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REVIEW

The complex G protein-coupled receptor kinase 2 (GRK2) interactome unveils new physiopathological targets

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GRK2 is a ubiquitous member of the G protein-coupled receptor kinase (GRK) family that appears to play a central, integrative role in signal transduction cascades. GRKs participate together with arrestins in the regulation of G protein-coupled receptors (GPCR), a family of hundreds of membrane proteins of key physiological and pharmacological importance, by triggering receptor desensitization from G proteins and GPCR internalization, and also by helping assemble macromolecular signalosomes in the receptor environment acting as agonist-regulated adaptor scaffolds, thus contributing to signal propagation. In addition, emerging evidence indicates that GRK2 can phosphorylate a growing number of non-GPCR substrates and associate with a variety of proteins related to signal transduction, thus suggesting that this kinase could also have diverse 'effector' functions. We discuss herein the increasing complexity of such GRK2 'interactome', with emphasis on the recently reported roles of this kinase in cell migration and cell cycle progression and on the functional impact of the altered GRK2 levels observed in several relevant cardiovascular, inflammatory or tumour pathologies. Deciphering how the different networks of potential GRK2 functional interactions are orchestrated in a stimulus, cell type or context-specific way is critical to unveil the contribution of GRK2 to basic cellular processes, to understand how alterations in GRK2 levels or functionality may participate in the onset or development of several cardiovascular, tumour or inflammatory diseases, and to assess the feasibility of new therapeutic strategies based on the modulation of the activity, levels or specific interactions of GRK2.
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Abbreviations: CDK, cyclin-dependent kinase; GIT, GRK-interacting protein; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GRK2ct, GRK2 C-terminal fragment; HF, heart failure

Introduction

G protein-coupled receptor kinases (GRKs) were initially identified as serine/threonine kinases that participate together with arrestins in the regulation of multiple GPCR. The GRKs constitute a group of protein kinases (seven members in mammals) that specifically recognize and phosphorylate agonist-activated GPCRs (Penela *et al.*, 2003; Premont and Gainetdinov, 2007). Arrestins then bind to the phosphorylated receptor, leading to uncoupling from heterotrimeric G proteins and receptor desensitization. As a result of β-arrestin binding, phosphorylated receptors are also targeted for clathrin-mediated endocytosis, a process that classically serves to re-sensitize and recycle receptors back to the plasma membrane (Reiter and Lefkowitz, 2006; Moore *et al.*, 2007).

Besides such 'negative', inhibitory role of GRKs/arrestins in GPCR signalling, recent and emerging evidence indicate that both GRKs and arrestins are able to interact with a variety of cellular proteins involved in signal transduction, thus contributing to signal propagation at defined cellular locations upon GPCR activation. In the case of β-arrestins, several laboratories have shown that these proteins can act as scaffold molecules that bring different signalling molecules into the receptor complex, such as c-Src, JNK-3, components of the Raf/MEK/ERK cascade, the cAMP phosphodiesterase PDE4, the Ral-GDS regulator of the cytoskeleton, components of the NfκB signalling pathway or the Mdm2 ubiquitin ligase, among others [reviewed in (Reiter and Lefkowitz, 2006; DeWire *et al.*, 2007; Premont and Gainetdinov, 2007)].

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Therefore, GRK-mediated arrestin recruitment is critical for triggering the modulation of important intracellular signalling cascades by GPCR, contributing to the overall cellular response to the presence of an agonist.

It should be stressed, however, that GRK's cellular role is not limited to promoting β-arrestin binding to activated GPCRs. GRKs are multidomain proteins with diverse cellular functions. In particular, the ubiquitous and prototypic GRK isoform, GRK2, is emerging as a key node in signal transduction pathways, displaying a very complex interactome, as we will discuss below. Moreover, the expression and function of GRK2 is tightly regulated and its levels and functionality altered in several pathological situations, thus suggesting that these changes may contribute to the onset or development of these pathologies (Penela *et al*., 2003; 2006; Premont and Gainetdinov, 2007; Ribas *et al*., 2007).

GRK2 is a multi-domain protein

GRK family members can be subdivided into three main groups based on sequence homology: visual GRK subfamily (GRK1 and GRK7), the β-adrenergic receptor kinase subfamily (GRK2/GRK3) and the GRK4 subfamily (GRK4, GRK5 and GRK6). GRK2, 3, 5 and 6 are ubiquitously expressed in mammalian tissues.

GRKs share a common structural architecture with a well-conserved, central catalytic domain (~270 aa), similar to that of other serine-threonine kinases, flanked by an N-terminal domain (~185 aa) and a variable-length carboxyl-terminal domain (~105–230 aa). The N-terminal domain has been proposed to be important for receptor recognition, for intracellular membrane anchoring and also contains an RH domain (regulator of G protein signalling homology domain) of ~120 aa. In the case of GRK2 and GRK3, the RH domain has been shown to specifically interact with Gαq family members, thus blocking its interaction with their effector, phospholipase C beta (PLCβ). The C-terminal region of GRK2 contains a pleckstrin homology domain (PH) with binding sites for the membrane phospholipid PIP2 and free Gβγ subunits and therefore is involved in its agonist-dependent translocation to the plasma membrane (Penela *et al*., 2003; Ribas *et al*., 2007).

The crystal structure of GRK2 in complex with G protein β1γ2 subunits provided new insights into GRK regulation, placing its three distinct regions (RH, kinase and PH domains) at the vertices of a triangle, an excellent example of how multiple modular domains are integrated in a single molecule to transduce and modulate signalling events (Lodowski *et al*., 2003). The crystal structure of GRK2 in complex with Gαq has also been solved. Residues in Gαq involved in its interaction with GRK2 are analogous to those implicated in association to its classical effector PLCβ, indicating that the GRK2 RH domain binds Gαq in a manner more similar to an effector-like interaction than an RGS-like one and making it possible to suggest a role for GRK2 as an effector of Gαq signalling-mediated pathways (Tesmer *et al*., 2005).

In addition to this general domain architecture, ongoing research is unveiling the localization of regions involved in interaction with different cellular proteins and of regulatory phosphorylation sites in the GRK2 structure [reviewed in (Penela *et al*., 2003; Ribas *et al*., 2007), also see below].

GRK2 phosphoryates non-GPCR substrates and displays a complex network of functional interactions

Recent data indicate that arrestins and GRKs can also regulate signalling mediated by other membrane receptor families, such as tyrosine kinase receptors for IGF-1, Insulin, PDGF or EGF (Hupfeld and Olefsky, 2007; Cipolletta *et al*., 2009). GRK2 phosphorylates PDGF-Rβ and reduces receptor activity without altering its down-regulation (Hildreth *et al*., 2004). Activation of EGF receptors promotes the recruitment of GRK2 and subsequent phosphorylation at tyrosine residues of GRK2 itself, increasing its catalytic activity and inducing opioid receptor transregulation (Chen *et al*., 2008).

In addition, a growing number of non-GPCR, non-plasma membrane receptor substrates are being identified for GRKs, particularly GRK2. These include tubulin, synucleins, phosducin, ribosomal protein P2, the inhibitory γ subunit of the type 6 retinal cyclic guanosine monophosphate (cGMP) phosphodiesterase, a subunit of the epithelial Na+ channel, the ERM family protein ezrin, the calcium-binding protein DREAM, IKappaBalpha or the p38 MAPK (Peregrin *et al*., 2006; Ribas *et al*., 2007; Patial *et al*., 2009) and references therein]. GRK2 also inhibits TGF-beta-mediated cell growth arrest and apoptosis by inducing Smad phosphorylation (Ho *et al*., 2005). Overall, these data suggest that GRK2 may act as an 'effector', participating in the regulation of diverse cellular phenomena through the phosphorylation of substrates that are very varied functionally (Figure 1).

Besides such phosphorylation-dependent processes, GRK2 may also contribute to modulate cellular responses in a phosphorylation-independent manner thanks to its ability to interact with a plethora of proteins involved in signalling and trafficking. In addition to the specific interaction with Gαq and Gβγ subunits discussed above, that may help to recruit GRK2 to the membrane and also block the interaction of these G protein subunits with cellular effectors, this kinase has been reported to associate with PI3K, clathrin, GIT, caveolin, MEK, AKT, and RKIP [see Ferguson (2007) and Ribas *et al*. (2007) for reviews and below]. Very recent reports have identified novel interactions of GRK2 with the RalA GTPase in HEK293 cells (Aziziyeh *et al*., 2009) and with the APC protein in osteoblasts (Wang *et al*., 2009)]. For instance, the interaction of GRK2 with PI3Kgamma has been proposed to facilitate PI3K recruitment to the membrane upon agonist stimulation, thus contributing to receptor endocytosis and desensitization (Naga Prasad *et al*., 2002). The GRK2/MEK association seems to be important in the control of chemokine induction of MAPK activation (Jimenez-Sainz *et al*., 2006). On the other hand, we have recently found that the interaction of GRK2 with GIT1, a scaffold protein involved in multiple cellular processes as cytoskeletal dynamics, membrane trafficking or cell adhesion is implicated in the modulation of cell migration in epithelial cells, as we will discuss below (Penela *et al*., 2008).

