1521-0111/98/6/751–760$35.00
MOLECULAR PHARMACOLOGY

https://doi.org/10.1124/molpharm.120.000111
Mol Pharmacol 98:751–760, December 2020

Special Section on RGS Proteins in Health and Disease — Minireview

Emerging Roles for Regulator of G Protein Signaling 2 in (Patho) physiology

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Received July 8, 2020; accepted September 8, 2020

ABSTRACT

Since their discovery in the mid-1990s, regulator of G protein signaling (RGS) proteins have emerged as key regulators of signaling through G protein–coupled receptors. Among the over 20 known RGS proteins, RGS2 has received increasing interest as a potential therapeutic drug target with broad clinical implications. RGS2 is a member of the R4 subfamily of RGS proteins and is unique in that it is selective for $G\alpha_q$. Despite only having an RGS domain, responsible for the canonical GTPase activating protein activity, RGS2 can regulate additional processes, such as protein synthesis and adenylate cyclase activity, through protein-protein interactions. Here we provide an update of the current knowledge of RGS2 function as it relates to molecular mechanisms of regulation as well as its potential role in regulating a number of physiologic systems and pathologies, including cardiovascular disease and central nervous system disorders, as well as various forms of cancer.

SIGNIFICANCE STATEMENT

Regulator of G protein signaling (RGS) proteins represent an exciting class of novel drug targets. RGS2, in particular, could have broad clinical importance. As more details are emerging on the regulation of RGS2 in various physiological systems, the potential utility of this small protein in therapeutic development is increasing.

Introduction

A vast number of physiologic processes are controlled by the large family of G protein–coupled receptors (GPCRs). These receptors mediate signal transduction through the activation of heterotrimeric ($\alpha\beta\gamma$) G proteins. Receptor activation induces a conformational change in $G\alpha$ that enables the exchange of GDP for GTP, rendering it active and able to mediate downstream signaling cascades. Recently, much effort is being devoted to the mechanisms regulating GPCR signaling. One key regulatory mechanism is mediated by the regulator of G protein signaling (RGS) protein superfamily. At least 20 RGS proteins have been identified since the mid-1990s, all containing a conserved 120-residue RGS domain. This domain is responsible for the canonical action of RGS proteins to serve as GTPase activating proteins (GAPs) on active, GTP-bound $G\alpha$ subunits. Through this action, RGS proteins modulate GPCR signaling by shortening the duration and amplitude of GPCR-mediated responses. In addition, many RGS proteins can regulate cellular functions through noncanonical mechanisms. RGS proteins achieve selectivity toward certain pathways through discrete tissue distribution and selectivity toward $G\alpha$ subtypes. Thus, they assist in fine-tuning GPCR signal transduction and represent a family of potential new drug targets.

RGS2 is a member of the largest family of RGS proteins, the R4 family. Like all the members of this family, RGS2 is a small protein with no additional domains apart from the RGS domain. Despite this, additional functions other than GAP activity have been attributed to RGS2, such as the participation in protein-protein interactions that affect both GPCR signaling and other processes. Emerging data from in vitro, in vivo, and human studies have identified a wide variety of physiologic roles for RGS2 (Fig. 1). Notably, low RGS2 protein levels have been observed and associated with a variety of disease states. In some cases, the mechanism by which RGS2 would contribute to disease has been established; however, much is yet to be discovered about the molecular mechanisms regulating RGS2 and how RGS2, in turn, affects physiologic function. Here, we summarize the key features pertaining to cellular functions regulated by RGS2, the regulation of RGS2 protein expression and activity, its physiologic roles, and implications for therapeutic development.

https://doi.org/10.1124/molpharm.120.000111.

ABBREVIATIONS: AC, adenylate cyclase; AD, Alzheimer's disease; AML, acute myeloid leukemia; AT1R, angiontensin II type 1 receptor; CNS, central nervous system; D2R, dopamine D2 receptor; EPS, extrapyramidal symptoms; GAP, GTPase activating protein; GPCR, G protein–coupled receptor; HDAC, histone deacetylase; 5-HT, serotonin; LABA, long-acting β-adrenergic receptor agonist; LRRK2, leucine-rich repeat kinase 2; PAR1, protease-activated receptor 1; PD, Parkinson's disease; PKC, protein kinase C; RGS, regulator of G protein signaling; SNP, single nucleotide polymorphism; VEGFB, vascular endothelial growth factor-B.

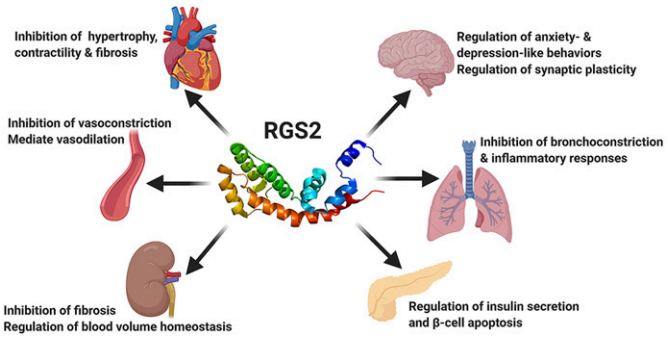

Fig. 1. Summary of RGS2 protein physiologic functions. RGS2 is widely expressed in various tissues, and there is support for a role in regulating diverse physiologic functions, including vaso- and bronchoconstriction, cardiac hypertrophy, CNS functions, and insulin secretion and β-cell physiology. Structure adapted from PDB: 4EKD, RGS2 in complex with $G\alpha_q$ (Nance et al., 2013). Figure created using Biorender (Biorender.com).

