Modulation of G Protein Signaling Normalizes Tumor Vessels

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Abstract

G protein–coupled biological processes are important for an ever-increasing number of human diseases and require fine-tuning through accessory molecules such as the regulators of G protein signaling (RGS). RGS5, a marker for tumor-resident pericytes, was recently established as playing a pivotal role in vascular maturation and vessel remodeling during carcinogenesis. Remarkably, tumors arising in a RGS5-deficient background display vessels with normalized morphology and an overall improved blood flow. Furthermore, these morphologic changes also lead to dramatic improvements in lymphocyte access to tumors and success of antitumor immunotherapy. Here, we consider the implications of these findings, and how they contribute to enhancing our understanding of remodeling angiogenic vessels as means for improving anticancer therapies. [Cancer Res 2009;69(2):396–9]

Modulation and Fine-Tuning: Regulator of G Protein Signaling Molecules Regulate G Protein–Coupled Signaling Pathways

G protein–coupled receptors (GPCR) trigger a vast number of essential biological functions. Typified by their heptahelical transmembrane structure, GPCRs activate intracellular heterotrimeric G proteins, which in turn stimulate various downstream effectors, resulting in cellular changes that affect immunity, neurotransmission, hormonal activity, and many other fundamental physiologic processes. Because of these broad-ranging effects, GPCRs have become a common target for the treatment of human diseases, and thus, the majority of pharmaceutical agents in the market currently act by manipulating GPCR function (1).

Proper functioning of the divergent family of GPCRs and associated trimeric G proteins is maintained through extensive regulation by other requisite signaling proteins. Regulator of G protein signaling (RGS) proteins represent a group of molecules that play a significant role in influencing GPCR signals. Essentially acting to switch off GPCR-activated pathways, RGS proteins are also known as GTPase-activating proteins due to their ability to bind activated Gα subunits and accelerate their intrinsic GTPase activity to deactivate G proteins (2). Although the biochemical properties of RGS proteins have been well documented, their physiologic functions and significance in vivo are only recently starting to emerge.

RGS5, one member of the RGS family, is dynamically regulated in various biological processes and has been implicated in blood pressure regulation, smooth muscle cell pathology, and tumor angiogenesis (3–5). Interestingly, RGS5 has emerged as a marker for pericytes, a smooth muscle cell–like population that lines vascular tubes and supports endothelial cells. One of the earliest reports to suggest that RGS5 expression was restricted to pericytes was a study that found RGS5 expression strongly correlated with the pericyte/vascular smooth muscle cell layer in developing mouse embryos (6). Bondjers and colleagues (6) showed that developing blood vessels in the central nervous system of mice deficient of platelet-derived growth factor-B (PDGF-B) or PDGF receptor-β (PDGFRβ) were almost completely devoid of pericytes, with a concurrent absence of RGS5 expression. This simultaneous loss of pericytes and RGS5 expression was highly indicative of a specific role of RGS5 in developing pericytes.

Pericytes Take Center Stage

Although overlooked for a long time, the key importance of pericytes in blood vessel development, maturation, and stabilization is now undisputed (7). The availability of genetic mouse models in recent years has sparked a keen interest in studying pericyte activity, and verified their fundamental role during vessel growth and pathologic angiogenesis. Indeed, pericytes are now recognized as potential targets for antiangiogenesis therapy (reviewed in ref. 8). Interestingly, RGS5 is the first marker for a very distinct population of tumor-resident pericytes. In a mouse insulinoma model, endothelial and perivascular cells were isolated based on CD31 and PDGFRβ expression and RGS5 was found to be highly up-regulated in PDGFRβ+ pericytes (5). These pericytes are unique because they are associated with a highly angiogenic vasculature, display an immature phenotype, and are possibly recruited from the bone marrow (9). A significant aspect of this study was that under aggressive combined antitumor therapy, RGS5 expression by pericytes was dramatically down-regulated. Therefore, RGS5 was established as not only being a marker for angiogenic tumor pericytes but the inverse correlation between tumor regression and RGS5 expression suggested that it may be manipulated to enhance tumor therapy.

RGS5 as a Key Modulator of Pericyte Maturation

The emerging role of RGS5 in developing and angiogenic pericytes prompted further analysis of RGS5 function in vivo and the development of RGS5 knockout models (3, 10, 11). Our own studies particularly addressed the role of RGS5 in the tumor environment by intercrossing RGS5-deficient mice with a mouse model for insulinoma. These RIP1-Tag5 (RT5) transgenic mice are characterized by defined stages of pancreatic islet cell carcinogenesis. Wild-type RT5 insulinomas displayed all the hallmark characteristics of angiogenic vessels, depicted by an aberrant arrangement of vessels that were tortuous and leaky and created a hypoxic environment. In the absence of RGS5, these features were dramatically changed so that the vessels became more regular and homogenous and less permeable and leaky, and oxygen supply to...
the tumors was significantly improved. Overall, the tumor vessels looked normalized, bearing resemblance to blood vessels in normal tissue (Fig. 1). Moreover, a closer look at the intratumoral pericytes revealed a shift in marker expression with the majority of pericytes co-staining with smooth muscle actin rather than PDGFRβ, indicative of a more mature phenotype (10).

