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Commentary

Regulator of G-protein signaling (RGS) proteins in cancer biology

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ABSTRACT

The regulator of G-protein signaling (RGS) family is a diverse group of multifunctional proteins that regulate cellular signaling events downstream of G-protein coupled receptors (GPCRs). In recent years, GPCRs have been linked to the initiation and progression of multiple cancers; thus, regulators of GPCR signaling are also likely to be important to the pathophysiology of cancer. This review highlights recent studies detailing changes in RGS transcript expression during oncogenesis, single nucleotide polymorphisms in RGS proteins linked to lung and bladder cancers, and specific roles for RGS proteins in multiple cancer types.

1. Introduction

Cancer is characterized by the uncontrolled growth of cells through increased proliferation and decreased apoptosis. Additionally, cancer cells can invade adjacent tissues and metastasize to non-adjacent organs and tissues. Uncontrolled growth, invasion, and metastasis are due to changes in cellular signaling pathways, and oncogenic transformation is often the direct result of mutations of the signaling molecules which constitute these pathways. In the past decade, G-protein coupled receptor (GPCR)-stimulated pathways have emerged as critical mediators of oncogenic signaling. GPCRs are a family of cell surface receptors which activate heterotrimeric G-proteins to transduce extracellular signals into the interior of a cell. Regulators of G-protein signaling (RGS) proteins are a highly diverse family of proteins containing an RGS domain which accelerates the deactivation of heterotrimeric G-proteins, thus modulating signaling initiated by GPCRs. There are over 20 mammalian RGS proteins ranging from small proteins comprised solely of an RGS domain to multi-domain proteins with functions in multiple signaling pathways. These additional domains serve to mediate interactions with other signaling proteins, allowing RGS proteins to serve as signaling scaffolds. This review examines recent studies focused on the involvement of RGS proteins in the initiation and progression of cancer.

2. G-protein signaling in cancer biology

G-protein coupled receptors (GPCRs) are known to mediate a wide variety of physiological processes, including sensory perception, immune responses, neurotransmission, and cardiovascular activity. Consequently, GPCRs are also linked to many disease states and serve as direct and indirect targets for roughly half of pharmaceuticals currently on the market [1]. GPCRs function to mediate ligand-dependent activation of heterotrimeric guanine nucleotide binding proteins (G-proteins). Heterotrimeric G-proteins consist of two functional signaling units: a guanine nucleotide-binding α-subunit and a βγ-subunit dimer. Upon ligand binding, conformational changes in the receptor activate a heterotrimeric G-protein by promoting the exchange of GDP for GTP in the Gα nucleotide-binding site. The active Gα and Gβγ subunits then dissociate and interact with various effector molecules, mediating cellular responses to GPCR activation. The G-protein deactivates when the Gα-subunit hydrolyzes GTP to GDP and reassociates with Gβγ. Thus, G-proteins are activated by receptor-stimulated nucleotide exchange and deactivated by GTPase activity.

Several recent reviews have described multiple roles for GPCR signaling in cancer [2–4]. GPCRs are expressed in cancerous tissues and mediate proliferation, survival from apoptotic signals, invasion, and metastasis and are activated by mitogens including lysophosphatidic acid (LPA), endothelin, thrombin, gastrin releasing peptide (GRP), thyroid stimulating hormone (TSH), cholecystokinin (CCK), angiotensin, stromal cell-derived factor-1 (SDF-1/CXCL12) and prostaglandins [5]. Many of these GPCR ligands are found in high concentrations in metastatic sites, resulting in autocrine/paracrine activation of their cognate GPCRs [6]. Further,

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several of the corresponding GPCRs are over-expressed in cancer cells: LPA receptors in ovarian, breast, colon, and prostate cancer (reviewed in Ref. [7]); endothelin receptors in colon and prostate cancers and melanoma (reviewed in Ref. [8]); TSH receptor in thyroid cancer [9]; protease-activated receptor 1 (PAR1) and prostaglandin EP receptors in breast, colon and prostate cancers [10,11], and CCK and CXCR4 receptors in lung and pancreatic cancers [12,13]. Finally, constitutively active GPCRs are encoded by cancer-causing viruses like Kaposi's sarcoma-associated herpes virus [14] and Epstein-Barr virus [15]. Currently, there are not any drugs targeted directly against GPCRs that are used clinically to treat cancer. However, Zhang et al. recently used an LPA analogue, α-bromophosphonate LPA (BrP-LPA), to inhibit both LPA receptors and the enzyme responsible for LPA production in breast cancer cells [16]. Treatment with BrP-LPA inhibited migration and invasion of MDA-MB-231 breast cancer cells *in vitro*. Further, BrP-LPA induced tumor regression in orthotopic breast tumor xenografts in mice. Reductions in tumor volume and vascularity induced by BrP-LPA were comparable to the effects seen with Taxol [16]. These data demonstrate that compounds targeting GPCRs could possibly serve as effective anti-cancer therapeutics.