RGS2 Regulation of Cellular Functions. RGS proteins present differential selectivity toward $G\alpha$ subtypes. Several RGS proteins can bind and act as GAPs for $G\alpha_q$, $G\alpha_{i/o}$, and $G\alpha_{12/13}$ with similar affinity (reviewed in, e.g., Zhang and Mende, 2011). Although several other RGS proteins are efficient GAPs for $G\alpha_q$, RGS2 is unique in that it is selective for $G\alpha_q$ over any other $G\alpha$ subunit. In fact, early studies demonstrated that RGS2 is able to bind and act as a GAP only on $G\alpha_q$ in vitro (Heximer et al., 1997). In addition, RGS2 shows much greater potency than the closely related RGS4 in inhibiting $G\alpha_q$-mediated phospholipase C activation, indicating that RGS2 is not only selective for $G\alpha_q$, but a more potent GAP for $G\alpha_q$ than its closely related family members. A few later studies were able to demonstrate GAP activity toward $G\alpha_i$ in reconstituted membrane systems (Ingi et al., 1998; Cladman and Chidiac, 2002). RGS2 has also been shown to regulate $G\alpha_i$ signaling in cardiomyocytes (Chakir et al., 2011); however, direct GAP activity toward $G\alpha_i$ or any other class of $G\alpha$ has yet to be demonstrated for RGS2 in vitro. The molecular basis for the selectivity of RGS2 for $G\alpha_q$ remained poorly understood until the crystal structure of RGS2 in complex with $G\alpha_q$ was determined (Nance et al., 2013). It showed that RGS2 adopts a unique conformational pose compared with that of published RGS-$G\alpha_{i/o}$ complexes. RGS2 forms extensive interactions with the $G\alpha_q$ α-helical domain, a stabilizing network of interactions that may explain the increased affinity of RGS2 for $G\alpha_q$ compared with that of RGS4 (Nance et al., 2013).

Although it contains no additional protein domains apart from the RGS domain, several non-GAP functions have been attributed to RGS2. These functions are mediated by the ability of RGS2 to interact with several proteins, apart from $G\alpha_q$. The interactions identified thus far, as well as their functional consequences, are summarized in Table 1. Although RGS2 does not act as a GAP on $G\alpha_s$, it can inhibit Gs-mediated signaling through a direct interaction with certain isoforms of adenylate cyclase (AC), the enzyme downstream of $G\alpha_s$ that is responsible for the production of the second messenger cAMP. Specifically, RGS2 interacts with, and inhibits, AC III, the predominant isoform in the olfactory system, as well as the cardiac isoforms AC V and VI (Sinnarajah et al., 2001). This interaction is mediated through the N-terminal region of RGS2 and unrelated to the actions of the RGS domain. This was demonstrated by the inability of RGS2 lacking the first 19 residues to either bind AC V or to suppress Gs-mediated signaling (Salim et al., 2003).

Apart from the interaction with AC, RGS2 also interacts with several GPCRs directly. Through this action, RGS2 exerts further selectivity for certain Gq-coupled receptors over others. Early studies identified an interaction with the third intracellular loop of the Gq-coupled M1 muscarinic acetylcholine receptor, which was specific for RGS2 over the closely related RGS16 (Bernstein et al., 2004). This interaction was also selective for the M1 receptor over the other muscarinic receptors (M2–5). Recently, through studies using bioluminescence resonance energy transfer in cells, RGS2 was shown to form a complex with the protease-activated receptor 1 (PAR1) as well (Kim and Ghil, 2020). This interaction seems dependent on concurrent interaction with $G\alpha_q$ and thus may be dependent on activation state of the receptor. Furthermore, RGS2 has been suggested to interact with, and selectively enhance inhibition of signaling through, the angiotensin II type 1 receptor (AT1R) (Matsuzaki et al., 2011). This study did not directly demonstrate binding of RGS2 to AT1R; however, it demonstrated that the RGS2 N terminus is an important determinant of the magnitude of suppression of AT1R Gq-mediated signaling. Thus, the N-terminal region of RGS2 appears to serve an important function in mediating protein-protein interactions with receptors, in addition to AC. It is also a key region for determining RGS2 protein stability as well as subcellular localization as described below.

An additional example of a noncanonical function of RGS2 is its ability to suppress total protein synthesis, through a direct interaction with the translation initiation factor Eukaryotic Initiation Factor 2ε (eIF2ε) (Nguyen et al., 2009). This action is mediated through a stretch in RGS2, partly overlapping with the RGS domain, but unrelated to GAP activity. The rate of total protein synthesis is directly involved in cell proliferation and growth, which can be induced by activation of GPCRs. RGS2 can suppress hypertrophy in neonatal rat cardiomyocytes, solely through its ability to suppress protein synthesis. Reintroduction of only the fragment of RGS2 responsible for this is enough to reverse hypertrophy (Chidiac et al., 2014). Altogether, although RGS2 is a seemingly simple protein, it is capable of several other functions in addition to acting as a GAP for $G\alpha$. These functions are mediated by protein-protein interactions, the majority of which occur through the N-terminal unstructured domain.

Regulation of RGS2 Protein Expression and Activity. Activity and expression of RGS proteins, including RGS2, is tightly spatially and temporally regulated. This can be achieved through transcriptional, epigenetic, and post-transcriptional mechanisms. A key regulatory mechanism controlling RGS2 protein levels is through rapid and constitutive degradation by the ubiquitin-proteasomal system. This system, critical for cell proliferation, differentiation, and survival, consists of a vast number of enzymes that couple a chain of ubiquitin molecules onto proteins to mark them for degradation by the 26S proteasome (Hershko and Ciechanover, 1998). Among these enzymes, the large family of E3 ligases (>600 known to date) recognizes substrates for ubiquitination and subsequent degradation through the 26S proteasome. In transfected cells, RGS2 has a protein half-life of ~20 minutes. Treatment of cells with the proteasome inhibitor MG-132 completely stabilizes RGS2 protein levels, indicating that proteasomal degradation

TABLE 1

Summary of RGS2 protein-protein interactions

Listed are confirmed interactions for RGS2 and their functional consequences. This list is not comprehensive, and more interactions are likely to be found in the future.

