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Role of G protein-coupled receptor kinase 2 (GRK2) in migration and inflammation

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ABSTRACT

G protein-coupled receptor kinase 2 (GRK2) emerges as a key modulator of G protein-coupled receptors and other plasma membrane receptors triggered by chemotactic messengers. In addition, GRK2 has been reported to interact with a variety of signal transduction proteins related to cell migration. Interestingly, the levels of expression and activity of this kinase are altered in several inflammatory disorders, thus suggesting that it may play an important role in the onset or progression of these pathologies. This review summarizes the mechanisms involved in the control of GRK2 expression and function and highlights novel functional interactions of this protein that might help to explain how altered GRK2 levels affects cell migration in different cell types and pathological settings.

Key words: GPCR.- Arrestins.- GIT.- p38MAPK.- MEK.

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RESUMEN

Papel de GRK2 (G protein-coupled receptor kinase 2) en migración e inflamación

La quinasa GRK2 (G protein-coupled receptor kinase 2) se perfila como un modulador clave de receptores acoplados a proteínas G y de otros receptores de membrana plasmática que responden a estímulos migratorios. Además, GRK2 es capaz de interaccionar con diferentes proteínas señalizadoras relacionadas con la migración celular. Por otra parte, puesto que los niveles de expresión y actividad de esta quinasa se encuentran alterados en distintas en enfermedades inflamatorias, se sugiere que GRK2 puede desempeñar un papel importante en el desencadenamiento o la progresión de estos procesos. Esta revisión resume los mecanismos implicados en el control de la expresión y función de GRK2 y resalta nuevas interacciones funcionales de esta proteína que pueden contribuir a explicar cómo las alteraciones en los niveles de GRK2 afectan a la migración de distintos tipos celulares y a diversas situaciones patológicas.

Palabras clave: GPCR.- Arrestinas.- GIT.- p38MAPK.- MEK.

INTRODUCTION

Signal-directed migration requires a spatio-temporal integration of information arising from mechanical cues and from diffusible molecules such as chemokines, bioactive lipids and growth factors. Failures in this process might result in aberrant migration leading to chronic inflammatory disorders, tumor metastasis, impaired wound healing or other diseases (1). Key messengers involved in cell migration (such as chemokines or sphingosine-1-phosphate, S1P) emit signals to the actin cytoskeleton and to adhesion structures by binding to plasma-membrane receptors of the GPCR family (2, 3).

GRK2 is a ubiquitous member of the G protein-coupled receptor kinase family, a group of seven serine/threonine protein kinases that specifically recognize and phosphorylate agonist-activated G protein-coupled receptors (GPCRs). GRK2-mediated receptor phosphorylation triggers the binding of cytosolic proteins termed arrestins, which block the ability of GPCR to interact with G proteins, leading to rapid 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, but may also help to promote the activation of additional signalling pathways by way of arrestins and/or GRKs acting as agonist-regulated adaptor scaffolds (4, 5). Interestingly, GRK2 expression and function has been shown to be altered in several inflammatory conditions (see below), thus suggesting that it may play an important role in the onset or progression of the inflammatory disease. Therefore, the understanding of the molecular mechanisms leading to altered GRK2 levels and functionality, as well as of the consequences of such changes on key cellular processes is a very active field of research.

Consistent with its role in GPCR desensitization, GRK2 has been shown to attenuate chemokine-induced migration in T cells and monocytes (revised in (6)), thus emerging as a relevant modulator of inflammatory responses. However, pro-migratory effects for β-arrestins and for a member of a distinct GRK subfamily, GRK6, have also been reported in response to different GPCR in several cell types (2, 6), thereby suggesting other unknown functional roles different to those related to GPCR desensitization.

