*Current Topics*

# Biologic Functions of the G12 Subfamily of Heterotrimeric G Proteins: Growth, Migration, and Metastasis†

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ABSTRACT: The G12 subfamily of heterotrimeric G proteins has been the subject of intense scientific interest for more than 15 years. During this period, studies have revealed more than 20 potential G12-interacting proteins and numerous signaling axes emanating from the G12 proteins, Gα12 and Gα13. In addition, more recent studies have begun to illuminate the various and sundry functions that the G12 subfamily plays in biology. In this review, we summarize the diverse range of proteins that have been identified as Gα12 and/or Gα13 interactors and describe ongoing studies designed to dissect the biological roles of specific Gα−effector protein interactions. Further, we describe and discuss the expanding role of G12 proteins in the biology of cells, focusing on the distinct properties of this subfamily in regulating cell proliferation, cell migration, and metastatic invasion.

Heterotrimeric guanine nucleotide binding proteins (G proteins)[1] transmit a variety of extracellular signals from cell surface G protein-coupled receptors (GPCRs) to intracellular effector molecules (*1*, *2*). G proteins consist of two functional signaling units, a guanine nucleotide binding α subunit and a $\beta\gamma$ subunit dimer. Upon receptor activation, the α subunit undergoes a conformational change that leads to the exchange of GTP for GDP and the dissociation of the α subunit from the $\beta\gamma$ dimer, allowing the subunits to engage their downstream effectors (*3*). Because of the array of extracellular signals that activate them and their increasingly large number of intracellular targets, G proteins have been implicated in many physiologic and pathophysiologic processes (*4*−*7*).

Heterotrimeric G proteins are classified according to the α subunit into four subfamilies: Gs, Gi, Gq, and G12. The G12 subfamily was the last of these to be identified and is comprised of only two members, Gα12 and Gα13. However, these α subunit proteins are expressed in virtually every tissue in the body (*8*, *9*) and in the 15 years since their discovery have been implicated in a wide variety of cellular events and responses. In this report, we review the recent advances in our understanding of the signal transduction pathways mediated by the G12 proteins.

## *Effectors of the G12 Subfamily*

Knowledge of cellular responses mediated by G12 proteins has outpaced our understanding of the specific mechanisms of G12-mediated signaling by several years. It was not until 1998 that the first downstream binding partner of the G12 subfamily, p115RhoGEF, was identified (*10*, *11*). Since then, a variety of experimental techniques, most notably the yeast two-hybrid system (*12*), have identified a diverse array of proteins that interact directly with Gα12 and/or Gα13 (Figure 1). These interacting proteins may serve as direct effectors of G12 proteins or as regulators of G12 signaling. Determining the roles of these specific protein−protein interactions in the various facets of G12-mediated signal transduction likely will occupy the field of G12 research for years to come.

This review will focus on proteins and signaling responses that lie downstream of the receptor-mediated activation of G12 proteins. Approximately 25 different GPCRs have been linked to the activation of the G12 subfamily; see Riobo and Manning (*13*) for a recent review. What follows is a discussion of the proteins that have been identified as direct interactors with G12 subfamily proteins, and just how these putative effector proteins may participate in G12-mediated biologies.

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[1] Abbreviations: G protein, heterotrimeric guanine nucleotide binding protein; GPCR, G protein-coupled receptor; PKC, protein kinase C; RhoGEF, Rho-specific guanine nucleotide exchange factor; LARG, leukemia-associated RhoGEF; RGS, regulator of G protein signaling; LPA, lysophosphatidic acid; E-cadherin, epithelial cadherin; N-cadherin, neural cadherin; ERM, ezrin, radixin, moesin; FERM, band 4.1, ERM; BTK, Bruton's tyrosine kinase; PP, protein phosphatase; AKAP, A-kinase anchoring protein; PKA, cAMP-dependent protein kinase; Hsp, heat shock protein; SNAP, soluble NSF-associated protein; JNK, c-jun N-terminal kinase; JLP, JNK-interacting leucine zipper protein.

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*RhoGEFs.* The most extensively characterized downstream mediators of signaling through the G12 subfamily are members of the RhoA family of monomeric GTPases. The members of this ubiquitously expressed family of proteins are known mainly for their role in regulating the actin cytoskeleton, but they also play important roles in dictating cell polarity, microtubule dynamics, membrane transport pathways, transcription factor activity, and cell growth (*14*). In addition, these proteins play pivotal roles in tumorigenesis and cancer progression (*15*). Gα12 and Gα13 stimulate Rho activity principally through direct interaction of the activated Gα protein with Rho-specific guanine nucleotide exchange factors, commonly denoted RhoGEFs, which include p115RhoGEF (*10*), PDZ-RhoGEF (*16*), and leukemia-associated RhoGEF (LARG) (*17*). In addition to the tandemly linked Dbl homology and pleckstrin homology domains that define most RhoGEFs, this family is characterized by the presence of an N-terminal regulator of G protein signaling (RGS)-like motif (*18*, *19*). Gα12 and Gα13 bind to RhoGEF through this motif, recruiting it to the membrane where it is able to promote Rho activation (*10*, *16*, *17*). The direct stimulation of RhoGEFs by G12 subfamily proteins appears to be an ancient signal transduction pathway, as this process mediates critical events in the development of *Drosophila* (*20*, *21*) and *Caenorhabditis elegans* (*22*). While binding of the activated Gα subunit is sufficient to induce RhoGEF activity in some cases [e.g., Gα13 activation of p115RhoGEF (*10*)], in other cases (e.g., Gα12 stimulation of LARG) activation of the RhoGEF protein requires both its interaction with the activated Gα subunit and its phosphorylation by a nonreceptor tyrosine kinase (*23*).