Interacting protein	Region of interaction	Functional consequence	Reference(s)
Gα subunits			
$G\alpha_q$	RGS domain; high affinity	GAP activity	e.g., Ingi et al., 1998; Nance et al., 2013
$G\alpha_{i1}$	RGS domain; low affinity	GAP activity	e.g., Ingi et al., 1998
$G\alpha_s$	RGS domain	Modulate signaling; no GAP activity	Roy et al., 2006
GPCRs			
AT1R	N terminus	Inhibition of AT1R signaling	Matsuzaki et al., 2011
PAR1	N terminus; third intracellular loop of PAR1; Gα dependent	Inhibition of PAR1 signaling	Ghil et al., 2014
PAR4	N terminus; third intracellular loop of PAR4; Gα dependent	Inhibition of PAR4 signaling	Kim and Ghil, 2020
M1R	N terminus; third intracellular loop of M1R	Inhibition of M1R signaling	Bernstein et al., 2004
α1AR	N terminus; third intracellular loop of α1AR	Inhibition of α1AR signaling	Hague et al., 2005
β2AR	N terminus; third intracellular loop of β2AR	Inhibition of β2AR signaling	Roy et al., 2006
MCH1R	Residues 28–80	Inhibition of MCH1R signaling	Miyamoto-Matsubara et al., 2010
Other			
AC III	N terminus; C1 region of AC III	Inhibits cAMP production	Sinnarajah et al., 2001
AC V	N terminus; C1 region of AC V	Inhibits cAMP production	Sinnarajah et al., 2001
AC VI	N terminus; C1 region of AC V	Inhibits cAMP production	Sinnarajah et al., 2001
eIF2Bε	Residues 79–115	Inhibits protein synthesis	Wang et al., 2018
PKGI-α	RGS2 phosphorylation	Inhibits RGS2 degradation; promotes RGS2 membrane localization	Tang et al., 2003; Osei-Owusu et al., 2007
PKC	Phosphorylation on Ser^{46}	Inhibits GAP activity in vitro	Cunningham et al., 2001
TRPV6	N terminus	Modulates channel activity	Schoeber et al., 2006
LRRK2	Undetermined	Control of neuronal process length, protective against neuronal toxicity	Dusonchet et al., 2014
Nek7	Undetermined	Mitotic spindle organization	de Souza et al., 2015
FBXO44	N terminus	E3 ligase; promotes RGS2 protein degradation	Sjögren et al., 2015
Teb4	N terminus	E3 ligase; promotes RGS2 protein degradation	Park et al., 2015

α1AR, α1-adrenoreceptor; β2AR, β2-adrenoreceptor; eIF2Bε, Eukaryotic Initiation Factor 2Bε; FBXO44, F-box only protein 44; M1R, muscarinic M1 receptor; MCH1R, melanin-concentrating hormone receptor 1; PAR4, protease-activated receptor 4; PKGI-α, cGMP-dependent protein kinase I-α; TRPV6, Transient Receptor Potential Vanilloid subfamily member 6.

plays an important role in its regulation (Sjögren et al., 2012). Selectively inhibiting RGS2 protein degradation could therefore be a viable strategy in disease states associated with low RGS2 protein levels, described in subsequent sections, and efforts have been made to identify the molecular machinery responsible for RGS2 protein degradation. RGS4 and RGS5, closely related to RGS2, are targeted for proteasomal degradation through the N-end rule pathway, where destabilizing residues at the very N terminus of the protein serves as a recognition signal, or degron, for their cognate E3 ligase (Lee et al., 2005). RGS2 has also been proposed as an N-end rule substrate, where Gln^2 is acetylated to create a degron for the E3 ligase (Park et al., 2015). This model is supported by the finding that a glutamine-to-leucine mutation in RGS2 (Q2L), identified in a hypertensive cohort, demonstrated enhanced proteasomal degradation (Yang et al., 2005; Bodenstein et al., 2007). This mutation would make RGS2 a direct target for the N-end rule pathway, as leucine is a primary destabilizing residue, whereas glutamine is a secondary destabilizing residue, requiring deamidation mediated by glutamine-specific N-terminal amidase to enter the N-end rule pathway (Wang et al., 2009; Sjögren and Neubig, 2010). However, later studies have failed to confirm the N-end rule pathway model of RGS2 protein degradation (Sjögren et al., 2015; Kanai et al., 2017).

An alternative model for RGS2 protein degradation was presented by our identification of a cullin-RING (Really Interesting New Gene) ligase that is able to degrade RGS2 in both transfected cells and mouse cardiomyocytes (Sjögren et al., 2015). The protein that recognizes RGS2 within this complex is F-box only protein 44. It is a member of the 69-member protein family of F-box proteins that have received increasing attention as drug targets in various diseases, ranging from several types of cancer to neurologic disorders, such as Parkinson's disease (Skaar et al., 2013; Wang et al., 2014). Whether these two models of RGS2 degradation—N-end rule pathway or F-box–mediated—are contradictory or whether RGS2 is degraded through alternate pathways depending on cell type or context is yet to be determined. Regardless, the importance of the N-terminal region of RGS2 for protein stability is clear. RGS2 has four isoforms, resulting from alternative translation starting at Met^1, Met^5, Met^{16}, and Met^{33}, respectively (Gu et al., 2008a), and the shorter variants (initiated at Met^{16} or Met^{33}) are protected from proteasomal degradation, further supporting the hypothesis that RGS2 is targeted for degradation through its N terminus (Kanai et al., 2017).