Interestingly, in addition to phosphorylate a variety of GPCR, recent data indicate that GRK2 is also able to phosphorylate other types of membrane receptors, such as the PDGF-receptor, and non-receptor substrates as tubulin, the ERM family protein ezrin, or the p38 MAPK (see (4, 5, 7) and references therein). Thus, GRK2 may participate in the regulation of diverse cellular phenomena through the phosphorylation of substrates that are very varied functionally. Moreover, GRK2 has been shown to interact with a variety of proteins involved in migration (MEK, Akt, PI3Kγ or GIT (reviewed in (5)). In this review we will briefly summarize the main mechanisms involved in the control of GRK2 expression and function, its reported alterations in inflammatory disorders and the emerging functional interactions of GRK2 with diverse molecules related to cell migration and inflammation (see Figure 1) that may shed new light on how GRK2 influences motility in different cellular types and on the physiological consequences of altering the cellular complement of this kinase.

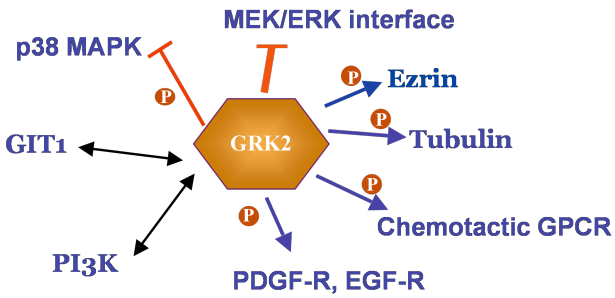

FIGURE 1.- ***GRK2 functionally interacts with multiple signalling proteins involved in cell migration.*** *See text for details.*

GRK2 STRUCTURE AND MODULATION

GRK family members can be subdivided into three main groups based on sequence homology: rhodopsin kinase or 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. 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 (4, 5).

In addition to this general domain architecture, ongoing research is unveiling the existence of regions involved in interaction with different cellular proteins and of regulatory phosphorylation sites in the GRK2

structure. Association with actinin, actin, calmodulin, caveolin, the Raf kinase inhibitor protein (RKIP) or with an undetermined microsomal component negatively modulates GRK kinase-dependent functions. In addition, phosphorylation of GRKs at different sites and by a variety of protein kinases has emerged as an important mechanism for regulation of their activity, the interaction with other proteins and even protein stability (4, 5).

REGULATION OF GRK2 BY PHOSPHORYLATION

Phosphorylation by second-messenger-modulated protein kinases (PKC and PKA).

PKC and PKA have been shown to modulate GRK activity and membrane targeting. "In vitro", PKC-mediated phosphorylation of GRK2 led to an enhanced phosphorylation of receptor but not soluble peptides, suggesting that PKC phosphorylation stimulated GRK2 translocation from the cytosol to the plasma membrane without affecting catalytic activity. It has been recently reported that phosphorylation of GRK2 by PKC takes place at serine 29 (8) within the calmodulin-binding region of GRK2. It has been suggested that in intact cells a pool of GRK2 may be tonically inhibited by forming a complex with calmodulin, and that PKC phosphorylation would release such inhibitory interaction and allow for GRK2 binding to the receptor substrate (8).

On the other hand, PKA activated by Gs-coupled receptors can directly phosphorylate GRK2, leading to an enhanced GRK2 activity toward β2-adrenergic receptors (9). The site of PKA phosphorylation on GRK2 was mapped at Serine 685, which is in close proximity to the Gβγ binding domain of GRK2. In fact, PKA phosphorylation does not affect the kinase activity, but rather enhances binding of GRK2 to Gβγ subunits, thereby facilitating membrane targeting of GRK2 and interaction with activated receptors.

Phosphorylation by c-Src and MAPK.

c-Src can directly phosphorylate GRK2 "in vitro" with high affinity and also promotes tyrosine phosphorylation of GRK2 upon agonist

stimulation of β2-adrenergic receptors (10). This process is dependent on the ability of β-arrestins to recruit c-Src (11). The activity of tyrosine-phosphorylated GRK2 is increased toward both soluble and membrane-bound substrates, suggesting a direct effect on its catalytic activity. Mutagenesis experiments have identified tyrosine residues within the RH region of GRK2 (amino acids 13, 86 and 92) that are critical for c-Src-mediated phosphorylation. Tyrosine phosphorylation also appears to enhance the interaction of GRK2 with Gαq (12) and to promote its degradation by the proteasome pathway (see below).

