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# Cellular deficiency in the RGS10 protein facilitates chemoresistant ovarian cancer

More than 30 regulators of G protein signaling (RGS) proteins encompass the RGS protein superfamily of critical regulators essential to cellular homeostasis. There is enormous structural and functional diversity among the RGS superfamily, and as such they serve a wide range of functions in regulating cell biology and physiology. Recent evidence has suggested roles for multiple RGS proteins in cancer initiation and progression, which has prompted research toward the potential modulation of these proteins as a new approach in cancer therapy. This article will discuss basic RGS molecular pharmacology, summarize the cellular functions and epigenetic regulation of RGS10, review ovarian cancer chemotherapy and describe the role of RGS10 in ovarian cancer survival signaling.

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## G protein signaling & RGS proteins

The canonical function of RGS proteins is to control the strength of G protein-coupled receptor (GPCR) signaling pathways. In this way, they operate as an 'off' switch to deactivate heterotrimeric G proteins. GPCRs activated by ligand binding trigger activation of their associated cellular G proteins by promoting nucleotide exchange of GDP for GTP in the G protein α subunit. The now-active Gα subunit dissociates from the Gβγ dimer, and both G protein components initiate signaling cascades by binding and regulating effector targets. The G proteins are inactivated by GTP hydrolysis, which returns the Gα subunit to its GDP-bound state and facilitates re-association with Gβγ.

Basal hydrolysis of GTP occurs very slowly in isolation, but RGS proteins dramatically accelerate the hydrolysis of GTP (Figure 1). Thus, RGS proteins accelerate the deactivation of GPCR-stimulated G proteins, which strongly blunts the amplitude and duration of G protein activity. The GTPase accelerating protein (GAP) activity of RGS proteins effectively terminates G protein signaling to downstream effectors. In addition to canonical GAP activity, RGS proteins may function as effector antagonists by sequestering active Gα subunits and blocking their ability to interact with downstream effectors, and by G protein-independent interactions with other signaling components [1,2].

## The composition of RGS10

RGS10 is enigmatic; its structure is little more than the universal characteristic RGS domain shared among all RGS proteins, yet loss of RGS10 expression has powerful effects on cells. With only approximately 167 amino acids in all, RGS10 remains among the smallest of the RGS protein family. The RGS domain is a nine α-helix, 120-aa structure responsible for $G\alpha_{i/o}$ selective GAP activity [3]. Additionally, RGS10 contains sites for regulatory palmitoylation and PKA-mediated phosphorylation (Figure 2), the latter of which has been shown to mediate nuclear localization [4,5]. While other RGS family members contain additional domains, such as a PDZ-binding domain or PH domain, the RGS10 protein lacks all these.

## RGS10's function: complex regulation of cell survival

Although the expression of RGS10 is ubiquitous, the highest levels are found in the brain

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**Key terms**

**G protein-coupled receptor:** Serpentine-like, seven-pass transmembrane domain receptors at the cell surface that initiate the transmission of extracellular signals into the cell to regulate a variety of processes important for homeostasis. Ligand binding to the GPCR causes a conformational change in the receptor that elicits the signaling cascade.

**G proteins:** Guanosine nucleotide-binding proteins that are critical mediators of signal transmission from the G protein-coupled receptor into intracellular signaling cascades involving second messenger proteins. G proteins are activated after conformational changes occur to the receptor, which causes an exchange of GDP for GTP, resulting in activation.

**RGS10:** RGS10 is a GTPase activating protein that regulates signaling through G α subunits involved in G protein-coupled receptor signaling through heterotrimeric G proteins.

**Ovarian cancer:** A term to represent uncontrolled, cancerous growths occurring in women who share an anatomical location – one or both ovaries.

