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REVIEW

Role of G12 proteins in oncogenesis and metastasis

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The G12 subfamily of heterotrimeric guanine nucleotide-binding proteins consists of two α subunits, Gα12 and Gα13. These proteins mediate signalling via G protein-coupled receptors and have been implicated in various physiological and pathophysiological processes. A number of direct and indirect effectors of Gα12 and Gα13 have been identified that mediate, or have been proposed to mediate, the diverse cellular responses accompanying activation of G12 proteins. This review describes the signalling pathways and cellular events stimulated by G12 proteins, with a particular emphasis on processes that are important in regulating cell migration and invasion, and could potentially be involved in the pathophysiology of cancer metastasis. Experimental findings directly implicating G12 proteins in the spread of metastatic disease are also summarized, indicating the importance of targeted inhibition of G12 signalling as a potential therapeutic option for locally advanced and metastatic disease.
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Keywords: G proteins; G12 subfamily; oncogenic transformation; migration; invasion; metastasis

Abbreviations: ASK-1, apoptosis signal regulating kinase-1; G protein, heterotrimeric guanine nucleotide-binding protein; GPCR, G protein-coupled receptor; JLP, JNK-interacting leucine zipper protein; JNK, c-Jun N-terminal kinase; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; MLC, myosin light chain; MZB, splenic marginal zone B; NPC, neural progenitor cell; non-RTK, non-receptor tyrosine kinase; PAR-1, protease-activated receptor-1; PDGF, platelet-derived growth factor; RGS, regulator of G protein signalling; RhoGEF, Rho-specific guanine nucleotide exchange factor; ROCK, Rho kinase; RTK, receptor tyrosine kinase; S1P, sphingosine-1-phosphate; SDF-1, CXC chemokine stromal cell-derived factor-1α; SRE, serum response element; VSMC, vascular smooth muscle cell

Heterotrimeric guanine nucleotide-binding proteins (G proteins) mediate extracellular signals from transmembrane G protein-coupled receptors (GPCRs) to engage intracellular effector pathways leading to a variety of cellular responses (Marinissen and Gutkind, 2001; Pierce *et al.*, 2002; Oldham and Hamm, 2007). G proteins consist of a guanine nucleotide-binding α subunit and a βγ subunit dimer. In its inactive state, the α subunit binds a GDP molecule. Upon receptor activation by an agonist, the engagement of the liganded receptor with the G protein triggers a conformational change in the α subunit that leads to the exchange of GDP for GTP, and dissociation of the α subunit from the βγ dimer, both of which can then signal to their downstream effectors (Fields and Casey, 1997).

Heterotrimeric G proteins are classified into four subfamilies based on the sequence similarity of the α subunits: Gs, Gi, Gq and G12. The G12 subfamily consists of two α subunits, Gα12 and Gα13. Activation of the G12 proteins impacts on several signalling pathways including those linking G proteins to monomeric GTPases, mitogen-activated protein kinases (MAPKs) and non-receptor tyrosine kinases (non-RTKs), among others. Thus, G12 proteins have been implicated in several physiological and pathophysiological processes (Dhanasekaran *et al.*, 1998; Rohrer and Kobilka, 1998; Offermanns, 2001; Dorsam and Gutkind, 2007). This review details our current understanding of how G12 proteins regulate some of these cellular events, in particular signalling pathways impacting oncogenesis and metastasis.

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Biological roles of G12 proteins

Gα12 and Gα13 are expressed in virtually every tissue in the body (Milligan *et al.*, 1992; Spicher *et al.*, 1994). Activation of these proteins impacts such cellular processes as growth and proliferation, cytoskeleton rearrangement, cell polarity, paracellular permeability, cell–cell adhesion, migration and invasion (Kurose, 2003; Kelly *et al.*, 2007). Several direct binding partners as well as indirect downstream effectors of Gα12/13 have been identified that have been implicated, and in some cases directly shown to be involved, in these and other biological events mediated by the G12 subfamily (Kelly *et al.*, 2007). Many of the effects of G12/13 signalling are mediated by the monomeric GTPase Rho. Gα12 and Gα13 activate Rho principally through direct interaction of the activated Gα subunit with Rho-specific guanine nucleotide exchange factors (RhoGEFs) (see Figure 1), which include p115RhoGEF (Hart *et al.*, 1998; Kozasa *et al.*, 1998), PDZ-RhoGEF (Fukuhara *et al.*, 1999) and leukemia-associated RhoGEF (LARG) (Fukuhara *et al.*, 2000a). Other proteins that directly interact with Gα12/13, and hence could serve as effectors include cadherins (Meigs *et al.*, 2001), radixin of the ezrin/radixin/moesin protein family (Vaiskunaite *et al.*, 2000; Liu and Voyno-Yasenetskaya, 2005), non-RTKs (Jiang *et al.*, 1998; Mao *et al.*, 1998; Shi *et al.*, 2000), protein phosphatases (Yamaguchi *et al.*, 2002; Zhu *et al.*, 2004; Zhu *et al.*, 2007), A-kinase anchoring proteins (AKAPs) (Diviani *et al.*, 2001; Niu *et al.*, 2001), the tight junction protein, zonula occludens-1 (Meyer *et al.*, 2002; Sabath *et al.*, 2008), Hsp90 (Vaiskunaite *et al.*, 2001) and regulators of G protein signalling RGS1, RGS16 and axin (Moratz *et al.*, 2000; Johnson *et al.*, 2003; Stemmle *et al.*, 2006), among others (Kurose, 2003; Kelly *et al.*, 2007).

Perhaps the most extensively studied roles of G12/13 are in cell growth and proliferation, and cell migration (described later). The G12 proteins also influence several other important cellular functions. G12/13 signalling is required for agonist-induced smooth muscle contraction (Gohla *et al.*, 2000; Hersch *et al.*, 2004). GPCRs that mediate vasoconstriction such as angiotensin II, endothelin-1 and thromboxane A_2 couple to both the Gq/11 and G12/13 subfamilies to stimulate myosin light chain (MLC) phosphorylation via Ca^{2+}-dependent activation of MLC kinase, and Rho/Rho kinase-mediated inhibition of myosin phosphatase, respectively (Gohla *et al.*, 2000). Phosphorylation of MLC leads to its interaction with actin and the generation of contractile force. A recent study utilizing conditional knockout of Gα12/13 in smooth muscle cells in mice demonstrated that G12/13 signalling is required for the development of salt-induced hypertension but not for the maintenance of basal blood pressure (Wirth *et al.*, 2008). G13-mediated signalling is important for platelet activation in hemostasis and thrombosis (Moers *et al.*, 2003). Platelets lacking Gα13 show defective shape change and aggregation *in vitro* and these defects are accompanied by reduced activation of RhoA and subsequently decreased MLC phosphorylation. Selective deletion of Gα13 in mouse platelets *in vivo* results in severely increased bleeding times and lack of formation of arterial thrombi in an experimentally induced thrombosis model (Moers *et al.*, 2003). In addition,