Although the different G12-responsive RhoGEFs have the same primary function of activating Rho, these proteins appear not to play wholly redundant roles in G12-mediated signaling. A study utilizing small interfering RNA to eliminate expression of individual RGS proteins in cultured kidney and prostate cells revealed that specific G12-coupled GPCRs require specific RhoGEFs for activation of Rho. In this study (*24*), signaling through the thrombin receptor was found to require LARG, whereas signaling through the lysophosphatidic acid (LPA) receptor was demonstrated to require PDZ-RhoGEF. These findings suggest that the G12-interacting RhoGEFs have overlapping yet unique functions in mediating G12-generated signals.

*RGS Proteins.* In addition to the family of RhoGEFs discussed above, Gα12 and Gα13 interact with several other proteins that possess RGS domains. Interestingly, many of these RGS proteins appear to interact preferentially with either Gα12 or Gα13. For example, RGS1 was shown to bind Gα12 but not Gα13 (*25*) and RGS16 was found to bind Gα13 but not Gα12 (*26*). More recently, Stemmle et al. (*27*) reported that the RGS domain of the protein axin selectively binds Gα12. As axin is an important regulator of the WNT/Frizzled signaling pathway that governs key cell growth and differentiation events, this interaction suggests a potential new role for G12 proteins in WNT signaling. Taken together, these studies suggest that in addition to stimulating GTP hydrolysis in proteins of the G12 subfamily, these RGS proteins may serve to regulate G12 signaling through other mechanisms and may provide a point of integration for signals emanating from G12 proteins and other heterotrimeric G proteins.

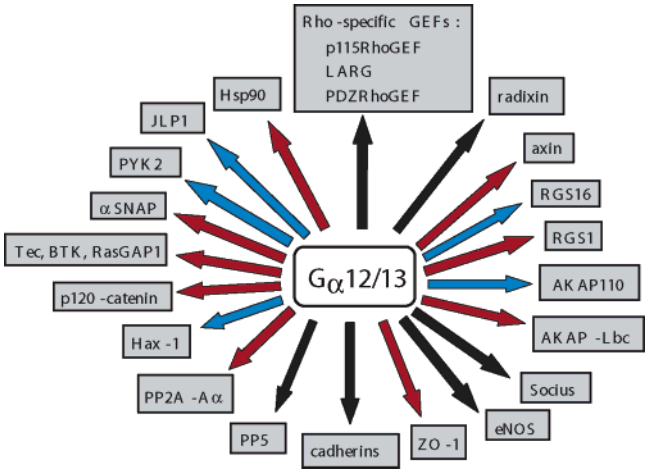


FIGURE 1: Proteins demonstrated to interact with G12 subfamily proteins. Direct interactions between G12 proteins and effector proteins are denoted with arrows, with red arrows indicating Gα12-specific interaction, blue arrows Gα13-specific interaction, and black arrows interaction with both Gα12 and Gα13. Abbreviations: PP, protein phosphatase; eNOS, endothelial nitric oxide synthase; RGS, regulator of G protein signaling; AKAP, A-kinase anchoring protein; ZO, zonula occludens; JLP, JNK-interacting leucine zipper protein; BTK, Bruton's tyrosine kinase; Hsp, heat shock protein.

*Cadherins.* Using a yeast two-hybrid screen for novel binding partners of mutationally activated Gα12, Meigs et al. (*28*) discovered an interaction between Gα12 and the cytoplasmic tail of the neural-specific cell surface glycoprotein cadherin-14. Biochemical analysis revealed that both Gα12 and Gα13 interact in an activation-dependent manner with the cytoplasmic tails of type I and type II classical cadherins, including epithelial E-cadherin, neural N-cadherin, and cadherin-14 (*28*). Cadherins mediate cell−cell adhesion and a variety of other cellular processes through their $Ca^{2+}$-dependent, homotypic binding to cadherins extending from neighboring cells (*29*). Interestingly, the binding of activated Gα12 to a distinct region within the cytoplasmic tail of E-cadherin triggers the release of the transcriptional activator β-catenin from the cadherin tail (*28*, *30*), attenuates the extracellular adhesive function of E-cadherin, and promotes cell migration (*30*).