and immune system. RGS10 is expressed in neurons and glial cells, and multiple hematopoietic cell types including macrophages and osteoclasts [6]. The function and physiologic significance of RGS10 has been explored by enhancing or eliminating protein expression in cells and mouse models. For instance, macrophages lacking RGS10 expression produce higher levels of proinflammatory cytokines upon activation, indicating that endogenous RGS10 normally suppresses proinflammatory macrophage responses [7]. Similarly, RGS10 suppresses microglial activation in response to lipopolysaccharide activation, blunting microglial proliferation and proinflammatory cytokine release and enhancing survival of neurons following inflammatory stress, reflected by enhanced microglial proliferation and increased inflammatory cytokine release in RGS10-deficient mice [8]. Mice lacking RGS10 are also hypersensitive to inflammatory-mediated neuronal cell death, while overexpression of RGS10 in a neuroblastoma cell line exerted a neuroprotective effect against inflammatory stress [9] RGS10's ability to increase neuronal survival reflects both inhibition of NF-κB-mediated proinflammatory cytokine production in microglia [8,10], as well as PKA-dependent direct survival effects in neurons [9]. Interestingly, the ability of RGS10 to enhance neuronal survival following inflammatory stress was dependent on its nuclear localization, suggesting a canonical G protein-dependent mechanism may not fully account for its activity [9]. In osteoclasts, RGS10 is highly expressed and is critical for survival of osteoclast survival and differentiation [11]. RGS10 facilitates calcium oscillations triggered by RANKL, an upstream activator of NF-κB that is a primary regulator of osteoclast differentiation [12]. Taken together, these reports suggest that RGS10 has complex, cell type-specific effects on cell function and survival in multiple tissues, likely through distinct mechanisms including PKA and NF-κB.

## Ovarian carcinoma

It is now accepted that '**ovarian cancer**' is the term for different cancer subtypes that share a common location – appearing on one or both ovaries. Furthermore, it is also acknowledged that these tumors actually arise from distinct locations, not the ovaries, and simply share this anatomical location during tumor progression, but not initiation. In regard to epithelial ovarian carcinoma, the most common type of ovarian cancer, different subtypes arise from the fallopian tube (high-grade serous), endometrium and/or retrograde menstruation (endometriod), metastasis from renal cell carcinoma and/or nests within the vagina (clear cell) and metastasis from the endocervix or GI tract (mucinous), which includes the colon, appendix and stomach [13].

Although in the past the different subtypes of epithelial ovarian cancer were treated as a single disease entity and received the same chemotherapy, this is rapidly changing. For example, patients with mucinous tumors are likely to be administered therapeutic agents efficacious against gastrointestinal tumors while serous tumors receive platinum and taxane combinations. This is reflective of 'precision' or 'personalized' medicine that is overtaking the field of oncology as a more suitable approach to patient care than the one-size-fits-all historic perspective. Breast cancer patient outcomes have benefited significantly from precision medicine resultant from the deluge of biomarkers, targeted therapeutics and surgical options.

## GPCRs in ovarian cancer

GPCRs are critical to maintaining homeostasis in cells. They are the fundamental link that receives and responds to appropriate signals coming from outside the cell. Without the presence of such receptors, cells would otherwise fail to benefit from critical communications and required nutrients. Their essential nature is underscored by the fact that over 800 independent GPCRs are known in existence. GPCR agonists and antagonists are also prominent in medicine, encompassing the bulk of all US FDA-approved, prescribed pharmaceuticals.

Although the majority of GPCRs function in olfaction, others are related to hormonal signaling and vital to the ovary. For example, there are GPCRs that regulate estrogen (GPR-30), gonadotropins (GnRH II receptor), LH (luteinizing hormone receptor) and FSH

