., 2012a; Tang et al., 2011; Yoda et al., 2015). The mechanism by which oncogenic activities of Gβγ are explained involves persistent activation of the PI3K/mTOR signaling pathway (Dbouk et al., 2012a; Khalil et al., 2016). Interestingly, Gβγ directly interacts with and activates PI3Ks (β and γ). Moreover, this signaling heterodimer also directly interacts with mTOR, facilitating GPCR signaling to AKT via mTORC2 (Robles-Molina et al., 2014), and it is well recognized that Gi-coupled GPCRs activate the PI3K/AKT/mTOR
signaling pathways. Both type-I β and γ PI3Ks are activated by Gβγ, leading to mTORC1 activation downstream of AKT. Moreover, a prominent role for mTORC2 in GPCR-dependent chemotaxis (Liu et al., 2010) and angiogenesis has been suggested (Dada et al., 2008; Ziegler et al., 2016).

The participation of Gβγ in tumor growth was initially revealed through the use of a construct that encoded the carboxyl-terminal region of GRK2, a widely used inhibitor of Gβγ signaling. Expression of this Gβγ interactor prevented prostate tumor growth in a murine model (Bookout et al., 2003). Similarly, inhibiting Gβγ signaling in breast cancer cells prevented tumor cell migration and metastatic dissemination in nude mice (Tang et al., 2011). Very recently, oncogenic Gβγ mutants were identified in a patient with a subtype of acute leukemia infiltrated into the bone marrow (Yoda et al., 2015).

PI3Kγ plays a relevant role in cancer progression by controlling chemotactic responses of immune cells. In murine models, experimental inhibition of this lipid kinase prevents tumor growth and metastatic dissemination (Martin et al., 2011; Schmid et al., 2011). Mechanistically, its inhibition reprograms tumor-associated macrophages to activate cytotoxic T cells, which inhibit tumor progression in murine models of pancreatic ductal adenocarcinoma (Kaneda et al., 2016). Moreover, PI3Kγ plays a demonstrated role in Kaposi Sarcoma herpes virus vGPCR-dependent oncogenesis, in which mTOR plays a central role in the paracrine non-cell autonomous, oncogenic pathway (Martin et al., 2011). In preclinical models, it has been demonstrated that cell transformation is induced by over-expression of PI3Kγ, whereas its inhibition attenuates oncogenic processes (Brazzatti et al., 2012).
GPCRs activate mTORC2; the macromolecular complex is an important upstream regulator of Rho GTPases and has been involved in GPCR-dependent chemotaxis (Dada et al., 2008; He et al., 2013; Hernandez-Negrete et al., 2007; Jacinto et al., 2004; Kuehn et al., 2011; Liu et al., 2010; Liu et al., 2014). Interestingly, mTORC2, the rapamycin-insensitive complex of mTOR, is known as the kinase that phosphorylates AKT at Ser-473, a critical residue whose phosphorylation is required to achieve the maximum activity of AKT (Sarbassov et al., 2005). Driving mutations and overexpression of type-I PI3Ks, as well as AKT, are frequently present in metastatic cancers with resistance to targeted therapies when the resistance occurs by a selection of clones in which mutation and aberrant expression of these proteins are linked to cancer dissemination (Juric et al., 2015).

Current findings implicate mTORC2 as a direct activator of Rac guanine nucleotide exchange factors, particularly P-Rex1, in response to GPCR activation acting through Gβγ. Gβγ interacts with mTORC2, putatively serving as a docking site to recruit it to the plasma membrane (Robles-Molina et al., 2014). As an interactor of mTOR, P-Rex1 was the first multi-domain protein with DEP domains known to be modulated by this multifunctional serine/threonine kinase (Hernandez-Negrete et al., 2007). Interestingly, an additional mTOR interactor containing DEP domains, named DEPTOR, behaves as an mTOR inhibitor whose expression is frequently reduced in multiple cancers (Peterson et al., 2009). Altogether, these findings suggest that P-Rex1 is a RacGEF that integrates PI3K and mTORC2 signaling outputs. Hypothetically, this might constitute a critical axis used by cells when recruited into the tumor stroma where they exert a paracrine effect stimulating tumor progression.