GRK2 activity is also regulated by p42/p44 MAPK. "In vitro" and "in situ" experiments revealed that ERK1 is able to phosphorylate recombinant GRK2 on serine 670 (13, 14), and both kinases are specifically co-immunoprecipitated in an agonist-dependent manner (13). Interestingly, Serine 670 lies within the Gβγ binding domain of GRK2, and MAPK phosphorylation of this site strongly impairs the GRK2/Gβγ interaction, thereby inhibiting kinase translocation and catalytic activity toward receptor membrane substrates.

REGULATION OF GRK2 EXPRESSION LEVELS

Little has been reported about the mechanisms governing GRK transcription. In aortic smooth muscle cells, 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 (15). However, whether these mechanisms apply to other cell types awaits further investigation.

On the other hand, regulation of GRK2 stability may provide an important mechanism for modulating its expression levels (Figure 2). GRK2 is rapidly degraded by the proteasome pathway, and β_2AR activation enhances GRK2 ubiquitination and turnover (16). Our laboratory has also shown that agonist-dependent binding of β-arrestin to GPCR supports GRK2 degradation by allowing the recruitment of c-Src

and the phosphorylation of GRK2 on critical tyrosine residues (11). MAPK-mediated GRK2 phosphorylation also triggers GRK2 degradation in a process which is again dependent on β-arrestin function (17). 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 (18). Mdm2 and GRK2 association and subsequent proteolysis is enhanced by β_2-adrenergic receptor stimulation and β-arrestin. On the contrary, activation of the PI3K/Akt pathway by agonists such as IGF-1 alters Mdm2-mediated GRK2 degradation, leading to enhanced GRK2 stability and increased kinase levels, what might be relevant in several pathophysiological contexts.

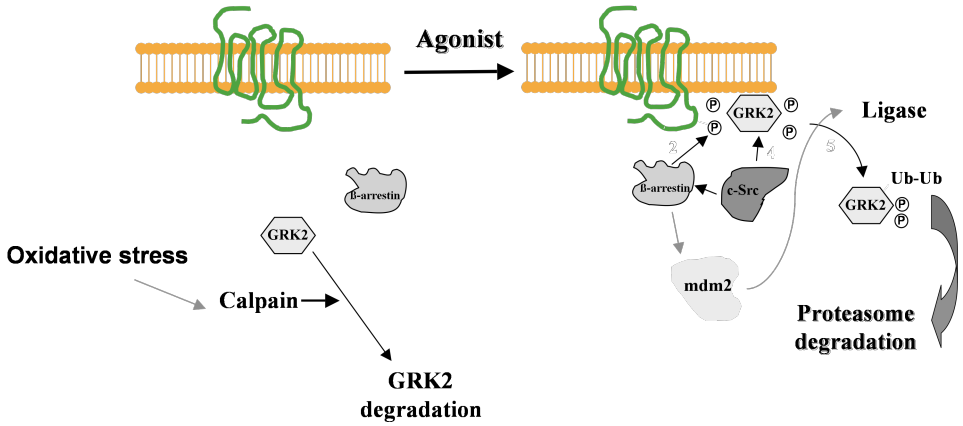

FIGURE 2.- ***Mechanisms involved in the regulation of GRK2 stability.*** *See text for details.*

GRK2 levels might also be downregulated by additional proteolytic pathways. Rheumatoid arthritis (RA) patients display significantly reduced GRK2 levels in peripheral blood mononuclear cells (PBMC) without significant changes in GRK mRNA levels. The same is observed in rats in an animal model of adjuvant arthritis (6). Proinflammatory cytokines as IL-6 and INFγ are elevated in patients with RA, and treatment of PBMC with these compounds strongly promotes GRK2 down-regulation.