G12 proteins appear to play a role in disrupting cadherin−β-catenin interaction and in downregulating the extracellular cell−cell adhesive function of cadherins. The mechanism of G12-induced release of β-catenin is not known, although studies utilizing targeted deletions within the E-cadherin cytoplasmic domain indicate that Gα12 and β-catenin bind to distinct regions of this domain (*31*), suggesting that G12 proteins do not simply displace β-catenin by competing for its binding site on the cadherin tail. Also, it is not known whether the negative regulation of cadherin function is solely due to G12-induced release of β-catenin or whether G12 proteins can modulate cadherin function in other ways. The recent report of an interaction between Gα12 and the cadherin-associated protein p120 catenin is of interest in this regard (*148*). The lone region of cadherin that has been identified as necessary for Gα12 interaction is a span of 11 consecutive residues within the E-cadherin cytoplasmic domain, ending 19 residues upstream of the C-terminus (*31*). Because p120 catenin binds to the cadherin cytoplasmic domain at a region much closer to the membrane-spanning

domain, it appears possible that cadherin serves as a scaffold for simultaneous binding of Gα12 and other proteins involved in the regulation of cadherin−β-catenin interaction and the extracellular adhesive function of cadherin. Finally, it is important to note that all of the effects of the G12 subfamily on the cadherin−β-catenin pathway have been demonstrated using constitutively active forms of Gα12 and Gα13. Thus, the physiologic significance of the G12−cadherin interaction in response to stimulation of GPCRs remains to be determined.

*Radixin.* Yeast two-hybrid screening also revealed an interaction between Gα13 and the protein radixin of the ERM (ezrin, radixin, moesin) family (*32*). ERM proteins provide dynamic cross-links between a number of plasma membrane proteins and the actin cytoskeleton. As a result, ERM proteins have been implicated in processes such as cell migration, adhesion, and division (*33*). Activated Gα13 was demonstrated to bind the N-terminal FERM (band 4.1, ERM) domain of radixin, causing radixin to shift to its "open", actin-binding conformation (*32*). Interaction between radixin and activated Gα12 was also observed in the same study. Whether Gα13 and/or Gα12 interacts with other FERM domain-containing proteins remains to be examined. Signaling of Gα13 through radixin appears to promote activity of the $Ca^{2+}$/calmodulin-dependent kinase CaMKII (*34*) and may play a role in Gα13-stimulated Rac activity (*34*) as well as Gα13-induced transformation of Rat-1 fibroblasts (*32*). It should be noted that activation of Rho is sufficient to induce ERM protein activation through stimulation of the phosphatidylinositol 4-phosphate 5-kinase (*35*). Furthermore, phosphorylation of the ERM proteins by Rho kinase stabilizes their activated conformation (*36*). Thus, Gα13 has the potential to affect the activity of radixin both directly through interaction with the FERM domain and indirectly through the activation of Rho.

*Nonreceptor Tyrosine Kinases.* Many studies have suggested a role for tyrosine kinases in Gα12- and Gα13-mediated signal transduction (*23*, *37*−*43*). Most of these studies have focused on the role of tyrosine kinase activity in the activation of Rho (*23*, *40*, *41*) (discussed above) or Rho-dependent actin cytoskeleton rearrangements (*37*−*39*, *42*, *43*). However, in some systems, stimulation of tyrosine kinase activity by members of the G12 subfamily also promotes Rho-independent effects, including the activation of PI-3-kinase signaling (*44*). Studies have demonstrated that Gα12 interacts with two members of the Tec family of nonreceptor tyrosine kinases: Bruton's tyrosine kinase (BTK) (*43*) and Tec (*23*). Interestingly, while Gα12 binding appears to promote BTK activity (*43*), binding of Gα12 to Tec appears to have no effect on its kinase activity (*23*). Furthermore, Gα13 binds and stimulates PYK2, a member of a different family of nonreceptor tyrosine kinases. Although Gα12 was not observed to bind PYK2, a kinase-deficient mutant of PYK2 was found to block the stimulation of a serum response element reporter gene by either Gα12 or Gα13 (*40*). Although many details of these signaling pathways remain to be elucidated, these studies suggest that members of the G12 subfamily regulate tyrosine kinase activity through direct interaction with kinase proteins.

*Protein Phosphatases.* In addition to the regulation of protein kinases, the G12 subfamily has been linked to the regulation of protein phosphatase activity. Interactions have been demonstrated between members of the G12 subfamily and both PP5 (*45*) and the scaffolding Aα subunit of PP2A (*46*). Interestingly, the characteristics of these interactions are very different. Gα12 and Gα13 interact with the tetratricopeptide repeat domain of PP5 in an activation-dependent fashion, stimulating PP5 activity (*45*). In contrast, only Gα12 appears to interact with PP2A Aα, and this interaction promotes PP2A activity independent of the activation state of Gα12 (*46*). Both PP5 (*47*) and PP2A (*48*) have many cellular targets, several of which have significant roles in tumorigenesis, cancer progression, and neurodegenerative diseases. Although the functional significance of G12 protein binding to these target protein phosphatases is still not clear, a recent study suggests a role for Gα12 as a regulatory subunit for the PP2A holoenzyme (*49*).