In this regard, P-Rex1 participates in the signaling cascade of CXCL12, which leads to Rac activation, cell migration, and an in vitro angiogenic effect (Carretero-Ortega et al., 2010).
Moreover, the recently-demonstrated participation of mTORC2 in the angiogenic effect of CXCL12 (Ziegler et al., 2016), together with the known interaction between P-Rex1 and this multimeric serine/threonine kinase (Hernandez-Negrete et al., 2007), further support the relevance of the Gβγ/PI3K/mTORC2/P-Rex1 pathway in angiogenesis. Current efforts are oriented to directly assess the activation of these signaling pathways in BMDC responding to chemotactic agonists present in the tumor microenvironment and how these pathways contribute to the protumoral properties of BMDC.

In this context, it is interesting that the CXCL12/CXCR4 pathway activates PI3Kγ and promotes chemotaxis of cells from the bone marrow (Chavakis et al., 2008). P-Rex1 is activated by Gβγ and PIP3 downstream of G protein coupled receptors (Welch, 2015), in addition to activation by growth factor receptors (Montero et al., 2011; Sosa et al., 2010) and a direct interaction with mTORC2 (Hernandez-Negrete et al., 2007). These interactions hypothetically integrate a critical signaling pathway involved in cell migration to the recruitment of different cell populations to the tumor stroma.

The family of P-Rex1 and P-Rex2 RacGEFs has been implicated in cancer progression (Berger et al., 2012; Fine et al., 2009; Goel et al., 2016; Lindsay et al., 2011; Lissanu Deribe et al., 2016; Montero et al., 2011; Pandiella and Montero, 2013; Qin et al., 2009; Sosa et al., 2010; Waddell et al., 2015); P-Rex1 is overexpressed in several types of cancer and thus has been considered a prognostic marker of poor patient survival in human breast carcinoma (Sosa et al., 2010). In murine models, this RacGEF contributes to metastasis in prostate cancer (Qin et al., 2009) and melanoma (Lindsay et al., 2011), and has been linked to anti-angiogenic therapy resistance in prostate cancer (Goel et al., 2016).
In the case of P-Rex2, this GEF mutation has been found in melanoma (Berger et al., 2012), and ectopic expression of the mutants accelerated oncogenesis of immortalized melanocytes in mice (Berger et al., 2012; Lissanu Deribe et al., 2016). An alternative mechanism by which this mutant RacGEF contributes to cancer is based on its resistance to regulation by PTEN, a tumor suppressor sensitive to inhibition by wild type P-Rex2 (Mense et al., 2015). An active area of research is how these observations can be translated into pharmacological strategies to inhibit Gβγ-dependent pathways in pathological settings such as cancer progression.

**Endosomes as platforms of Gβγ signaling**

Agonist-bound receptors activate GDP-bound heterotrimeric G proteins. As a consequence, signaling-ready Gβγ is released. The effects of time and space are now appreciated as critical factors defining new levels of complexity to GPCR signaling (Lohse and Hofmann, 2015). At the plasma membrane, different agonist-stabilized conformations can bias the activated pathways. Initial responses elicited at the plasma membrane are followed by a second wave of signaling by GPCR interactors such as arrestins. Independent trafficking routes for receptors and activated G proteins eventually lead to what has been considered a third wave of signaling at endosomal compartments, where novel effector systems may be locally activated (Murphy et al., 2009). The cell specific context of these waves of signaling elicits specific integrated actions. Various examples highlight the importance of endosomal trafficking, broadening possibilities of heterotrimeric G protein signaling in time and space. Gβγ, in particular, follows internalization routes that define the activation of signaling complexes at endosomal compartments (Garcia-Regalado et al., 2008), or alternatively, Gβγ can follow translocation routes independent of endosomal trafficking (Saini et al., 2007). LPA receptors promote Gβγ trafficking to endosomes.
where it assembles a PI3K/AKT signaling complex (Garcia-Regalado et al., 2008). This is a Gi-dependent process that involves Gβγ interaction with Rab11. Whether Gβγ signaling from endosomes contributes to chemotactic cell migration is currently unknown.

**Gβγ activates ERK-signaling cascades**

Multiple studies have revealed the diverse signaling pathways linking GPCR signaling to ERK activation. Rather unexpectedly, Gβγ activates ERK through Ras (Crespo et al., 1994), providing the first demonstration that GPCRs promote Gβγ-dependent proliferation involving protein interactions instead of diffusible second messengers.

In addition, activation of PI3Kγ via Gβγ has been linked to the proliferative effect of GPCRs (Lopez-Ilasaca et al., 1997). In oncogenic settings, the GPCR signaling specificity of this lipid kinase has provided a rational basis for the treatment of Kaposi Sarcoma in preclinical models (Martin et al., 2011). In this case, a virally encoded GPCR that selectively activates PI3Kγ stimulates AKT and mTOR, generating a paracrine circuit directly linked to the carcinogenic effect. Intriguingly, in breast cancer cells P-Rex1 integrates PI3K signaling to ERK via a Rac/PAK/Raf/MEK pathway. This involves activating mutations of the catalytic subunit of PI3Kα and overexpression of HER2 growth factor receptors (Ebi et al., 2013).