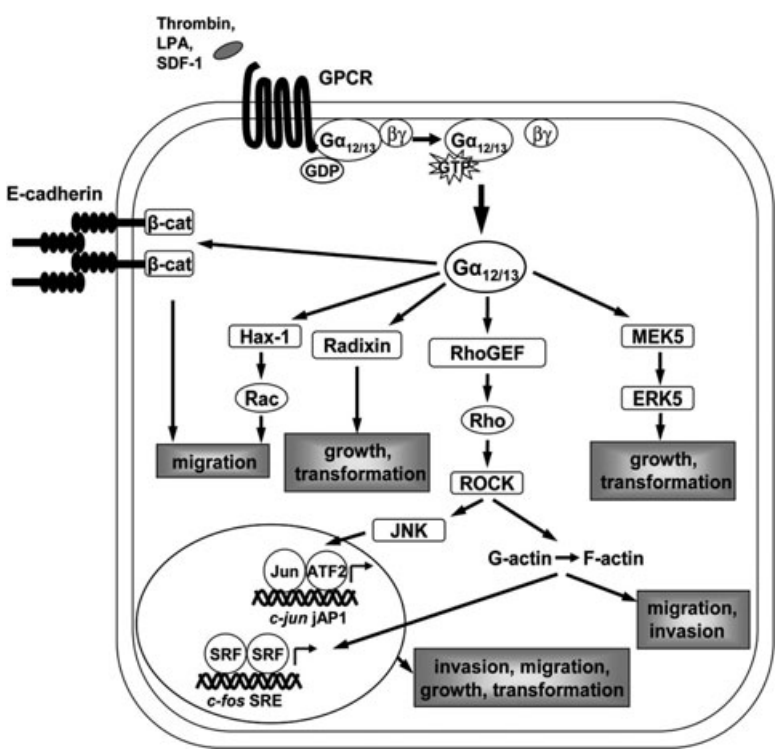

Figure 1 Schematic of Gα12/13 effectors and signalling pathways impacting cell growth and transformation, migration and invasion. β-cat, beta-catenin; ATF2, activating transcription factor-2; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; jAP1, c-*jun* AP1-like response element; JNK, c-Jun N-terminal kinase; LPA, lysophosphatidic acid; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; RhoGEF, Rho-specific guanine nucleotide exchange factor; ROCK, Rho kinase; SDF-1, CXC chemokine stromal cell-derived factor-1α; SRE, serum response element; SRF, serum response factor.

G12/13 mediate receptor-induced cardiac hypertrophic responses by activating a Gα12/13-Rho-c-Jun N-terminal kinase (JNK) pathway (Maruyama *et al.*, 2002). JNK is a MAPK that is an indirect effector of G12/13 signalling, usually activated downstream of Rho GTPases (Goldsmith and Dhanasekaran, 2007). The G12 proteins also mediate protease-activated receptor-1 (PAR-1)-induced vascular endothelial barrier permeability via Rho and Rho kinase (ROCK) activation (McLaughlin *et al.*, 2005).

While the G12 proteins clearly play a role in cell growth and proliferation (discussed below), they have also been implicated in apoptotic pathways. Constitutively activated Gα13 triggers apoptosis via a pathway involving Rho activation (Althoefer *et al.*, 1997). Gα12 appears to regulate apoptosis in epithelial cells by activating JNK and protein phosphatase 2A (PP2A) leading to loss of expression of the anti-apoptotic protein, Bcl-2 (Yanamadala *et al.*, 2007). Gα12 and Gα13 have also been shown to stimulate apoptosis via two MAPK pathways, one by activating apoptosis signal regulating kinase-1 (ASK-1) and the other by activating MAPK kinase kinase 1 (MEKK1), both leading to JNK activation (Berestetskaya *et al.*, 1998). Interestingly, Gα13 forms a complex with ASK-1 and regulates apoptosis by reducing the rate of ASK-1 degradation (Kutuzov *et al.*, 2007). Formation of the Gα13-ASK-1 complex is enhanced by coexpression of JNK-interacting leucine zipper protein (JLP) suggesting that JLP may be acting as a scaffolding protein to form a macromolecular complex (Kutuzov *et al.*, 2007). JLP was identified earlier as physically interacting with Gα13 and leading to increased Gα13-mediated JNK activation (Kashef *et al.*, 2005). In this regard, it is also important to note that Gα13 signals via p115RhoGEF and activates JNK to regulate primitive endoderm formation in murine embryonic carcinoma cells (Jho *et al.*, 1997; Lee *et al.*, 2004). An interaction between Gα13 and JLP is required for this process (Kashef *et al.*, 2006).

G12 proteins in physiological cell migration

The role of G12 proteins in regulating physiological cell migration was first identified in studies in *Drosophila*. Genetic ablation of *concertina*, the single ortholog of Gα12 and Gα13 in *Drosophila*, impairs cell shape changes that underlie mesoderm internalization during gastrulation in flies (Parks and Wieschaus, 1991). An essential role of Gα12/13 has also been demonstrated in cell shape changes and migration events that occur during gastrulation in zebrafish (Lin *et al.*, 2005). In mice, deletion of Gα13 has been shown to disrupt organization of the vascular system, resulting in lethality at approximately day 10.5 of embryogenesis (Offermanns *et al.*, 1997; Ruppel *et al.*, 2005). Embryonic fibroblasts cultured from these mice display a reduced chemokinetic response to thrombin, the agonist for PARs. Thus, this defect in cell migration may be responsible for the failed angiogenesis (Offermanns *et al.*, 1997). Quite recently, a role for Gα12/13 was demonstrated in the development of the central nervous system (Moers *et al.*, 2008). Gα12/13 appear to mediate stop signals which are required for the proper positioning of migrating cortical plate neurons and Purkinje cells during development. Conditional knockout of Gα12 and Gα13 in the nervous system of mice results in neuronal ectopia of the cerebral and cerebellar cortices due to overmigration of these cells (Moers *et al.*, 2008). Another study showed that the orphan GPCR, GPR56, is highly expressed in neural progenitor cells (NPCs) and negatively regulates NPC migration via a Gα12/13-Rho pathway (Iguchi *et al.*, 2008). Interestingly, loss of the mouse *GPR56* gene causes neuronal ectopia in the cerebral cortex (Li *et al.*, 2008). Thus, GPR56 may couple to G12/13 to regulate the migration of cells during cortical development.

Changes in cytoskeletal dynamics required for cell migration are coordinated in large part by Rho GTPases (Ras, Rac, Cdc42 and Rho) (Raftopoulou and Hall, 2004), and signalling by G12 proteins is responsible for many of the effects on cell movement that accompany Rho activation. Polymerization of actin and assembly of focal adhesions are important for cell shape changes and cell contraction during migration, and Gα12 and Gα13 stimulate the formation of stress fibres and focal adhesions in a Rho-dependent manner (Buhl *et al.*, 1995; Gohla *et al.*, 1998; 1999). Such Rho-dependent signalling pathways appear to be important during gastrulation in *Drosophila*, as noted above a process dependent on *concertina* function (Barrett *et al.*, 1997; Nikolaidou and Barrett, 2004), and during the corresponding process in zebrafish (Lin *et al.*, 2005; Solnica-Krezel, 2005). Rho activation by Gα12/13 is also required, as mentioned above, for regulating NPC migration (Iguchi *et al.*, 2008).

Besides their role in regulating cell migration during embryonic development, the G12 subfamily is also involved in controlling the migration of lymphocytes, neutrophils and vascular smooth muscle cells (VSMCs) (Xu *et al.*, 2003; Rieken *et al.*, 2006; Tan *et al.*, 2006; Takashima *et al.*, 2008) (see Table 1). The CXC chemokine stromal cell-derived factor-1α (SDF-1) induces cell migration in T lymphocytes by binding to the GPCR, CXCR4, to activate a Gα13-Rho signalling axis (Tan *et al.*, 2006). On the other hand, Gα12/13 likely regulate the number of splenic marginal zone B (MZB) cells, a specialized population of B lymphocytes, by exerting an inhibitory

Table 1 GPCRs that activate G12/13 signalling to regulate the migration and invasion of mammalian cells