Further studies into the intricate mechanisms regulating RGS2 protein levels and function have used nonsynonymous

single nucleotide polymorphisms (SNPs) identified through genomic studies. RGS proteins need to localize to the plasma membrane to act as GAPs for Gα. In the case of RGS2, this membrane targeting is mediated through a stretch in the N-terminal region. One SNP results in a R44H mutation, which was determined to completely block RGS2 plasma membrane targeting (Gu et al., 2008b). A subsequent study identified a second mutation, D40Y, resulting in similarly impaired plasma membrane targeting (Phan et al., 2017). Hence, apart from playing a key role in targeting RGS2 for protein degradation and protein-protein interactions, the N terminus also plays a crucial role in targeting RGS2 to the plasma membrane. Additional SNPs have been found in both the coding region and the 3′-untranslated region of RGS2. In many cases, the functional effects on RGS2 protein levels and/or function have yet to be determined, but some have been extensively studied in terms of physiologic impact. Some of these will be described in the following sections in the context of their possible clinical relevance.

In addition to interacting with, and modulating signaling through, certain GPCRs, as described above, RGS2 expression is also, in turn, regulated by GPCR activity. One example is the induction of RGS2 expression by long-acting β-adrenergic receptor agonists (LABAs) used in asthma treatment (Holden et al., 2014). We discovered that RGS2 protein levels are increased by protein kinase C (PKC) activation occurring downstream of Gq-coupled GPCRs. These increased levels enhanced the ability of RGS2 to suppress G protein–mediated signaling and may serve as a general negative feedback loop for Gq-mediated signal transduction (Raveh et al., 2014). This also demonstrated that RGS2 activity is directly correlated with its expression levels. Whether the PKC-mediated increase in RGS2 protein levels is a result of direct phosphorylation is yet to be determined. In contrast to our findings, PKC phosphorylation of Ser^{46} was found to inhibit RGS2 GAP activity toward $G\alpha_{11}$ in vitro (Cunningham et al., 2001). Thus, the exact role of PKC regulation of RGS2 is still unclear and may depend on cellular context. Altogether, although much progress has been made in deciphering the mechanisms regulating RGS2 protein levels and activity, much is yet to be discovered.

RGS2 Is a Regulator of Cardiovascular Function. The most well defined physiologic role for RGS2 is its regulation of the cardiovascular system. RGS2 is highly expressed in both the vasculature and heart, as well as in the kidney. Early studies in $RGS2^{-/-}$ mice shed light on the role of RGS2 in suppressing blood pressure, as they demonstrated both hypertension and prolonged responses to vasoconstrictor signaling by Gq-coupled GPCRs, such as AT1R and the purinergic receptor P2Y (Heximer et al., 2003; Hercule et al., 2007). However, regulation of blood pressure by RGS2 was later demonstrated to be more complex in nature. RGS2 is an effector of NO-mediated vasodilation in vascular smooth muscle cells (Tang et al., 2003; Sun et al., 2005; Obst et al., 2006). Phosphorylation of RGS2 on Ser^{46} and Ser^{60} by protein kinase G (PKG) induces translocation to the plasma membrane and modestly increases GAP activity of RGS2 toward $G\alpha_q$ (Osei-Owusu et al., 2007), suggesting that activation of the NO-cGMP-PKG axis to induce vasodilation may be, at least in part, mediated by increased RGS2 function. Yet another study found that the regulation of blood pressure by RGS2 might not originate solely from the vasculature. In a cross-transplantation study, restoring RGS2 expression in the kidney was sufficient to restore normal blood pressure in $RGS2^{-/-}$ mice (Gurley et al., 2010). Although this study did not determine whether the effects of RGS2 expression originated from the kidney epithelium or vasculature, it indicates that the mechanisms mediating the effects of RGS2 on blood pressure homeostasis are more intricate than originally proposed.

Recent investigations have expanded on the role of RGS2 in the regulation of cardiovascular homeostasis. It appears that RGS2 plays an adaptive role in controlling uterine blood flow during pregnancy. $RGS2^{-/-}$ mice do not display increased blood flow normally associated with utero-placental perfusion during pregnancy (Koch et al., 2019). Furthermore, both $RGS2^{-/-}$ and $RGS5^{-/-}$ mice were more sensitive to α-adrenergic stimulation before pregnancy, with this sensitivity sustained through midpregnancy for $RGS2^{-/-}$ mice. Treatment of wild-type and $RGS5^{-/-}$ mice with the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester increased sensitivity to α-adrenergic stimulation to similar levels as $RGS2^{-/-}$, implying a possible mechanism for RGS2 dysfunction. This study reveals that RGS2 may play an important role in the regulation of vascular function during pregnancy (Koch et al., 2019). Further support for this was provided by a recent study demonstrating that RGS2 mRNA levels were reduced in the placenta during preeclampsia (Perschbacher et al., 2020). RGS2 was identified as a histone deactylase (HDAC) 9–dependent gene using immortalized human HTR8/SVneo trophoblasts. HDAC9 was reduced in human placentas affected by preeclampsia, suggesting a mechanism for the observed reduction in placental RGS2 mRNA levels. The same study also demonstrated that female mice with reduced RGS2 expression within the feto-placental unit displayed increased diastolic blood pressure and heart rate during the last week of gestation compared with wild-type mice, further supporting a protective role for RGS2 in preeclampsia (Perschbacher et al., 2020).