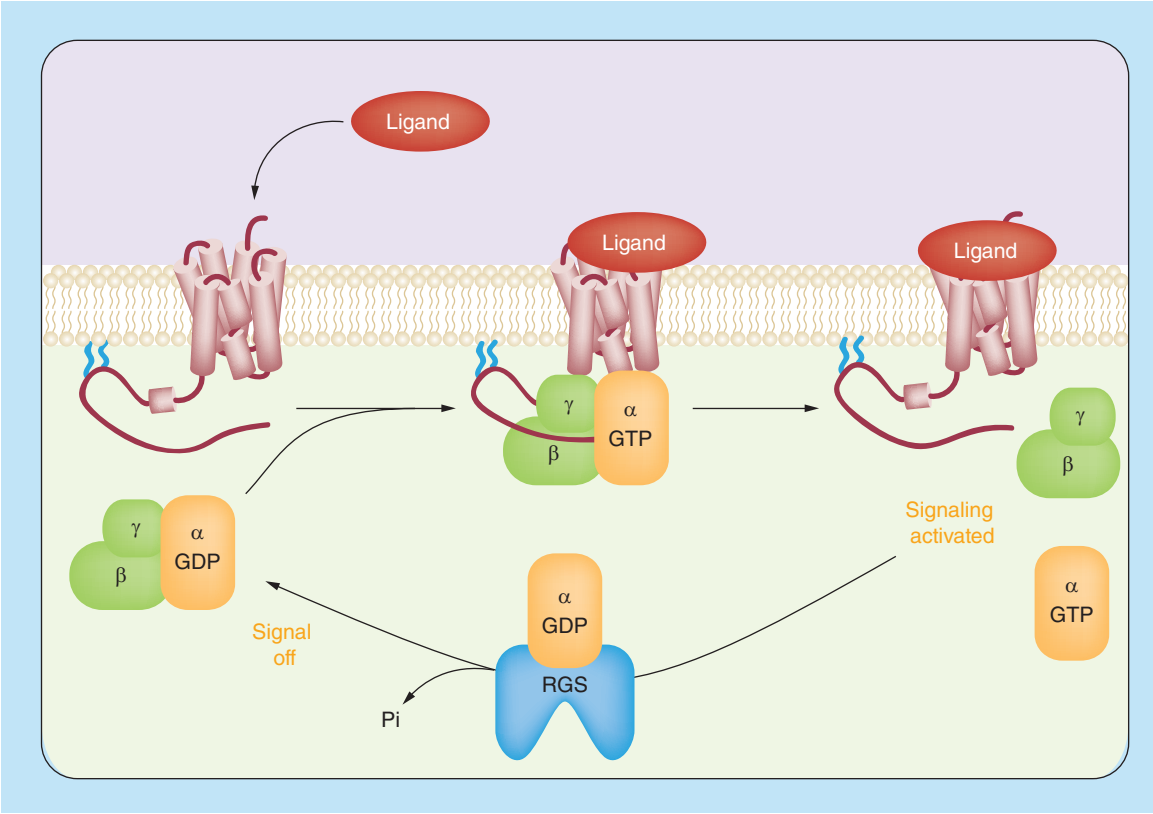


**Figure 1. The canonical role of regulators of G protein signaling proteins.** Ligand binding triggers conformational changes in the G protein-coupled receptor, which translates to G protein mediation of signaling activation in the cell. Regulators of G protein proteins blunt the signal through hydrolysis of GTP.

(follicle-stimulating hormone receptor). The fluctuation of LH and FSH regulates reproduction by controlling the development of follicles. Thus, their GPCRs are critical, but whether these GPCRs are involved in tumorigenesis in ovarian cancer is debated [14]. On the other hand, there are numerous GPCRs that are implicated in other types of cancer [15,16]. For example, mutations in the melanocortin 1 receptor increases the risk of melanoma, particularly among lightly pigmented individuals who receive damaging UV radiation.

Cancer cells transform 'normal' signaling pathways considerably, until they are aberrantly adjusted to maintain an out-of-control rate of cellular proliferation. During the progression from a normal cell to a cancerous one, many protein-level changes occur, including the expression and function of G protein-coupled receptors. For example, the lysophosphatidic acid receptors (LPARs), which are seven-pass, transmembrane receptors located at the cell surface, display significantly altered levels of expression among cancer patient samples, compared with normal ovary. Some LPARs are significantly diminished (LPA1 and LPA4), while others are significantly enhanced (LPA2 and LPA3), suggesting disparate roles for signaling and subsequent outcomes using the same signaling input (lysophosphatidic acid [LPA]) [15]. Although the expression of LPA receptors is not a biomarker of outcome, the 39-gene expression signature resultant from LPA stimulation of ovarian cancer cells does represent worsened prognosis, suggesting that GPCR ligands produce powerful information.

Even though changes in the expression of LPA receptors are not clinically useful biomarkers for ovarian cancer, their function clearly has disastrous effects. In a mouse model where the LPA1, LPA2 and LPA3 receptors were individually overexpressed in ovarian cancer cells, animals bearing LPAR-tumors fared considerably worse than LacZ-tumor controls. LPA receptor expression increased the volume of ovarian tumor-formed ascites, the percentage of mice developing ascites, primary tumor volume, the growth rate of the primary tumor and the extent of metastatic lesions [16].