**Inhibition of Gβγ signaling offers pharmacological potential in cancer treatment**

Aberrant chemotactic cell migration in cancer not only involves transformed cells but also multiple other types that constitute the stroma and those that enhance metastatic behavior. Since cancer cell dissemination is the most life threatening stage of cancer progression, pharmacological prevention of this aberrant process represents one of the most challenging yet
beneficial interventions to mechanistically fight cancer progression. GPCRs are essential in the normal and aberrant dissemination of multiple cells throughout the organism. Since Gβγ is a common actor of the chemotactic signaling pathways frequently altered in cancer, its ability to recognize multiple effectors via distinct interacting motifs may be pharmacologically targeted to prevent aberrant cell migration. Thus, characterization of chemotactic Gβγ-dependent signaling pathways offers unique opportunities to investigate promising pharmacological strategies with the ultimate goal to develop inhibitors that differentially block aberrant Gβγ-effectors. Keeping active those relevant to the physiological effects of this fundamental multitasking heterodimer, the aim is to inhibit those linked to cancer progression, related to inflammation, and involved in the recruitment of proangiogenic mediators that contribute to tumor angiogenesis and metastatic dissemination of tumor cells (Smrcka, 2013). Experimental evidence already shows that specific interactions between Gβγ and its effectors, along with the corresponding functional effects, can be blocked with small molecules selected by virtual docking (Bonacci et al., 2006). Moreover, inhibiting some of these specific interactions prevents invasive behavior of cancer cells (Tang et al., 2011). Therefore, further understanding of Gβγ-dependent signaling networks offers a growing potential for pharmacological intervention that aims to inhibit aberrant cell migration in cancer settings.
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Wrote or contributed to the writing of the manuscript: JVP, IBV, RDCV, and GRC.
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Footnotes

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Legends for Figures

Figure 1. GPCR signaling to Rho GTPases plays a critical role in tumor microenvironment.

Cancer progression relies on the communication among different cell populations recruited into the tumor stroma. These include fibroblasts and endothelial cells from surrounding tissues and bone marrow derived cells (BMDC), such as macrophages, leukocytes, monocytes, endothelial progenitor cells, and other myeloid precursors. Chemokines secreted by stromal and cancer cells are key mediators of inflammation, angiogenesis, and immunosuppression, contributing to tumor growth, invasion and metastasis. Among them, several GPCR agonists in the tumor microenvironment activate signaling pathways to Rho GTPases via $G_\alpha$ and $G_\beta_\gamma$ subunits and their RhoGEF effectors. The leading edge of migrating cells contributing to tumor progression is amplified in the inset to highlight the role of $G_\beta_\gamma$ as an activator of RhoGEFs that regulate actin cytoskeleton dynamics leading chemotactic cell migration.

Figure 2. GPCR-dependent chemotaxis in individual migrating cells. A general model of chemotactic cell migration is based on studies of fast-moving individual cells, such as neutrophils and *Dictyostelium discoideum* amoebas (Devreotes and Horwitz, 2015). Accordingly, a chemotactic gradient is sensed by GPCRs located at the proximity of incoming stimuli (A). Then, $G_\beta_\gamma$ is locally released, recruiting PI3K and RhoGEFs (B). These effectors of $G_\beta_\gamma$ promote a spatiotemporal restricted activation of Rho GTPases resulting in polarized morphological changes based on the dynamics of the actin cytoskeleton (C). In this context, cell migration occurs as an integrated effect of protrusions, adhesion and contraction at the advancing front, followed by rear edge retraction. Maintained cell migration involves translocation of signaling proteins (D). The process is positively influenced by vesicle trafficking (Murphy et al.,
In this regard, G\(\beta\gamma\) trafficking, mediated by its interaction with Rab11, activates a PI3K-AKT signaling axis at early endosomes (EE) (Garcia-Regalado et al., 2008). Whether G\(\beta\gamma\)-trafficking leads to endosomal activation of RhoGEFs is currently being investigated.