In addition to vascular regulation by RGS2, there is also ample evidence that RGS2 has a crucial role in regulating cardiac function through G protein-dependent and -independent mechanisms. Although they display no detrimental cardiac phenotype under normal conditions, $RGS2^{-/-}$ mice display augmented mortality, cardiac hypertrophy, and cardiac fibrosis in response to pressure overload (Takimoto et al., 2009). In line with this observation, RNA interference–mediated RGS2 knockdown in neonatal ventricular myocytes exacerbates phenylephrine- and endothelin-1–induced hypertrophy (Zhang et al., 2006). Additionally, in vitro overexpression of RGS2 inhibits cardiomyocyte hypertrophic effects induced by α1- or β-adrenergic receptor activation (Nunn et al., 2010). Although all of these effects could be attributed to the effects of RGS2 on Gq- and Gs-mediated signaling, a later study demonstrated that the 37-residue stretch in RGS2 responsible for inhibiting de novo protein synthesis ($RGS2^{eb}$) inhibits cardiac hypertrophy comparably to full-length RGS2 (Lee et al., 2017).

Although it did not directly focus on RGS2, a recent study found that vascular endothelial growth factor-B (VEGFB) reduced markers of hypertrophy in angiotensin II–treated rat cardiomyocytes. The reduced angiotensin II–mediated intracellular Ca^{2+} responses by VEGFB was determined to likely be due to stabilization of PKG and its downstream effectors, including RGS2. Angiotensin II treatment reduced RGS2

levels in cardiomyocytes, an effect that was partially abated upon cotreatment with VEGFB (Shen et al., 2018). Altogether, these data suggest that RGS2 may serve protective roles in cardiac function through multiple mechanisms and may be a potential drug target in cardiac, as well as vascular, diseases associated with overactive GPCR signaling.

Data from human studies also support a role for RGS2 in the regulation of cardiovascular function. Several SNPs associated with reduced RGS2 protein levels and/or function have been linked to hypertension in several different ethnic groups (Yang et al., 2005; Riddle et al., 2006). One example is the SNP resulting in the Q2L mutation found in a Japanese hypertensive cohort. This mutation causes reduced expression of RGS2 due to enhanced proteasomal degradation (Bodenstein et al., 2007; Park et al., 2015; Phan et al., 2017), demonstrating that reduced RGS2 protein levels have an impact in humans as well as in murine models. Further support for the effect of altered RGS2 protein levels for cardiovascular functions is provided by evidence that RGS2 expression is increased in patients with Bartter's/Gitelman's syndrome, a disorder characterized by low blood pressure (Calò et al., 2004). Finally, low RGS2 protein levels have also been associated with nonresponsiveness to antihypertensive treatment (Semplicini et al., 2010), suggesting RGS2 as a key regulator of blood pressure homeostasis in humans as well as in animal models.

Roles for RGS2 in the Central Nervous System. RGS2 is widely expressed in the central nervous system (CNS) and has been linked to a number of CNS disorders, including anxiety and depression, as well as recently proposed links to Parkinson's and Alzheimer's diseases. Although most studies thus far have only demonstrated association of RGS2 with disease states, with little to no mechanistic analysis, it is likely that RGS2 could also be a potential therapeutic target for the treatment of several CNS diseases.

The first evidence of a link between RGS2 and anxiety came from early studies in RGS2$^{-/-}$ mice, demonstrating enhanced anxiety as measured by the dark/light preference test (Oliveira-Dos-Santos et al., 2000). Subsequent research identified a quantitative trait locus in mice, containing the *RGS2* gene, that was associated with anxiety-related behavior (Yalcin et al., 2004). More recently, RGS2$^{-/-}$ mice were demonstrated to show enhanced fear learning, indicative of anxiety-like behaviors. There was also decreased neurotransmitter concentrations in RGS2$^{-/-}$ mice as well as lower expression of the serotonin (5-HT) receptor 5-HT$_{2C}$. In contrast, 5-HT$_{2A}$ showed increased expression (Raab et al., 2018). In addition to the evidence provided by knockout models, mice heterozygous or homozygous for an SNP in the 3′-untranslated region of the RGS2 gene, rs4606, which causes decreased RGS2 expression, show enhanced anxiety- and depression-like behaviors (Lifschytz et al., 2012). The rs4606 SNP was also associated with reduced expression of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, specifically in the raphe nucleus, identifying a potential mechanism for RGS2-mediated anxiety-like behaviors (Lifschytz et al., 2012).

After these initial studies, four SNPs associated with both panic disorder and agoraphobia were identified in the RGS2 gene in humans (Leygraf et al., 2006). An additional nine SNPs were later associated with behavioral inhibition in children, which is a familial and developmental risk factor for anxiety disorders (Smoller et al., 2008). Four of these were also found to be associated with introversion in adults, another risk factor for anxiety disorders. Furthermore, two SNPs were associated with increased activity in the amygdala and left insular cortex, regions associated with anxiety, in response to an emotional test. In addition, rs4606 was deemed a risk factor for anxiety (Smoller et al., 2008). Further studies have solidified the association between rs4606 and various anxiety disorders in both children and adults and suggest a relationship between low RGS2 levels and general anxiety disorders (Koenen et al., 2009; Stein et al., 2014; Asselmann et al., 2018).