Another GPCR, the chemokine receptor CXCR2, promotes tumorigenesis in ovarian cancer. It does this via modulating several proteins, p21, CDK4/6, cyclin D1, cyclin A and cyclin B1, all which aid cellular progression through the cell cycle. Furthermore, the overexpression of CXCR2 in high-grade serous ovarian carcinoma is a prognostic of poor survival outcomes [17]. This further suggests that GPCR expression can medi-

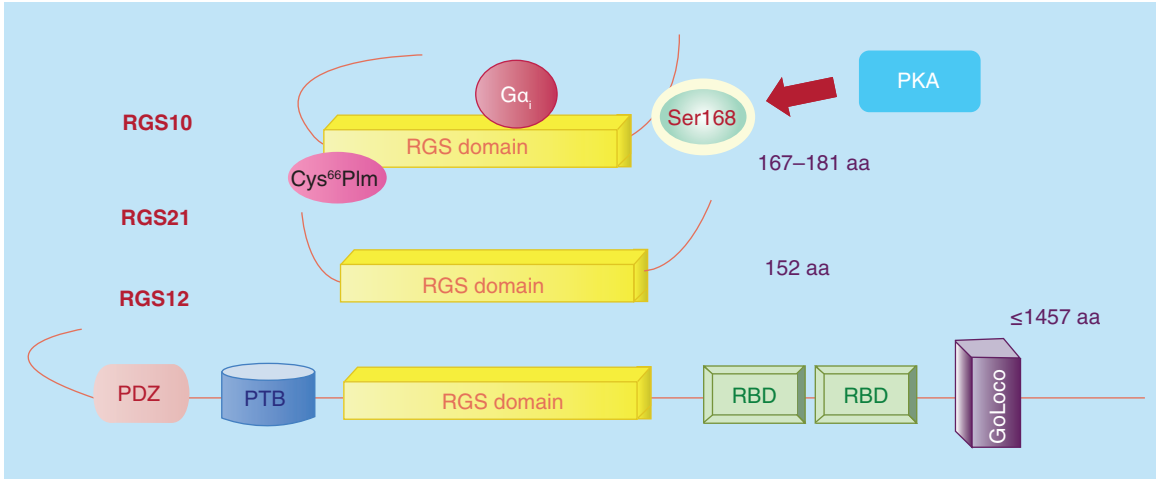


**Figure 2. RGS10 contains only the universal regulators of G protein signaling domain.** The composition of RGS proteins varies tremendously. Here, the size and organization of RGS10 is compared against the smallest family member, RGS21 (152 amino acids), as well as the largest subfamily member, RGS12 (splice variants ≤1457 amino acids). All RGS proteins share the universal RGS domain, which accelerates GTPase activity of Gα proteins (RGS10 is shown binding to $G\alpha_i$). Palmitoylation of RGS10 at $Cys^{66}$ significantly potentiates its GAP activity. PKA phosphorylates RGS10 at serine 168, without altering its GAP activity. Both RGS10 and RGS12 belong to the D/R12 subfamily based on phylogenetic analysis and have several splice variants, which explains the range of amino acids. RGS12 has a domain present in PDZ, PTB, two Raf-like RBDs and a G protein regulatory (GoLoco) motif. PDZ: PSD-95, D1g and ZO-1/2; PTB: Phosphotyrosine-binding domain; RBD: Ras-binding domain; RGS: Regulators of G protein signaling.

ate significantly negative impacts on malignant cells. Together, these data demonstrate a strong role for multiple GPCRs and, by association, their negative regulation by RGS proteins, in ovarian tumorigenesis and aggressiveness.

## Chemotherapy regimens & chemoresistance development

The vast majority of patients presenting to the clinic with ovarian cancer will receive a platinum-based chemotherapy regimen and respond to treatment (Table 1 [18]). However, for most patients this response is short-lived which makes chemoresistance the remaining issue impeding cure in ovarian cancer. Although traditional cytotoxic chemotherapy regimens work quite well in most patients, the disease is only in remission for 18–24 months. It returns with a vengeance and usually becomes resistant to platinum therapy, which is the first-line of treatment for this malignancy. In fact, the selection of treatment is based on whether or not a patient is responsive to platinum therapy (carboplatin or cisplatin) and the period before resistance was acquired [19].