In addition to canonical GPCR signaling, transactivation of receptor tyrosine kinases (RTKs), in which G-protein activation stimulates activation of RTKs, has recently emerged as a G-protein signaling mechanism that is associated with cancer progression. GPCR-stimulated transactivation has been linked to the hormone therapy refractory forms of prostate [17] and breast cancer [18]. Many cancers over-express RTKs, particularly members of the epidermal growth factor family, as well as their cognate ligands [19]. In recent years, RTK-targeted drugs such as Herceptin (trastuzumab) for breast cancer, Iressa (Gefitinib) for lung cancer, and Gleevec (Imatinib mesylate) for myelogenous leukemia, have emerged as cancer therapeutics [20,21]. Most RTK-targeting drugs work by blocking ligand binding or inhibiting receptor tyrosine kinase or downstream kinase activity [22–24]. Several models of GPCR-mediated RTK transactivation have been described and can involve both ligand-dependent and -independent mechanisms [25]. Further studies will be required to define specific mechanisms of GPCR-mediated RTK transactivation in order to determine the contribution of GPCRs to RTK signaling and to develop these pathways as therapeutic targets.

In addition to changes in the GPCRs themselves, altered expression and activity of heterotrimeric G-proteins is also known to contribute to tumorigenesis [6,26]. Heterotrimeric G-protein α-subunits are classified into four families based on homology and effector interactions: Gi, Gs, Gq, and G12. Expression of constitutively active members of all four Gα families induces transformation of rodent fibroblasts [27]. Constitutively active Gi G-proteins, particularly Gαi2, have been found in human endocrine tumors [28]. Further, tumor cells expressing constitutively active Gαi2 exhibit faster cell growth and tumor formation, while the expression of dominant negative Gαi2 attenuates cell growth and tumor formation [29]. Growth-promoting hormones such as thyroid stimulating hormone and growth hormone releasing hormone (GHRH) activate Gαs-coupled receptors; these pathways are up-regulated in thyroid tumors [9] and pituitary adenomas [30], respectively. Further, Gαs is constitutively active in a subset of pituitary tumors [31]. G12 is a critical regulator of the cytoskeleton and promotes invasion/migration of prostate, breast, and ovarian cancer cells (reviewed in Ref. [32]). G12 was first identified as a transforming gene in a screen of A soft tissue sarcoma-derived cDNA library [33]. Further, over-expression of either wild-type or mutationally activated forms of the protein are capable of transforming NIH 3T3 cells [34] and elevated levels of G12/13 G-proteins are found in cancerous tissue compared with matched, non-transformed tissue (reviewed in Ref. [35]). Finally, over-expression of Gq has been demonstrated to transform NIH3T3 cells [36], and activating mutations of Gαq are associated with uveal melanoma [37]. These studies demonstrate a role for heterotrimeric G-protein signaling in the cancer progression and metastasis.

3. RGS proteins regulate G-protein signaling

GPCRs and G-proteins mediate a wide variety of signals and their activity is finely tuned by multiple regulatory proteins. One critical regulatory point in the G-protein cycle is the deactivation of G-proteins by GTP hydrolysis which is enhanced by GTPase activating proteins (GAPs) (Fig. 1). A group of proteins which function as heterotrimeric G-protein GAPs were identified over a decade ago in yeast, worms, and mammals and termed Regulator of G-protein Signaling proteins. Each RGS protein contains a ~120 amino acid domain, termed the RGS box that is responsible for GAP activity. RGS proteins are capable of accelerating GTPase activity up to 1000-fold [38] and have been demonstrated to have profound physiological effects. In addition to functioning as GAPs for heterotrimeric G-proteins, RGS domains are also capable of serving as effector antagonists by competitively binding activated Gα-subunits or effector enzymes [39,40] or kinetic scaffolds by promoting rapid cycling of Gα-subunits between active and inactive states [41,42]. In the retina, RGS9-1 functions to terminate visual signaling cascades [43]. In the heart, RGS2 attenuates angiotensin signaling to regulate blood pressure [44]. The absence of either of these RGS proteins leads to bradyopsia and hypertension, respectively. Thus, RGS proteins are critical to physiological signal transduction cascades.