**RGS5 and Antitumor Therapy**

*RGS5*-deficient RT5 mice exhibited accelerated tumor development and reduced survival. This is most likely a consequence of the normalized vasculature, which improves blood flow and intratumoral oxygen supply. The huge clinical implications of the morphologic changes in the blood vessels became only apparent when we analyzed how the tumor-bearing mice respond to therapy. We had previously shown that the highly angiogenic and disorganized vasculature within RT5 tumors creates a major obstruction for infiltrating immune effector cells, making tumors unresponsive to immunotherapy (12). Interestingly, by creating a proinflammatory, antiangiogenic intratumoral environment, the tumor vasculature could be remodeled to assume a more normal appearance and open tumors for immune-mediated cancer destruction (12). Remarkably, the normalized vasculature in *RGS5*-deficient RT5 tumors showed a striking resemblance to vessels under therapy. This finding prompted us to assess the responsiveness of *RGS5* knockout tumors for immunotherapy. We found that whereas there was no significant increase in the level of spontaneous host immune cells infiltrating the tumor parenchyma, activated tumor-specific T cells adoptively transferred into tumor-bearing *RGS5*-deficient mice were able to penetrate the tumors more effectively than wild-type tumors (Fig. 1). Furthermore, mice that had received this treatment exhibited substantially prolonged survival compared with mice that did not undergo adoptive transfer (10). Thus, in our model, removing RGS5 from the tumor microenvironment leads to normalization of the tumor vasculature and consequently improved clinical outcomes in response to immune therapy.

**Antiangiogenesis Therapy: What Is New?**

Although remodeling of vessels in *RGS5*-deficient tumors is a prerequisite for therapeutic success, this approach is clearly different from classic antiangiogenic therapy. Antiangiogenic cancer therapy is based on a simple but logical concept: destroy the tumor vasculature to deprive the tumor of nutrients and oxygen and ultimately induce tumor regression. Vascular endothe-
lial growth factor (VEGF) is central to the process of angiogenesis and is the primary target for antiangiogenic drugs, such as bevacizumab (13). Although the long-term survival benefits have not been as affirmative as initially hoped (14), greater success was seen when administered in combination with chemotherapy (15). A key finding in this context is that VEGF/VEGFR blockade leads transiently to vessel stabilization and reduced vascular permeability (16), a phenomenon described as vessel normalization (17), which increases drug penetration and explains superior clinical outcomes of combination therapies. These studies show some similarities with our findings in RGS5 knockout tumors with regard to overall vascular morphology, diminished tumor hypoxia, and reduced vessel permeability. Interestingly, VEGF signal blockade temporarily increases pericyte coverage of tumor vessels, which is mediated through Ang1/Tie2 signaling (16). Although anti-VEGF therapy ultimately destroys tumor vessels, this temporary shift in vessel morphology supports tumor cell eradication in combination therapies for a narrow window in time. In contrast, RGS5 deficiency in tumors causes a permanent shift in pericyte maturation and a long-lasting improvement of the aberrant vascular morphology. Although this would be detrimental on its own, it dramatically improves the outcome of immunotherapy (10). Importantly, angiogenic activity is reduced without interception or modulation of VEGFR/VEGFR signals. Emerging evidence suggests that inhibition of VEGF signaling can stimulate alternate proangiogenic molecules, such as ligands of the fibroblast growth factor family, to compensate for the lack of angiogenic signals and trigger a secondary phase of tumor angiogenesis (18). Also of note is the recent revelation that some tumors, particularly glioblastomas, respond to withdrawal of angiogenic signals by adapting a highly invasive mode and migrate along blood vessels in brain parenchyma (19). Therefore, it is highly desirable to identify alternative ways to modulate tumor vasculature that do not interfere with the complex regulatory network of proangiogenic factors.

Implications and Future Directions
Our recent studies on the role of pericytes and RGS5 in the tumor environment have provided new insights both on mechanisms of blood vessel maturation and on therapeutic manipulation of the tumor vasculature. Importantly, however, our findings stress the significance of blood vessels as a barrier for lymphocyte extravasation. Despite an increasing number of publications on the crucial role of tumor vessels in antitumor immunity (20, 21), this is still frequently overlooked. Our data are supported by a recent study by Buckanovich and colleagues who reported that the tumor endothelial barrier in ovarian cancer is in part mediated by the endothelin receptor B (ET$_{BR}$), which interferes with T-cell adhesion to the endothelium. Blocking signaling through ET$_{BR}$ activates tumor vessels and enhances lymphocyte access into tumors (21). For RGS5, it remains to be shown how modulation of pericyte maturity and improved lymphocyte adhesion or migration may be linked. Pericytes exert diverse biological functions and are reportedly implicated in lymphocyte adhesion (22). However, currently, it is not known whether the effect of pericyte maturation manifests at a cellular level or has broader effects on the tumor microenvironment.

Importantly, manipulating RGS5 at best will only support other forms of therapy because it is unable to reduce tumor burden. To date, immune-based forms of therapy have been ineffective in exerting tumor control. Our findings highlight the importance of vessel normalization for immunotherapy and thus hold promise for improving existing immune-mediated anticancer strategies. The potential to enhance the outcome of other anticancer strategies such as radiation and chemotherapy in the absence of RGS5 needs to be explored. RGS5 cell type specificity and its crucial role in the tumor microenvironment make it a prime candidate for pharmaceutical intervention. However, to fully capitalize on this potential, the regulatory functions of RGS5 in various signaling cascades need to be elucidated. The intratumoral effects observed in the RGS5-deficient mice provide a strong basis for pursuing these studies with a focus on tumor-resident pericytes.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 7/24/2008; revised 9/15/2008; accepted 9/23/2008.

Grant support: National Health and Medical Research Council (458627), Western Australian Institute for Medical Research, and University of Western Australia (R. Ganss).

We thank M. Jugold, F. Kiessling, P. Rigby, H.M. Marti, T. Rabie, S. Kaden, H-J. Grüne, G.J. Hämmerling, and B. Arnold for collaborative work on RGS5.

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Cancer Res 2009; 69: (2). January 15, 2009 398 www.aacrjournals.org


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