*A-Kinase Anchoring Proteins (AKAPs).* Two studies have reported interactions between members of the G12 subfamily and A-kinase anchoring proteins (AKAPs) (*50*, *51*). Classically, members of the AKAP family of proteins bind the regulatory subunit of the cAMP-dependent protein kinase (PKA), anchoring it at specific subcellular locations (*52*). However, AKAPs can also serve as scaffolds for the assembly of multiple cellular signaling components, allowing them to coordinate signal transduction through many pathways at once (*52*). Gα12 interacts with AKAP-Lbc (*50*). AKAP-Lbc has a Dbl homology domain that functions as a Rho family-specific guanine nucleotide exchange factor. As observed with the RGS domain-containing RhoGEFs discussed above, activation of Gα12 causes translocation of AKAP-Lbc to the plasma membrane, inducing activation of Rho. Interestingly, AKAP-Lbc can also serve as a scaffold that integrates signals from PKA and PKC into the activation of protein kinase D (*53*); a role of G12 signaling in this pathway is currently unknown. A second AKAP, termed AKAP110, has been shown to interact with Gα13 but not Gα12 (*51*). Moreover, coexpression of activated Gα13 with this testis-specific AKAP in human embryonic kidney cells triggered the release of the catalytic subunits of PKA from AKAP110, inducing PKA activity (*51*). These data suggest a novel pathway by which Gα13 can regulate PKA activity independent of cAMP.

*Zonula Occludens Proteins.* Immunofluorescence studies in cultured epithelial cells have suggested that Gα12 is concentrated at tight junctions (*54*). Subsequent studies revealed that Gα12 interacts directly with the resident tight junctional proteins zonula occludens-1 and zonula occludens-2 in a manner dependent upon the Src homology 3 (SH3) domain of the zonula occludens protein (*55*). The functional significance of this interaction was suggested by the finding that ectopic expression of activated Gα12 in Madin-Darby canine kidney cells triggered an increase in paracellular permeability and disrupted the polarized distribution of zonula occludens-1 and the $Na^+$−$K^+$-ATPase (*55*, *56*).

*Hsp90.* Studies have also demonstrated that Gα12 interacts directly with heat shock protein 90 (Hsp90) (*57*, *58*). This interaction appears to be important for the localization of Gα12 to lipid rafts (*58*) and the ability of Gα12 to engage its downstream signaling partners (*57*). This interaction with Hsp90 appears to be specific for Gα12, as Hsp90 displayed essentially no affinity for Gα13 (*57*). Moreover, it is

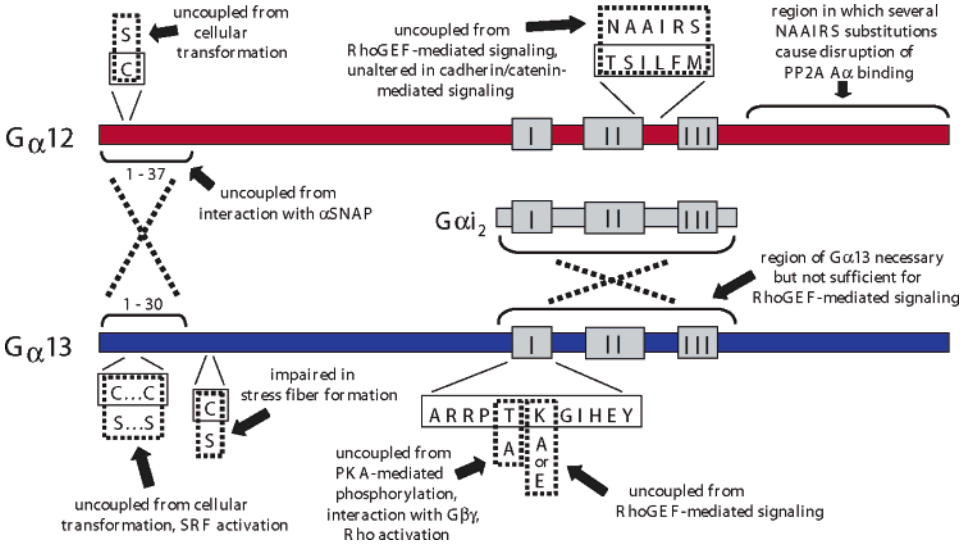


FIGURE 2: Mutations of G12 proteins impacting effector binding and biologic function. Primary structures of Gα12 (red) and Gα13 (blue) are shown with N-termini at the left. The single-letter code for native amino acids (text within solid boxes) and mutationally substituted amino acids (text outside solid boxes) are shown. Dashed boxes and lines indicate areas in which amino acid substitutions have been engineered. The three Switch regions (I, II, and III) for Gα12, Gα13, and $G\alpha i_2$ are indicated. Abbreviations: SRF, serum response factor; PKA, protein kinase A; PP, protein phosphatase. Cursory descriptions of mutants are denoted with solid arrows. See the text for additional details and literature citations for individual mutants.

interesting to note that Gα13 is absent from lipid rafts (*58*), suggesting that Hsp90-mediated localization of Gα12 to lipid rafts may contribute to differences in the respective signaling functions of Gα12 and Gα13.