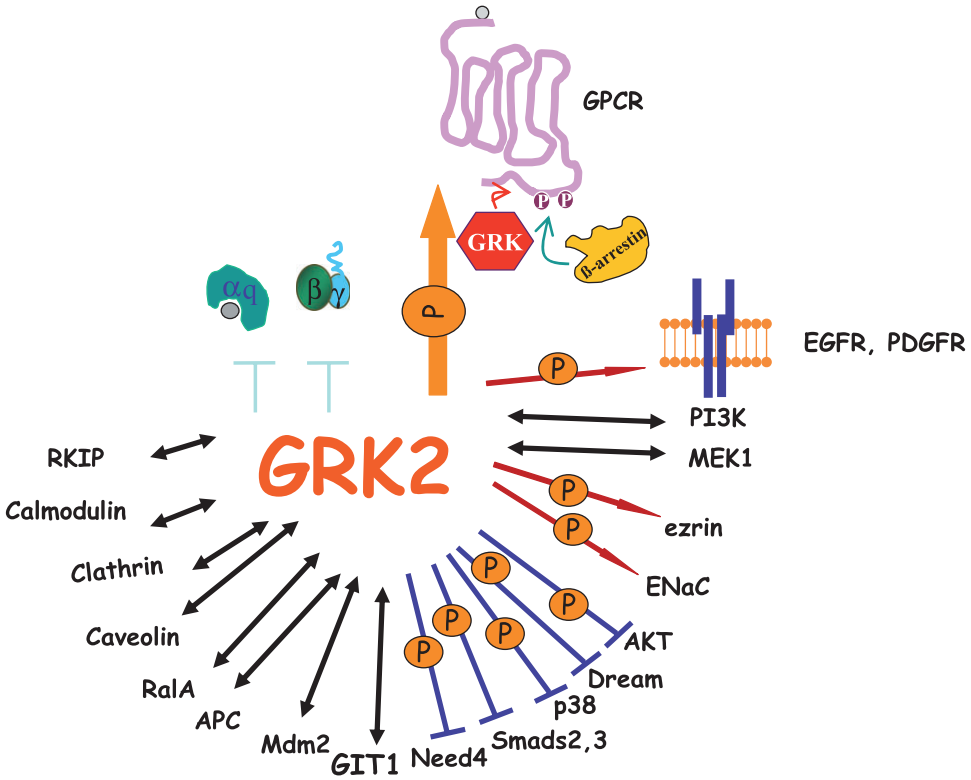

Figure 1 The complex GRK2 interactome. In addition to its 'classical' role triggering GPCR phosphorylation and β-arrestin binding, GRK2 can modulate cell signalling by interacting with Gαq and Gβγ subunits. Furthermore, emerging evidence indicate that GRK2 phosphoryates diverse non-GPCR substrates and displays a complex network of functional interactions with proteins involved in signal transduction. The P symbol denotes that GRK2 has been shown to phosphorylate the indicated proteins. The molecular target nomenclature in text and figure legends conforms to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al.*, 2008). See text for details. GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase.

Other functional interactions have been shown to be involved in the regulation of GRK expression levels, localization, and activity (Penela *et al.*, 2003; Reiter and Lefkowitz, 2006; Premont and Gainetdinov, 2007). The association of GRK2 with α-actinin, clathrin, calmodulin, caveolin or RKIP appear to participate in controlling GRK2 activity and in determining the complex subcellular distribution of the kinase.

Phosphorylation by different kinases has been shown to either enhance (PKA, PKC, Src) or decrease (ERK) membrane targeting and/or the catalytic activity of GRK2 (Sarnago *et al.*, 1999; Elorza *et al.*, 2000; Penela *et al.*, 2003), thus opening the possibility of transmodulation by different signalling pathways. A recent work has put forward S-nitrosylation of GRK2 as a novel mechanism to inhibit its activity (Whalen *et al.*, 2007). Phosphorylation of GRK2 at given tyrosine or serine residues is also emerging as a key mechanism to dynamically modulate its interaction with cellular partners. Tyrosine phosphorylation by c-Src appears to enhance the interaction of GRK2 with Gαq (Ribas *et al.*, 2007) and with the GIT1 scaffold protein. On the other hand, ERK1/2 phosphorylates GRK2 on S670, strongly impairing the GRK2/Gβγ interaction (Pitcher *et al.*, 1999) and inhibiting kinase translocation and catalytic activity towards receptor membrane substrates, while also modulating GRK2 interaction with GIT1 [(Penela *et al.*, 2008) see also below].

Regarding the regulation of GRK2 expression little has been reported about the mechanisms governing GRK transcription. In aortic smooth muscle cells, we found that agents that induce physiological vasoconstriction and hypertrophy markedly enhance GRK2 promoter activity, whereas pro-inflammatory cytokines promote the opposite effect, suggesting that the expression of GRK2 is strongly controlled at the transcriptional level by the interplay between various signal transduction pathways (Ramos-Ruiz *et al.*, 2000). TGF-β induces GRK2 expression in hepatocarcinoma cells (Ho *et al.*, 2005) and in vascular smooth muscle cells (Guo *et al.*, 2009). However, whether these mechanisms apply to other cell types awaits further investigation.

Regulation of GRK2 stability may provide an important mechanism for modulating its expression levels. We have shown that GRK2 is rapidly degraded by the proteasome pathway, and that GRK2 ubiquitination and turnover is enhanced by β_2AR activation as a result of phosphorylation of GRK2 by c-Src and MAPK in a β-arrestin-dependent manner (Penela *et al.*, 1998; 2001; Elorza *et al.* 2003). More recently, we have shown that Mdm2, an E3-ubiquitin ligase involved in the control of cell growth and apoptosis, plays a key role in GRK2 degradation (Salcedo *et al.*, 2006). Mdm2 and GRK2 association and subsequent proteolysis are facilitated by the β-arrestin scaffold function upon β_2-adrenergic receptor stimulation. On the contrary, activation of the PI3K/Akt pathway by agonists such as IGF-1 alters Mdm2 phosphorylation and triggers its nuclear localization thus hampering Mdm2-mediated GRK2 degradation and leading to enhanced GRK2 stability and increased kinase levels (Salcedo *et al.*,

2006). It is tempting to suggest that deregulated activity of the PI3K/Akt pathway in pathophysiological contexts characterized by increased cell proliferation and survival would lead to GRK2 up-regulation in human tumour malignancies (Metaye *et al*., 2005), and we are currently very actively investigating this possibility.

This complex GRK2 'interactome' puts forward that this kinase lies at the crossroads of multiple signalling pathways. However, there are a number of important questions that remain to be addressed. How are such interactions modulated by extracellular signals? Which of them are relevant in physiological and pathological situations characterized by changes in GRK2 expression levels and/or functionality? Are there new interactions of GRK2 that underlie its participation in key biological functions? In this context, we will focus on the recently reported roles of GRK2 in cell migration and cell cycle progression and discuss the functional impact of the altered GRK2 levels observed in several relevant cardiovascular, inflammatory or tumour pathologies.

GRK2 in cardiovascular cells: functional consequences

GRK2 plays a key role in the control of beta-adrenergic signalling in the heart. Hemizygous GRK2 mice expressing 50% less protein than control littermates are hyper-responsive to catecholamines and present increased cardiac contractility and function. The opposite happens with transgenic mice overexpressing different levels of this kinase (Koch *et al*., 1998) in which the adrenergic cardiac response is impaired. The relationship between increased GRK2 protein levels and end-stage heart failure (HF) has been established in animal models and in patients afflicted with different heart conditions. Increased left ventricular GRK2 mRNA and activity were reported in patients with ischemic or idiopathic dilated cardiomyopathy, cardiac ischemia, volume overload and left ventricular hypertrophy (Penela *et al*., 2006; Premont and Gainetdinov, 2007). Interestingly, in some animal models the development of overt HF is preceded by an elevation of GRK2 levels that correlates with progressive impaired myocardial contractility and β-adrenergic receptor (βAR) responsiveness [reviewed in (Penela *et al*., 2006)]. More interestingly, GRK2 levels are elevated post-infarction only in animals that develop heart failure (Theilade *et al*., 2003). The levels and/or activity of GRK2 are also elevated in lymphocytes of patients with cardiac failure (Iaccarino *et al*., 2005). This has lead to the possibility that lymphocyte GRK2 could be used as surrogate readout of myocardial levels of GRK2.

The presence of beta-agonists has been reported to up-regulate GRK2 mRNA, whereas ischemia might promote GRK2 degradation by the proteasome in some experimental models [reviewed in Penela *et al*. (2006)]. However, there is limited knowledge of the mechanisms modulating GRK2 expression in cardiovascular cells in pathological settings.