RGS proteins are divided into eight subfamilies based upon RGS domain homology and common domain structures (Fig. 2). The distinct combination of domains creates highly regulated, multifunctional proteins which can carry out complex signaling tasks. The R4 family is the simplest structurally, comprised of only the RGS domain and a small, N-terminal extension involved in receptor selectivity [45]. In contrast, the R7, R12, and GEF families contain additional functional domains that dictate subcellular localization, assemble multi-protein complexes, and directly regulate the activity of other signaling molecules. For example, the Dishevelled/Egl-10/Pleckstrin (DEP) domain of the R7 family targets these proteins to the plasma membrane or nucleus. R12 family GoLoco domains function as guanine nucleotide dissociation inhibitors, thus preventing activation of Gi family heterotrimeric G-proteins

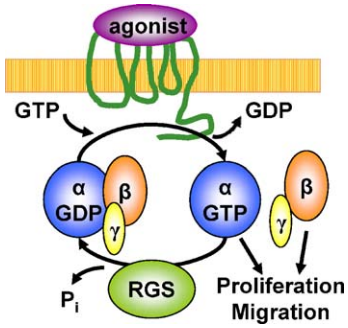

Fig. 1. RGS proteins accelerate the GTPase activity of heterotrimeric G-protein Gα-subunits. In their inactive state, the α-subunit of a heterotrimeric G-protein is bound to GDP. Upon agonist activation, conformational changes in the receptor induce the Gα-subunit to release GDP and bind GTP. The binding of GTP causes dissociation of the α-subunit and the βγ dimer, allowing them to interact with effector molecules and propagate signaling cascades associated with cellular growth, survival, migration and invasion. G-protein signaling is deactivated when the α-subunit hydrolyzes GTP to GDP and the reassociates with the βγ dimer. RGS proteins function to accelerate the GTPase activity of the α-subunit, thereby inhibiting downstream activity.

[46]. The GEF family contains a Rho guanine nucleotide exchange factor (GEF) domain which activates the small G-protein Rho, thus linking G12/13 activation with Rho activation. Accessory domains, such as Rac binding domain, Dishevelled homology (DH), Pleckstrin homology (PH), and PSD-95/Discs-large/ZO-1 homology (PDZ), mediate protein–protein interactions, leading to the formation of signaling complexes and providing signaling specificity. For example, RGS12 enhances nerve growth factor-stimulated MAP kinase pathways by binding of Raf and MEK2 [41] and the N-terminal domain of RGS2 binds M1 muscarinic acetylcholine receptors to selectively regulate Gq-mediated signaling [47]. Therefore, RGS proteins can serve as inhibitors of G-protein signaling, serve as effectors, or act as scaffold proteins to assemble receptors, G-proteins, and effectors together into a signaling complex. RGS proteins are themselves highly regulated. Post-translational modifications of RGS proteins including phosphorylation, palmitoylation, and sumoylation modulate GAP activity, alter subcellular localization, and influence protein stability and protein–protein interactions [48]. Thus, RGS proteins are complex signaling molecules that are involved in a variety of functions and interactions (Fig. 3).

In recent years several approaches have been used to determine the physiological roles of RGS proteins. Many investigators have over-expressed RGS proteins in a given system, but this method is limited in that over-expression of a protein changes the stoichiometry of signaling molecules and may not reflect endogenous specificity. RGS knock-out animals have also become available for the most widely expressed RGS proteins, including RGS2 [49], RGS4 [50], RGS5 [51], and RGS9 [43]; however, these models are best used to study changes in normal physiological processes. Several groups, including ours, have utilized RGS-insensitive Gα-subunits to determine the significance of RGS protein regulation of G-protein signaling pathways [52], but this method does not identify specific roles for RGS proteins. While these studies have enhanced our understanding of the physiological role of RGS proteins, much work remains to be done to determine the role of RGS proteins in cancer.

4. Changes in expression of RGS transcripts and proteins in oncogenesis

The transformation of normal cells into cancerous cells requires concerted changes in the expression of multiple genes. These genetic changes result in the activation of proto-oncogenes and the inactivation of tumor suppressor genes allowing unregulated cell growth. Many recent studies have attempted to identify proto-oncogenes and tumor suppressors using multiplex gene microarray technology to compare the genetic profiles of matched samples of cancerous and normal tissues. Multiple RGS proteins were identified as differentially expressed genes in a variety of cancers including ovarian cancer [53], melanoma [54,55], renal cell carcinoma [54,56,57], lymphoma [58,59], hepatocellular carcinoma [60,61], prostate cancer [62,63], breast cancer [64,65], thyroid cancer [66,67], pancreatic cancer [68], leukemia [59,69,70], and glioma [71]. These changes in RGS expression between normal and cancerous tissues are summarized in Table 1. Notable changes

Table 1
Changes in RGS transcript expression associated with carcinogenesis.