GPCR	*Cell type*	*References*
PAR-1	Embryonic fibroblasts	Offermanns *et al.*, 1997
	Breast cancer cells	Kelly *et al.*, 2006a
	Prostate cancer cells	Kelly *et al.*, 2006b
LPA receptor	Fibroblasts	Goulimari *et al.*, 2005
		Goulimari *et al.*, 2008
	Ovarian cancer cells	Bian *et al.*, 2006
	Embryonic cortical neurons	Moers *et al.*, 2008
CXCR4	T lymphocytes	Tan *et al.*, 2006
S1P receptor	MZB cells	Rieken *et al.*, 2006
	VSMCs	Takashima *et al.*, 2008
	Glioblastoma cells	Malchinkhuu *et al.*, 2008
	Embryonic cortical neurons	Moers *et al.*, 2008
Thromboxane	Breast cancer cells	Kelly *et al.*, 2006a
A_2 receptor	Prostate cancer cells	Kelly *et al.*, 2006b
GPR56	Neural progenitor cells	Iguchi *et al.*, 2008

GPCR, G protein-coupled receptor; LPA, lysophosphatidic acid; MZB, splenic marginal zone B; PAR-1, protease-activated receptor-1; S1P, sphingosine-1-phosphate; VSMC, vascular smooth muscle cell.

effect on sphingosine-1-phosphate (S1P)-induced migration of these cells. Mice that lack the Gα12/13 subunits show significantly reduced numbers of MZB cells suggesting that, besides affecting peripheral MZB cell maturation, loss of Gα12/13 causes disinhibition of S1P-induced promigratory signalling (Rieken *et al.*, 2006). G12 proteins dictate morphologic polarity in neutrophils that is necessary for their migration in uniform concentrations of attractants. This function of G12 and G13 is mediated by activation of Rho with subsequent stimulation of the Rho-dependent protein kinase, p160-ROCK and myosin II to generate myosin-based contraction of the trailing edge of the migrating cell (Xu *et al.*, 2003). Gα12/13 subunits also control cell polarity and directed migration of fibroblasts in a Rho-dependent manner (Goulimari *et al.*, 2005; 2008). In VSMCs, S1P activates G12/13 signalling via the S1P(2) receptor, leading to activation of Rho and inhibition of both platelet-derived growth factor (PDGF)-induced Rac activation and migration of VSMCs (Takashima *et al.*, 2008). These disparate effects of Rho activation reflect the intricacy of Rho signalling mechanisms impacting on cell migration (Sahai and Marshall, 2002).

Cell migration is a complex process (Friedl and Wolf, 2003; Raftopoulou and Hall, 2004; Van Haastert and Devreotes, 2004), and while in some cell types G12 proteins regulate migration via stimulation of Rho, in other cell types they promote migration via processes independent of Rho signalling (Meigs *et al.*, 2002; Radhika *et al.*, 2004) (Figure 1). In fibroblasts, Gα13-stimulated migration requires interaction of the Gα subunit with Hax-1, a cytoskeleton-associated, cortactin-interacting protein. Coexpression of Hax-1 with constitutively active Gα13 attenuates the ability of Gα13 to stimulate Rho activity and leads to a significant reduction in stress fibres, while at the same time potentiating Rac activity (Radhika *et al.*, 2004). In another example, the ability of Gα12 to negatively regulate the adhesive function of cadherin enhances cell migration, and this function of Gα12 is independent of Rho activation (Meigs *et al.*, 2002). The primary mechanism for the increased migration in this instance appears to be due to the ability of the Gα12-E-cadherin interaction to trigger the release of β-catenin from the cytoplasmic tail of E-cadherin and thereby reverse E-cadherin-mediated suppression of migration (Meigs *et al.*, 2002).

In addition to mediating signals from GPCRs to impact on cell migration, in some cellular contexts G12 proteins may mediate signals from RTKs to regulate migration. An intriguing recent study demonstrated that Gα13 is required for RTK-induced migration of fibroblast and endothelial cells, and this function of Gα13 is independent of GPCR signalling (Shan *et al.*, 2006). The mechanism through which Gα13 might be involved in this process without involvement of a GPCR is still unclear, but the finding suggests that G12 proteins may be coordinating promigratory signals from GPCRs and RTKs.

G12 proteins in oncogenic transformation and cancer

Soon after their discovery, several studies established a role for both Gα12 and Gα13 in oncogenic transformation (Chan *et al.*, 1993; Xu *et al.*, 1993). Interestingly, the wild-type form of Gα12 was identified as a transforming oncogene due to its ability to promote focus formation in NIH3T3 mouse fibroblasts, revealing the G12 subfamily as the only class of heterotrimeric G proteins that is transforming when overexpressed as a wild-type form (Chan *et al.*, 1993). Subsequent studies utilizing overexpression or mutationally activated forms of Gα12/13 have confirmed the ability of these proteins to transform fibroblasts (Jiang *et al.*, 1993; Xu *et al.*, 1993; Vara Prasad *et al.*, 1994; Voyno-Yasenetskaya *et al.*, 1994). Furthermore, overexpression of GPCRs that couple to G12 proteins such as PAR-1 (Martin *et al.*, 2001) and M1 muscarinic acetylcholine receptor (Fromm *et al.*, 1997), or stimulation of GPCRs by agonists (Aragay *et al.*, 1995; Marinissen *et al.*, 2003; Radhika *et al.*, 2005) has been shown to promote cell growth and transformation through endogenous G12 signalling. These studies led to the hypothesis that GPCRs may signal through G12 proteins to promote tumorigenesis and tumour cell growth (Radhika and Dhanasekaran, 2001). Recently, it was demonstrated that the expression of G12 proteins themselves is significantly upregulated in tissue specimens from patients with adenocarcinoma of the breast and prostate (Kelly *et al.*, 2006a,b). Interestingly, a growth-promoting effect of G12 proteins was not observed in any of the breast and prostate cancer cell lines examined in this study (Kelly *et al.*, 2006a,b), suggesting that G12-mediated effects on cell proliferation may be cell type-specific, and much more pronounced in fibroblasts than in epithelial-derived cells.

Importantly, the levels of several G12-coupled receptors are elevated in various cancers and contribute to tumour cell growth and metastasis when activated by circulating or locally produced agonists (Dorsam and Gutkind, 2007). The G12/13-coupled protease-activated receptor, PAR-1, is overexpressed in highly invasive breast carcinoma cell lines and tissue specimens (Even-Ram *et al.*, 1998), and in advanced-stage prostate cancer patient samples (Daaka, 2004). Thrombin stimulates invasion of cancer cells *in vitro* (Henrikson *et al.*, 1999; Shi *et al.*, 2004; Kelly *et al.*, 2006a,b), and antisense-mediated downregulation of PAR-1 blocks cancer cell invasion (Even-Ram *et al.*, 1998). Receptors for bio-active lipids, such as lysophosphatidic acid (LPA) and S1P, are involved in cancer cell proliferation and migration (Dolezalova *et al.*, 2003; Mills and Moolenaar, 2003). LPA is secreted by ovarian cancer cells and, by acting on the LPA receptors, sets up an autocrine loop that promotes both growth and survival of the cancer cells (Mills and Moolenaar, 2003). LPA receptors couple to G12/13 (Riobo and Manning, 2005), and it was recently shown that G12 proteins are critical regulators of LPA-stimulated migration of ovarian cancer cells (Bian *et al.*, 2006). In another human cancer cell line, a positive feedback mechanism of Rho/ROCK activation mediated via the Rho effector, Dia1 (a Diaphanous-related formin) and the RhoGEF, LARG, appears to be important for LPA-stimulated invasion (Kitzing *et al.*, 2007). The G12/13-coupled chemokine receptor, CXCR4, is aberrantly overexpressed in many malignant tumours including breast, prostate and lung cancer (Balkwill, 2004). CXCR4 binds to and is activated only by SDF-1. Interestingly, the expression of SDF-1 has been observed to be high in the organs where these tumour

cells most frequently metastasize (Muller *et al.*, 2001; Balkwill, 2004). Thus, activation of G12/13 signalling by several GPCRs likely plays an important role in tumour progression. As mentioned earlier, Gα13 has been implicated in transducing promigratory signals from RTKs (Shan *et al.*, 2006). Interestingly, these RTKs, *viz*, platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) are overexpressed in highly metastatic cancers (Ferrara *et al.*, 2003; Jechlinger *et al.*, 2006; Normanno *et al.*, 2006).

