Review Article

Theme: Heterotrimeric G Protein-based Drug Development: Beyond Simple Receptor Ligands

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Regulator of G Protein Signaling 17 as a Negative Modulator of GPCR Signaling in Multiple Human Cancers

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Abstract. Regulators of G protein signaling (RGS) proteins modulate G protein-coupled receptor (GPCR) signaling networks by terminating signals produced by active G α subunits. RGS17, a member of the RZ subfamily of RGS proteins, is typically only expressed in appreciable amounts in the human central nervous system, but previous works have shown that RGS17 expression is selectively upregulated in a number of malignancies, including lung, breast, prostate, and hepatocellular carcinoma. In addition, this upregulation of RGS17 is associated with a more aggressive cancer phenotype, as increased proliferation, migration, and invasion are observed. Conversely, decreased RGS17 expression diminishes the response of ovarian cancer cells to agents commonly used during chemotherapy. These somewhat contradictory roles of RGS17 in cancer highlight the need for selective, high-affinity inhibitors of RGS17 to use as chemical probes to further the understanding of RGS17 biology. Based on current evidence, these compounds could potentially have clinical utility as novel chemotherapeutics in the treatment of lung, prostate, breast, and liver cancers. Recent advances in screening technologies to identify potential inhibitors coupled with increasing knowledge of the structural requirements of RGS-G α protein-protein interaction inhibitors make the future of drug discovery efforts targeting RGS17 promising. This review highlights recent findings related to RGS17 as both a canonical and atypical RGS protein, its role in various human disease states, and offers insights on small molecule inhibition of RGS17.

KEYWORDS: cancer; drug discovery; GPCR; G protein; regulator of G protein signaling.

INTRODUCTION

G protein-coupled receptors (GPCRs) are the largest class of proteins in the human genome and regulate various physiological processes, ranging from chemosensation to neurotransmission (1). Due to their evolutionarily conserved function as small molecule binding proteins, GPCRs have proved to be useful targets for the development of therapeutic agents. Currently, one third to one half of drugs marketed in the USA act on a GPCR, targeting diseases like hypertension, asthma, schizophrenia, and prostate cancer. Interestingly, over 30% of these drugs elicit their effects by binding to one of only 50 receptors, which represents only ~13% of the non-olfactory GPCR-ome, leaving ample room for future GPCR-targeted drug discovery efforts (2). Furthermore, as G protein-mediated signaling events have been

clinically validated for therapeutic use, proteins downstream of these receptors have gained attention as potential sites of chemical intervention, such as the regulator of G protein signaling (RGS) protein family. Inhibition of RGS proteins by small molecules represents a means by which to enhance GPCR signals by increasing the lifetimes of GTP-bound, active G α subunits. One member of the RGS family that has recently emerged as a potential drug target is RGS17, as it has been implicated in a number of the most common forms of cancer, including lung, breast, prostate, and liver cancers (3–5).

Guanine Nucleotide-Binding Protein (G Protein) Signaling

GPCRs exert their effects by acting as guanine nucleotide exchange factors (GEFs) on G protein α subunits, thereby translating extracellular stimuli into intracellular signaling cascades. G α subunits can be grouped together based on primary sequence identity, their downstream signaling partners, and their sensitivity to RGS protein activity. The inhibitory G α subunits G α_i , G α_o , and G α_z result in inhibition of adenylyl cyclases (AC), decreased cellular cAMP levels, and are sensitive to RGS-mediated GAP activity, whereas the stimulatory G α_s family activates AC, increasing intracellular cAMP, and are insensitive to RGS proteins (6). The activation of the G $\alpha_{q/11}$ family, which is also

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sensitive to regulation by RGS family members, results in increased phospholipase C (PLC) activity, ultimately resulting in calcium mobilization (7). Finally, the $G_{\alpha_{12/13}}$ family activates RhoGEF, which acts as a GAP for $G_{\alpha_{12/13}}$ subunits and a GEF for the small GTPase Rho, linking GPCR signaling to Rho-mediated cellular events, such as cytoskeletal rearrangements and cell division (8). Upon stimulation with ligand, a ternary complex is formed between the ligand, GPCR, and $G\alpha\beta\gamma$ heterotrimer, where GDP is exchanged for GTP in the $G\alpha$ subunit, which then dissociates from the obligate $G\beta\gamma$ dimer (9). Both the $G\beta\gamma$ and GTP-bound $G\alpha$ are then able to initiate signaling cascades through interaction with downstream effectors, such as AC, PLC, ion channels, and RhoGEF. In order to terminate signaling, $G\alpha$ hydrolyzes GTP to GDP *via* its intrinsic GTPase activity, and $G\alpha$ -GDP then associates with $\beta\gamma$, reforming the inactive $G\alpha\beta\gamma$ heterotrimer, thus terminating signaling (Fig. 1).