Platinum drugs are effective killers of rapidly dividing ovarian cancer cells. They bind DNA and form mostly intrastrand cross-links, which creates DNA adducts, preventing DNA synthesis and transcription. However, platinum drugs are vulnerable to resistance mechanisms due to their facilitated transport into the cell. In addition, sooner or later cells will initiate mech-

Table 1. Chemotherapy used in combination to treat ovarian cancer.

| Commonly used drugs | Platinum resistant | Salvage therapy |
|---|---|---|
| Carboplatin or cisplatin | Paclitaxel or docetaxel | Altretamine |
| Cyclophosphamide | Etoposide | Capecitabine |
| Paclitaxel or docetaxel | Gemcitabine | Cyclophosphamide or ifosfamide |
| Bevacizumab | Liposomal doxorubicin | Irinotecan |
| Gemcitabine | Topotecan | Melphalan |
| Doxorubicin | | Oxaliplatin |
| Olaparib | | (Nab- or) paclitaxel |
| | | Pemetrexed |
| | | Vinorelbine |

anisms to inactivate the drug or the damage it creates (e.g., DNA repair). This creates a scenario whereby an effectively working drug becomes useless and salvage therapy must be administered. Ultimately the majority of patients succumb to chemoresistant disease, even with salvage treatment [19].

To overcome this scenario, PARP inhibitors (olaparib, eliparib, rucaparib, niraparib, among others), which interfere with DNA repair at sites of DNA damage, were utilized in this setting. Although disappointing setbacks emerged after failing to achieve significant improvement in overall survival during clinical trials, PARP inhibitors are still being pursued therapeutically and olaparib did receive approval by the US FDA in late 2014. For a while the enthusiasm for PARP inhibition dimmed, but olaparib has refueled enthusiasm and demonstrated that patients with mutations in BRCA1/2 may receive benefit from PARP inhibition.

Also ongoing is research dedicated to understanding previously undetected or underappreciated chemoresistance mechanisms. For example, the presence of abundant E3 ubiquitin ligase facilitates platinum resistance, but its knockdown can also cause ovarian cancer cells to become 24-fold more sensitive to cisplatin [20]. In addition, cystathionine-β-synthase (a sulfur metabolism enzyme in primary serous) silencing in an orthotopic model resistant to cisplatin was able to resensitize the ovarian cancer cells to cisplatin [21]. Another promising area reported the treatment with an inhibitor of NEDD8-activating enzyme, a ubiquitin-like modifier-activating enzyme, which produced synergistic effects against ovarian tumors in combination with platinum agents [22]. With so many diverse mechanisms responsible, the clinical panacea might only occur after establishing a formulary with abundant targeted options chosen according to individualized precision medicine.

## Novel roles for RGS10 in ovarian cancer chemoresistance

Recent work performed by our groups highlights the role of RGS10 in chemoresistance. In our model, loss of RGS10 enhances survival among chemoresistant ovarian cancer cells. We have observed loss of RGS10 expression in multiple chemoresistant ovarian cancer cell lines [23,24]. We have also demonstrated that we are able to manipulate the sensitivity of ovarian cancer cells to paclitaxel, cisplatin and even vincristine by modulating RGS10 expression [23]. Loss of RGS10 expression in ovarian cancer cells resulted in enhanced cell viability and increased activation of AKT, a key survival pathway in ovarian cancer. The mechanism of RGS10 regulation of cell survival in ovarian cancer is not fully understood, and its ability to regulate specific upstream oncogenic GPCRs such as LPAR and CXCR2 remains to be defined.