The ability of G12 proteins to transform fibroblasts appears to be primarily dependent on the activation of Rho proteins (Fromm *et al.*, 1997; Martin *et al.*, 2001; Kumar *et al.*, 2006a), although in certain contexts other G12 effectors may be involved (see below; Figure 1). Besides their importance in controlling actin cytoskeleton rearrangement and cell polarity, as noted above, Rho GTPases also regulate microtubule dynamics, transcription factor activity and aspects of cell growth (Etienne-Manneville and Hall, 2002). Stimulation of Rho GTPases by G12 proteins impacts on several signalling pathways that include MAPK signalling cascades, and G12 proteins coordinate the activation and/or inhibition of different MAPKs (Collins *et al.*, 1996; Voyno-Yasenetskaya *et al.*, 1996; Nagao *et al.*, 1999; Arai *et al.*, 2003; Dermott *et al.*, 2004). Gα12 and Gα13 stimulate the activity of the MAPK, JNK (Goldsmith and Dhanasekaran, 2007); a major outcome of JNK activation in cells is the phosphorylation of the transcription factor c-Jun, leading to increased transcription of genes involved in proliferation and survival, cell motility and invasion, among others (Karin *et al.*, 1997). Thus, many of the biological consequences of Gα12/13 activation, including growth, differentiation and cellular transformation, could potentially be due to an impact on c-Jun activity (Jho *et al.*, 1997; Marinissen *et al.*, 2003; Radhika *et al.*, 2005) (Figure 1). Stimulation of Rho by G12 proteins has also been shown to lead to cellular transformation via other signalling pathways such as activation of PDGFα receptor and STAT3 (Kumar *et al.*, 2006a,b), induction of transcription of COX2 (Dermott *et al.*, 1999; Slice *et al.*, 1999) and Egr-1 (Vara Prasad *et al.*, 1994; Vara Prasad and Dhanasekaran, 1999), and by regulation of transcription from serum response element (SRE) (Fromm *et al.*, 1997). There is some evidence that effector pathways other than Rho GTPases, such as extracellular signal-regulated kinase 5 (ERK5) activation (Fukuhara *et al.*, 2000b), may contribute to the transforming potential of G12 proteins. This is particularly important as Gα12 and Gα13 are more potent stimulators of cellular transformation than overexpressed or mutationally activated RhoA (Fromm *et al.*, 1997). In this regard, activation of Rac is required for Gα13-mediated SRE-dependent gene transcription induced by radixin (Liu and Voyno-Yasenetskaya, 2005). Radixin is involved in cross-linking of the actin cytoskeleton to the plasma membrane, and it has been shown to interact with Gα13 and to play a role in Gα13-induced transformation of Rat-1 fibroblasts (Vaiskunaite *et al.*, 2000). Thus, other G12-effector signalling axes besides the signalling pathways leading to Rho activation may be required for G12-stimulated oncogenic transformation and is an important area for further study.

G12 proteins in cancer invasion and metastasis

G protein-coupled receptors that couple to Gα12/13 as well as downstream effectors of Gα12/13 have been implicated in tumorigenesis and cancer progression (Sahai and Marshall, 2002; Dorsam and Gutkind, 2007). The findings noted above indicating the importance of G12 proteins in cell migration during development, and the fact that G12 protein expression is upregulated in some human cancers, provided hints that the G12 subfamily of heterotrimeric G proteins may have a role in metastasis. Indeed, a direct role of G12 proteins in cancer invasion and metastasis has been recently demonstrated (Kelly *et al.*, 2006a,b). Expression of constitutively activated Gα12 and Gα13, or activation of Gα12/13 signalling by PAR-1 as well as thromboxane A_2 receptor, induces a striking increase in breast and prostate cancer cell invasion *in vitro* (Kelly *et al.*, 2006a,b) (Table 1). Furthermore, blocking downstream signalling via G12 reduces breast cancer metastasis *in vivo* and results in a significant increase in metastasis-free survival of mice (Kelly *et al.*, 2006a). In these studies 4T1 mouse mammary carcinoma cells were employed; when implanted in the mammary fat pad of recipient mice these cells form tumours and metastasize in a manner similar to human breast cancer (Aslakson and Miller, 1992; Smith *et al.*, 2004). Inhibition of G12 signalling in the 4T1 cells through expression of a dominant-negative form of p115RhoGEF reduces the rate of metastatic dissemination of the cells from the primary tumour following their implantation in the mouse mammary fat pad. Interestingly, when the same cells are introduced directly into the bloodstream, inhibition of G12 signalling has no effect on the ability of these cells to metastasize (Kelly *et al.*, 2006a). These findings indicate that G12 signalling is important in the early steps of the metastasis process and appears to promote cancer metastasis by stimulating tumour cell invasion and entry into the bloodstream.

As noted above, many of the effects of G12 proteins on cell growth, transformation and migration are mediated by Rho proteins which have a well-described role in tumorigenesis and metastasis. RhoA and RhoC are overexpressed in several tumours including colon, breast, lung and pancreas (Fritz *et al.*, 1999; Sahai and Marshall, 2002) and their expression levels correlate positively with the progression of the tumour (Suwa *et al.*, 1998; Fritz *et al.*, 1999). Thus, activation of Rho by G12 proteins appears to be a crucial signalling mechanism for regulating cancer metastasis. In fact, it has been demonstrated that G12 signalling leading to invasion in several breast and prostate cancer cell lines requires activation of Rho and its downstream effector ROCK (Kelly *et al.*, 2006a,b; unpublished observations) (see Figure 1). Similarly, G12-mediated migration of ovarian cancer cells also depends on activation of Rho/ROCK signalling (Bian *et al.*, 2006). On the other hand, the G12/13-Rho signalling pathway mediates S1P-induced inhibition of migration of glioblastoma cells (Malchinkhuu *et al.*, 2008), revealing the complexity of cell invasion and migration and the different mechanisms by which Rho can regulate these processes.

The involvement of the two subtypes of G12 proteins in cancer cell invasion may be complicated and cell type-specific. For example, activated Gα12 inhibits, rather than promotes, the invasion of the inflammatory breast cancer cell

line SUM149 (Patrick Kelly, unpublished observations). Also in this regard, a recent study showed that expression of constitutively active Gα13 or activation of G13-coupled receptors by lysophosphatidylcholine inhibits chemokine-stimulated invasion of melanoma cells *in vitro* and impairs their metastasis in mice. This effect of activated Gα13 in melanoma cells appears to be mediated by a reduction in the levels of Rho-GTP due to high p190RhoGAP activity (Bartolome *et al.*, 2008). In addition, G12 signalling independent of Rho activation may also potentially lead to invasion and cancer progression. In the T47D breast cancer cell line, expression of a Gα12 mutant that is uncoupled from Rho-mediated signalling (Meigs *et al.*, 2005) induces a small but significant increase in invasion (Kelly *et al.*, 2006a). This finding suggests that, while Rho activation by G12 proteins is necessary for stimulating cancer cell invasion, other effectors of Gα12/13 may also be required for this function. As mentioned above, Gα12 and Gα13 interact with members of the cadherin superfamily of cell adhesion proteins, most notably E-cadherin, and negatively regulate its adhesive function (Meigs *et al.*, 2002). E-cadherin is required for cells to maintain their epithelial character and several studies have shown that loss of E-cadherin function in cancer is associated with a transition to a more aggressive, mesenchymal phenotype (Conacci-Sorrell *et al.*, 2002; Thiery, 2002). Thus, G12 signalling could promote cancer metastasis by inhibiting E-cadherin function. Taken together, these studies suggest that the G12 proteins possibly regulate cancer cell invasion and migration via several mechanisms.