Regulators of G Protein Signaling

RGS proteins, as GTPase acceleration proteins (GAPs), function to expedite signal termination by increasing the rate of GTP hydrolysis and decreasing the lifetime of $G\alpha$ -GTP by orders of magnitude (10). The defining feature of the RGS family, which is composed of 20 canonical members, is the presence of a highly conserved, approximately 120 amino acid region that binds activated $G\alpha$ subunits, termed the RGS Homology (RH) domain. This domain is composed of nine α helices, $\alpha 1$ -9, that form a two-lobed structure composed of the bundle and terminal subdomains (Fig. 2a) (11). Aside from the RH domain, RGS proteins can contain a number of accessory domains, leading to their subdivision into four distinct families based on sequence similarity and the inclusion of these additional domains, as shown in Fig. 2b. Additionally, there are approximately 11 noncanonical RGS-like proteins, including GPCR kinases (GRKs), RhoGEFs, and sorting nexins, that contain RH domains but ostensibly perform important functions other than or in addition to acting as $G\alpha$ GAPs.

The RZ Family

The RZ family is composed of four members, each of which was shown to be highly homologous to RGSZ1 upon their initial discovery. The members of this family, RGS17 (RGSZ2), 19 (GAIP), 20 (RGSZ1), and Ret-RGS, are encoded by three genes *Rgs17*, *Rgs19*, and *Rgs20*. *Rgs20* undergoes alternative splicing, giving rise to RGS20 and Ret-RGS (12,13). As compared to other RGS families, the RZ family proteins are small and relatively simple. Each member contains a short N-terminal poly-cysteine (pCys) string, an RH domain, and a very short C-terminus (13). The pCys string serves as a substrate for palmitoylation in RGS19, anchoring the protein in the membrane (14), and this mechanism is likely conserved in all members of the family, based on conservation of this sequence and their identification as membrane-bound proteins (15,16). Additionally, all members of the RZ family can bind to $G\alpha_z$, though some family members are capable of binding additional $G\alpha$ subtypes (13,17,18).

RGS19, the first identified member of the RZ family, was discovered in 1995 *via* yeast-two hybrid (Y2H) screening that employed $G\alpha_{i3}$ as bait, and its discovery was notable because it was the first time a mammalian RGS- $G\alpha$ protein-protein interaction had been observed (19). RGS19 and RGS17 share 50% amino acid identity and 75% similarity with the bulk of the divergence occurring at the extreme N-termini and the region between the pCys string and the RH domain. Additionally, unique to RGS19 is a C-terminal PDZ binding motif that enables GIPC binding, which may act as a scaffold to regulate RGS19 recruitment (20,21). Functionally, recent work has begun to show possible connections between RGS19 and nociception and pain due to its ability to regulate serotonergic and opiate signals (22,23).

RGS20 was first identified due its GAP activity toward $G\alpha_z$, and subsequent efforts determined that it, in fact, had higher affinity for $G\alpha_z$ than other $G\alpha_{i/o}$ proteins, leading to its initial description as RGSZ1 (16,24). Of all the RZ family members, RGS20 most closely resembles RGS17, as these two proteins have 53% amino acid identity and 72% similarity. Notably, the pCys string is perfectly conserved between RGS20 and 17, though RGS20 harbors a 31 residue N-terminal extension that RGS17 lacks. A significant body of evidence exists relating RGS20 function to the regulation of opioid signaling through the μ -opioid receptor (μ OR) (25–27). As noted above, Ret-RGS is a splice variant of the gene that also encodes for RGS20, resulting Ret-RGS being 147 residues longer than RGS20. Though Ret-RGS contains the pCys string common to RZ members, it also contains a putative membrane spanning domain, potentially further tethering it to cellular membranes (15). Ret-RGS is the RZ family member most distinct from RGS17, as the proteins' primary sequences are only 33% identical and 44% similar, though the lower degree of similarity can be almost completely attributed to Ret-RGS's extended N-terminus.

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Gene Structure

Like other RGS proteins, RGS17 was first identified during Y2H screening for its ability to interact with an activated $G\alpha$ subunit, namely constitutively active mutants of $G\alpha_o$ (13,28). *Rgs17* is located on murine chromosome 10 and at position 6q25.3 in humans (29). Subsequent work identified that in humans *Rgs17* can be transcribed into mRNAs varying in length from 2 to 8 kb, but as only a single cDNA for RGS17 has been detected, it is presumed that these differences occur in untranslated regions (10).