*Other G12 Binding Partners*. In addition to the proteins and pathways discussed above, G12 proteins have been shown to interact with several additional proteins (Figure 1). Gα12 has been reported to interact with the Ras-specific GTPase activating protein $RasGAP1^m$ to downregulate Ras signaling (*43*), αSNAP (soluble NSF-associated protein) to regulate the membrane localization of cadherins (*59*), and endothelial nitric oxide synthase to regulate NO biosynthesis (*60*). Gα13 has been reported to interact with Hax-1 to regulate Rac activity (*61*) and JLP1 [c-jun N-terminal kinase (JNK)-interacting leucine zipper protein 1] to regulate JNK activity (*62*). Finally, both Gα12 and Gα13 have been reported to bind Socius, a protein that associates with Rho-like Rnd proteins to regulate disassembly of stress fibers (*63*).

### *Indirect Effectors of G12 Signaling*

G12-mediated signaling has been shown to stimulate the activity of a diverse set of downstream proteins that have not been demonstrated to interact directly with Gα12 or Gα13. These include phospholipase D (*64*−*67*), phospholipase C-$\epsilon$ (*68*−*70*), phospholipase $A_2$ (*71*), JNK and p38MAPK (*72*, *73*), and NF-$\kappa$B (*74*). G12-mediated signaling has also been shown to trigger phosphorylation of vasodilator-stimulated phosphoprotein (*75*), as well as phosphorylation of the focal adhesion proteins paxillin, focal adhesion kinase, and p130 Crk-associated substrate (*76*). Although RhoA proteins have been implicated in each of the G12-mediated events described above, other downstream effectors of G12 proteins may play significant roles in these processes as well. In addition to the responses described above, G12 proteins have been reported to promote signaling through GSK-3$\beta$ (*77*), stimulate ERK5 (*78*), and control $Na^+-H^+$ exchange (*79*, *80*).

### *Structural Features of G12 Proteins Critical for Signaling Pathways*

Efforts to elucidate the structural features of the G12 subfamily proteins have been impeded by the poor yield of these proteins, despite a variety of expression and purification strategies that have been employed (*81*, *82*). In recent work, Kreutz et al. (*83*) replaced the 46 N-terminal amino acids of each G12 protein with residues 1−28 of the α subunit $G\alpha i_1$ and succeeded in purifying these chimeric Gα12 and Gα13 variants from baculovirus-infected insect cells. Subsequent crystallization and structural analysis of these proteins revealed several differences between Gα12 and Gα13 (*83*), such as the presence of an additional helix in Gα13, that may explain their selective interaction with certain effector proteins (see Figure 1). In another study, bacterially expressed chimeras comprised of several regions of Gα13 interspersed with regions of $G\alpha i_1$ have allowed the crystal structure of a dimer between this chimera and the G protein regulatory region of p115RhoGEF to be examined (*18*). This work has identified several domains and a number of specific amino acids within Gα13 and p115RhoGEF that lie at the interface of these two proteins.

Mutational studies of Gα12 and Gα13 also have provided important clues about structure−function aspects of these proteins. Chimeras of Gα13 and $G\alpha i_2$ were used to demonstrate that the Switch regions of Gα13 (Figure 2), which are regions common to all Gα subunits that undergo an activation-triggered conformational change thought to be critical for many Gα−effector interactions (*84*−*86*), are necessary but not sufficient for signaling through Rho-mediated pathways (*85*). Also, mutation of a key lysine residue within the Switch I region of Gα13 (Figure 2) hindered Gα13−RhoGEF interaction as well as the ability of Gα13 to stimulate transcriptional activation mediated by serum response factor (*87*); charge-reversal substitutions of single amino acids in the Switch II region of Gα13 produced a similar result (*88*). In another study, a mutant of Gα12

was isolated that displays selective uncoupling from different effector proteins. To engineer this mutant, six native amino acids of Gα12 slightly overlapping the Switch II region (Figure 2) were replaced with the "filler" sequence Asn-Ala-Ala-Ile-Arg-Ser. This "NAAIRS" mutant of Gα12 was impaired in its RhoGEF binding and unable to stimulate Rho-mediated pathways but retained normal interaction with cadherin and the ability to regulate extracellular cadherin function and β-catenin localization (*89*). This NAAIRS substitution approach was also used to identify regions of Gα12 required for binding to the scaffolding (Aα) subunit of PP2A; the C-terminal region of Gα12 was found to harbor most of the residues critical for this interaction (*49*). In another study, replacement of the 37 N-terminal amino acids of Gα12 with the 30 N-terminal amino acids from Gα13 abolished the ability of Gα12 to interact with the effector αSNAP (*59*). These latter two examples are of particular interest as they involve regions of Gα12 distinct from the Switch regions. These studies suggest that unexpected domains and surfaces of Gα12 and Gα13 will be found to participate in binding the various effector proteins linked to the G12 subfamily.