In addition to anxiety-related disorders, there is also a tenuous link between RGS2 and Parkinson's disease (PD) and Parkinson-like disease states. A phenotypic study suggested that genetic variations in the *RGS2* gene are associated with susceptibility to Parkinson-like extrapyramidal symptoms (EPS) induced by antipsychotic drugs. Again, rs4606 was found to be associated with Parkinson-like EPS in patients with schizophrenia being treated with a typical antipsychotic drug alone or in combination with risperidone (Greenbaum et al., 2007). The G allele of rs4606 was later shown to be overrepresented in patients without EPS in a cohort of African American and Caucasian patients with schizophrenia, indicating a possible protective effect of this allele (Greenbaum et al., 2009). However, there are conflicting results from these human genetic screens on the impact of genetic variations in the RGS2 gene and Parkinson-like EPS in schizophrenia. Some studies have found no link between any haplotype of rs4606 and EPS or movement disorders (Al Hadithy et al., 2009; Bakker et al., 2012), whereas others have deemed the G allele to be detrimental (Higa et al., 2010). Thus, in humans, the impact of genetic variations in the *RGS2* gene on Parkinson-like EPS in schizophrenia remains unclear.

Apart from human genetic screens, a few studies have provided in vitro mechanistic data on the possible involvement of RGS2 in PD. Mutations in leucine-rich repeat kinase 2 (LRRK2) is a common marker of PD. Small interfering RNA knockdown of RGS2 in LRRK2-expressing *Caenorhabditis elegans* increased the survivability of dopaminergic neurons. Follow-up experiments elucidated that the RGS domain and LRRK2 coimmunoprecipitate with each other even with PD-associated LRRK2 mutations. RGS2 decreases both the GTPase and kinase activity of LRRK2, resulting in an inhibition of LRRK2-dependent neurite shortening. It was also found that RGS2 expression is decreased in the striatum of patients with mutant LRRK2 and sporadic PD compared with control, providing a clinical link between RGS2 and PD (Dusonchet et al., 2014).

Dopamine is a central regulator of motor functions and reward and plays a central role in the pathology of addiction, schizophrenia, and PD. A 2016 study found that RGS2 negatively modulates dopamine D2 receptor (D2R) signaling in neuroblastoma N2A cells (Luessen et al., 2016). In addition, RGS2 knockdown was found to increase constitutive D2R internalization while preventing quinpirole-induced internalization. The latter effect is due to a tighter interaction between D2R and β-arrestin resulting in less β-arrestin dissociation from the membrane. Although this study does not look into the implications of RGS2 in PD concerning these interactions, demonstrating that RGS2 directly alters dopamine signaling and receptor internalization provides strong justification for future studies (Luessen et al., 2016).

Finally, there is also a suggested association between RGS2 levels and the progression of Alzheimer's disease (AD).

A genomewide transcriptomic study identified lower RGS2 mRNA levels being correlated with increased sensitivity to amyloid-$\beta_{1–42}$ treatment in cell culture. Accumulation of amyloid-β plaques is one of the primary markers of AD, and these results imply that RGS2 may play a protective role against this hallmark of the disease. Furthermore, RGS2 mRNA levels were decreased in cells isolated from patients with AD compared with healthy controls. In contrast to these results, however, lower RGS2 levels were also correlated with better results for the Mini Mental State Examination and AD Assessment Scale, two tests that indicate cognitive ability. These cognitive effects were attributed to enhanced muscarinic signaling, enhanced melatonin production, and enhanced EIF2Bε-mediated translation, all processes regulated by RGS2 (Hadar et al., 2016). Taken together, low RGS2 mRNA correlated with both detrimental and beneficial outcomes in AD, and more studies are warranted to decipher the mechanistic basis for the role that RGS2 might play in the progression of this disease.

RGS2 as a Potential Cancer Target. As described above, RGS2 can mediate effects on cellular functions through several different mechanisms in addition to being a negative regulator of G protein signaling. These additional functions may contribute to and be associated with a number of malignancies. Although the specific mechanisms by which RGS2 is involved in cancer progression is less understood than in the cardiovascular system, emerging evidence suggests an important regulatory role in several types of cancer, including breast, prostate, acute myeloid leukemia, bladder, ovarian, and colorectal cancer.

Breast cancer is the second deadliest cancer in the United States (Siegel et al., 2019), with several subtypes, many of which lack effective treatments. A proposed role for RGS2 as a tumor suppressor has recently emerged. RGS2 is downregulated in breast cancer cells and human tumor samples, and overexpression of RGS2 can inhibit MCF-7 breast cancer cell growth (Lyu et al., 2015). In addition, both mRNA and protein levels of RGS2 were shown to be downregulated in breast invasive carcinoma of no special type, the most common subtype of invasive breast cancer (Wang et al., 2018). This same study also demonstrated that patients with lower RGS2 expression levels had a significantly poorer overall survival rate (Wang et al., 2018). The mechanisms involved in RGS2 suppression and how RGS2, in turn, regulates breast cancer progression are still under investigation. One proposed regulatory mechanism is mediated through the deubiquitinating enzyme monocyte chemotactic protein–induced protein 1 that was demonstrated to protect RGS2 from degradation in MCF-7 cells (Lyu et al., 2015). However, it is still uncertain whether this mechanism occurs endogenously in breast cancer, as these studies were performed using overexpression systems. Overall, these studies provide support for RGS2 as a tumor suppressor and a potential target for breast cancer treatment. However, other studies seem to contradict these data (Kelly et al., 2006a; Xie et al., 2009). Hence, the role of RGS2 as a breast cancer tumor suppressor warrants further investigation.