Although the issue of whether elevated GRK2 expression is a precipitating factor for congestive heart failure or an initial adaptive process that eventually turns pathologic has not been solved, the available data underline the importance of GRK2 levels as a marker of predisposition to cardiac dysfunction and supports the idea that GRK2 offers a potential therapeutic target. Several mouse models seem to support this concept. The inhibition of GRK2 activity by overexpression of GRK2ct (also termed βARKct), a construct encompassing the Gβγ binding domain of GRK2 that inhibits endogenous GRK2 by competing with the kinase for Gβγ-mediated membrane translocation, has provided a successful approach for restoring cardiac function in animals with HF. GRK2ct transgenic animals show enhanced basal and isoproterenol-stimulated contractility, suggesting that the level of GRK2 activity directly modulates βAR-dependent cardiac responses *in vivo* (Rockman *et al*., 1998). Moreover, overexpression of GRK2ct rescues cardiac dysfunction and improves survival in many different mouse models of heart failure, although it does not prevent cardiac deterioration in Gαq or dominant-negative CREB transgenic mice despite the fact that normal βAR signalling was restored [reviewed in (Penela *et al*., 2006)].

These results indicate that the potential benefits of gene therapy based on GRK2ct delivery would depend on the aetiology of the heart failure, and suggest that GRK2ct effects go beyond the modulation of the β1AR/adenylyl cyclase signalling module. Indeed, chronic adrenergic activation appears to be more detrimental than beneficial for heart disease: increased adrenergic activation is a direct cause of cardiomyopathy; chronic elevations in adrenergic signals achieved by transgenic overexpression of beta1-receptors, Gαs proteins or adenylyl cyclase, or by means of chronic agonist treatment in mice evolve to cardiac dysfunction; genetic polymorphisms that enhance sympathetic tone are independent risk factors for HF; and, most importantly, β-blockers represent a successful standard treatment for this disease (Lymperopoulos *et al*., 2007; Dorn, 2009b). It should be noted that over-expression of the GRK2ct peptide may also affect endogenous GRK2 activity towards other GPCR different from β1-adrenergic in the heart, and this construct could also titrate Gβγ dimers from different signalling mediators and thus down-regulate other signalling routes. The beneficial effects of GRK2ct might be also related to its ability to disrupt the interaction of GRK2 with some of its partners such as RKIP, PI3K/Akt, tubulin, ezrin or GIT1. Finally, GRK2 inhibition may favour recently identified alternative cardio-protective signalling pathways that may help improve cardiovascular function and survival outcomes (Noma *et al*., 2007). These pathways have been shown to be more dependent on the arrestin-mediated branch downstream of GPCRs. GRK2 inhibition could lead to a 'signalling switch' that would selectively increase signalling to certain cardioprotective routes downstream of GPCRs what could compensate the cardiotoxic effects of the concomitant over-activation of second messenger-dependent pathways.

Recent experiments using GRK2-deficient mice have also shed new light on the role of this kinase in the heart (Dorn, 2009a). GRK2-deficient mice are embryonic lethal and display a marked cardiac hypoplasia (Jaber *et al*., 1996). This observation initially suggested that GRK2 was required for the proper development and maintenance of heart structure. However, mice with specific ablation of GRK2 in cardiomyocytic cells are viable (Matkovich *et al*., 2006). This result unmasks the fact that it is not myocardial GRK2 protein, but rather an extra-cardiac effect of GRK2 deficiency what accounts for a

hampered cardiac development. These cardiomyocyte-deficient GRK2 mice are, however, more prone to cardiac damage induced by adrenergic stimulation when adult, arguing against a beneficial role of decreased GRK2 in cardiac dysfunction and adding to the controversy of whether GRK2 inhibition could be beneficial or harmful in HF. A subsequent report has succeeded in shedding some light on this issue. By developing mice with an inducible GRK2-deficiency in cardiomyocytes, Raake *et al*. have been able to elegantly demonstrate that deleting GRK2 either prior to or 10 days after myocardial infarction impedes HF and improves survival (Raake *et al*., 2008). This report clearly demonstrates a causal role for GRK2 in cardiac remodelling and dysfunction, and identifies this kinase as a target for therapeutic intervention. Moreover, the same group further established that long-term silencing GRK2 by means of adeno-associated viruses injected in mouse myocardium increases contractility, prevents remodelling, and, more importantly, halts the neurohumoral vicious circle of increased catecholamines and aldosterone characteristic of HF (Rengo *et al*., 2009).

It should also be noted that, in addition to modulating β-adrenergic receptors in the heart, α2-adrenergic receptors, which negatively regulate the release of catecholamines by the adrenal gland, are also desensitized by GRK2. As adrenal GRK2 is also up-regulated in heart failure, it would trigger a catecholamine overdrive, which *per se* is detrimental for cardiac function in the long term. Therefore, the pathological effects of GRK2 in heart failure might rely on locally impaired contractility as well as on unbalanced systemic homeostasis due to excessive desensitization of neuro-humoral receptors. Interestingly, a recent report has demonstrated the efficacy of inhibiting GRK2 by expressing GRK2ct in rat adrenal glands using adenoviral vectors (Lymperopoulos *et al*., 2007; Dorn, 2009a). This strategy restores α2-receptor signalling, decreases circulating catecholamine levels, and improves cardiac function after myocardial infarction. Overall, these data support GRK2 inhibition as an integrated approach to therapies that target catecholaminergic pathways in heart failure.

In addition to HF, several clinical manifestations of hypertension have been associated to increased GRK2 expression. GRK2 is selectively up-regulated in lymphocytes from young borderline hypertensive patients while remained unaltered in normotensive subjects (Gros *et al*., 1997; Cohn *et al*., 2009). These changes paralleled impaired βAR responsiveness, key to vasodilatation responses. One of the caveats of this study was whether the changes observed in lymphocytes accurately mirrored those occurring in the vasculature. This fact was confirmed in spontaneously hypertensive rats and in salt-sensitive hypertensive Dahl rats, identifying GRK2 as a point of convergence in hypertension of different aetiologies (Gros *et al*., 2000). Elevated GRK2 expression in the vasculature is not only a mere marker of the hypertensive state but rather a precipitating factor of hypertension. Transgenic mice with vascular smooth muscle-targeted overexpression of GRK2 display attenuated vascular βAR signalling and vasodilatation while α-adrenergic-mediated vasoconstriction remains unaltered. This leads to elevation of resting blood pressure and vascular remodelling (Eckhart *et al*., 2002). Inhibition of vascular smooth muscle GRK2 by cell-specific gene ablation also enhances α1D-adrenergic receptor constriction (Cohn *et al*., 2008). Recently, both endothelin-1 receptor signalling and endothelial eNOS production, which are instrumental for the maintenance of portal tension, have been shown to be impaired by elevated GRK2 levels (Liu *et al*., 2005). This new role of endothelial GRK2 in vascular homeostasis could be relevant in human primary hypertension, but whether GRK2 up-regulation in this condition is confined to vascular smooth muscle cells or also extended to the endothelium remains to be established.

Overall, these studies suggest that enhanced vascular GRK2 might be an important contributor to the pathogenesis/maintenance of human essential hypertension. Interestingly, it has been reported that GRK2 positively modulates the functionality of the epithelial Na+ channels (ENaC), present in diverse salt-scavenging epithelia, the hyperactivity of which is directly related to the development of hypertension (Dinudom *et al*., 2004).

GRK2 interactome in epithelial and immune cell migration: physiopathological implications

Signal-directed migration requires a spatio-temporal integration of information arising from mechanical cues and from diffusible molecules including growth factors and molecules acting through GPCR such as chemokines and bioactive lipids like sphingosine-1-phosphate (S1P). Failures in this process might result in aberrant migration leading to chronic inflammatory disorders, tumour invasion and metastasis, impaired wound healing or other diseases (Ridley *et al*., 2003). In this regard, in addition to participate in the regulation and signalling of a variety of GPCR related to migration processes, GRK2 has been shown to interact with a variety of proteins involved in migration such as MEK, Akt, ezrin, PI3Kγ or GIT. Consistently, recent evidence suggests that GRK2 plays an important, complex role in epithelial and immune cell migration.