Other mutations have been shown to uncouple G12 proteins not from specific effectors but from certain biological responses. Substitution of the native Cys residue at position 11 within Gα12 for a Ser residue (Figure 2), a mutation shown to disrupt palmitoylation of Gα12, blunted the ability of the protein to trigger a transformed phenotype when expressed in cultured mouse fibroblasts (*90*). In Gα13, Cys-to-Ser substitutions at positions 14 and 18 (Figure 2) abolished the ability of the protein to signal through serum response factor and to stimulate stress fiber formation (*91*). In a separate study, the same substitutions disrupted the ability of Gα13 to trigger cellular transformation, whereas a distinct Cys-to-Ser mutation (at position 37; see Figure 2) selectively disrupted the ability of Gα13 to cause stress fiber formation (*92*). Also, a naturally occurring variant of Gα13, harboring a nonsense mutation that causes its truncation within the Switch III region, was found to lack the ability to cause cellular transformation (*93*). In addition, mutation of a putative PKA phosphorylation site within the Switch I region of Gα13 (Figure 2) has revealed PKA-mediated modification of this protein as a potential mechanism for blocking the Gα13-Rho signaling axis (*149*). Further studies characterizing the effector binding properties of NAAIRS-substituted and other types of Gα12 and Gα13 mutants should provide useful molecular "tools" for dissecting the roles of specific Gα−effector interactions in G12-mediated physiologic and pathologic processes.

### *Physiologic and Pathologic Significance of the G12 Subfamily*

Soon after their discovery, both Gα12 and Gα13 were demonstrated to harbor the ability to induce oncogenic transformation in fibroblasts (*94*, *95*). These findings have led to the hypothesis that cell surface GPCRs may signal through the G12 proteins to promote tumorigenesis and tumor cell growth (*96*). Co-incident with this discovery, studies of the *Drosophila* protein Concertina, which was later revealed to be an ortholog of the G12 subfamily, demonstrated that this protein was required for the cellular shape changes and movements that occur during normal gastrulation (*97*). Thus, it became apparent that the G12 subfamily also mediates cell shape change and migration; as a result, most studies on the biologic significance of the G12 subfamily have focused on either this function or the ability of G12 proteins to promote cell growth and transformation. However, with advances such as the recent development of conditional knockout mice, new and more complex functions of the G12 subfamily have begun to emerge (Table 1).

*G12 Subfamily in Cell Proliferation and Transformation.* As mentioned above, the earliest identified function of the G12 subfamily was the ability to promote growth and induce neoplastic transformation. Using an expression cloning approach to identify putative oncogenes in a Ewing's sarcoma-derived cell line, Aaronson and colleagues identified Gα12 as a transforming oncogene by virtue of its ability to stimulate the formation of foci in NIH3T3 mouse fibroblasts (*94*). Interestingly, the wild-type form of Gα12 promoted this transformed phenotype, revealing the G12 subfamily as the only class of heterotrimeric G proteins that are transforming when overexpressed as a wild-type form (*94*). Subsequently, several studies confirmed and expanded upon these findings (*94*, *95*, *98*, *99*). Interestingly, most of these studies utilized overexpression or mutational activation of G12 subfamily proteins in nontransformed rodent fibroblasts to demonstrate the transforming effects of the G12 pathway (*94*, *95*, *98*−*101*). However, other studies have demonstrated that overexpression of GPCRs such as PAR-1 (*102*) and the M1 muscarinic acetylcholine receptor (*103*) promotes fibroblast growth and transformation through the endogenous G12 proteins. Furthermore, Aragay et al. (*104*) demonstrated that stimulation of PAR-1 receptors in the 1321N1 astrocytoma cell line induced cell growth in a Gα12-dependent fashion. Taken together, these studies suggest that even at physiologic levels the G12 proteins are able to drive cell growth and have led to the hypothesis that GPCRs may signal through the G12 proteins to promote tumorigenesis and tumor cell growth (*96*).

The signaling pathways downstream of G12 that promote cell growth and neoplastic transformation have been studied extensively. Many of the growth promoting and transforming effects of the G12 proteins appear to be mediated by the RhoA family of monomeric G proteins (*102*, *103*, *105*, *106*). Stimulation of RhoA downstream of G12 proteins promotes the activation of JNK (*107*), p38 MAPK (*108*), STAT3 (*109*), and the PDGFα receptor (*106*) as well as serum response element-regulated transcription (*110*), NF-κB-regulated transcription (*111*), and expression of COX-2 (*112*, *113*). Each of these pathways has been implicated in G12-triggered cellular transformation; however, both Gα12 and Gα13 are much more potent stimulators of fibroblast transformation than overexpressed or mutationally activated RhoA (*103*). In addition, Gα13-induced transformation appears to be independent of RhoA in at least one system (*32*). As a result, it has been suggested that at least some of the potent transforming potential of the G12 subfamily is mediated through proteins distinct from RhoA, such as Rac (*32*, *34*), ERK5 (*78*), and the cadherin−β-catenin complex (*28*, *30*).