**Figure 3. RhoGEFs identified as G\(\alpha\) or G\(\beta\gamma\) interactors.** GPCRs activate Rho GTPases via direct interactions between G\(\beta\gamma\), GTP-bound G\(\alpha_{12/13}\) or G\(\alpha_q\) with the indicated RhoGEFs. These RhoGEF have a modular organization characterized by the presence of a catalytic DH (Dbl-homology) domain followed by a PH (Pleckstrin-homology) domain predicted with the SMART analysis tool ([http://smart.embl-heidelberg.de/](http://smart.embl-heidelberg.de/)).

The list of GPCR-regulated RhoGEFs is based on studies showing, at least by coimmunoprecipitation or other equivalent assays, interaction between the indicated G proteins and RhoGEFs. G\(\alpha_{12/13}\)-regulated RhoGEFs: p115RhoGEF/ARHGEF1 (Hart et al., 1998), LARG/ARHGEF12 (Fukuhara et al., 2000), PDZ-RhoGEF/ARHGEF11 (Fukuhara et al., 1999), MCF2/Dbl/ARHGEF21 (Jin and Exton, 2000), ARHGEF2/GEF-H1 (Meiri et al., 2014) and ARHGEF28/RGNEF (Masia-Balague et al., 2015). G\(\alpha_q\)-regulated RhoGEFs: p63RhoGEF/ARHGEF25 (Lutz et al., 2005; Lutz et al., 2007), TRIO/ARHGEF23 (Rojas et al., 2007; Vaque et al., 2013), and ARHGEF28/RGNEF (Masia-Balague et al., 2015). G\(\beta\gamma\)-regulated RhoGEFs: P-Rex1 (Welch et al., 2002) and P-Rex2 (Donald et al., 2004), PLEKHG2 (Ueda et al., 2008), p114RhoGEF/ARHGEF18 (Niu et al., 2003), ARHGEF5/TIM (Wang et al., 2009) and MCF2/Dbl/ARHGEF21(Nishida et al., 1999).

As shown at the bottom, the cycle of Rho GTPases is regulated by GEFs, GAPs and GDIs. G protein regulated RhoGEFs activate Rho GTPases by promoting exchange of GDP for GTP. GTPase-Activating Proteins (GAPs) are negative regulators that help the GTPase to hydrolyze GTP to GDP. Guanine Nucleotide Dissociation Inhibitors (GDIs) inhibit GDP
dissociation, keeping the GTPase in an inactive state.

**Figure 4. P-Rex1 as a putative chemotactic scaffold.** Directional cell migration is the most conspicuous consequence of a precise choreography of Rho GTPases activated at the right time and place by chemotactic GPCR-regulated RhoGEFs. This sophisticated process is putatively more efficient with the intervention of multidomain proteins acting as chemotactic scaffolds. Accumulating evidences suggest that P-Rex1, a Rac1-specific GEF, which has been associated with metastatic processes, has the inherent attributes to perform this role. Besides its reported interactions with phosphoinositides and Gβγ (Welch, 2015), it also interacts with additional signaling proteins having a relevant role in migratory responses. S1P1 receptor carboxyl-terminal tail interacts with P-Rex1-PDZ domains, maintaining a longer chemotactic effect due to an internalization delay (Ledesma-Sanchez et al., 2010). Gβγ and P-Rex1 interact with mTORC2, a multimeric serine/threonine kinase involved in AKT activation and cell migration (Hernandez-Negrete et al., 2007; Robles-Molina et al., 2014). Finally, type I PKA interacts with P-Rex1-PDZ domains, phosphorylates the GEF at its first DEP domain and exerts an indirect effect on the carboxyl-terminal region (via a putative PKA-regulated kinase), resulting in inhibitory intramolecular interactions (Chavez-Vargas et al., 2016). In conclusion, P-Rex1 is a putative chemotactic scaffold that integrates the actions of diverse signaling proteins including receptors, G protein subunits, lipid second messengers and important serine-threonine kinases such as mTOR (mechanistic target of rapamycin) and PKA (cyclic AMP-dependent protein kinase).
Figure 1

Invasion

Tumor cell migration

ECM degradation

Fibroblast

Mesenchymal stromal cell

Invasive tumor

BMDC recruitment

BMDC migration

SDF-1

CCL2

SDF-1

CXCR4

CXCR2

CCR2

Thrombin

SIP

PAR1

Secondary tumor

Osteoblast

BMDC

Angiogenesis

Endothelial cell migration

IL-8

SDF-1

IL-8

CXCR4

CXCR2

SIP1

EP2

Primary tumor

Metastasis

Cytoskeleton rearrangement

GTP

GDP

Rho

Rho GEFs

Rho GDP

Rho GTP
Figure 3
Figure 4