Normal Tissue Distribution

The endogenous tissue distribution of *Rgs17* is largely variable depending on the animal species and methodology employed, but the overall consensus is that RGS17 is found in the central nervous system. In humans, *Rgs17* mRNA can be detected in the nucleus accumbens (NAc), parahippocampal gyrus, and putamen, but the highest levels of expression are observed in the cerebellum, though overall *Rgs17* is expressed to a much lower degree than other RGS family members (30). Low levels of human *Rgs17* is also observed in

Fig. 1. GPCR-G protein activation cycle. Upon ligand binding to a GPCR, $G\alpha\beta\gamma$ binds the receptor, where GDP on the $G\alpha$ subunit is exchanged for GTP, leading to dissociation of this complex. $G\alpha$ and $\beta\gamma$ are then free to activate downstream signaling pathways. Signaling is terminated when an RGS protein binds the $G\alpha$ -GTP, leading to GTP hydrolysis to GDP. RGS then dissociates from $G\alpha$ -GDP, which is sequestered by $\beta\gamma$, reforming the heterotrimer and priming the cycle for reactivation upon future GPCR-ligand binding events. Adopted from PDB Structures: 1AGR ($G\alpha$, RGS), 3SN6 (GPCR, $G\alpha$, $\beta\gamma$) (11,73)

the testis (13,30). In mice, *Rgs17* exists in the cerebral cortex and to a higher extent in the striatum and NAc (31). In rats, *Rgs17* can be detected in the frontal cortex, striatum, NAc, and, interestingly, atrial myocytes (32,33). Moreover, *Rgs17* expression can be induced in cultured rat smooth muscle cells by platelet-derived growth factor DD (PDGF-DD), indicating a link between GPCR and receptor tyrosine kinase signaling (34). Additionally, *Rgs17* levels are subject to regulation by neurotransmitter signaling through dopamine receptors. Genetic knockout of the D_1 dopamine receptor (D_1R) leads to decreased *Rgs17* expression in the medial frontal cortex of mice; however, when D_1R signaling is reduced *via* prenatal cocaine exposure in rabbits, increased *Rgs17* expression is observed (31). In rats, prenatal exposure to the D_2R agonist quinpirole results in increased *Rgs17* expression in the frontal cortex, striatum, and NAc (33). Taken together, the tissue expression discrepancies exhibited between species highlight the importance of working with human tissue, preferably primary, whenever possible and that findings from rodent models may not always be directly translatable to human health.

GTPase Accelerating Protein Activity

After RGS17 was discovered and identified as being a member of the RZ family, it was proposed that RGS17 would be specific for $G\alpha_z$, similar to RGS20. Early work demonstrated that RGS17 can, in fact, bind and accelerate the GTPase activity of $G\alpha_z$, but unlike RGS20, it is not necessarily specific for this subtype. RGS17 is capable of binding $G\alpha_{i1-3}$, $G\alpha_o$, and $G\alpha_z$ and displays a preference for

$G\alpha_z$ and $G\alpha_o$ subunits in GAP assays involving purified proteins. Oddly, in assays using membrane preparations, RGS17 displays preferential binding to $G\alpha_i$ and $G\alpha_o$ rather than $G\alpha_z$, implying that these interactions may be more relevant in a cellular context. At equimolar concentrations, RGS17 shows faster GTPase acceleration than RGS20 on all inhibitory $G\alpha$, though neither acts as quickly as RGS4 (13). Additionally, RGS17 has been shown to bind $G\alpha_q$ using both immunoprecipitation and surface plasmon resonance, though *in vitro* GAP assays have been unable to detect RGS17-mediated $G\alpha_q$ GTPase acceleration (13,35). Interestingly, RGS17 is capable of reducing calcium flux elicited by the thyrotropin-releasing hormone receptor, which couples to $G\alpha_{q/11}$. This has led to the hypothesis that RGS17 may physically occlude interactions between $G\alpha_{q/11}$ -GTP and its downstream effectors, thereby acting as an effector antagonist (13). RGS17 has also been shown to regulate signals generated by other GPCRs coupled to inhibitory G proteins, most notably the D_2R , M_2 acetylcholine receptor, and μOR (13,36). In fact, *in vivo* at the μOR , RGS17 has been shown to regulate signaling through $G\alpha_z$ in murine periaqueductal grey matter (PAG), and mice lacking RGS17 show increased antinociception and faster tolerance development in response to opioids (36).