GRK2 is highly expressed in different cellular types of the immune system and emerges as an important regulator of cell responses during inflammation (Vroon *et al*., 2006). GRK2 phosphorylates manifold chemokine receptors such as CCR5, CCR2b, CXCR4, CXCR2 and chemotactic receptors for substance P, S1P or formyl-peptide, responsible for leukocyte trafficking to the inflammatory foci, T cell egression from lymphoid organs, leukocyte activation or proliferation (Vroon *et al*., 2006). According to the classical, 'negative' role of GRK2 in GPCR modulation, it was hypothesized that GRK2 levels would be important in determining the rate and extent of GPCR desensitization at the leading edge, where high concentrations of chemokines and chemokine receptors are present. Consistently, splenocytes and T lymphocytes isolated from GRK2+/– mice display increased agonist-induced activation of ERK and PI3K/Akt pathways (Vroon *et al*., 2004) and increased migration when compared with wild-type littermates in response to certain chemokines (CCL5, CXCL12). In agreement with these data, it has been described that the GRK2 and GRK5 transcriptional down-regulation caused by activation of the Toll-like receptor (TLR)-4 pathway lowers chemokine receptor desensitization augmenting the migratory response of polymorphonuclear leukocytes (PMNs) (Fan

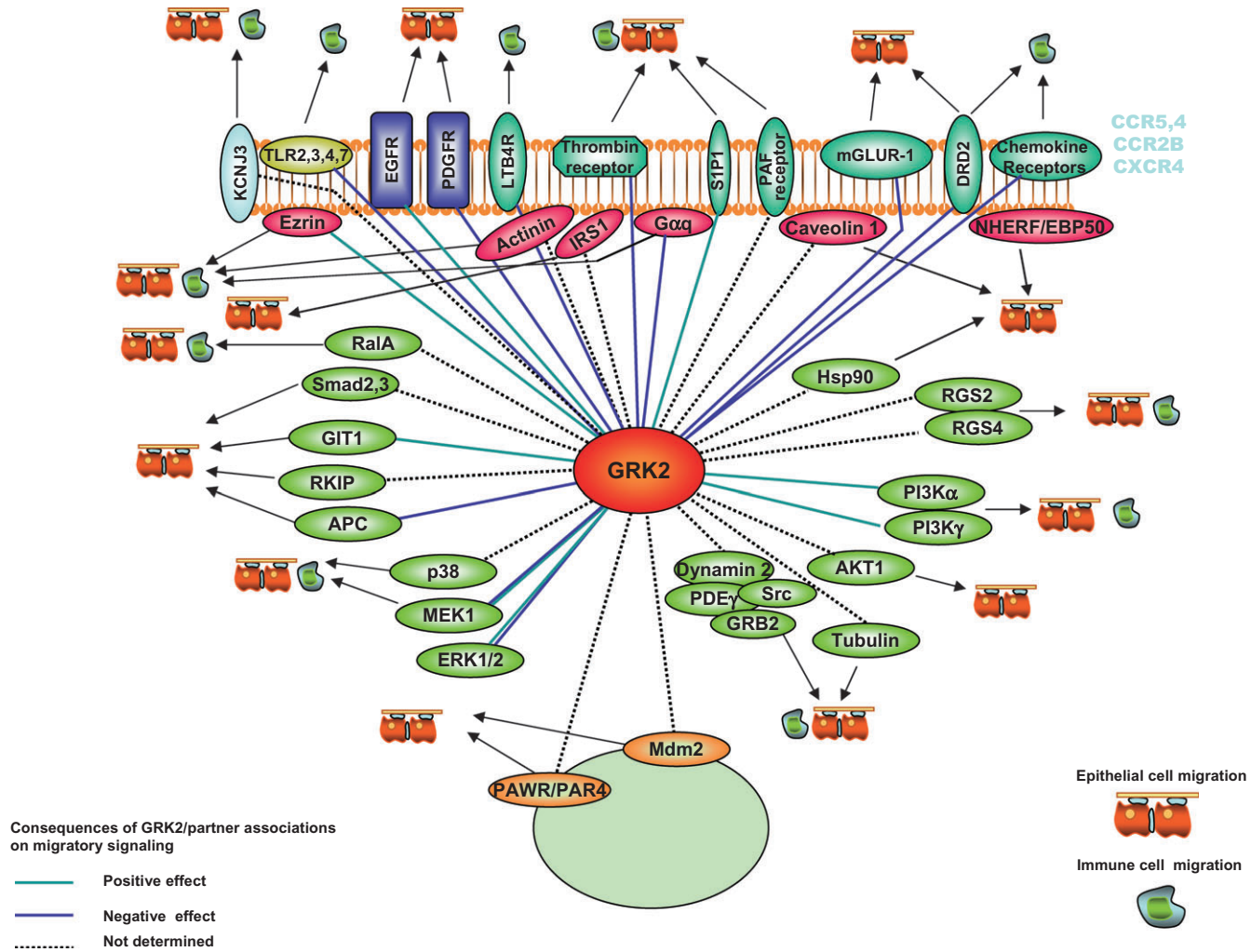

Figure 2 GRK2 interactome involved in cell migration. Schematic representation of signalling molecules/pathways relevant for migration that have been shown to functionally interact with GRK2 (Pathway Studio 6.0, ResNet MedScan Database, Ariadne Genomics). GRK2 interacts with diverse receptors, molecular switches, structural proteins, scaffolding molecules and kinases related to cell motility and chemotaxis. The overall effect on cell migration of the different interactions of GRK2 with its partners will be dependent on the cell type and the signalling context. See text for details. GRK, G protein-coupled receptor kinase.

et al., 2003). The modulation of chemokine-mediated induction of ERK activity by altered GRK2 levels seems to involve both kinase-dependent and independent functions, the later related to the ability of GRK2 to interfere the MEK/ERK interface (Jimenez-Sainz *et al*., 2006). Whether other functional interactions of GRK2 (Figure 2) may also modify immune cell migration remains to be investigated (Penela *et al*., 2009).

These observations suggest an important role of GRK2 levels in cells of the immune system that can have functional consequences in the development and progression of inflammatory diseases. Accordingly, a decrease of GRK2 protein expression (~55%) and kinase activity was found in peripheral blood mononuclear cells of patients with rheumatoid arthritis compared with healthy subjects [reviewed in (Vroon *et al*., 2006)]. The decline of GRK2 is a direct consequence of the pathology as demonstrated in animal models of experimental arthritis, because diseased mice specifically show a reduction in GRK2 levels in splenocytes and mesenteric lymph node cells.

Transient down-regulation of GRKs in immune cells during inflammation may represent an initially adaptive mechanism to facilitate cell response, whereas chronic GRK2 down-regulation would lead to an aberrant inflammatory outcome. However, whether GRK2 expression levels are determinant for the progression of RA still requires further evidence. Expression of GRK2 in leukocytes from patients with multiple sclerosis (MS) was also decreased by 40% compared with that of healthy individuals (Vroon *et al*., 2005). GRK2 levels appear to have a direct impact in the clinical course of experimental MS, as the onset of the relapsing-remitting experimental autoimmune encephalomyelitis in hemizygous GRK2+/– mice was significantly accelerated, concomitant with a higher initial infiltration of T cells into the brain. Curiously, these animals display lower inflammatory infiltrates in the long term and do not develop relapses in the disease compared with wild-type animals. Therefore, the effects of altered GRK2 expression are far from being well defined, as many different cell types that are responsible for the progress of the disease, not only from

the immune system but also from the inflamed tissue, can respond differently to GRK2 changes. For instance, whereas in T cells and monocytes decreased GRK2 levels lead to enhanced migration, consistent with its classical role in GPCR desensitization [revised in Vroon *et al*. (2006); DeFea (2007); Penela *et al*. (2009)] preliminary data suggest that in other cell types and in response to other stimuli (granulocytes stimulated with LTB4 or IL-8), decreased GRK2 levels do not affect chemotactic responses (Tarrant *et al*., 2008), suggesting that the effect of GRK2 on cell migration would be dependent on the precise stimuli or cell type.

In this context, we have recently investigated a potential role for GRK2 in epithelial cell migration. GRK2 promotes changes in actin cytoskeleton and paxillin localization consistent with enhanced focal adhesion turnover and higher cell motility. Moreover, GRK2 promotes increased migration towards fibronectin in different epithelial cell lines models and in fibroblasts. These effects were independent of GRK2 kinase activity, as they were also observed upon expression of a catalytically inactive mutant. Therefore, and contrary to that what has been described in immune cells, increased GRK2 expression facilitates migration towards fibronectin and GRK2 down-regulation impairs migration (Penela *et al*., 2008).

How do GRK2 levels modulate the cellular response to fibronectin, which acts through an integrin receptor? We have found that fibronectin induces sphingosine kinase activity and the subsequent release of S1P, what in turn triggers migration via Gi-coupled S1P1-receptors (Penela *et al*., 2008). Interestingly, GRK2 appears to affect this process at least at two levels. First, *trans*-modulation of S1P1 receptors by fibronectin is potentiated by GRK2 expression, as enhanced GRK2 levels lead to increased S1P production, by yet unknown mechanisms. In addition, GRK2 facilitates the activation of the ERK pathway and migration in response to fibronectin and S1P (Penela *et al*., 2008). Therefore, contrary to the classical paradigm, GRK2 would be playing an overall 'positive' role in cell signalling in epithelial cells and fibroblasts (Figure 2).