Gα12 and Gα13 appear to similarly affect the migration and invasion of several cancer cell types (Bian *et al.*, 2006; Kelly *et al.*, 2006a,b; Bartolome *et al.*, 2008; Malchinkhuu *et al.*, 2008), suggesting that they have overlapping functions. For example, the effect of separately blocking signalling via G12 and G13 leads to a similar degree of inhibition of LPA-induced migration of ovarian cancer cells as that observed upon expression of dominant-negative p115RhoGEF that blocks downstream signalling by both G12 proteins. (Bian *et al.*, 2006). The functions of G12 and G13, however, are not completely redundant during embryonic development. Gα13-deficient mice are embryonic lethal at around E10, where as Gα12-deficient mice are apparently normal (Gu *et al.*, 2002). Thus, embryos are able to develop normally in the presence of a full complement of wild-type Gα13 alleles even if both Gα12 alleles are disrupted. Embryos lacking both Gα12 and Gα13 die between E8 and E8.5. Although having a single allele each of Gα12 and Gα13 is sufficient for survival, in the absence of a Gα12 allele one allele of Gα13 is not enough for the embryos to survive beyond E9.5 (Gu *et al.*, 2002). This suggests that there is some functional overlap between Gα12 and Gα13 during early development. This is interesting, both in terms of understanding fully the roles of G12 and G13 signalling in cancer, and for designing drug targets to inhibit metastasis.

Concluding remarks

The G12 subfamily of heterotrimeric G proteins impacts a variety of cellular functions and physiological processes. The dysregulation of some of these processes clearly underlies the pathophysiology of tumour development and progression. The studies highlighted in this review provide compelling evidence that Gα12 and Gα13 play pivotal roles in many aspects of cancer invasion and metastasis. Various downstream effectors that are important in G12/13-induced cell growth and transformation, migration and invasion have been identified. Rho GTPase, in particular, appears to be a principle downstream target of G12/13 signalling leading to these cellular responses, but other effectors of G12 proteins are likely important for many of the consequences of their activation. A complete understanding of the signalling pathways triggered by G12/13 requires the elucidation of specific effectors that directly interact with the Gα proteins and the downstream molecules that these effectors engage. Although Gα12 and Gα13 affect many similar biologies, they may do so via different signalling mechanisms. For example, Hax-1 only interacts with Gα13 and not Gα12, and, as discussed earlier, this interaction is important for Gα13-stimulated migration of fibroblasts (Radhika *et al.*, 2004). In this regard, the identification of mutational variants of Gα12/13 that are selectively uncoupled from specific effectors (Meigs *et al.*, 2005) provides valuable tools to decipher the importance of specific downstream pathways in particular G12-mediated biologies; such information will be required to fully understand the role of G12 proteins in migration and invasion.

Although *in vitro* experiments provide information about the mechanism and the molecular players involved in the various signalling pathways triggered by G12/13, *in vivo* experiments are required for accurate modeling of the impact of activation of G12 proteins on physiological and pathophysiological processes. The importance of such studies has been recently demonstrated with the development of Gα12/13 conditional knockout mice (Ruppel *et al.*, 2005; Moers *et al.*, 2008; Wirth *et al.*, 2008), and with the use of the dominant-negative RhoGEF construct *in vivo* (Kelly *et al.*, 2006a). With these advances in experimental techniques and the application of siRNA technology to G12 signalling (Andreeva *et al.*, 2006; Bartolome *et al.*, 2008), new and more complex functions of G12/13 signalling have begun to emerge. These approaches will also aid in understanding further the role of G12 proteins in oncogenesis and metastasis.

From a clinical perspective, pharmacologic inhibition of G12 signalling could be an effective therapeutic option for controlling cancer metastases. This may be achieved by targeting a specific G12/GPCR interaction in a particular type of cancer, or by inhibiting specific downstream signalling pathway(s) activated by Gα12/13. Therefore, a complete understanding of the biological role of G12 proteins in cell growth, invasion and cancer progression is imperative and will constitute a major aspect of G12 research in the years to come.

References

Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–209.

Althoefer H, Eversole-Cire P, Simon MI (1997). Constitutively active Galphaq and Galpha13 trigger apoptosis through different pathways. *J Biol Chem* **272** (39): 24380–24386.

Andreeva AV, Vaiskunaite R, Kutuzov MA, Profirovic J, Skidgel RA, Voyno-Yasenetskaya T (2006). Novel mechanisms of G protein-dependent regulation of endothelial nitric-oxide synthase. *Mol Pharmacol* **69** (3): 975–982.

Aragay AM, Collins LR, Post GR, Watson AJ, Feramisco JR, Brown JH *et al*. (1995). G12 requirement for thrombin-stimulated gene expression and DNA synthesis in 1321N1 astrocytoma cells. *J Biol Chem* **270** (34): 20073–20077.

Arai K, Maruyama Y, Nishida M, Tanabe S, Takagahara S, Kozasa T *et al*. (2003). Differential requirement of G alpha12, G alpha13, G alphaq, and G beta gamma for endothelin-1-induced c-Jun NH2-terminal kinase and extracellular signal-regulated kinase activation. *Mol Pharmacol* **63** (3): 478–488.

Aslakson CJ, Miller FR (1992). Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res* **52** (6): 1399–1405.

Balkwill F (2004). Cancer and the chemokine network. *Nat Rev Cancer* **4** (7): 540–550.

Barrett K, Leptin M, Settleman J (1997). The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in Drosophila gastrulation. *Cell* **91** (7): 905–915.

Bartolome RA, Wright N, Molina-Ortiz I, Sanchez-Luque FJ, Teixido J (2008). Activated G(alpha)13 impairs cell invasiveness through p190RhoGAP-mediated inhibition of RhoA activity. *Cancer Res* **68** (20): 8221–8230.

Berestetskaya YV, Faure MP, Ichijo H, Voyno-Yasenetskaya TA (1998). Regulation of apoptosis by alpha-subunits of G12 and G13 proteins via apoptosis signal-regulating kinase-1. *J Biol Chem* **273** (43): 27816–27823.

Bian D, Mahanivong C, Yu J, Frisch SM, Pan ZK, Ye RD *et al*. (2006). The G12/13-RhoA signaling pathway contributes to efficient lysophosphatidic acid-stimulated cell migration. *Oncogene* **25** (15): 2234–2244.

Buhl AM, Johnson NL, Dhanasekaran N, Johnson GL (1995). G alpha 12 and G alpha 13 stimulate Rho-dependent stress fiber formation and focal adhesion assembly. *J Biol Chem* **270** (42): 24631–24634.

Chan AM, Fleming TP, McGovern ES, Chedid M, Miki T, Aaronson SA (1993). Expression cDNA cloning of a transforming gene encoding the wild-type G alpha 12 gene product. *Mol Cell Biol* **13** (2): 762–768.

Collins LR, Minden A, Karin M, Brown JH (1996). Galpha12 stimulates c-Jun NH2-terminal kinase through the small G proteins Ras and Rac. *J Biol Chem* **271** (29): 17349–17353.

Conacci-Sorrell M, Zhurinsky J, Ben-Ze'ev A (2002). The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest* **109** (8): 987–991.

Daaka Y (2004). G proteins in cancer: the prostate cancer paradigm. *Sci STKE* **2004** (216): re2.

Dermott JM, Reddy MR, Onesime D, Reddy EP, Dhanasekaran N (1999). Oncogenic mutant of Galpha12 stimulates cell proliferation through cycloxygenase-2 signaling pathway. *Oncogene* **18** (51): 7185–7189.