Family A/RZ	
RGS17/RGSZ2	↑ in prostate cancer [99]; ↑ in lung cancer [99]
RGS19/GAIP	↑ in ovarian cancer [53]; regulates wnt/β-catenin signaling [100]; binding partner GIPC down-regulated in primary kidney tumors, colorectal tumors, gastric cancer, and prostate cancer [101]
RGS20/RGSZ1	↑ in melanoma [102]
Family B/R4	
RGS1	↑ in melanoma [55]; ↑ in head and neck squamous cell carcinoma [103]; ↑ in adult T-cell leukemia [70]; ↑ in renal cell carcinoma [54]; ↑ in ovarian cancer [54]; ↑ in cervical cancer [104]; ↑ in mantle cell lymphoma [81]
RGS2	↓ in ovarian cancer [53]; ↑ in breast cancer [65]; ↑ in fibrolamellar carcinoma [105]; ↓ in prostate cancer [17]; ↓ in acute myeloid leukemia [69]; ↑ in mantle cell lymphoma [81]
RGS3	↑ in docetaxel resistant breast cancers [106]; ↑ associated with enhanced glioma cell motility [71]; ↑ in soft tissue sarcomas [107]
RGS4	↑ associated with enhanced glioma cell motility [71]; ↑ in thyroid carcinoma [66]; ↓ in ovarian cancer [53]
RGS5	↑ in hepatocellular carcinoma [61]; ↑ in breast cancer, melanoma, multiple myeloma, ovarian cancer, and acute myeloid leukemia [98]; ↑ in fibrolamellar carcinoma [105]
RGS8	N/A
RGS13	↓ in mantle cell lymphoma [59]; ↑ in B- and T-cell lymphoma [108]
RGS16	↑ in pediatric high hyperdiploid acute lymphoblastic leukemias [109]; ↑ in pineal parenchymal tumors [110]; p53 target gene in colorectal cancer [111]
RGS18	N/A
Family C/R7	
RGS6	↑ in ovarian cancer [53]; SNPs associated with bladder cancer risk [80]
RGS7	N/A
RGS9	N/A
RGS11	Increased expression associated with Oxaliplatin resistance in colorectal cancer [112]
Family D/R12	
RGS10	N/A
RGS12	N/A
RGS14	N/A
Family E/RA	
Axin 1/Axin 2	Mutations associated with gastric cancer [113], renal cell carcinoma [114], intrahepatic cholangiocarcinomas [115], adenoid cystic carcinoma [116], cerebellar medulloblastomas [117], oral squamous cell carcinoma [118]; colorectal cancer [119]; ↓ in colorectal cancer [72]; ↓ in non-small cell lung cancer [120]; ↓ in ovarian endometroid adenocarcinoma [121]; ↓ in breast cancer [122]; ↓in sporadic medulloblastomas [123]
Family GEF/RF	
p115-RhoGEF	N/A
PDZ-RhoGEF	SNP is linked to a reduced risk of lung cancer in Mexican Americans [78]
LARG	N/A

RGS proteins are emerging as a family of proteins that is linked to the initiation and progression of cancer. These are reports from the literature of changes in RGS transcript expression that have been linked to specific types of cancer. RGS proteins are organized according to subfamily.