More recently, several groups have begun to examine the biologic significance of G12-stimulated cell growth and neoplastic transformation in human cancers. The earliest of these studies demonstrated that Gα12 and Gα13 expression is stronger in cell lines derived from human breast, prostate,

Table 1: Biologic Functions of the G12 Family of Heterotrimeric G Proteins

| function | cell type or tissue | references |
|---|---|---|
| promotes growth | fibroblasts | *99, 100, 102* |
| | astrocytoma cells | *104* |
| induces cell transformation | fibroblasts | *94, 95, 98, 99, 100, 102* |
| inhibits growth | breast CA cells | *115* |
| mediates cell shape changes of gastrulation | mesoderm | *97, 120* |
| mediates cell polarity and migration | fibroblasts | *61, 126* |
| | neutrophils | *119* |
| | breast CA cells | *30* |
| | lymphocytes | *117, 118* |
| | endothelial cells | *121, 122* |
| promotes cancer cell invasion | prostate CA cells | *116* |
| | breast CA cells | *115* |
| | ovarian CA cells | *134* |
| promotes in vivo metastasis | breast CA cells | *115* |
| promotes platelet aggregation | platelets | *140* |
| promotes smooth muscle tension | smooth muscle cells | *141* |
| promotes myocardial hypertrophy | cardiomyocytes | *72* |
| increases paracellular permeability | MDCK cells | *55* |
| induces apoptosis | COS-7 and CHO-K1 cells | *146, 147* |
| induces neurite retraction | PC-12 and N1E-115 cells | *38, 39* |
| mediates neuronal differentiation | P19 cells | *144* |

and colon cancers compared to cell lines derived from nontransformed human tissue (*114*). This finding suggested that the G12 proteins are upregulated during neoplastic transformation of these common forms of cancer. Subsequently, this observation was confirmed in actual human tissues; analysis of Gα12 expression in histopathologic specimens from patients with adenocarcinoma of the breast and prostate revealed consistently higher levels of Gα12 protein compared to benign breast or prostate epithelial cells (*115, 116*). Interestingly, however, when the role of G12 signaling in breast and prostate cancer cell growth was examined, G12 signaling did not appear to promote, and in some cases even inhibited, tumor cell growth (*115, 116*). Thus, while it is clear that in some cell types Gα12 and Gα13 may be important promoters of proliferation, it appears that these G12-mediated effects may be cell-type-specific.

*G12 Subfamily in Physiologic Cell Migration.* The role of the G12 subfamily in cell movement was first identified through developmental studies in *Drosophila*. Genetic ablation of *Concertina*, the single ortholog of Gα12 and Gα13 in *Drosophila*, impairs the cell shape changes that underlie mesoderm internalization during *Drosophila* gastrulation (*97*). Subsequent studies in other systems have also revealed a role for G12 proteins in cell movement and migration. Several studies have suggested a role for the G12 subfamily in regulating lymphocyte and neutrophil migration (*117*−*119*). Suppression of Gα12 and Gα13 function in zebrafish blocks a distinct subset of the cellular shape changes and migration events required for vertebrate gastrulation (*120*). Deletion of Gα13 in mice impairs the organization of the vascular system, resulting in lethality at approximately day 10.5 of embryogenesis (*121, 122*). Embryonic fibroblasts cultured from these mice display a reduced chemokinetic response to several GPCR ligands; this defect in cell migration may underlie the failed angiogenesis (*121*). Interestingly, expression of Gα12 did not rescue the defect in fibroblast migration, indicating that Gα12 and Gα13 have overlapping but not identical biologic functions (*121*).

Many of the effects of G12 proteins on cell movement and migration have been attributed to the ability of the G12 subfamily to activate Rho. Gα12 and Gα13 stimulation of RhoA induces actin stress fiber formation and cell contraction (*37, 123, 124*). Such a pathway appears to contribute to the cell shape changes required for gastrulation in *Drosophila* (*20, 21*) and in zebrafish (*125*). Recent reports also have suggested that the activation of RhoA proteins by the G12 subfamily dictates cell polarity in migrating neutrophils (*119*) and in fibroblasts (*126*), suggesting that this pathway may explain the effects of G12 proteins on cell migration. However, cell migration is a complex, cell-type-specific process (*127*−*129*), and in some cell types, the G12 subfamily appears to promote cell migration independent of Rho activation (*30, 61*). Thus, there is still much to be understood about the role of the G12 subfamily in cell movement and migration.