The dynamic association of GRK2 with GIT-1 has been shown to underlie the effects of the kinase on epithelial cell migration (Penela *et al*., 2008). GIT-1, first described as a GRK-interacting protein (Premont *et al*., 1998), is a multidomain protein with ARF-GAP activity, able to interact with Rac modulators, paxillin and MEK1. GIT-1 plays important roles in receptor endocytosis and cell motility, and it can promote migration by increasing focal adhesion turnover, delivering active PAK to the leading edge and also by acting as a scaffold for ERK activation in focal adhesions (reviewed in Hoefen and Berk, 2006). Interestingly, S1P stimulation dynamically modulates the GRK2/GIT1 association. S1P-mediated Src-like activation first triggers GRK2 phosphorylation at tyrosine residues and promotes GRK2/GIT interaction; subsequent phosphorylation of GRK2 by MAPK at S670 disrupts the complex and allows additional rounds of dynamic GRK2/GIT1 association that favour cell migration by promoting an efficient and localized activation of the Rac/PAK/MEK/ERK signalling cascade. Mutants unable to engage in such dynamic processes (such as the GRK2-S670A mutant, which displays a very strong association with GIT1) can not substitute for wild-type GRK2 and impair cell signalling and migration in response to these stimuli. These results highlight the functional relevancy of the dynamic GRK2/GIT1 interaction (Penela *et al*., 2008).

Decreased GRK2 levels in hemizygous mice result in delayed wound healing rate *in vivo* (Penela *et al*., 2008), consistent with a physiological role for GRK2 as a regulator of coordinated integrin and GPCR-directed epithelial cell migration. These data put forward the interesting notion that altered GRK2 expression levels might alter migratory responses in pathological conditions. Aberrant epithelial cell motility plays a key role in cancer progression and metastasis. S1P and integrin signalling, as well as other GPCRs such as chemokine receptors or protease-activated receptors are involved in these processes (Milstien and Spiegel, 2006; Dorsam and Gutkind, 2007). Increased S1P-receptor activity is common in breast and other solid tumours correlating with metastasis and chemoresistance, whereas overexpressed β1 and α6β4 integrins promote carcinoma invasion (Brockbank *et al*., 2005). Likewise, CXCR4 and CXCR2 are functionally over-expressed in breast tumours, ovarian cancer and melanoma, among others (Dorsam and Gutkind, 2007). Certain signalling pathways instrumental in many cancers cause the up-regulation of GRK2 protein levels in malignant cell lines (Ho *et al*., 2005; Salcedo *et al*., 2006). In addition, preliminary data indicate that GRK2 protein levels can be either up-regulated in tissue samples of patients with granulosa cell tumours and with differentiated thyroid carcinoma (Metaye *et al*., 2002, 2008), or down-regulated in a subgroup of prostate tumours (Prowatke *et al*., 2007). Altogether these results suggest that altered GRK2 expression in specific tumour cells may affect migration in response to particular stimuli and play a role in carcinogenesis. This hypothesis is further supported by the observed cooperation of GRK2 with known oncogenes in '*in vitro*' transformation assays (Meloni *et al*., 2006) and by the emerging role of GRK2 in cell cycle progression (see below). A detailed characterization of GRK2 expression levels in different types of tumours and further insight on the effects of altered GRK2 expression in tumour progression are needed to further define its role in this process.

GRK2 and cell cycle progression

As discussed above, GRK2 knockout mice are embryonic lethal at day 9–12 (Jaber *et al*., 1996) and display marked cardiac abnormalities as a result of extra-cardiac GRK2 functions (Matkovich *et al*., 2006). In addition, germline GRK2 ablation promotes generalized embryo growth retardation and additional alterations from normal development. These features support the idea that this protein plays a critical role in basic cellular functions such as cell proliferation, differentiation or migration during development. In this regard, emerging evidence points at a role for GRK2 as both an extrinsic and intrinsic cell-cycle regulator (Figure 3). GRK2 expression has been reported to have distinct impacts on cell proliferation and mitogenic signalling depending on both the cell type and the mitogenic stimuli analysed. GRK2 inhibits TGF-mediated cell growth arrest and apoptosis in human hepatocarcinoma cells (Ho *et al*., 2005). On the other hand, GRK2 attenuates serum- or PDGF-induced proliferation of thyroid cancer cell lines (Metaye *et al*., 2008) and smooth

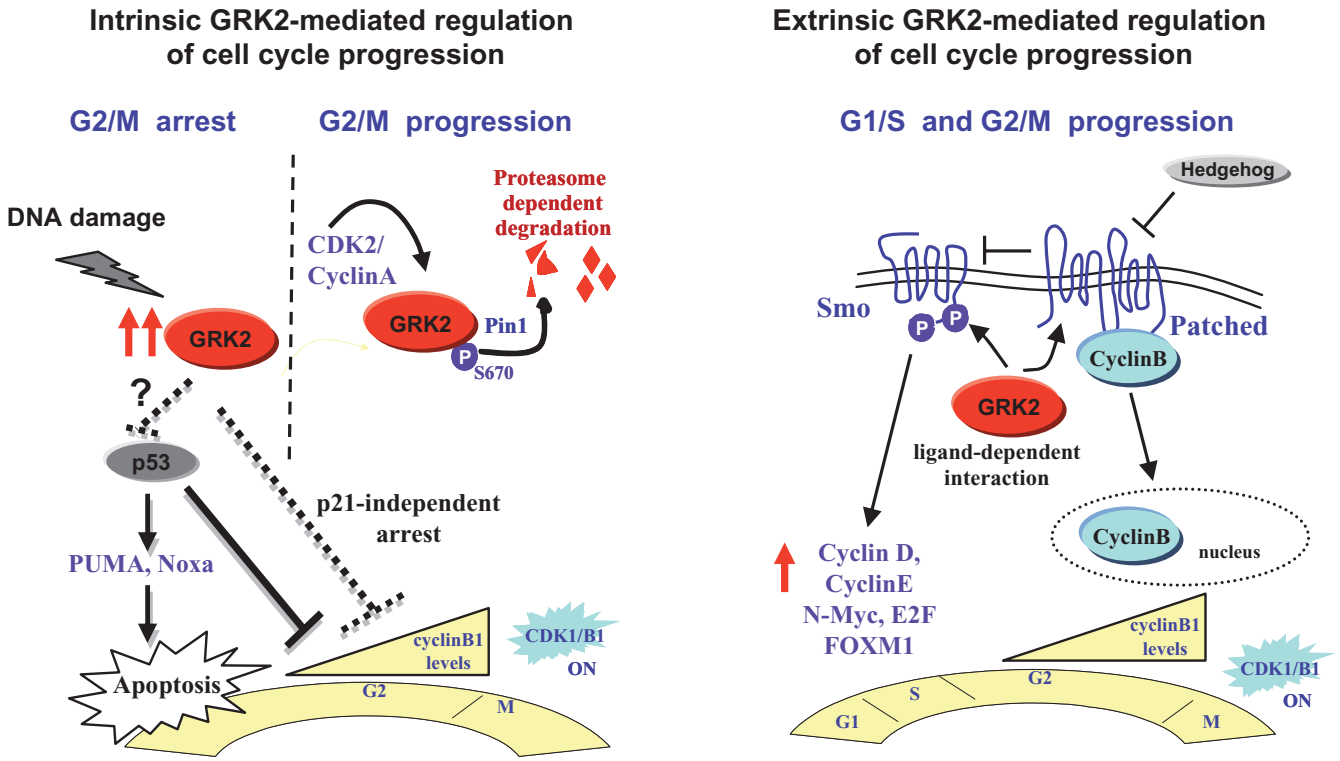

Figure 3 GRK2 interactome involved in the modulation of cell cycle progression. GRK2 is linked to diverse regulatory networks acting at specific stages of the cell cycle. In response to both extrinsic and intrinsic cues, GRK2 protein plays a critical role in driving cell progression through G1/S and G2/M transitions in a kinase-dependent and independent manner. GRK2 is part of an intrinsic pathway that ensures timely progression of cell cycle at G2/M by means of its functional interaction with CDK2/cyclinA and Pin1 (Penela *et al.*, 2010). Such pathway is disrupted upon DNA damage, when GRK2 appears to turn into a pro-arresting factor that promotes increased cell survival and to dampen p53-dependent responses by mechanisms that remain to be established (dotted lines/question mark). On the other hand, GRK2 contributes to the Hedgehog/Smoothened-triggered control of cell proliferation by promoting Smo activity and relieving the Patched-dependent inhibition of cyclin B (Jiang *et al.*, 2009). CDK, cyclin-dependent kinase; GRK, G protein-coupled receptor kinase.

muscle cells (Peppel *et al.*, 2000), respectively, whereas its expression increases MAPK signalling in response to EGF in HEK-293 cells (Wan *et al.*, 2003) and GRK2 kinase activity is required for IGF-1-triggered proliferation and mitogenic signalling in osteoblasts (Bliziotes *et al.*, 2000) We and other groups have found that GRK2 potentiate Smoothened receptor signalling and cooperates with Smoothened to transform the fibroblastic cell line C3H10T1/2 in a focus formation assay (Chen *et al.*, 2004; Meloni *et al.*, 2006; Molnar *et al.*, 2007). Moreover, knock-down of a GRK2 ortholog has been reported to cause growth arrest in zebrafish accompanied by abnormalities in somitogenesis, the hematopoyetic system and in patterning of the eyes and neural tube, what finally compromise the survival of the organism (Jiang *et al.*, 2009). As developmental arrest can be partially rescued by expression of a GRK2 kinase-inactive mutant, this phenotype relies both on kinase-dependent and independent processes. Moreover, such phenotype resembles deficiencies in cell-cycle progression and cell differentiation caused by impaired Smoothened signalling. The ability of GRK2 to interact with Patched and to relieve the Patched-induced cytosolic retention of cyclin B appears to underlie the effect of GRK2 in developing brain and eyes, while the phosphorylation-mediated regulation of Smoothened could be responsible for the role of GRK2 in somite patterning (Jiang *et al.*, 2009). Such GRK2 regulatory roles depend on extrinsic cues promoting cell division, as the GRK2-mediated phosphorylation of Smoothened and the interaction with the Patched/cyclin B pathway are both promoted by the Hedgehog ligand.