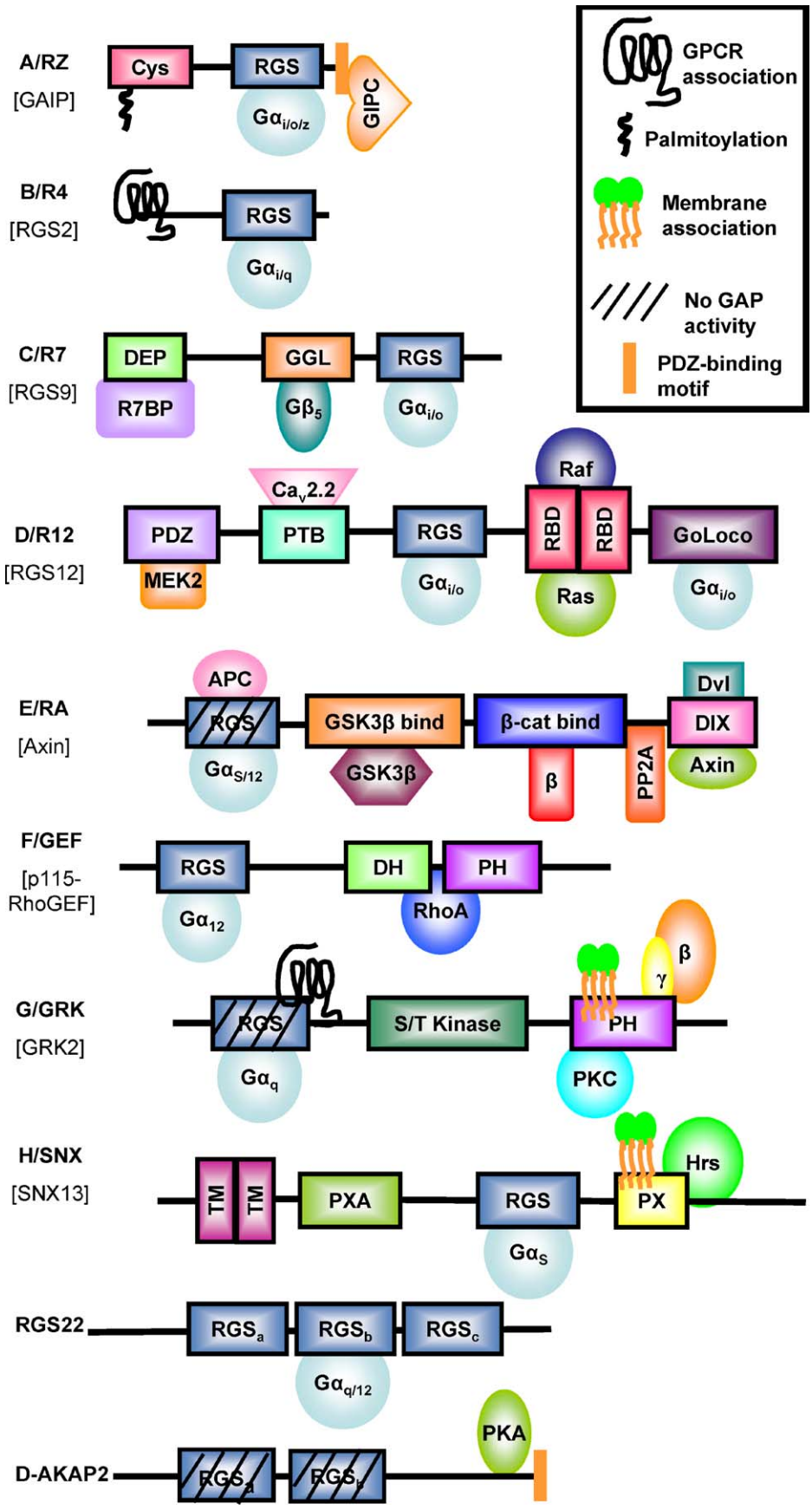

Fig. 2. RGS subfamilies and common interacting proteins. RGS proteins are divided into eight subfamilies based on RGS domain homology and accessory domains. Domains outlined in black are part of the RGS protein, and common binding partners are shown with no black outline. G-protein specificity of RGS domain GAP activity is indicated on the Gα-subunits. A/RZ family RGS proteins are characterized by an N-terminal cysteine string motif which can be reversibly palmitoylated and is implicated in membrane/

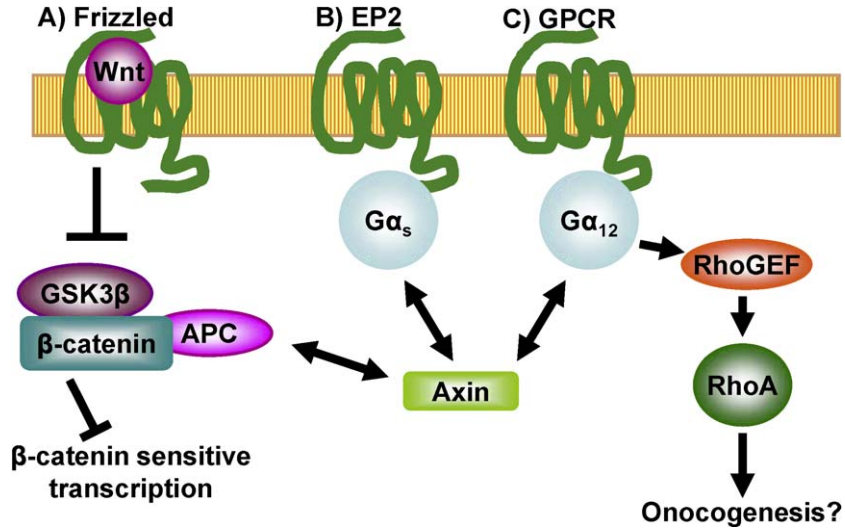

Fig. 3. Role of axin in Wnt and GPCR signaling cascades. The atypical RGS protein axin has been reported to play a role in multiple signaling cascades. Blunted arrows indicate inhibition, single-headed arrows indicate activation, and double-headed arrows indicate association. (A) In the Wnt pathway, axin blocks β-catenin-sensitive transcription by scaffolding its destruction complex. (B) In colon cancer cells Gαs binding to axin may disrupt the β-catenin destruction complex, resulting in transcription of β-catenin-sensitive genes. (C) The RGS domain of axin competes with p115-RhoGEF for Gα12 in MDA-MB231 breast cancer cells, possibly attenuating the oncogenic G12/Rho signaling axis.

include significant down-regulation of RGS2 in androgen-independent prostate cancer [17] and acute myeloid leukemia [69], axin in metastatic colorectal cancer [72], and up-regulation of RGS5 in hepatocellular carcinoma [61] and the vasculature of renal cell carcinoma [56].

4.1. RGS SNPs in cancer

Single nucleotide polymorphisms (SNPs) are genetic mutations that occur in translated and untranslated regions such as promoters, either affecting the primary structure or expression levels of the encoded protein. SNPs in genes such as p53 [73] and epidermal growth factor family receptors [74] are commonly associated with cancer. RGS SNPs have previously been linked to several human diseases including schizophrenia [75], anxiety and panic disorders [76], celiac disease [77], and hypertension [77]. These studies suggest that genetic variation in RGS proteins may play a significant role in the pathophysiology of multiple human diseases.