Dermott JM, Ha JH, Lee CH, Dhanasekaran N (2004). Differential regulation of Jun N-terminal kinase and p38MAP kinase by Galpha12. *Oncogene* **23** (1): 226–232.

Dhanasekaran N, Tsim ST, Dermott JM, Onesime D (1998). Regulation of cell proliferation by G proteins. *Oncogene* **17**: 1383–1394 (11 Reviews).

Diviani D, Soderling J, Scott JD (2001). AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation. *J Biol Chem* **276** (47): 44247–44257.

Dolezalova H, Shankar G, Huang MC, Bikle DD, Goetzl EJ (2003). Biochemical regulation of breast cancer cell expression of S1P2 (Edg-5) and S1P3 (Edg-3) G protein-coupled receptors for sphingosine 1-phosphate. *J Cell Biochem* **88** (4): 732–743.

Dorsam RT, Gutkind JS (2007). G-protein-coupled receptors and cancer. *Nat Rev Cancer* **7** (2): 79–94.

Etienne-Manneville S, Hall A (2002). Rho GTPases in cell biology. *Nature* **420** (6916): 629–635.

Even-Ram S, Uziely B, Cohen P, Grisaru-Granovsky S, Maoz M, Ginzburg Y *et al*. (1998). Thrombin receptor overexpression in malignant and physiological invasion processes. *Nat Med* **4** (8): 909–914.

Ferrara N, Gerber HP, LeCouter J (2003). The biology of VEGF and its receptors. *Nat Med* **9** (6): 669–676.

Fields TA, Casey PJ (1997). Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. *Biochem J* **321** (Pt 3): 561–571.

Friedl P, Wolf K (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* **3** (5): 362–374.

Fritz G, Just I, Kaina B (1999). Rho GTPases are over-expressed in human tumors. *Int J Cancer* **81** (5): 682–687.

Fromm C, Coso OA, Montaner S, Xu N, Gutkind JS (1997). The small GTP-binding protein Rho links G protein-coupled receptors and Galpha12 to the serum response element and to cellular transformation. *Proc Natl Acad Sci USA* **94** (19): 10098–10103.

Fukuhara S, Murga C, Zohar M, Igishi T, Gutkind JS (1999). A novel PDZ domain containing guanine nucleotide exchange factor links heterotrimeric G proteins to Rho. *J Biol Chem* **274** (9): 5868–5879.

Fukuhara S, Chikumi H, Gutkind JS (2000a). Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G(12) family to Rho. *FEBS Lett* **485** (2–3): 183–188.

Fukuhara S, Marinissen MJ, Chiariello M, Gutkind JS (2000b). Signaling from G protein-coupled receptors to ERK5/Big MAPK 1 involves Galpha q and Galpha 12/13 families of heterotrimeric G proteins. Evidence for the existence of a novel Ras AND Rho-independent pathway. *J Biol Chem* **275** (28): 21730–21736.

Gohla A, Harhammer R, Schultz G (1998). The G-protein G13 but not G12 mediates signaling from lysophosphatidic acid receptor via epidermal growth factor receptor to Rho. *J Biol Chem* **273** (8): 4653–4659.

Gohla A, Offermanns S, Wilkie TM, Schultz G (1999). Differential involvement of Galpha12 and Galpha13 in receptor-mediated stress fiber formation. *J Biol Chem* **274** (25): 17901–17907.

Gohla A, Schultz G, Offermanns S (2000). Role for G(12)/G(13) in agonist-induced vascular smooth muscle cell contraction. *Circ Res* **87** (3): 221–227.

Goldsmith ZG, Dhanasekaran DN (2007). G protein regulation of MAPK networks. *Oncogene* **26** (22): 3122–3142.

Goulimari P, Kitzing TM, Knieling H, Brandt DT, Offermanns S, Grosse R (2005). Galpha12/13 is essential for directed cell migration and localized Rho-Dia1 function. *J Biol Chem* **280** (51): 42242–42251.

Goulimari P, Knieling H, Engel U, Grosse R (2008). LARG and mDia1 Link G{alpha}12/13 to Cell Polarity and Microtubule Dynamics. *Mol Biol Cell* **19** (1): 30–40.

Gu JL, Muller S, Mancino V, Offermanns S, Simon MI (2002). Interaction of G alpha(12) with G alpha(13) and G alpha(q) signaling pathways. *Proc Natl Acad Sci USA* **99** (14): 9352–9357.

Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG *et al*. (1998). Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. *Science* **280** (5372): 2112–2114.

Henrikson KP, Salazar SL, Fenton JW II, Pentecost BT (1999). Role of thrombin receptor in breast cancer invasiveness. *Br J Cancer* **79** (3–4): 401–406.

Hersch E, Huang J, Grider JR, Murthy KS (2004). Gq/G13 signaling by ET-1 in smooth muscle: MYPT1 phosphorylation via ETA and CPI-17 dephosphorylation via ETB. *Am J Physiol Cell Physiol* **287** (5): C1209–C1218.

Iguchi T, Sakata K, Yoshizaki K, Tago K, Mizuno N, Itoh H (2008). Orphan G protein-coupled receptor GPR56 regulates neural progenitor cell migration via a G alpha 12/13 and Rho pathway. *J Biol Chem* **283** (21): 14469–14478.

Jechlinger M, Sommer A, Moriggl R, Seither P, Kraut N, Capodiecci P

et al. (2006). Autocrine PDGFR signaling promotes mammary cancer metastasis. *J Clin Invest* **116** (6): 1561–1570.

Jho EH, Davis RJ, Malbon CC (1997). c-Jun amino-terminal kinase is regulated by Galpha12/Galpha13 and obligate for differentiation of P19 embryonal carcinoma cells by retinoic acid. *J Biol Chem* **272** (39): 24468–24474.

Jiang H, Wu D, Simon MI (1993). The transforming activity of activated G alpha 12. *FEBS Lett* **330** (3): 319–322.

Jiang Y, Ma W, Wan Y, Kozasa T, Hattori S, Huang XY (1998). The G protein G alpha12 stimulates Bruton's tyrosine kinase and a rasGAP through a conserved PH/BM domain. *Nature* **395** (6704): 808–813.

Johnson EN, Seasholtz TM, Waheed AA, Kreutz B, Suzuki N, Kozasa T *et al*. (2003). RGS16 inhibits signalling through the G alpha 13-Rho axis. *Nat Cell Biol* **5** (12): 1095–1103.

Karin M, Liu Z, Zandi E (1997). AP-1 function and regulation. *Curr Opin Cell Biol* **9** (2): 240–246.

Kashef K, Lee CM, Ha JH, Reddy EP, Dhanasekaran DN (2005). JNK-interacting leucine zipper protein is a novel scaffolding protein in the Galpha13 signaling pathway. *Biochemistry* **44** (43): 14090–14096.

Kashef K, Xu H, Reddy EP, Dhanasekaran DN (2006). Endodermal differentiation of murine embryonic carcinoma cells by retinoic acid requires JLP, a JNK-scaffolding protein. *J Cell Biochem* **98** (4): 715–722.

Kelly P, Moeller BJ, Juneja J, Booden MA, Der CJ, Daaka Y *et al*. (2006a). The G12 family of heterotrimeric G proteins promotes breast cancer invasion and metastasis. *Proc Natl Acad Sci USA* **103** (21): 8173–8178.

Kelly P, Stemmle LN, Madden JF, Fields TA, Daaka Y, Casey PJ (2006b). A role for the G12 family of heterotrimeric G proteins in prostate cancer invasion. *J Biol Chem* **281** (36): 26483–26490.

Kelly P, Casey PJ, Meigs TE (2007). Biologic functions of the G12 subfamily of heterotrimeric g proteins: growth, migration, and metastasis. *Biochemistry* **46** (23): 6677–6687.