Production of reactive oxygen species (ROS) has been detected in different cells treated with these cytokines, suggesting that ROS could mediate the decrease in GRK2 levels observed in these experimental models of inflammation. In fact, it has been recently described that exposure of lymphocytes to oxidative stress results in decreased GRK2 levels in T lymphocytes (19). Interestingly, the H_2O_2-induced decreases in GRK2 protein were prevented by inhibiting calpain activity, but not proteasome function. "In vitro" proteolysis experiments show that purified m-calpain promotes partial degradation of GRK2 in a calcium-dependent way (19). It is tempting to suggest that calpain-mediated degradation of GRK2 could also play a role in other situations characterized by altered calpain activity and GRK levels, such as hypertension. Overall, these results indicate the occurrence of different proteolytic pathways for GRK2 down-regulation (proteasome- and calpain dependent). How these mechanisms are activated and contribute to the control of GRK2 stability in a given cell type and physiological condition is an interesting area of future research.

GRK2 AND INFLAMMATORY DISORDERS

GRK2, which is highly expressed in different cellular types of the immune system, emerges as an important regulator of the immune cell responses during inflammation. GRK2 phosphorylates manifold chemokine receptors such as CCR5, CCR2b, CXCR4, CXCR2 and chemotactic receptors for substance P, S1P or formyl-peptide, responsible of leukocyte trafficking to the inflammatory foci, T cell egression from lymphoid organs, leukocyte activation or proliferation (6). Interestingly, a decrease of GRK2 protein expression (~55%) and kinase activity was found in peripheral blood mononuclear cells of patients with rheumatoid arthritis (RA), as compared with kinase levels in healthy subjects (20). The decline of GRK2 is a direct consequence of the pathology as demonstrated in animal models of experimental arthritis (21), which specifically show a reduction in GRK2 levels in splenocytes and mesenteric lymph node cells. Down-regulation of GRKs in immune cells during inflammation may represent initially an adaptive mechanism to facilitate cell response, but chronic GRK2 down-regulation can lead to an aberrant inflammatory

response. Indeed, T cells from GRK2$^{+/-}$ mice, that display a 50% reduction in GRK2 levels, showed a significant increase in signalling and chemotaxis toward CCR1 and CCR5 receptor ligands compared to wild-type T cells (22). However, whether GRK2 expression levels are determinant for the progression of RA still requires further evidence. Interestingly, such notion was indeed demonstrated for another inflammatory disorder, i.e. multiple sclerosis (MS) (23). Expression of GRK2 in leukocytes from patients suffering from MS, but not from neurological disorders without an inflammatory component, was also decreased by circa 40% compared with kinase levels from healthy individuals. GRK2 levels have a direct impact in the clinical course of experimental MS. Thus, the onset of the relapsing-remitting experimental autoimmune encephalomyelitis (EAE) in hemizygous GRK2$^{+/-}$ mice was significantly accelerated, what is paralleled by 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 of the disease compared to wild-type animals. Reactive microglia and neuronal damage secondary to excessive glutamate signalling are important components of diverse neurological disorder that course with inflammation. Interestingly, GRK2 expression levels in brain also modulates the strength of microglia-mediated inflammation, what might determine the sensitivity to cerebral stroke by means of rate-limiting the extent of local release of inflammatory mediators that could attract T cells (24). Therefore, the effects of altered GRK2 expression are far to be clear-cut in terms of inflammation, since many different cell types, not only from the immune system but also from the inflamed tissue, are responsible for the course of the disease and can respond distinctly to GRK2 changes.

Brain GRK2 also modulates the strength of microglia-mediated inflammation and its protein expression levels might determine the sensitivity to cerebral stroke (24). Reactive microglia and neuronal damage secondary to excessive glutamate signalling are important components of AD pathology. Recently, a study aimed to evaluate peripheral lymphocyte GRK2 expression in patients suffering from AD has shown a positive correlation between kinase protein levels and the severity of the disease (25).