Recently, RGS SNPs have also been reported in lung cancer and bladder cancer. A variant allele in the gene encoding PDZ-RhoGEF was found to confer a 40% reduction in the risk of lung cancer in Mexican American males [78]. The Ser1416Gly mutation is in the C-terminus of PDZ-RhoGEF, the region of the protein that mediates homo- and hetero-oligomerization, which attenuates the ability of the protein to mediate guanine nucleotide exchange/activation of Rho. Further, this mutation reportedly reduces PDZ-RhoGEF activation of serum response element-dependent genes that are activated by transfection of RhoGEF proteins. The reduction in lung cancer risk was apparent in smokers, but not in non-smokers, suggesting a gene/environment interaction. Lung cancer risk varies significantly among different ethnic groups, with Mexican Americans having a lower incidence rate than Caucasians [79]. Interestingly, the number of Mexican Americans who were homozygous for the protective, PDZ-RhoGEF variant allele was over double the number of Caucasian participants.

SNPs in RGS6 have been linked to a significant decrease in the risk of developing bladder cancer [80]. Berman et al. analyzed the occurrence of 12 non-coding SNPs in genes encoding RGS2, RGS5, RGS6, RGS11, and RGS17, as well as changes in transcript level, alternative splicing events, and protein translation efficiency for each of these alleles. The single most protective allele, a variant of RGS6, was correlated with a 34% decrease in bladder cancer risk and a three-fold greater translation rate. Similarly to the PDZ-RhoGEF variant in lung cancer, this protective effect was most evident in smokers. These reported SNPs suggest that mutations in RGS proteins could have profound effects on the etiology of cancer.

5. RGS protein function in cancer

In addition to correlative studies demonstrating that changes in RGS gene expression are linked to cancer, there have also been several studies demonstrating functions of RGS proteins in cancer.

protein interactions and intracellular localization. The scaffolding protein GAIP interacting protein, C-terminus (GIPC) binds RGS19 at the C-terminus. The B/R4 family contains the simplest RGS proteins with a short N-terminal region that is required for receptor co-localization. C/R7 family members are characterized by Dishevelled/Egl-10/Pleckstrin (DEP) domains, which bind syntaxin-like proteins such as R7 binding protein (R7BP) to mediate intracellular localization, and Gγ-like (GGL) domains which bind $G\beta_5$-subunits. The D/R12 family varies greatly. RGS10 is the smallest, with little more than an RGS domain, while RGS12 and RGS14 have tandem Ras binding domains (RBD) and C-terminal GoLoco motifs (GoLoco) which serve as guanine nucleotide dissociation inhibitors (GDIs) for $G\alpha_{i/o}$-subunits. RGS12 has additional N-terminal motifs, including a Psd-95/Dlg/ZO1 (PDZ) domain, which binds mitogen-activated protein kinases (MEK2), and a phosphotyrosine binding (PTB) domain which has been shown to bind N-type calcium channels ($Ca_v2.2$). Members of the E/RA family are negative regulators of the Wnt signaling pathway. Axin binds adenomatous polyposis coli (APC), β-catenin (β-cat bind), and glycogen synthase kinase-3β (GSK-3β bind) to form the β-catenin destruction complex. Other interacting partners include phosphatase PP2A at the C-terminal end of the protein, and Dishevelled (Dsh) at the DIX domain. The DIX domain also mediates axin oligomerization. The F/GEF family RGS proteins are RhoA specific guanine nucleotide exchange factors (GEFs) with canonical Dbl homology (DH) and Pleckstrin homology (PH) domains. Leukemia-associated RhoGEF (LARG) and PDZ-RhoGEF also have N-terminal PDZ domains. The G/GRK family consists of the G-protein coupled receptor kinases, each with an N-terminal RGS domain that binds $G\alpha_q$. The serine/threonine kinase domain (S/T kinase) phosphorylates GPCRs to initiate internalization. Three sorting nexins make up the H/SNX family and are characterized by an RGS domain located between phosphatidylinositol-binding (PX) and PX-associated (PXA) domains. The PXA, PX, and transmembrane domains (TM) mediate membrane association, and binding to hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) links SNX to the endocytic machinery. SNX13 (aka RGS-PX1) has also been reported to serve as a GAP for $G\alpha_s$, but these findings have not been confirmed. Dual specific-A Kinase Anchoring Protein-2 (D-AKAP2) and RGS22, which both include multiple RGS domains, do not fall under any of the eight families.

5.1. RGS2

RGS2 is one of the best characterized RGS genes with respect to cancer. Changes in expression have been linked to breast cancer [65], prostate cancer [17], acute myeloid leukemia [69], ovarian cancer [53], mantle cell lymphoma [81], and bladder cancer [80]. Further, the RGS2 gene is localized to chromosome 1q, a region of the genome that is commonly altered in solid tumors [82,83]. The following section will discuss the role of RGS2 in prostate cancer, and acute myeloid leukemia.