Noncanonical Functions and Interactions

Aside from its canonical role as a GAP toward activated $G\alpha$ subunits, a number of unique or atypical functions of RGS17 have been described, some of which seem to be mediated by the pCys string as opposed to the RH domain.

Fig. 2. RGS homology domain and the RGS protein family. **a** The RH domain is composed of nine α -helices, forming a structure of two distinct lobes: the terminal lobe containing both the N- and C-termini (α 1-3, 8, 9) and the bundle domain containing a four-helix, anti-parallel bundle (α 4-7). $G\alpha$ subunits engage the bottom of the structure, largely through contacts made with the bundle domain PDB: 1ZV4 (RGS17). **b** Domain composition and identified members of the different families of RGS proteins. RZ and R4 proteins are the simplest RGS proteins, composed of an RH domain with short N-terminal regions and are approximately 190–240 residues long. The R7 family contains a few accessory domains and is much longer than RZ/R4 members at 470–675 residues. The R12 family is the largest and most complex set of RGS proteins at 500–1000+ residues, except for RGS10, which is closer to the R4 family in length but is grouped in the R12 family based on RH sequence identity. pCys poly-Cysteine string, RH RGS homology, AH amphipathic helix, DEP disheveled/Egl-10/pleckstrin domain, GGL G protein γ -like, PDZ Psd-95/DlgA/ZO1 domain, PTB phosphotyrosine-binding domain, RBD Raf-like Ras binding domain, GOLoco $G\alpha_{i/o}$ loco

The most well-established noncanonical function of RGS17 is its ability to act as a scaffold in a complex surrounding the μ OR. RGS17, as well as RGS19 and 20, interacts with histidine triad nucleotide binding protein 1 (HINT1) through its pCys string, as first identified *via* Y2H screening for proteins that directly bind to RGS20 (25). The formation of this complex is dependent on the presence of Zn^{2+} . RGS17's pCys string coordinates two Zn^{2+} , each of which is coordinated by four cysteine residues, forming a structure known as a zinc ribbon (37). The HINT1-RGS17 complex then engages the μ OR and recruits protein kinase C (PKC) γ to the plasma membrane, where PKC γ phosphorylates the receptor, preventing further activation as a means of desensitization (38). The HINT1-RGS17 association with the receptor appears to be mediated by RGS17 rather than HINT1, as RGS17 is able to interact directly with μ OR intracellular regions, namely the C-terminus and intracellular loop 3. Moreover, the formation of this RGS-receptor complex is not specific to the μ OR, as RGS17 is capable of binding peptides derived from intracellular portions of serotonin ($1A$ and $2A$), dopamine (D_2), and cannabinoid (CB_1) receptors, as determined using surface plasmon resonance (37). Furthermore, this interaction seems to be

relevant *in vivo* as RGS17 and RGS20 both co-precipitate with the μ OR in mouse PAG synaptosomal preparations (36). RGS17 also contains two PDZ binding domains at residues 61–64 and 75–79 that bind to the N-terminal PDZ domain of neural nitric oxide synthase, which functions to couple NMDA glutamate receptor signals to μ OR (39). In addition to binding HINT1, the pCys string of RGS17, 19, and 20 mediates interaction with GAIP-interacting protein N-terminus (GIPN), an E3 ubiquitin ligase that degrades $G\alpha_{i3}$. This suggests that RZ RGS proteins can serve as a scaffold to link activated $G\alpha$ subunits to ubiquitin-dependent proteasomal degradation *in vitro* (40). This function is notable because it complements the overall role of RGS proteins as negative regulators of $G\alpha$ signaling using a GAP-independent mechanism.

Post-translational Modification

Though the RZ family consists of little more than a pCys string and an RH domain, RGS17 is subject to modification and regulation through a number of post-translational modifications. The first post-translational modification of an RZ family member identified was the palmitoylation of RGS19 on its pCys string, which largely serves to regulate intracellular trafficking and localization. Palmitoylation involves a reversible reaction between Cys residues on the RGS protein and the carboxylic acid moiety of the 16-carbon fatty acid palmitate, the addition of which tethers the RGS protein to membranes. This serves to concentrate RGS proteins to the same subcellular compartments as $G\alpha$ subunits, which also exist as lipid-modified proteins within cells, though unmodified RGS proteins are able to exist in the cytosolic fraction of cells (14). It is assumed that this mechanism holds true for other members of the RZ family, considering that the pCys string is perfectly conserved between RGS19 and RGS17.