*G12 Subfamily in Tumor Cell Invasion and Metastasis.* The pathophysiology of cancer invasion and metastasis shares many features and mechanisms with that of the biological process of gastrulation (*130*−*133*). Hence, it was a logical step to explore the potential role of the G12 proteins in cancer cell invasion. Work in our laboratories has shown that G12 signaling promotes both prostate (*116*) and breast (*115*) cancer cell invasion in vitro. Similarly, Bian and colleagues found that the G12 proteins were critical regulators of LPA-induced ovarian cancer cell migration in vitro (*134*). In all of these studies, the activation of Rho appeared to be critical for the invasion promoting effects of the G12 proteins. Interestingly, as with the effects on cell growth, these invasion stimulating effects may be cell-type-specific. We have recently found that expression of the activated form of Gα12 inhibits the invasion of the inflammatory breast cancer line SUM149 (unpublished observation). Moreover, stimulation of sphingosine-1-phosphate receptor-2 in glioblastoma cell lines inhibits cell migration through a Rho-dependent pathway likely mediated by the G12 proteins (*135*). The reasons for these disparate effects are currently unknown; however, they may reflect the complex effects of Rho proteins on cell invasion (*136*) .

Although in vitro assays can provide mechanistic insight into cancer progression and spread, the complexity of the metastatic process demands in vivo experimentation for accurate modeling (*137*). When implanted in the mammary fat pad of recipient mice, 4T1 mouse mammary carcinoma cells grow and metastasize in a manner similar to that of human breast cancer (*130, 138, 139*). Inhibition of G12 signaling in the 4T1 cells through expression of a dominant negative form of the p115-RhoGEF reduced the rate of metastatic dissemination of the cells following their implantation in the mouse mammary fat pad (*115*). Interestingly, when the same cells were introduced directly into the bloodstream, inhibition of G12 signaling had no effect on the ability of these cells to metastasize (*115*). This result implies that G12 function is not required in the later steps of the metastatic cascade. Taken together, these in vivo results correlate well with the in vitro findings suggesting that G12 signaling promotes metastasis by stimulating invasion by cells within the primary tumor. Further, from a clinical perspective, these studies suggest that drugs targeting

the G12 proteins may provide effective therapies for slowing the invasion of cancer and reducing the morbidity and mortality associated with certain cancers.

*Other Physiologic Roles of the G12 Subfamily.* There are other physiologic processes in which a role for G12 signaling has been reported; the most extensively studied of these is platelet activation. Selective deletion of Gα13 in mouse platelets results in mice with increased bleeding times and reduced sensitivity to experimentally induced thrombosis (*140*). This phenotype appears to result from a platelet defect that is characterized by reduced sensitivity to various aggregation-inducing stimuli and a marked reduction in the extent of Rho activation at all levels of platelet activation (*140*). In addition, G12 signaling has been implicated in the maintenance of smooth muscle tension (*141*, *142*), control of vascular permeability (*143*), development of myocardial hypertrophy (*72*), formation of primitive endoderm (*144*, *145*), and stimulation of apoptotic pathways (*146*, *147*).

### *Future Directions*

In this review, we have attempted to highlight the many recent advances in our understanding of the G12 subfamily of heterotrimeric G proteins, yet above all, we hope that this review underscores the fact that there are many questions about the G12 proteins that remain to be answered. Given the number and diversity of proteins that have been identified in the past 15 years as downstream binding partners of G12 proteins, it is reasonable to predict that additional interacting proteins will be identified. However, in the coming years, it will be important to move beyond this identification phase of study and begin to dissect how these pathways interact with one another and how together they are able to explain the biology of the G12 proteins. One area of research that has shown promise toward this end is the identification of mutant G12 proteins that are selectively uncoupled from particular signaling "arms". As an example, our recent work has revealed a six-amino acid substitution mutant of Gα12 that is impaired in RhoGEF binding and signaling through Rho-mediated pathways yet retains normal signaling through the cadherin−β-catenin pathway (*89*). This and other selectively uncoupled mutants should provide useful reagents for determining the biologic roles of specific G12−effector interactions. Furthermore, Gα12 or Gα13 mutants that are uncoupled from multiple effector proteins will be valuable, as they may reveal shared structural features of effectors that are important for interaction with G12 subfamily proteins.

In addition, the study of the biologic functions of the G12 subfamily remains a nascent field. Even a cursory analysis of the receptors which activate G12 proteins, and the pathways emanating from G12 proteins, would suggest that these proteins have the potential to be a signaling nexus for many important biologic processes. The development of genetic models of G12 signaling (*120*−*122*) and the recent application of siRNA technology to the G12 field (*60*) should facilitate these types of studies. Also, dominant-negative variants of effector proteins, such as RhoGEF proteins truncated to remove Rho-activating domains while retaining G protein regulatory domains (*40*), should continue to reveal novel roles for G12 proteins in cellular signaling pathways. Thus, while the role of the G12 proteins in cell proliferation, migration, and invasion is apparent, with additional study many new physiologic and pathophysiologic roles of the G12 proteins are likely to emerge. Taken together with the mechanistic studies described above, we believe these studies will lead to a comprehensive understanding of the G12 family of heterotrimeric G proteins.

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