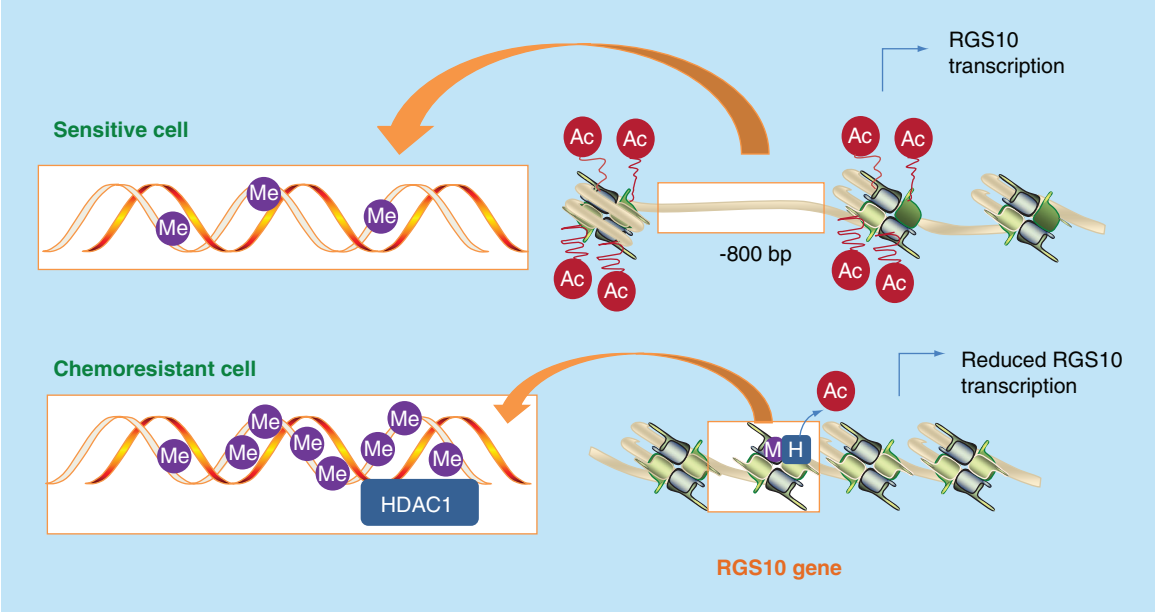


**Figure 3. Epigenetic modifications to the RGS10 gene and promoter region that regulate the gene's expression.** In chemotherapy-sensitive cancer cells, histone acetylation opens up the nucleosome conformation surrounding the promoter region of the RGS10 gene, allowing transcription factors to bind and transcribe the gene. DNA methylation is enhanced in genomic DNA approximately 800 basepairs upstream of the RGS10 transcriptional start site in chemoresistant ovarian cancer cells. DNA methylation is required to recruit HDAC1, which release acetylation and result in more tightly packed chromatin at the RGS10 promoter, thereby reducing RGS10 expression.

## Epigenetic regulation of RGS10 expression

The expression of RGS10 is dynamically regulated in multiple systems, allowing its activity to be highly responsive to biological signals. Unlike many RGS proteins, which are typically regulated by protein degradation, RGS10 is transcriptionally regulated in multiple systems. In the neural and immune systems highlighted above, RGS10 expression is acutely regulated by upstream signals: RGS10 is silenced by inflammatory signals such as LPA and TNF-α [8], while RGS10 expression is induced by RANKL activation in osteoclasts [12]. We have also shown that RGS10 is suppressed in multiple models of ovarian cancer chemoresistance [23]. Furthermore, we have determined that epigenetic silencing by histone deacetylation and DNA methylation of the *RGS10* promoter is critical in the loss of RGS10 expression in chemoresistant ovarian cancer cells lines (Figure 3) [24]. Inhibitors of histone deacetylase and DNA methyltransferase enzymes synergistically enhance RGS10 expression in chemoresistant cell lines [25]. Chemoresistant A2780-AD ovarian cancer cells display reduced RGS10 expression and *RGS10* proximal promoters show increases in DNA methylation and marked reduction in histone acetylation compared with chemosensitive counterparts [24]. Further, HDAC-1 enzymes are specifically recruited to *RGS10* promoters in chemoresistant cells without an accompanying increase in overall HDAC1 expression [24]. Inhibition of DNA methyl transferase activity blocks recruitment of HDAC1 to *RGS10* promoters, suggesting that DNA methylation is a prerequisite for HDAC recruitment [25]. These results are consistent with a well-established role for epigenetic silencing in establishing chemoresistance in ovarian cancer [26], and suggest that *RGS10* silencing contributes to this mechanism.

## Conclusion & future perspective

Taken together, the discoveries reviewed herein are making the RGS10 protein less enigmatic. Although the functional role of RGS10 in ovarian cancer chemoresistance was previously unknown, we have elucidated the outcome of RGS10 silencing in this model, as well as the epigenetic mechanisms regulating it. In this context, the presence of RGS10 has a significant impact on cell survival. Future studies and current ongoing investigations are further elucidating novel features of RGS10 and will be reported in due time.

### Financial & competing interests disclosure

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### Executive summary

- Although RGS10 is among the smallest of the RGS superfamily of proteins and has little more than the universal regulators of G protein signaling (RGS) domain, novel functions mediated by this protein are emerging.
- RGS10 has interestingly complex, but cell type-specific effects on function and survival, possibly via distinct mechanisms including PKA and NF-κB.
- RGS10 plays a role in chemoresistance in ovarian cancer. Loss of RGS10 enhances survival among these cells.
- RGS10 is epigenetically regulated. In chemoresistant cells, RGS10 proximal promoters show increases in DNA methylation and reduction in histone acetylation compared with chemosensitive cells.

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