In addition to covalent modification by lipids, RGS17 is also a substrate for phosphorylation. When it was first identified, RGS17 was noted for containing a number of putative sites for phosphorylation, as its primary protein sequence contains six potential casein kinase sites and three PKC sites (13). RGS17 was also identified in a large-scale search for proteins containing phosphotyrosine residues in murine brain samples. RGS17 can be phosphorylated on Y137 at the base of α 5, though the kinase responsible for this modification and its functional consequence have yet to be determined (41). Additionally, RGS19 is phosphorylated on Ser151 by mitogen-activated protein kinase 1, increasing its GAP activity toward $G\alpha_{i3}$. This residue lies between in loop between α 5 and α 6 in the RH domain and is conserved across the RZ family, indicating that all members of the family are likely substrates (42).

RGS17 can also be covalently linked to sugars. In the mouse brain, RGS17 exists as a glycoprotein that purifies with the fraction containing glycosylated proteins. Furthermore, when immunoblotted, RGS17 is observed as a series of bands of varying molecular weights, and the higher molecular weight species are sensitive to glycosidase treatment (36). The location and functional implications of these modifications have yet to be explored.

In addition to lipidation, phosphorylation, and glycosylation, RGS17 is also a substrate for sumoylation by SUMO1, 2, and 3 and is detected in mouse synaptosomes in its sumoylated form. K90 in $\alpha 3$ and K121 in $\alpha 4$ are two potential sumoylation sites in RGS17. The sumoylated forms preferentially coimmunoprecipitate with $G\alpha$ and μ OR, meaning that this modification possibly changes function of RGS17 from a GAP to a scaffold or effector antagonist (43). Additionally, RGS17 contains two SUMO interaction motifs, one of which (residues 64–67) is able to noncovalently associate with SUMO and other sumoylated proteins, leaving open the possibility of RGS17 forming even higher order SUMO-dependent scaffolding complexes (44).

RGS17 also serves as a substrate for ubiquitination at K147, located between $\alpha 5$ and $\alpha 6$, as found during a large-scale proteomic effort. Ubiquitinated RGS17 could be detected in murine brain and kidney tissues, but not liver, heart, or muscle (45). The exact function of RGS17 ubiquitination is unknown, but this modification likely marks RGS17 for degradation through the proteasome.

RGS17 AND DISEASE

Lung and Prostate Cancer

RGS17's first link to cancer was its identification as a potential marker for familial lung cancer, as a susceptibility locus was tracked to chromosome 6q23-25, the genomic location of *Rgs17*. Further work showed that RGS17 is often overexpressed in both lung and prostate cancers by 8.3- and 7.5-fold, respectively (3,46). Furthermore, it has been shown that knockdown of RGS17 in lung cancer-derived cultured cells decreases tumor volume by 59–75% in a mouse xenograft model of cancer. Moreover, RGS17 overexpression causes increased expression of proteins with cAMP response elements (CRE) in their promoter region. These results indicate that the proliferative effect observed in RGS17-dependent cancers is likely due to RGS17's GAP activity toward inhibitory $G\alpha$ subunits, resulting in increased activity of the PKA-CREB pathway. Increased RGS17 would lead to decreased $G\alpha_{i/o}$ signaling, decreased AC inhibition, increased formation of cAMP, increased PKA activity, and CREB activation, ultimately altering the transcription of CRE-regulated genes (3). In some lung cancer cell lines, it has been shown that RGS17 protein levels can be regulated by microRNAs (miRNA, miR), which are short, non-coding RNA sequences that regulate translation of their target mRNA sequences. In lung cancer, there is evidence that the specific miRNA that regulates expression of RGS17 is Hsa-mir-182, expression of which drastically reduces the amount of endogenous RGS17. In fact, expression ectopic of Hsa-mir-182 recapitulates what is observed when RGS17 is specifically knocked down using synthetic shRNA, and increased Hsa-mir-182 is sufficient to reduce the growth and proliferation of lung cancer *in vitro* (47).