In addition, GRK2 has been recently shown to establish a complex network of novel functional interactions during cell cycle progression that are critical for timely G2/M transition (Penela *et al.*, 2010). We have found that GRK2 levels are controlled intrinsically by the cell-cycle machinery under normal conditions and in response to DNA damage, and differentially contribute either to cell cycle progression or cell arrest in a receptor-independent manner. GRK2 protein levels are transiently down-regulated during the G2/M transition by a mechanism involving CDK2-mediated phosphorylation of GRK2 at S670, what drives binding to the prolyl-isomerase Pin1 and subsequent degradation. Preventing GRK2 phosphorylation at this residue impedes normal GRK2 down-regulation and markedly delays cell cycle progression (Penela *et al.*, 2010). Interestingly, the 'default' GRK2 protein decay in G2 is prevented in the presence of DNA damaging agents that trigger cell cycle arrest such as doxorubicin. Moreover, in cells with higher steady-state levels of the kinase, increased stabilized GRK2 levels in such conditions inversely correlate with the p53 response and the induction of apoptosis (Penela *et al.*, 2010), strongly suggesting that GRK2 contributes to

orchestrate G2/M checkpoint mechanisms, helping to restrict the apoptotic fate of arrested cells. As it has been reported that GRK2 is up-regulated in the context of oncogenic signalling (Ho *et al*., 2005; Metaye *et al*., 2005; Salcedo *et al*., 2006), it is tempting to suggest that inhibition of GRK2 expression might sensitize cells to drug-induced DNA damage.

Concluding remarks

The increasingly complex GRK2 'interactome' puts forward this kinase is a relevant signalling node of the cellular transduction network. The intricacy of this network of functional interactions and the participation of this protein in basic cellular processes as migration and cell cycle progression or cardiovascular cell functionality predicts that alterations in GRK2 levels and/or activity, as those reported in several relevant cardiovascular, inflammatory or cancer pathologies, may have important effects in human disease. This makes of this kinase a potentially interesting diagnostic marker and therapeutic target.

However, further research is needed to better understand how the potential GRK2 interactomes are orchestrated depending on the specific stimulus, the cell type or the physiological context. The modulation of these potential GRK2 interactions by extracellular signals and its spatial and temporal integration remains to be established, and the overall physiological and pathological implications arising from this emerging GRK2 interaction map are far from being elucidated.

In this regard, we need to better understand the mechanisms and signals governing the expression and activity of GRK2 and other GRKs during the progression of different diseases. It would also be important to assess the impact of such alterations in the complex integrated network of GRK cellular functions, as it is likely that altered GRK2 expression would differentially affect the function of its interaction partners and impair homeostasis in distinct ways, depending on the cell type involved. The combined use of cellular and animal models with altered GRK2 complement/functionality in specific cell types or situations would be also critical to unveil key cellular and physiological processes controlled by this protein.

In this context, the availability of specific GRK2 inhibitors would be very helpful. However, to our knowledge, no specific and potent inhibitors of GRK2 amenable for animal or cellular studies are currently accessible. As for other protein kinases, the search for ATP –mimetic inhibitors of GRK2 poses the problems of specificity and side effects. Given the highly conserved catalytic domain of GRKs, finding isoform-specific inhibitors of this kind may prove particularly challenging. Alternative strategies to inhibit GRK2 activity have been attempted using peptides corresponding either to beta2-adrenergic receptor regions reported to interact with this kinase (Winstel *et al*., 2005) or to a stretch of residues corresponding to the catalytic domain of GRK2 and GRK3 (Anis *et al*., 2004) or based on RNA aptamers (Mayer *et al*., 2008). As discussed above, a gene therapy approach based on the delivery of the inhibitory GRK2ct domain, that would inhibit endogenous GRK2 by competing with the kinase for

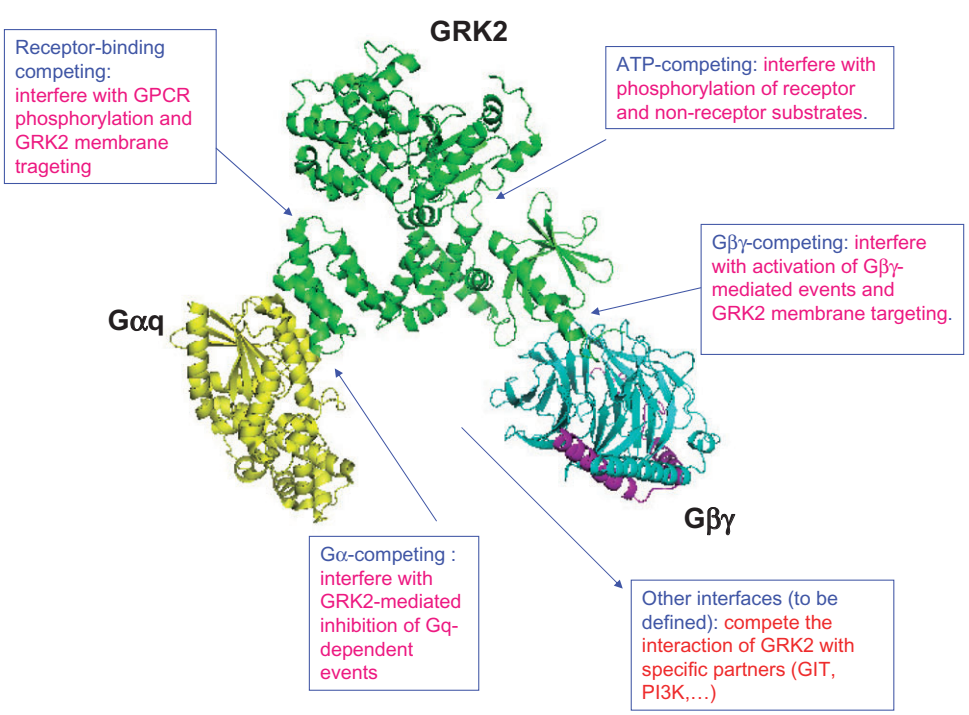

Figure 4 Targeting the GRK2 interactome. A better knowledge of the determinants of the functional interactions of GRK2 with different signalling proteins may help to design novel strategies in order to specifically target GRK2 interfaces with selective partners (receptors, G protein subunits or other components of the GRK2 interactome) relevant for particular cellular functions. The structure of GRK2 in complex with Gβγ and Gαq (Tesmer *et al*., 2005) is shown. See text for details. GRK, G protein-coupled receptor kinase.

Gβγ-mediated membrane translocation, has been useful in different transgenic mice heart failure experimental models, preventing hypertrophy and the progressive deterioration of cardiac function. However, whether these effects are due only to GRK2 inhibition or also involve sequestration of Gβγ subunits remains to be clearly established (Penela *et al*., 2006).

As growing evidence supports that other important functions of GRK2 (as epithelial cell migration or cell cycle progression) are dependent on protein-dependent scaffold roles, targeting of these functions would requires reducing GRK2 levels, as they would be only partially modified by inhibition of GRK2 catalytic activity. In addition to the potential use of siRNA-based approaches for specifically down-regulating kinase expression, the recent knowledge of the GRK2 structure and the emerging identification of interaction domains and surfaces with given cellular proteins will help to design peptides or molecules able to impair or disrupt such interactions selectively, in order to target specific functions of this kinase (Figure 4). Finally, a better understanding of the molecular mechanisms regulating GRK2 expression and function may also be used to indirectly modulate kinase activity *in vivo*, by agents able to modulate its gene expression, stability or functionality.

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Conflict of interest

None.