The role of RGS2 in prostate cancer seems to be complex and dependent on cancer stage. In clinical samples, RGS2 protein levels are downregulated by moderate hypoxia during primary prostate cancer development, whereas they are upregulated in late stages (Linder et al., 2018). Therefore, RGS2 protein levels might be used as a prognostic maker to distinguish between primary and advanced disease. In addition, by analyzing a cohort of patients with advanced prostate cancer, a correlation was found between high RGS2 level and poor patient survival and metastasis (Linder et al., 2018). Furthermore, RGS2 knockdown in LNcap prostate cancer cells decreased migration and induced epithelial cell morphology and behavior (Linder et al., 2018). All these data indicate that RGS2 contributes to metastasis in androgen-sensitive prostate cancer. However, in the androgen-independent stage, RGS2 is proposed to play a tumor suppressor role. Microarray data demonstrated decreased RGS2 protein levels in prostate carcinoma (Wolff et al., 2012). The proposed mechanism for RGS2 downregulation is through hypermethylation of the *RGS2* promotor region. Furthermore, increased RGS2 expression levels inhibit androgen-independent cell growth as well as tumor growth in a xenograft mouse model. This is consistent with microarray data showing that RGS2 is one of the most plausible candidates causing repressed androgen-independent prostate tumor growth (Jennbacken et al., 2009; Wolff et al., 2012). The inhibition of cell growth induced by RGS2 may be attributed to the observation that RGS2 can attenuate androgen-independent androgen receptor activity (Cao et al., 2006). However, the mechanism by which RGS2 exerts these effects is currently unknown, as GAP activity cannot explain the suppression. Further support for a protective role of RGS2 in androgen-independent prostate cancer is provided by the demonstration that overexpression of RGS2 can inhibit oxytocin-, transforming growth factor-β1–, and epidermal growth factor–induced PC3 cell migration (Caggia et al., 2018). In contrast, another group found no effect of RGS2 overexpression on thrombin-stimulated cell invasion in PC3 cells or another androgen-insensitive cell line, DU145 (Kelly et al., 2006b). Furthermore, a recent study showed that higher RGS2 protein levels were found to be prognostic for poor survival in castration-resistant prostate cancer and that *RGS2* gene expression levels positively correlated with androgen receptor expression and activity (Linder et al., 2020). Thus, the role of RGS2 in prostate cancer is complex and may depend on the stage of cancer progression.

Apart from breast and prostate cancer, RGS2 has also been proposed as a tumor suppressor in acute myeloid leukemia (AML). RGS2 was expressed at significantly lower levels in patients with AML in comparison with normal bone marrow, especially in patients with an internal tandem duplication in FMS-like tyrosine kinase 3, the most common mutation in AML (Schwäble et al., 2005). In addition, RGS2 overexpression inhibits FLT3-ITD–induced cell proliferation and growth induced by this mutation and antagonizes differentiation blockade induced by this mutation, which is an important step for malignant transformation (Schwäble et al., 2005). The mechanism by which RGS2 is downregulated or by which mechanism RGS2 achieves the effects in AML is not yet defined.

Repression of RGS2 has been proposed to be associated with other less common cancer types such as bladder cancer, ovarian cancer, and colorectal cancer. RGS2 expression levels were found to be significantly lower in tumor tissues than in matched normal tissues, and low RGS2 expression was correlated with reduced overall survival in patients with bladder cancer (Ying et al., 2015). RGS2 suppression could be attributed to a higher percentage of promotor hypermethylation because of increased expression of Ubiquitin Like

With PHD And Ring Finger Domains 1 (UHRF1), a regulator of DNA methylation (Ying et al., 2015). Epigenetic suppression of RGS2 expression has also been observed in chemoresistant ovarian cancer. RGS2 expression level is slightly lower in ovarian cancer cells, and overexpression of RGS2 can inhibit lipopolysaccharide-mediated downstream signaling, which is important for ovarian cancer cell growth (Hurst et al., 2009). In this instance, RGS2 expression is suppressed in chemoresistant ovarian cancer cells, in part due to accumulation of HDAC and DNA methyltransferase at the RGS2 promotor region (Cacan, 2017). However, it is still unknown if downregulation of RGS2 is the cause of the progression and chemoresistance in ovarian cancer. Finally, low RGS2 mRNA and protein levels have been associated with poor survival in stage II and III colorectal cancer (Jiang et al., 2010). This study only demonstrated association, without dissecting potential mechanisms for RGS2 suppression or how RGS2 would suppress colorectal cancer progression. However, it is yet another example of low RGS2 levels correlating with disease progression.

Although the above examples demonstrate downregulation of RGS2 and a possible tumor suppressor role, this may not hold true for all cancer types. For instance, upregulation of RGS2 is related to poor survival in patients with lung adenocarcinoma (Yin et al., 2016). In contrast, downregulation of RGS2 is associated with increased invasion and metastasis of human non-small cell lung cancer cells caused by loss of Mediator Complex Subunit 1 (Kim et al., 2012). These studies emphasize that RGS2 may play different roles depending on the cancer subtype, even in the same tissue. Another example of how overexpression of RGS2 may promote cancer progression is demonstrated by mantle cell lymphoma. In a cDNA array study, RGS2 was found one of four genes suggested to promote tumor progression in both the blastoid variant and common mantle cell lymphoma (Zhu et al., 2002). Additionally, RGS2, among several signal transduction genes in the Ras, mitogen-actived protein kinase, and phosphoinositide 3-kinase pathways, is overexpressed in both primary and metastatic fibrolamellar carcinoma tumors (Kannangai et al., 2007). Collectively, these studies demonstrate, by association, that RGS2 expression levels can be one of the factors promoting tumorigenesis; however, further mechanistic analysis is warranted to determine whether altered RGS2 expression is a cause or effect in these cancers.

Although progress has been made on investigating the association between altered RGS2 expression and cancer progression, there still exists a large gap in knowledge concerning the molecular mechanisms underlying changed RGS2 expression levels. Furthermore, in the majority of cancers, we do not know whether the change in RGS2 mRNA and protein levels is a driver in cancer progression or if it is a result of altering other important tumor suppressors or oncogenes. Finally, the mechanisms by which RGS2 may alter proliferation, migration, or invasion of cancer cells are currently unknown. Increased knowledge of all of these concepts will enhance our understanding of the diverse phenotypes related to RGS2 in different cancer types. Dissection of the mechanisms involved will aid in future cancer therapy development.