Hepatocellular Carcinoma (HCC)

Similar to what has been observed in prostate and lung cancers, RGS17 mRNA is detectable in rat HCC tissue, but not normal whole liver tissue or hepatocytes. Likewise, in 5 of

7 human HCC samples analyzed, RGS17 mRNA was significantly overexpressed as compared to patient-matched control tissue ($p=0.011$), though when all seven samples were analyzed together, no statistical significance was observed ($p=0.061$). Again similar to previous reports of RGS17 in cancer, increased expression correlates to increased cellular proliferation in HepG2 cells, and knockdown of RGS17 *via* RNA interference results in decreased cellular proliferation. Additionally, decreased RGS17 is correlated with decreased intracellular cAMP levels, presumably through increased $G\alpha_{i/o}$ -mediated inhibition of AC. Interestingly, the work performed in the HCC cancer model could not detect changes in protein expression levels in the presence of Hsa-mir-182 overexpression. In fact, in HCC, it seems that RGS17 protein stability might be regulated by proteosomal degradation, as the presence of proteasome inhibitor MG132 results in increased RGS17 *in vitro* (4). The presence of proteosomal degradation of RGS17 further validates reports that RGS17 is a substrate for ubiquitination *in vivo* (45). The fact that Hsa-mir-182 did not regulate RGS17 protein levels in HCC could be due to a cell line or tissue type-dependent phenomenon, though a thorough examination of this hypothesis has yet to be realized (4). In addition to proteosomal degradation, it is possible that RGS17 levels are epigenetically regulated. In HCC tissues that show copy number losses on chromosome 6q, decreased methylation of CpG sites in *Rgs17* is observed, likely leading to increased RGS17 expression (48).

Breast Cancer

Recently, a number of findings relating RGS17 to breast cancer have begun to emerge. Similar to prostate, lung, and liver cancers described above, RGS17 can be upregulated in cancerous *versus* noncancerous tissue. Using immunohistological staining, RGS17 protein was found in 96% of cancerous samples, whereas it was only detectable in 57% of normal samples. Furthermore, RGS17 expression was absent or very low in 12 of 28 normal samples, and low in the remaining 16, but 85% (74 of 87) of cancerous samples had moderate to high expression (5). Additionally, in breast cancer, RGS17 expression is positively correlated with p63 expression, a protein that can be over expressed in a number of cancers, including breast, lung, and prostate cancers (5,49,50). RGS17 knockdown *via* RNA interference inhibited cancer cell migration in a wound healing assay and invasion in a Boyden chamber assay, recapitulating results seen in HCC and lung cancers (3–5). In breast cancer tissue, a novel miRNA, miR-32, capable of modulating RGS17 expression was identified, and it was also shown that this miRNA is specifically downregulated in cancerous breast tissue as compared to surrounding normal tissue. Overexpression of miR-32 causes decreased RGS17 expression and reductions in cancer cell proliferation, migration, and invasion (5). In breast cancer cells, the mechanism by which RGS17 is initially upregulated remains unknown, but *in vitro* work has shown that one possible mechanism is by chromosomal rearrangements. In MCF7 cells, chromosomal instability can result in a chromosomes 3 and 6 rearrangement, placing the *IRAI* promoter upstream of the RGS17 coding sequence, though the consequence of this on transcript level has yet to be identified (51). Additionally, RGS17 is upregulated in MCF-7

cells after treatment with ionizing radiation, though the ultimate consequence of this increase remains unknown (52).

Ovarian Cancer

In ovarian cancer, it appears that RGS17 is capable of mediating chemoresistance, the ability of malignancies to grow in the presence of chemotherapeutic drugs. When cancerous cell lines are exposed to chemotherapeutic agents (cisplatin [*cis*-diamminedichloroplatinum (II)], vincristine, or paclitaxel), a loss of RGS17 expression is observed in cells that become chemoresistant. Moreover, knockdown of RGS17 expression *via* RNA interference is sufficient to increase cell survival and decrease the growth inhibition response following challenge with these compounds. Conversely, overexpression of RGS17 leads to increased sensitivity to drug treatment, though the effect is less pronounced (53). Mechanistically, RGS17 in ovarian cancer cells appears to modulate the PI3K/AKT survival pathway, rather than the cAMP-PKA-CREB pathway like in HCC, lung, and prostate cancers (3,53). Lysophosphatidic acid (LPA) can act in an autocrine manner, such that binding to one of its receptors activates $G\alpha_i$ proteins, resulting in the phosphorylation and activation of protein kinase B (Akt) and the promotion of cell survival. Increased RGS17 results in decreased Akt activation following treatment with LPA, thus representing a mechanism for growth arrest. Therefore, the loss of RGS17 promotes increased growth and survival through increased $G\alpha_i$ -mediated activation of the Akt signaling axis (53).

Acute Myeloid Leukemia (AML)

Recent work has implicated a possible role for RGS17 in AML chemoresistance that could prove similar to that identified in ovarian cancer. The expression of miR-363 is inversely related to response to chemotherapy, and increased miR-363 is evident in bone marrow samples from patients with chemoresistant AML. Most importantly, RGS17 has been identified as a target gene of miR-363 (54). It is tempting to speculate that increased miR-363 would correlate to decreased RGS17 levels, increased Akt activation, and ultimately, diminished response to chemotherapeutic agents, though this hypothesis has yet to be tested. Alternatively, analysis of miR-363 levels in chemosensitive and resistant ovarian cancer cells could prove to be of merit.