References

Alexander SPH, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edition (2008 revision). *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Anis Y, Leshem O, Reuveni H, Wexler I, Ben Sasson R, Yahalom B *et al*. (2004). Antidiabetic effect of novel modulating peptides of G-protein-coupled kinase in experimental models of diabetes. *Diabetologia* **47**: 1232–1244.

Aziziyeh AI, Li TT, Pape C, Pampillo M, Chidiac P, Possmayer F *et al*. (2009). Dual regulation of lysophosphatidic acid (LPA1) receptor signalling by Ral and GRK. *Cell Signal* **21**: 1207–1217.

Bliziotes M, Gunness M, Zhang X, Nissenson R, Wiren K (2000). Reduced G-protein-coupled-receptor kinase 2 activity results in impairment of osteoblast function. *Bone* **27**: 367–373.

Brockbank EC, Bridges J, Marshall CJ, Sahai E (2005). Integrin beta1 is required for the invasive behaviour but not proliferation of squamous cell carcinoma cells in vivo. *Br J Cancer* **92**: 102–112.

Chen W, Ren XR, Nelson CD, Barak LS, Chen JK, Beachy PA *et al*. (2004). Activity-dependent internalization of smoothened mediated by beta-arrestin 2 and GRK2. *Science* **306**: 2257–2260.

Chen Y, Long H, Wu Z, Jiang X, Ma L (2008). EGF Transregulates Opioid Receptors through EGFR-mediated GRK2 Phosphorylation and Activation. *Mol Biol Cell* **19**: 2973–2983.

Cipolletta E, Campanile A, Santulli G, Sanzari E, Leosco D, Campiglia P *et al*. (2009). The G protein coupled receptor kinase 2 plays an essential role in beta-adrenergic receptor-induced insulin resistance. *Cardiovasc Res* **84**: 407–415.

Cohn HI, Harris DM, Pesant S, Pfeiffer M, Zhou RH, Koch WJ *et al*. (2008). Inhibition of vascular smooth muscle G protein-coupled receptor kinase 2 enhances alpha1D-adrenergic receptor constriction. *Am J Physiol* **295**: H1695–H1704.

Cohn HI, Xi Y, Pesant S, Harris DM, Hyslop T, Falkner B *et al*. (2009). G protein-coupled receptor kinase 2 expression and activity are associated with blood pressure in black Americans. *Hypertension* **54**: 71–76.

DeFea KA (2007). Stop that cell! Beta-arrestin-dependent chemotaxis: a tale of localized actin assembly and receptor desensitization. *Annu Rev Physiol* **69**: 535–560.

DeWire SM, Ahn S, Lefkowitz RJ, Shenoy SK (2007). Beta-arrestins and cell signaling. *Annu Rev Physiol* **69**: 483–510.

Dinudom A, Fotia AB, Lefkowitz RJ, Young JA, Kumar S, Cook DI (2004). The kinase Grk2 regulates Nedd4/Nedd4-2-dependent control of epithelial Na+ channels. *Proc Natl Acad Sci U S A* **101**: 11886–11890.

Dorn GW, 2nd (2009a). GRK mythology: G-protein receptor kinases in cardiovascular disease. *J Mol Med* **87**: 455–463.

Dorn GW, 2nd (2009b). Novel pharmacotherapies to abrogate postinfarction ventricular remodeling. *Nat Rev Cardiol* **6**: 283–291.

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

Eckhart AD, Ozaki T, Tevaearai H, Rockman HA, Koch WJ (2002). Vascular-targeted overexpression of G protein-coupled receptor kinase-2 in transgenic mice attenuates beta-adrenergic receptor signaling and increases resting blood pressure. *Mol Pharmacol* **61**: 749–758.

Elorza A, Penela P, Sarnago S, Mayor F, Jr (2003). MAPK-dependent degradation of G protein-coupled receptor kinase 2. *J Biol Chem* **278**: 29164–29173.

Elorza A, Sarnago S, Mayor F, Jr (2000). Agonist-dependent modulation of G protein-coupled receptor kinase 2 by mitogen-activated protein kinases. *Mol Pharmacol* **57**: 778–783.

Fan G, Ballou LM, Lin RZ (2003). Phospholipase C-independent activation of glycogen synthase kinase-3beta and C-terminal Src kinase by Galphaq. *J Biol Chem* **278**: 52432–52436.

Ferguson SS (2007). Phosphorylation-independent attenuation of GPCR signalling. *Trends Pharmacol Sci* **28**: 173–179.

Gros R, Benovic JL, Tan CM, Feldman RD (1997). G-protein-coupled receptor kinase activity is increased in hypertension. *J Clin Invest* **99**: 93.

Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS, Feldman RD (2000). G-protein-coupled receptor kinase activity in hypertension: increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* **35**: 38–42.

Guo J, Chen H, Ho J, Mancini J, Sontag T, Laporte SA *et al*. (2009). TGFbeta-induced GRK2 expression attenuates AngII-regulated Vascular Smooth Muscle Cell proliferation and migration. *Cell Signal* **21**: 899–905.

Hildreth KL, Wu JH, Barak LS, Exum ST, Kim LK, Peppel K *et al*. (2004). Phosphorylation of the platelet-derived growth factor receptor-beta by G protein-coupled receptor kinase-2 reduces receptor signaling and interaction with the Na(+)/H(+) exchanger regulatory factor. *J Biol Chem* **279**: 41775–41782.

Ho J, Cocolakis E, Dumas VM, Posner BI, Laporte SA, Lebrun JJ (2005). The G protein-coupled receptor kinase-2 is a TGFbeta-inducible antagonist of TGFbeta signal transduction. *Embo J* **24**: 3247–3258.

Hoefen RJ, Berk BC (2006). The multifunctional GIT family of proteins. *J Cell Sci* **119**: 1469–1475.

Hupfeld CJ, Olefsky JM (2007). Regulation of receptor tyrosine kinase signaling by GRKs and beta-arrestins. *Annu Rev Physiol* **69**: 561–577.

Iaccarino G, Barbato E, Cipolletta E, De Amicis V, Margulies KB, Leosco D *et al*. (2005). Elevated myocardial and lymphocyte GRK2 expression and activity in human heart failure. *Eur Heart J* **26**: 1752–1758.

Jaber M, Koch WJ, Rockman H, Smith B, Bond RA, Sulik KK *et al*. (1996). Essential role of beta-adrenergic receptor kinase 1 in cardiac development and function. *Proc Natl Acad Sci U S A* **93**: 12974–12979.

Jiang X, Yang P, Ma L (2009). Kinase activity-independent regulation of cyclin pathway by GRK2 is essential for zebrafish early development. *Proc Natl Acad Sci U S A* **106**: 10183–10188.

Jimenez-Sainz MC, Murga C, Kavelaars A, Jurado-Pueyo M, Krakstad BF, Heijnen CJ *et al*. (2006). G protein-coupled receptor kinase 2 negatively regulates chemokine signaling at a level downstream from g protein subunits. *Mol Biol Cell* **17**: 25–31.

Koch WJ, Lefkowitz RJ, Milano CA, Akhter SA, Rockman HA (1998). Myocardial overexpression of adrenergic receptors and receptor kinases. *Adv Pharmacol* **42**: 502–506.

Liu S, Premont RT, Kontos CD, Zhu S, Rockey DC (2005). A crucial role for GRK2 in regulation of endothelial cell nitric oxide synthase function in portal hypertension. *Nat Med* **11**: 952–958.

Lodowski DT, Barnhill JF, Pitcher JA, Capel WD, Lefkowitz RJ, Tesmer JJ (2003). Purification, crystallization and preliminary X-ray diffraction studies of a complex between G protein-coupled receptor kinase 2 and Gbeta1gamma2. *Acta Crystallogr D Biol Crystallogr* **59**: 936–939.

Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ (2007). Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. *Nat Med* **13**: 315–323.

Matkovich SJ, Diwan A, Klanke JL, Hammer DJ, Marreez Y, Odley AM *et al*. (2006). Cardiac-specific ablation of G-protein receptor kinase 2 redefines its roles in heart development and beta-adrenergic signaling. *Circ Res* **99**: 996–1003.

Mayer G, Wulffen B, Huber C, Brockmann J, Flicke B, Neumann L *et al*. (2008). An RNA molecule that specifically inhibits G-protein-coupled receptor kinase 2 in vitro. *RNA* **14**: 524–534.

Meloni AR, Fralish GB, Kelly P, Salahpour A, Chen JK, Wechsler-Reya RJ *et al*. (2006). Smoothened signal transduction is promoted by G protein-coupled receptor kinase 2. *Mol Cell Biol* **26**: 7550–7560.

Metaye T, Gibelin H, Perdrisot R, Kraimps JL (2005). Pathophysiological roles of G-protein-coupled receptor kinases. *Cell Signal* **17**: 917–928.

Metaye T, Levillain P, Kraimps JL, Perdrisot R (2008). Immunohistochemical detection, regulation and antiproliferative function of G-protein-coupled receptor kinase 2 in thyroid carcinomas. *J Endocrinol* **198**: 101–110.