Kitzing TM, Sahadevan AS, Brandt DT, Knieling H, Hannemann S, Fackler OT *et al*. (2007). Positive feedback between Dia1, LARG, and RhoA regulates cell morphology and invasion. *Genes Dev* **21** (12): 1478–1483.

Kozasa T, Jiang X, Hart MJ, Sternweis PM, Singer WD, Gilman AG *et al*. (1998). p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. *Science* **280** (5372): 2109–2111.

Kumar RN, Ha JH, Radhakrishnan R, Dhanasekaran DN (2006a). Transactivation of platelet-derived growth factor receptor alpha by the GTPase-deficient activated mutant of Galpha12. *Mol Cell Biol* **26** (1): 50–62.

Kumar RN, Shore SK, Dhanasekaran N (2006b). Neoplastic transformation by the gep oncogene, Galpha12, involves signaling by STAT3. *Oncogene* **25** (6): 899–906.

Kurose H (2003). Galpha12 and Galpha13 as key regulatory mediator in signal transduction. *Life Sci* **74** (2–3): 155–161.

Kutuzov MA, Andreeva AV, Voyno-Yasenetskaya TA (2007). Regulation of apoptosis signal-regulating kinase 1 degradation by G alpha13. *FASEB J* **21** (13): 3727–3736.

Lee YN, Malbon CC, Wang HY (2004). G alpha 13 signals via p115RhoGEF cascades regulating JNK1 and primitive endoderm formation. *J Biol Chem* **279** (52): 54896–54904.

Li S, Jin Z, Koirala S, Bu L, Xu L, Hynes RO *et al*. (2008). GPR56 regulates pial basement membrane integrity and cortical lamination. *J Neurosci* **28** (22): 5817–5826.

Lin F, Sepich DS, Chen S, Topczewski J, Yin C, Solnica-Krezel L *et al*. (2005). Essential roles of G{alpha}12/13 signaling in distinct cell behaviors driving zebrafish convergence and extension gastrulation movements. *J Cell Biol* **169** (5): 777–787.

Liu G, Voyno-Yasenetskaya TA (2005). Radixin stimulates Rac1 and Ca2+/calmodulin-dependent kinase, CaMKII: cross-talk with Galpha13 signaling. *J Biol Chem* **280** (47): 39042–39049.

McLaughlin JN, Shen L, Holinstat M, Brooks JD, Dibenedetto E, Hamm HE (2005). Functional selectivity of G protein signaling by agonist peptides and thrombin for the protease-activated receptor-1. *J Biol Chem* **280** (26): 25048–25059.

Malchinkhuu E, Sato K, Maehama T, Mogi C, Tomura H, Ishiuchi S *et al*. (2008). S1P(2) receptors mediate inhibition of glioma cell migration through Rho signaling pathways independent of PTEN. *Biochem Biophys Res Commun* **366** (4): 963–968.

Mao J, Xie W, Yuan H, Simon MI, Mano H, Wu D (1998). Tec/Bmx non-receptor tyrosine kinases are involved in regulation of Rho and serum response factor by Galpha12/13. *EMBO J* **17** (19): 5638–5646.

Marinissen MJ, Gutkind JS (2001). G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* **22** (7): 368–376.

Marinissen MJ, Servitja JM, Offermanns S, Simon MI, Gutkind JS (2003). Thrombin protease-activated receptor-1 signals through Gq- and G13-initiated MAPK cascades regulating c-Jun expression to induce cell transformation. *J Biol Chem* **278** (47): 46814–46825.

Martin CB, Mahon GM, Klinger MB, Kay RJ, Symons M, Der CJ *et al*. (2001). The thrombin receptor, PAR-1, causes transformation by activation of Rho-mediated signaling pathways. *Oncogene* **20** (16): 1953–1963.

Maruyama Y, Nishida M, Sugimoto Y, Tanabe S, Turner JH, Kozasa T *et al*. (2002). Galpha(12/13) mediates alpha(1)-adrenergic receptor-induced cardiac hypertrophy. *Circ Res* **91** (10): 961–969.

Meigs TE, Fields TA, McKee DD, Casey PJ (2001). Interaction of Galpha 12 and Galpha 13 with the cytoplasmic domain of cadherin provides a mechanism for beta -catenin release. *Proc Natl Acad Sci USA* **98** (2): 519–524.

Meigs TE, Fedor-Chaiken M, Kaplan DD, Brackenbury R, Casey PJ (2002). Galpha12 and Galpha13 negatively regulate the adhesive functions of cadherin. *J Biol Chem* **277** (27): 24594–24600.

Meigs TE, Juneja J, DeMarco CT, Stemmle LN, Kaplan DD, Casey PJ (2005). Selective uncoupling of G alpha 12 from Rho-mediated signaling. *J Biol Chem* **280** (18): 18049–18055.

Meyer TN, Schwesinger C, Denker BM (2002). Zonula occludens-1 is a scaffolding protein for signaling molecules. Galpha(12) directly binds to the Src homology 3 domain and regulates paracellular permeability in epithelial cells. *J Biol Chem* **277** (28): 24855–24858.

Milligan G, Mullaney I, Mitchell FM (1992). Immunological identification of the alpha subunit of G13, a novel guanine nucleotide binding protein. *FEBS Lett* **297** (1–2): 186–188.

Mills GB, Moolenaar WH (2003). The emerging role of lysophosphatidic acid in cancer. *Nat Rev Cancer* **3** (8): 582–591.

Moers A, Nieswandt B, Massberg S, Wettschureck N, Gruner S, Konrad I *et al*. (2003). G13 is an essential mediator of platelet activation in hemostasis and thrombosis. *Nat Med* **9** (11): 1418–1422.

Moers A, Nurnberg A, Goebbels S, Wettschureck N, Offermanns S (2008). Galpha12/Galpha13 deficiency causes localized overmigration of neurons in the developing cerebral and cerebellar cortices. *Mol Cell Biol* **28** (5): 1480–1488.

Moratz C, Kang VH, Druey KM, Shi CS, Scheschonka A, Murphy PM *et al*. (2000). Regulator of G protein signaling 1 (RGS1) markedly impairs Gi alpha signaling responses of B lymphocytes. *J Immunol* **164** (4): 1829–1838.

Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME *et al*. (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410** (6824): 50–56.

Nagao M, Kaziro Y, Itoh H (1999). The Src family tyrosine kinase is involved in Rho-dependent activation of c-Jun N-terminal kinase by Galpha12. *Oncogene* **18** (31): 4425–4434.

Nikolaidou KK, Barrett K (2004). A Rho GTPase signaling pathway is used reiteratively in epithelial folding and potentially selects the outcome of Rho activation. *Curr Biol* **14** (20): 1822–1826.

Niu J, Vaiskunaite R, Suzuki N, Kozasa T, Carr DW, Dulin N *et al*. (2001). Interaction of heterotrimeric G13 protein with an A-kinase-

anchoring protein 110 (AKAP110) mediates cAMP-independent PKA activation. *Curr Biol* **11** (21): 1686–1690.

Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR *et al*. (2006). Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* **366** (1): 2–16.

Offermanns S (2001). In vivo functions of heterotrimeric G-proteins: studies in Galpha-deficient mice. *Oncogene* **20** (13): 1635–1642.

Offermanns S, Mancino V, Revel JP, Simon MI (1997). Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency. *Science* **275** (5299): 533–536.

Oldham WM, Hamm HE (2007). How do receptors activate G proteins? *Adv Protein Chem* **74**: 67–93.

Parks S, Wieschaus E (1991). The Drosophila gastrulation gene concertina encodes a G alpha-like protein. *Cell* **64** (2): 447–458.

Pierce KL, Premont RT, Lefkowitz RJ (2002). Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* **3** (9): 639–650.