Neurological Disorders

As RGS17 is expressed to the highest degree in the brain in healthy individuals, it comes as no surprise that RGS17 has also been indicated in various neurological conditions. Unfortunately, many of its potential roles have been identified *via* large-scale screening efforts, and there is little to no mechanistic insight into its exact role. For example, RGS17 expression is decreased by nearly an order of magnitude in clinical depression, as determined *via* RNA microarray analysis of postmortem brain samples from patients with and without a history of major depressive disorder (55). There also has been an association of single nucleotide polymorphisms (SNPs) at chromosome 6q25, the location of *Rgs17*, with bipolar disorder, though a definite role of RGS17

has yet to be established (56). RGS17 may also be involved in addiction and drug abuse. Differences in RGS17 expression levels have been correlated to morphine preference differences observed between C57BL/6J and DBA/2J mice (57). DBA/2J mice exhibit higher levels of RGS17 protein and mRNA expression in the NAc, midbrain, and brainstem, possibly explaining the decreased reward and, therefore, decreased preference for morphine as compared to C57BL/6J mice in a two-bottle test (58). In humans, *Rgs17* SNPs are associated with substance abuse, most notably one SNP that results in lowered RGS17 expression is correlated with increased alcohol, marijuana, and opioid dependence in both African and European Americans (59). Additionally, one study found that *Rgs17* SNPs have been associated with smoking initiation in an Asian population (60).

RGS17 and Metastatic Disease

As noted above, reduction of RGS17 activity *via* RNA interference is able to reduce the migratory and invasive phenotypes of cells derived from HCC, lung, and breast cancers, implying that RGS17 could be involved in metastatic processes (3–5). It is very likely that these observations are due aberrant signaling, as RGS17's canonical role is to negatively regulate inhibitory $G\alpha$ signaling. An abundance of RGS17 could lead to persistent inhibition $G\alpha_{i/o}$, leading to an imbalance in $G\alpha_{i/o}/G\alpha_s$ signaling and ultimately excessive AC-mediated cAMP production, as has been shown in both lung cancer and HCC cells (3,4). Excessive cAMP would then lead to CREB activation through PKA, resulting in excessive transcription of CREB target genes, which has also been observed in lung cancer cells (3). This could lead to increased levels of CREB target genes that are directly involved in metastasis and anchorage-independent cell growth, such as vascular endothelial growth factor (VEGF), type IV collagenases, or cyclin D1, though this is somewhat speculative as only cyclin D1 expression as been experimentally shown to decrease in response RGS17 knockdown (3,61–63).

CHEMICAL INHIBITION OF RGS PROTEINS

RGS- $G\alpha$ Druggability

Since their discovery in the mid 1990s, RGS proteins have remained of great interest for drug discovery and development due to their ability to modulate GPCR signaling cascades. Traditionally, protein-protein interactions (PPIs) have been categorized as undruggable, but recent successes in the field challenge this assumption (64). In fact, recently PPI inhibitors have even begun to enter clinical trials, such as SAR1118 for dry eye and navitoclax for cancer (65,66). As RGS proteins have no intrinsic catalytic activity and exert their function by binding activated $G\alpha$ subunits, previous drug discovery efforts have primarily focused on identifying molecules capable of inhibiting the $G\alpha$ -RGS PPI (67). The most apparent means to achieve this would be by identifying molecules capable of binding directly to the residues that form the interaction surface of the $G\alpha$ or RGS. This interface, also referred to as the A site, has been the subject of numerous previous efforts to design inhibitors

targeting RGS4, a member of the R4 family. Using the previously solved structure of the RGS4-G α complex, Jin and coworkers designed cyclic peptides that mimicked the G α switch I region, inhibiting the RGS4-G α interaction with micromolar potency (67). This work proved that inhibition of the interaction was possible, but as peptides generally tend to make poor drugs, alternative methods to identify inhibitors were sought (68).

Ultimately, high-throughput screening against RGS proteins has proved the most fruitful in identifying lead compounds with inhibitory activity toward these PPIs, with methodologies ranging from bead-based flow cytometry and luminescence to colorimetric monitoring of G α GTPase activity (68–71). Interestingly, screening against RGS4 has often identified cysteine-reactive compounds that bind covalently to a site distinct from the A site (72,73). This site is closer to a region that has been termed the B site that binds endogenous phospholipids to regulate GAP activity, establishing the hypothesis that inhibition of the RGS-G α PPI can be achieved through molecules that act allosterically to the actual interaction interface (74,75).