Metaye T, Menet E, Guilhot J, Kraimps JL (2002). Expression and activity of G protein-coupled receptor kinases in differenciated thyroid carcinoma. *J Clin Endocrinol Metab* **87**: 3279–3286.

Milstien S, Spiegel S (2006). Targeting sphingosine-1-phosphate: a novel avenue for cancer therapeutics. *Cancer Cell* **9**: 148–150.

Molnar C, Holguin H, Mayor F, Jr, Ruiz-Gomez A, de Celis JF (2007). The G protein-coupled receptor regulatory kinase GPRK2 participates in Hedgehog signaling in Drosophila. *Proc Natl Acad Sci U S A* **104**: 7963–7968.

Moore CA, Milano SK, Benovic JL (2007). Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol* **69**: 451–482.

Naga Prasad SV, Laporte SA, Chamberlain D, Caron MG, Barak L, Rockman HA (2002). Phosphoinositide 3-kinase regulates beta2-adrenergic receptor endocytosis by AP-2 recruitment to the receptor/beta-arrestin complex. *J Cell Biol* **158**: 563–575.

Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J *et al*. (2007). Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest* **117**: 2445–2458.

Patial S, Luo J, Porter KJ, Benovic JL, Parameswaran N (2009). G-protein coupled receptor kinases mediate TNFalpha-induced NFkappaB signaling via direct interaction with and phosphorylation of IkappaBalpha. *Biochem J* **425**: 169–178.

Penela P, Ruiz-Gomez A, Castano JG, Mayor F, Jr (1998). Degradation of the G protein-coupled receptor kinase 2 by the proteasome pathway. *J Biol Chem* **273**: 35238–35244.

Penela P, Elorza A, Sarnago S, Mayor F, Jr (2001). Beta-arrestin- and c-Src-dependent degradation of G-protein-coupled receptor kinase 2. *Embo J* **20**: 5129–5138.

Penela P, Ribas C, Mayor F, Jr (2003). Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. *Cell Signal* **15**: 973–981.

Penela P, Murga C, Ribas C, Tutor AS, Peregrin S, Mayor F, Jr (2006). Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. *Cardiovasc Res* **69**: 46–56.

Penela P, Ribas C, Aymerich I, Eijkelkamp N, Barreiro O, Heijnen CJ *et al*. (2008). G protein-coupled receptor kinase 2 positively regulates epithelial cell migration. *Embo J* **27**: 1206–1218.

Penela P, Ribas C, Aymerich I, Mayor F, Jr (2009). New roles of G protein-coupled receptor kinase 2 (GRK2) in cell migration. *Cell Adh Migr* **3**: 19–23.

Penela P, Rivas V, Salcedo A, Mayor F Jr (2010). G protein–coupled receptor kinase 2 (GRK2) modulation and cell cycle progression. *Proc Natl Acad Sci U S A* **107**: 1118–1123.

Peppel K, Jacobson A, Huang X, Murray JP, Oppermann M, Freedman NJ (2000). Overexpression of G protein-coupled receptor kinase-2 in smooth muscle cells attenuates mitogenic signaling via G protein-coupled and platelet-derived growth factor receptors. *Circulation* **102**: 793–799.

Peregrin S, Jurado-Pueyo M, Campos PM, Sanz-Moreno V, Ruiz-Gomez A, Crespo P *et al*. (2006). Phosphorylation of p38 by GRK2 at the docking groove unveils a novel mechanism for inactivating p38MAPK. *Curr Biol* **16**: 2042–2047.

Pitcher JA, Tesmer JJ, Freeman JL, Capel WD, Stone WC, Lefkowitz RJ (1999). Feedback inhibition of G protein-coupled receptor kinase 2 (GRK2) activity by extracellular signal-regulated kinases. *J Biol Chem* **274**: 34531–34534.

Premont RT, Claing A, Vitale N, Freeman JL, Pitcher JA, Patton W *et al*. (1998). β2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein. *Proc Natl Acad Sci U S A* **95**: 14082–14087.

Premont RT, Gainetdinov RR (2007). Physiological roles of G protein-coupled receptor kinases and arrestins. *Annu Rev Physiol* **69**: 511–534.

Prowatke I, Devens F, Benner A, Grone EF, Mertens D, Grone HJ *et al*. (2007). Expression analysis of imbalanced genes in prostate carcinoma using tissue microarrays. *Br J Cancer* **96**: 82–88.

Raake PW, Vinge LE, Gao E, Boucher M, Rengo G, Chen X *et al*. (2008). G protein-coupled receptor kinase 2 ablation in cardiac myocytes before or after myocardial infarction prevents heart failure. *Circ Res* **103**: 413–422.

Ramos-Ruiz R, Penela P, Penn RB, Mayor F, Jr (2000). Analysis of the human G protein-coupled receptor kinase 2 (GRK2) gene promoter: regulation by signal transduction systems in aortic smooth muscle cells. *Circulation* **101**: 2083–2089.

Reiter E, Lefkowitz RJ (2006). GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab* **17**: 159–165.

Rengo G, Lymperopoulos A, Zincarelli C, Donniacuo M, Soltys S, Rabinowitz JE *et al*. (2009). Myocardial adeno-associated virus serotype 6-betaARKct gene therapy improves cardiac function and normalizes the neurohormonal axis in chronic heart failure. *Circulation* **119**: 89–98.

Ribas C, Penela P, Murga C, Salcedo A, Garcia-Hoz C, Jurado-Pueyo M *et al*. (2007). The G protein-coupled receptor kinase (GRK) interactome: role of GRKs in GPCR regulation and signaling. *Biochim Biophys Acta* **1768**: 913–922.

Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G *et al*. (2003). Cell migration: integrating signals from front to back. *Science* **302**: 1704–1709.

Rockman HA, Chien KR, Choi DJ, Iaccarino G, Hunter JJ, Ross J *et al*. (1998). Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. *Proc Natl Acad Sci U S A* **95**: 7000–7005.

Salcedo A, Mayor F, Jr, Penela P (2006). Mdm2 is involved in the ubiquitination and degradation of G-protein-coupled receptor kinase 2. *Embo J* **25**: 4752–4762.

Sarnago S, Elorza A, Mayor F, Jr (1999). Agonist-dependent phosphorylation of the G protein-coupled receptor kinase 2 (GRK2) by Src tyrosine kinase. *J Biol Chem* **274**: 34411–34416.

Tarrant TK, Rampersad RR, Esserman D, Rothlein LR, Liu P, Premont RT *et al*. (2008). Granulocyte chemotaxis and disease expression are differentially regulated by GRK subtype in an acute inflammatory arthritis model (K/BxN). *Clin Immunol* **129**: 115–122.

Tesmer VM, Kawano T, Shankaranarayanan A, Kozasa T, Tesmer JJ (2005). Snapshot of activated G proteins at the membrane: the Galphaq-GRK2-Gbetagamma complex. *Science* **310**: 1686–1690.

Theilade J, Strom C, Christiansen T, Haunso S, Sheikh SP (2003). Differential G protein receptor kinase 2 expression in compensated hypertrophy and heart failure after myocardial infarction in the rat. *Basic Res Cardiol* **98**: 97–103.

Vroon A, Heijnen CJ, Kavelaars A (2006). GRKs and arrestins: regulators of migration and inflammation. *J Leukoc Biol* **80**: 1214–1221.

Vroon A, Heijnen CJ, Lombardi MS, Cobelens PM, Mayor F, Jr, Caron MG *et al*. (2004). Reduced GRK2 level in T cells potentiates chemotaxis and signaling in response to CCL4. *J Leukoc Biol* **75**: 901–909.

Vroon A, Kavelaars A, Limmroth V, Lombardi MS, Goebel MU, Van Dam AM *et al*. (2005). G protein-coupled receptor kinase 2 in multiple sclerosis and experimental autoimmune encephalomyelitis. *J Immunol* **174**: 4400–4406.

Wan KF, Sambi BS, Tate R, Waters C, Pyne NG (2003). The inhibitory γ subunit of the type 6 retinal cGMP phosphodiesterase functions to link c-Src and G-protein-coupled receptor kinase 2 in a signaling unit that regulates p42/p44 mitogen-activated protein kinase by epidermal growth factor. *J Biol Chem* **278**: 18658–18663.

Wang L, Gesty-Palmer D, Fields TA, Spurney RF (2009). Inhibition of WNT signaling by G protein-coupled receptor (GPCR) kinase 2 (GRK2). *Mol Endocrinol* **23**: 1455–1465.

Whalen EJ, Foster MW, Matsumoto A, Ozawa K, Violin JD, Que LG *et al*. (2007). Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2. *Cell* **129**: 511–522.

Winstel R, Ihlenfeldt HG, Jung G, Krasel C, Lohse MJ (2005). Peptide inhibitors of G protein-coupled receptor kinases. *Biochem Pharmacol* **70**: 1001–1008.