Radhika V, Dhanasekaran N (2001). Transforming G proteins. *Oncogene* **20** (13): 1607–1614.

Radhika V, Hee Ha J, Jayaraman M, Tsim ST, Dhanasekaran N (2005). Mitogenic signaling by lysophosphatidic acid (LPA) involves Galpha12. *Oncogene* **24** (28): 4597–4603.

Radhika V, Onesime D, Ha JH, Dhanasekaran N (2004). Galpha13 stimulates cell migration through cortactin-interacting protein Hax-1. *J Biol Chem* **279** (47): 49406–49413.

Raftopoulou M, Hall A (2004). Cell migration: Rho GTPases lead the way. *Dev Biol* **265** (1): 23–32.

Rieken S, Sassmann A, Herroeder S, Wallenwein B, Moers A, Offermanns S *et al*. (2006). G12/G13 family G proteins regulate marginal zone B cell maturation, migration, and polarization. *J Immunol* **177** (5): 2985–2993.

Riobo NA, Manning DR (2005). Receptors coupled to heterotrimeric G proteins of the G12 family. *Trends Pharmacol Sci* **26** (3): 146–154.

Rohrer DK, Kobilka BK (1998). G protein-coupled receptors: functional and mechanistic insights through altered gene expression. *Physiol Rev* **78** (1): 35–52.

Ruppel KM, Willison D, Kataoka H, Wang A, Zheng YW, Cornelissen I *et al*. (2005). Essential role for Galpha13 in endothelial cells during embryonic development. *Proc Natl Acad Sci USA* **102** (23): 8281–8286.

Sabath E, Negoro H, Beaudry S, Paniagua M, Angelow S, Shah J *et al*. (2008). Galpha12 regulates protein interactions within the MDCK cell tight junction and inhibits tight-junction assembly. *J Cell Sci* **121** (Pt 6): 814–824.

Sahai E, Marshall CJ (2002). RHO-GTPases and cancer. *Nat Rev Cancer* **2** (2): 133–142.

Shan D, Chen L, Wang D, Tan YC, Gu JL, Huang XY (2006). The G protein G alpha(13) is required for growth factor-induced cell migration. *Dev Cell* **10** (6): 707–718.

Shi CS, Sinnarajah S, Cho H, Kozasa T, Kehrl JH (2000). G13alpha-mediated PYK2 activation. PYK2 is a mediator of G13alpha -induced serum response element-dependent transcription. *J Biol Chem* **275** (32): 24470–24476.

Shi X, Gangadharan B, Brass LF, Ruf W, Mueller BM (2004). Protease-activated receptors (PAR1 and PAR2) contribute to tumor cell motility and metastasis. *Mol Cancer Res* **2** (7): 395–402.

Slice LW, Walsh JH, Rozengurt E (1999). Galpha(13) stimulates Rho-dependent activation of the cyclooxygenase-2 promoter. *J Biol Chem* **274** (39): 27562–27566.

Smith MC, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnica-Worms D *et al*. (2004). CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res* **64** (23): 8604–8612.

Solnica-Krezel L (2005). Conserved patterns of cell movements during vertebrate gastrulation. *Curr Biol* **15** (6): R213–R228.

Spicher K, Kalkbrenner F, Zobel A, Harhammer R, Nurnberg B, Soling A *et al*. (1994). G12 and G13 alpha-subunits are immunochemically detectable in most membranes of various mammalian cells and tissues. *Biochem Biophys Res Commun* **198** (3): 906–914.

Stemmle LN, Fields TA, Casey PJ (2006). The regulator of G protein signaling domain of axin selectively interacts with Galpha12 but not Galpha13. *Mol Pharmacol* **70** (4): 1461–1468.

Suwa H, Ohshio G, Imamura T, Watanabe G, Arii S, Imamura M *et al*. (1998). Overexpression of the rhoC gene correlates with progression of ductal adenocarcinoma of the pancreas. *Br J Cancer* **77** (1): 147–152.

Takashima S, Sugimoto N, Takuwa N, Okamoto Y, Yoshioka K, Takamura M *et al*. (2008). G12/13 and Gq mediate S1P2-induced inhibition of Rac and migration in vascular smooth muscle in a manner dependent on Rho but not Rho kinase. *Cardiovasc Res* **79** (4): 689–697.

Tan W, Martin D, Gutkind JS (2006). The Galpha13-Rho signaling axis is required for SDF-1-induced migration through CXCR4. *J Biol Chem* **281** (51): 39542–39549.

Thiery JP (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* **2** (6): 442–454.

Vaiskunaite R, Adarichev V, Furthmayr H, Kozasa T, Gudkov A, Voyno-Yasenetskaya TA (2000). Conformational activation of radixin by G13 protein alpha subunit. *J Biol Chem* **275** (34): 26206–26212.

Vaiskunaite R, Kozasa T, Voyno-Yasenetskaya TA (2001). Interaction between the G alpha subunit of heterotrimeric G(12) protein and Hsp90 is required for G alpha(12) signaling. *J Biol Chem* **276** (49): 46088–46093.

Van Haastert PJ, Devreotes PN (2004). Chemotaxis: signalling the way forward. *Nat Rev Mol Cell Biol* **5** (8): 626–634.

Vara Prasad MV, Dhanasekaran N (1999). GTPase deficient mutant of G(alpha13) regulates the expression of Egr-1 through the small GTPase Rho. *Oncogene* **18** (8): 1639–1642.

Vara Prasad MV, Shore SK, Dhanasekaran N (1994). Activated mutant of G alpha 13 induces Egr-1, c-fos, and transformation in NIH 3T3 cells. *Oncogene* **9** (8): 2425–2429.

Voyno-Yasenetskaya TA, Pace AM, Bourne HR (1994). Mutant alpha subunits of G12 and G13 proteins induce neoplastic transformation of Rat-1 fibroblasts. *Oncogene* **9** (9): 2559–2565.

Voyno-Yasenetskaya TA, Faure MP, Ahn NG, Bourne HR (1996). Galpha12 and Galpha13 regulate extracellular signal-regulated kinase and c-Jun kinase pathways by different mechanisms in COS-7 cells. *J Biol Chem* **271** (35): 21081–21087.

Wirth A, Benyo Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S *et al*. (2008). G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med* **14** (1): 64–68.

Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, Kelly K *et al*. (2003). Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* **114** (2): 201–214.

Xu N, Bradley L, Ambdukar I, Gutkind JS (1993). A mutant alpha subunit of G12 potentiates the eicosanoid pathway and is highly oncogenic in NIH 3T3 cells. *Proc Natl Acad Sci USA* **90** (14): 6741–6745.

Yamaguchi Y, Katoh H, Mori K, Negishi M (2002). Galpha(12) and Galpha(13) interact with Ser/Thr protein phosphatase type 5 and stimulate its phosphatase activity. *Curr Biol* **12** (15): 1353–1358.

Yanamadala V, Negoro H, Gunaratnam L, Kong T, Denker BM (2007). Galpha12 stimulates apoptosis in epithelial cells through JNK1-mediated Bcl-2 degradation and up-regulation of IkappaBalpha. *J Biol Chem* **282** (33): 24352–24363.

Zhu D, Kosik KS, Meigs TE, Yanamadala V, Denker BM (2004). Galpha12 directly interacts with PP2A: evidence FOR Galpha12-stimulated PP2A phosphatase activity and dephosphorylation of microtubule-associated protein, tau. *J Biol Chem* **279** (53): 54983–54986.

Zhu D, Tate RI, Ruediger R, Meigs TE, Denker BM (2007). Domains necessary for Galpha12 binding and stimulation of protein phosphatase-2A (PP2A): Is Galpha12 a novel regulatory subunit of PP2A? *Mol Pharmacol* **71** (5): 1268–1276.