NOVEL GRK2 FUNCTIONAL INTERACTIONS MIGHT BE RELATED TO THE ROLE OF THIS KINASE IN INFLAMMATION AND CELL MIGRATION

GRK2 as a modulator of the MEK/ERK interface upon chemokine challenge

We have recently reported, using GRK2-transfected cells or splenocytes from heterozygous GRK2 mice, that elevated levels of GRK2 can inhibit chemokine-mediated induction of ERK activity and, on the contrary, that decreased levels of GRK2 promote a more robust ERK activation upon agonist treatment. Neither the kinase activity of GRK2 nor its interaction with G protein subunits is necessary for this inhibitory effect and no changes were observed in the extent of MEK activation in our experimental settings. Interestingly, we have found that GRK2 and MEK1 are present in the same multimolecular complex and that this interaction correlates with an inhibition of ERK activation, that involves a direct or coordinate interaction with MEK (26). Thus, this association seems to be important in the control of chemokine induction of MAPK activation hence for the extent of chemotactic cell motility. By binding to MEK, GRK2 could interfere (at the cellular level or at defined cellular locations) with MEK association to proteins important for its cellular compartimentation, internalization, or activity, such as MEK-ERK scaffolds. Therefore, changes in GRK2 expression in pathological conditions would alter chemokine receptor signaling at different levels.

GRK2/p38 MAPK

We have found that GRK2 directly phosphorylates p38α at the Thr123 residue located at the entrance of the p38 docking groove (7). Mimicking phosphorylation of this residue interferes with the binding and phosphorylation of well-established p38 substrates (ATF2, MEF2, MK2), and also with the ability of p38 to bind and become phosphorylated by MKK6 in vitro and in cells. This phosphorylation has an impact on p38-dependent cellular functions, since changing GRK2 levels alters the secretion of TNFα upon LPS stimulation, this process being increased in $GRK2^{+/-}$ murine macrophages.

Kinases upstream p38 have been classically involved in its activation by means of phosphorylation at the activation loop, but this work defines GRK2 as a kinase upstream of p38 and also as an "inactivating MAP2K", what may have important consequences in the regulation of p38 activation subsequent to stimuli that potentiate GRK2 activity, such as GPCRs. Accordingly, one may envisage a scenario where previous GPCR stimulation could lead to an increased pool of pT123-p38 and thus serve to dampen subsequent p38 stimulation by other stimuli. In support of this notion, p38 activity happens to be reduced in end stage failing myocardium in humans while the elevation of GRK2 levels is an early event in cardiac failure (see ref. (27) for a review). In striking accordance, activation of p38 has long been shown to be critical for the development of various inflammatory processes including rheumatoid arthritis, pulmonary illnesses and inflammatory bowel disease (28). Precisely, as mentioned above, a significant reduction of GRK2 levels was described in animal models of adjuvant-induced arthritis (21), or human rheumatoid arthritis and multiple sclerosis (20, 23), what also points to a negative correlation between GRK2 levels and p38 activity, and a functional role for GRK2 in the control of p38 activation, that might be altered in inflammatory conditions.

GRK2/GIT

The GIT family of proteins display a complex domain structure, including a zinc-finger motif, three ankyrin repeats present in the N-terminal region of the protein, a Spa2-homology domain (SHD), a coiled-coiled domain and a paxillin-binding site (PBS). As a result of such multidomain architecture, GIT proteins can interact with a variety of signalling molecules involved in multiple cellular processes as cytoskeletal dynamics, membrane trafficking, cell adhesion and signal scaffolding (29). Therefore, the GRK2/GIT1 interaction may be involved in the modulation of cell migration processes (5). However, only recently the functional consequences and the modulation of such interaction by extra-cellular signals have been addressed. Our laboratory has found that GRK2 positively regulates epithelial cell migration by mechanisms involving coordinated fibronectin and S1P-mediated signalling and the modulation of the Rac/PAK/MEK/ERK1/2 pathway in response to S1P and adhesion,

based on the dynamic regulation of the interaction of GRK2 with GIT1 (Penela et al., EMBO J., in press). These results suggest that the overall contribution of GRK2 to cell migration would be cell-type specific and stimulus-specific. This possibility is currently being investigated in our group.

In sum, the complex GRK2 "interactome" with multiple signaling proteins involved in inflammation and cell migration (summarized in Figure 1), its reported alterations in inflammatory disorders and the network of mechanisms controlling its activity and expression levels suggest key functional roles for GRK2 in inflammation, that deserve to be explored in the future.

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