RGS17 Inhibition

Due to its role in lung, liver, breast, and prostate cancers, our research group has interest in the development of small molecules capable of inhibiting the RGS17-G α interaction. We hypothesize that chemical inhibition of RGS17 would recapitulate the reduction in invasion, migration, and tumor size in cancer that is observed when RGS17 expression is reduced *via* RNA interference (3–5). Additionally, specific chemical inhibitors of RGS17 could serve as tool compounds to help unravel the cancer type-specific functions of RGS17 that have been previously reported (3,53). RGS17 merits further evaluation as a potential drug target due to its relatively narrow pattern of expression in normal human tissue and its specific upregulation in the cancers of interest. As RGS17 is generally relegated to CNS tissues (30), we hypothesize that potential side effects of an inhibitor could be mitigated if the compound is large (>400 Da) and/or sufficiently hydrophilic, and thus incapable of crossing the blood-brain barrier.

To this end, we have pursued high-throughput screening, as in the past, it has been successful in identifying inhibitors of

Fig. 3. Chemical inhibitors of RGS17 and potential sites for inhibitor selectivity. **a** Chemical structures of previously identified RGS17 inhibitors. The RL-series of compounds was discovered using a luminescent bead-based screen of 1300 compounds against RGS17-G α_o PPI (66). The UI inhibitors were identified using a colorimetric assay of RGS4-induced G α GTPase activity and further work identified their activity toward RGS17 (65). **b** Residues unique to RGS17 as opposed to other RZ family members could facilitate identification of binding contacts that confer specificity for RGS17. Residues unique to RGS17's primary sequence are shown in *green sticks*. Residues that are shared or are extremely similar (Asp v. Glu, for example) with one RZ family member are indicated as *yellow sticks*. Residues that are completely conserved across the RZ family are indicated in *grey*, and the side chains are not shown

other RGS proteins (68). Initial efforts in the screening of ~3500 compounds have identified six compounds capable of inhibiting RGS17-G α formation *in vitro* with micromolar affinity, though issues with RGS protein specificity or the presence of potentially reactive chemical moieties have lessened the promise of these compounds (Fig. 3a) (69,70). In order to increase the chances of success of identifying specific RGS17 inhibitors that lack reactive functional groups, larger chemical libraries need to be tested against RGS17 and ongoing efforts in our lab are aimed at doing exactly that. As other members of the RGS family are involved in important physiological processes, such as heart rate regulation and vision, pan-RGS inhibition could be deleterious. Thus, identification of molecules that specifically inhibit RGS17 is of the utmost importance. As noted before, the RH domain of RGS17, 19, and 20 is highly conserved, but there are a number of residues unique to RGS17. As shown in Fig. 3b, many of these divergent residues are located in the terminal subdomain, especially $\alpha 9$. Additionally, there are a few RGS17-specific residues in the bundle subdomain, distal to the G α interface and near the region identified as the B site in RGS4, which makes the discovery of RGS17-specific compounds more promising (Fig. 3b) (75). Future efforts will focus on exploring the druggability of this site in RGS17, potentially using fragment-based screening and structure-based methods, as this paradigm is beginning to gain traction in PPI inhibition drug discovery programs (76).

CONCLUSION

RGS17 is able to negatively regulate GPCR signaling through a variety of mechanisms, from its activity as G α GAP to targeting G α subunits for proteosomal degradation to promoting receptor desensitization. It has been implicated in regulating proliferation, migration, and invasion in some of the most common forms of human cancer, including lung, breast, prostate, and liver cancers. This information coupled with RGS17's expression in only a limited number of human tissues makes it a potential target for the development of a new class chemotherapeutic agents. Specific RGS17 inhibitors incapable of permeating the blood-brain barrier would have few predicted on-target adverse effects, though the identification of such molecules is needed for pre-clinical validation of this hypothesis. As all previously identified RGS17 inhibitors lack specificity and/or contain potentially reactive moieties, future work remains to be done in the area of RGS17 inhibition with small molecules. Though preliminary work has been performed to meet this goal, future efforts must focus on the screening of larger, more diverse compound libraries, as increasing the area of chemical space interrogated will increase the likelihood of success. Additionally, alternative drug development methodologies employing *a priori* knowledge and structure-based screening paradigms may be fruitful in accelerating the identification of RGS17 inhibitors.

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