The majority of prostate cancers progress from androgen-dependent to androgen-independent cell growth, making hormone ablation therapy ineffective and reducing treatment options. Cao et al. demonstrated that RGS2 expression is specifically down-regulated in androgen-independent prostate cancer cell lines and tissue samples compared with their androgen-dependent counterparts [17], which may result in greater signaling through these GPCR-mediated pathways. Additionally, over-expression of RGS2 decreases Androgen receptor (AR) and MAP kinase activity in androgen-independent prostate cancer cell lines. RGS2 effects on these pathways were only partially blocked by a GAP-deficient mutant, suggesting that non-RGS domain functions of RGS2 may play a role in regulation of androgen-independent signaling [17].

Internal tandem duplications (ITD) in the fetal liver tyrosine kinase-3 (Flt3) receptor is one of the most common mutations in acute myeloid leukemia (AML) and is found in over 30% of AML cases [84]. The presence of Flt3-ITD mutants results in an increase in growth factor independent proliferation, clonal growth, and resistance to radiation-induced apoptosis of AML cells. Additionally, Flt3-ITD mutations are associated with increased expression of pro-proliferative genes and decreased expression of pro-differentiation genes. Schwable et al. showed that the presence of Flt3-ITD mutations decreases RGS2 expression [69]. Further, over-expression of RGS2 in Flt3-ITD expressing cells reduces the level of Flt3-dependent phosphorylation of Akt and GSK-3. Finally, RGS2 antagonizes the differentiation block induced by expression of Flt3-ITD mutants, a critical event in transformation of myeloid cells. Thus, RGS2 opposes oncogenic pathways in different forms of cancer.

5.2. RGS-RhoGEF proteins

The small G-protein Rho plays an integral role in many normal physiological and pathophysiological processes and has been demonstrated to mediate actin rearrangements and stress fiber formation, smooth muscle contraction, cell rounding, neurite retraction, gene transcriptional activity, and cell cycle progression [85]. Rho is required for cellular migration in a variety of cancers, and therefore plays a role in invasion and metastasis [86]. Further, there is evidence linking Rho signaling to cellular proliferation and survival through effects on the cell cycle and transcription [87]. Like heterotrimeric G-protein α-subunits, small monomeric G-proteins are active when bound to GTP and inactive when bound to GDP; they are activated by nucleotide exchange and deactivated by nucleotide hydrolysis. Thus, the activity of Rho and other small monomeric G-proteins is controlled by a tightly regulated array of guanine nucleotide exchange factors (GEFs), GDP dissociation inhibitors (GDIs), and GAPs. Rho GEFs effectively activate Rho by catalyzing the exchange of GDP for GTP.

RGS-RhoGEFs, including leukemia-associated RhoGEF (LARG), PDZ-RhoGEF, and p115-RhoGEF, contain an RGS domain that binds activated Gα12/13 and a GEF domain that activates Rho by catalyzing the exchange of GDP for GTP. This domain structure allows RGS-RhoGEFs to have a dual role as both RGS proteins and as G-protein stimulated effectors, linking GPCR signaling with downstream Rho activity [88]. In the past five years, several studies have demonstrated a role for RGS-RhoGEFs in cancer. In 2004, Wang et al. demonstrated that LPA and thrombin utilize LARG and PDZ-RhoGEF, respectively, to activate Rho in PC-3 prostate cancer cells [89]. As described above, a non-coding SNP in PDZ-RhoGEF was linked to a reduced risk of lung cancer in Mexican Americans [78]. Additionally, over-expression of p115-RhoGEF, PDZ-RhoGEF, or LARG induces transformation of NIH-3T3 cells [88]. RGS-RhoGEFs likely undergo complex regulation and participate in multiple signaling interactions. They are capable of associating with receptor tyrosine kinases through their PDZ domains and form hetero- and homo-oligomers via their C-terminal tails, the removal of which enhances GEF activity [90]. RGS-RhoGEF proteins have the potential to be critical regulators of cancer initiation and progression.

5.3. Axin

The Wnt signaling cascade regulates proliferation, differentiation, and motility and plays a critical role in development [91]. Further, aberrant Wnt signaling has been strongly linked to colon cancer, hepatocellular carcinoma, ovarian cancer, prostate cancer, and melanoma [92]. Wnt ligands bind two families of cell surface receptors, Frizzled and low-density-related lipoprotein receptor 5/6, setting off a signaling cascade that controls the stability of the transcriptional regulator and oncogene β-catenin. The accumulation of β-catenin leads to transcription of target genes such as c-jun, c-myc, and cyclin D1. Axin, an atypical RGS protein, serves as a molecular scaffold for a β-catenin destruction complex, binding directly to adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β), and β-catenin. This axin-based complex localizes constitutively active GSK-3β such that it can phosphorylate β-catenin, marking it for ubiquitination and subsequent degradation. Binding of Wnt-family ligands to cell surface Frizzled receptors destabilizes the β-catenin destruction complex, preventing the degradation of β-catenin and allowing it to accumulate and translocate to the nucleus where it regulates transcription. Many cancers express mutated forms of APC which are incapable of binding axin [93], resulting in enhanced β-catenin stability and greater β-catenin-dependent transcription. Further, axin participates in the anti-oncogenic TGFβ pathway as a binding partner for SMAD3. Thus, axin is a critical regulator of pathways required for antagonism of β-catenin activity and is a tumor suppressor [91].