Additional Clinical Implications. Apart from the clinical implications described above, RGS2 may play a role in several other areas. Like in the cardiovascular system, airway constriction is also regulated through Gq-coupled activation. RGS2 has been proposed to play a significant role in suppressing Gq signaling and subsequent inflammatory responses and airway remodeling. RGS2 expression levels are reduced in patients with asthma (Jiang et al., 2015), and overexpression of RGS2 reduces intracellular free calcium flux elicited by histamine, methacholine, leukotrienes, and other Gq-coupled spasmogens in primary human airway smooth muscle cells. In line with this, $RGS2^{-/-}$ mice display enhanced airway smooth muscle contractility (Holden et al., 2011; Xie et al., 2012). In addition, the standard-of-care treatment with corticosteroids and LABAs synergistically induces RGS2 expression in both the human bronchial epithelial cell line BEAS-2B and primary bronchial epithelial cells (Holden et al., 2014), possibly explaining the beneficial effects of the combination treatment compared with corticosteroid treatment alone. This is further supported by the fact that protection against Gq-mediated increases in intracellular free calcium, after LABA plus corticosteroid treatment, is dependent on RGS2 (Holden et al., 2011). RGS2 also plays a role in inflammatory responses elicited by Gq. In a model of airway inflammation induced by inhaled lipopolysaccharide, $RGS2^{-/-}$ mice displayed increased airway hyperreactivity in response to the muscarinic receptor agonist methacholine, compared with wild-type mice (George et al., 2018). $RGS2^{-/-}$ mice also displayed enhanced airway hyperresponsiveness in a model of house dust mite–induced airway inflammation (George et al., 2017). In addition, recruitment of inflammatory cells, neutrophils, and eosinophils to the bronchoalveolar lavage fluid was significantly increased in $RGS2^{-/-}$ mice (George et al., 2017). Altogether, these data suggest that RGS2 plays a protective role against airway hyperreactivity and may be a promising target in suppressing aberrant activation of Gq signaling and downstream inflammatory responses in asthma.

Additional suggested physiologic functions for RGS2 include, but are not limited to, kidney fibrosis progression regulation (Jang et al., 2014), brown adipose tissue function and differentiation (Klepac et al., 2019), and serving as a key regulator of pancreatic β-cell function (Dong et al., 2017). In a 2017 study, $RGS2^{-/-}$ mice were found to have enhanced β-cell apoptosis and dysregulated insulin secretion in response to glucose challenge. Based on these and additional data in β cells and isolated β islets, the authors suggested that RGS2 serves a protective role against β-cell loss and may be a promising drug target in the treatment of both type 1 and type 2 diabetes (Dong et al., 2017). Although the data on these additional functions are limited to only a few or even a single publication, the common theme among these studies is that low RGS2 protein levels, and thereby function, are detrimental to physiologic function.

Future Perspectives for Therapeutic Targeting. From all the aforementioned studies it seems that RGS2 may be a promising drug target with broad clinical implications. RGS2 protein levels and/or function is altered in a wide range of pathologies, and in the majority of scenarios low RGS2 protein levels correlate with detrimental effects on physiologic function. RGS proteins have received increasing attention as potential drug targets in the past couple of decades. As crucial regulators of GPCR signaling, they represent a novel point of intervention for targeting G protein–mediated responses. GPCRs are the primary target of a large number of clinically used drugs. Many of these are associated with side effects, due to the widespread distribution of any given GPCR and the fact

that a single receptor can initiate a wide range of G protein–dependent and –independent signaling cascades. Thus, in recent years efforts have been made to develop allosteric modulators or biased agonists that would direct signaling through the receptor in a more fine-tuned manner. It is feasible to imagine that an RGS protein modulator would serve a similar function. Taking into account G protein selectivity and tissue distribution, a specific RGS protein would differentially affect signaling cascades initiated by a GPCR. Furthermore, in the absence of receptor activation, an RGS modulator would not have an effect on G protein signaling. Taken together, RGS proteins could have big therapeutic potential, serving similar roles as allosteric modulators at GPCRs.

Identifying viable small molecule drug leads targeting RGS2, as for all RGS proteins, represents a challenge, in particular when the goal is to enhance function. RGS2 proteins are not highly amenable to small molecule binding, and although several RGS protein inhibitors have been developed (Roman et al., 2007; Blazer et al., 2010, 2011; Bodle et al., 2017, 2018), enhancing function by means of a small molecule would be even more difficult. We have applied unbiased screening approaches to identify small molecules that increase RGS2 protein levels and thereby function. Through these efforts, we identified digoxin and other cardiotonic steroids to selectively enhance RGS2 protein levels by extending protein half-life (Sjögren et al., 2012). Interestingly, just a modest increase in RGS2 protein levels (2–3-fold) was sufficient to inhibit G protein–mediated signaling both in vitro and in vivo and was cardioprotective in a mouse model of cardiac injury (Sjögren et al., 2016). These data served as proof of concept that increased RGS2 protein levels correlate with increased protein function and that mechanisms that mediate the regulation of RGS2 steady-state level could be targeted as a therapy in a wide range of pathologies. As more of these mechanisms are revealed, the potential for RGS2 as an emerging drug target grows.

Authorship Contributions

Participated in research design: McNabb, Zhang, Sjögren.

Performed data analysis: McNabb, Zhang, Sjögren.

Wrote or contributed to the writing of the manuscript: McNabb, Zhang, Sjögren.

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