In colon cancer cells, the Gs-coupled EP2 receptor mediates the mitogenic effect of prostaglandin E2 (PGE2), which induces proliferation and transcription of β-catenin-sensitive genes. Castellone et al. demonstrated that PGE2-stimulated β-catenin-dependent gene transcription is mediated by Gαs. Axin was co-immunoprecipitated with active Gαs, indicating that axin may be an effector of PGE2-stimulated Gαs. Over-expression of the RGS domain of axin almost completely abolished the proliferative response to PGE2. Further, stimulation with PGE2 or expression of constitutively active Gαs was associated with reduced GSK-3β binding to axin, suggesting that Gαs association with axin may disrupt the β-catenin destruction complex, resulting in greater β-catenin-sensitive gene transcription [94]. These data suggest that there are points of intersection between heterotrimeric G-protein signaling and the wnt signaling pathway that are mediated by the atypical RGS protein axin.

While axin contains an RGS domain, it does not appear to stimulate GTPase activity of Gα-subunits, although it is capable of binding Gαs and Gα12. In MDA-MB 231 breast cancer cells, the axin RGS domain competes with p115-RhoGEF for Gα12. Binding of axin blocks Rho-mediated cell rounding induced by expression of constitutively active Gα12 [95]. As discussed above, G12, p115-RhoGEF, and Rho are known mediators of oncogenesis. It is possible that axin may serve to attenuate the G12/Rho signaling

axis via effector antagonism (Fig. 3). If axin is mutated or otherwise compromised in cancer cells, its inhibition of Rho signaling would be alleviated, allowing for greater Rho signaling and activation of oncogenic pathways. Further study will be required to determine the significance of the interaction between axin, G12, and Rho.

5.4. RGS5

Angiogenesis is a critical step in the establishment of a solid tumor, allowing the tumor access to growth factors, nutrients, and oxygen [96]; many cancer therapies now target this process. In 2004, two groups identified RGS5 as an up-regulated gene in a microarray screen of hepatocellular carcinoma [56,61]. Further, Furuya et al. determined that, rather than being expressed in tumor cells themselves, RGS5 is actually found in the pericytes of tumor blood vessels (though not in normal kidney vasculature), indicating that RGS5 may play a role in tumor neovascularization [56].

Tumor vasculature is typically underdeveloped and is characterized by the presence of immature pericytes, tumor hypoxia, and chaotic, leaky vessels. RGS5 expression has been shown to attenuate calcium and ERK signaling downstream of sphingosine-1-phosphate (S1P), angiotensin II, PDGF and endothelin-1, all of which are critical to vascular maturation (reviewed in Ref. [97]). In a mouse model of pancreatic islet cancer, RGS5 deletion resulted pericyte maturation and vascular normalization, leading to decreased tumor hypoxia and vessel leakiness compared with wild-type tumor vasculature [68]. The more stable vasculature allows for the growth of larger tumors. At later stages, RGS5 deletion resulted in increased tumor burden and earlier death. While underdeveloped vasculature causes tumor hypoxia, increased vessel leakiness, and decreased access to nutrients, more chaotic vasculature also reduces immune system access. Interestingly, though RGS5 deficient mice had an increased tumor burden, they had better response rates to injections of tumor-specific immune cells, indicating that RGS5 attenuation of vascular maturation may protect the tumor from immune attack. Finally, RGS5 has been identified as a broadly expressed tumor antigen, suggesting roles in multiple forms of cancer [98].

6. Conclusions

In the past decade, GPCRs and their cognate ligands have been shown to play a significant role in the initiation and progression of cancer; consequently, it is likely that regulators of GPCRs are also important to the regulation of oncogenic pathways. In this review, we present evidence that the RGS family of proteins play a role in multiple types of cancer. The transcription of over a dozen RGS genes is altered during oncogenesis and mutations in RGS genes have been shown to confer a reduced risk of lung and bladder cancers. Further, specific roles for RGS proteins have been demonstrated in prostate cancer, acute myeloid leukemia, ovarian cancer, colon cancer, and tumor angiogenesis. Further studies will serve to define specific roles of RGS proteins in cancer and lead to a better overall understanding of the signaling pathways regulating oncogenesis.

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