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Review

Molecular Cancer Therapeutics

RAC1: An Emerging Therapeutic Option for Targeting Cancer Angiogenesis and Metastasis

Hemant K. Bid[1], Ryan D. Roberts[1], Parmeet K. Manchanda[2], and Peter J. Houghton[1]

Abstract

Angiogenesis and metastasis are well recognized as processes fundamental to the development of malignancy. Both processes involve the coordination of multiple cellular and chemical activities through myriad signaling networks, providing a mass of potential targets for therapeutic intervention. This review will focus on one master regulator of cell motility, RAC1, and the existing data with regard to its role in cell motility, including particular roles for tumor angiogenesis and invasion/metastasis. We also emphasize the preclinical investigations carried out with RAC1 inhibitors to evaluate the therapeutic potential of this target. Herein, we explore potential future directions as well as the challenges of targeting RAC1 in the treatment of cancer. Recent insights at the molecular and cellular levels are paving the way for a more directed and detailed approach to target mechanisms of RAC1 regulating angiogenesis and metastasis. Understanding these mechanisms may provide insight into RAC1 signaling components as alternative therapeutic targets for tumor angiogenesis and metastasis. *Mol Cancer Ther; 12(10); 1925–34. ©2013 AACR.*

Introduction

RAC1 is a member of the Rho GTPase family, which includes RHO, RAC1, and CDC42. These proteins classically regulate the machinery that controls the assembly and disassembly of cytoskeletal elements (reviewed in ref. 1). RAC1 activity, as a modulator of the cytoskeleton, is critical for a number of normal cellular activities including phagocytosis, mesenchymal-like migration, axonal growth, adhesion and differentiation of multiple cell types as well as reactive oxygen species (ROS)-mediated cell killing (reviewed in ref. 2). RAC1 also plays a major role in the moderation of other signaling pathways involved in cellular growth and cell-cycle regulation (3), the formation of cell–cell adhesions (4), and the process of contact inhibition (5). These RAC-1–mediated activities appear central to the processes that underlie malignant transformation including tumorigenesis, angiogenesis, invasion, and metastasis. As such, some have posited that this protein and its partners may prove effective targets for drugs aimed at disrupting these pathways that mediate malignancy.

Authors' Affiliations: [1]Center for Childhood Cancer, Nationwide Children's Hospital; and [2]Department of Radiation Oncology, James Cancer Center, The Ohio State University, Columbus, Ohio

Note: H.K. Bid, R.D. Roberts, and P.K. Manchanda contributed equally to this work.

Corresponding Author: Peter J. Houghton, Center for Childhood Cancer, The Research Institute, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH 43205. Phone: 614-355-2670; Fax: 614-355-2927; E-mail: Peter.Houghton@nationwidechildrens.org

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RAC1 appears to be deregulated in both expression and activity in a variety of tumor cells (6). RAC1 hyperactivation and overexpression seems to correlate well with aggressive growth and other malignant characteristics in several different tumor types. This correlative evidence, coupled with our ever-improving appreciation for the mechanistic role that RAC1 plays in a number of malignancy-related processes, has piqued the interest of several groups who have speculated on the plausibility of targeting RAC1 and associated intracellular machinery for the potential antitumor effects that manipulating these pathways might have. This review will focus on potential roles of RAC1 in angiogenesis and metastasis and will summarize efforts that have been made to target these pathways. We attempt to present in an organized fashion multiple lines of evidence that illustrate how RAC1 activity contributes to these complex processes and offer an assessment of how we might exploit this target to disrupt the process of malignant progression.

RAC1 Signaling

RAC1 signaling pathways play an important role in the pathobiology of various processes that promote tumor progression. Consistent with this, growing evidence from animal studies clearly indicates that RAC1 signaling contributes to tumorigenesis. RAC1 primarily activates p21-activated kinases, such as PAK1, PAK2, and PAK3. PAKs are serine/threonine kinases that phosphorylate and activate actin-binding LIM kinases, LIMK1 and LIMK2, which in turn phosphorylate and inactivate cofilin (Fig. 1). Cofilin binds actin filaments, reversing the process of polymerization brought on by ARP2/3 activity, converting F-actin filaments into

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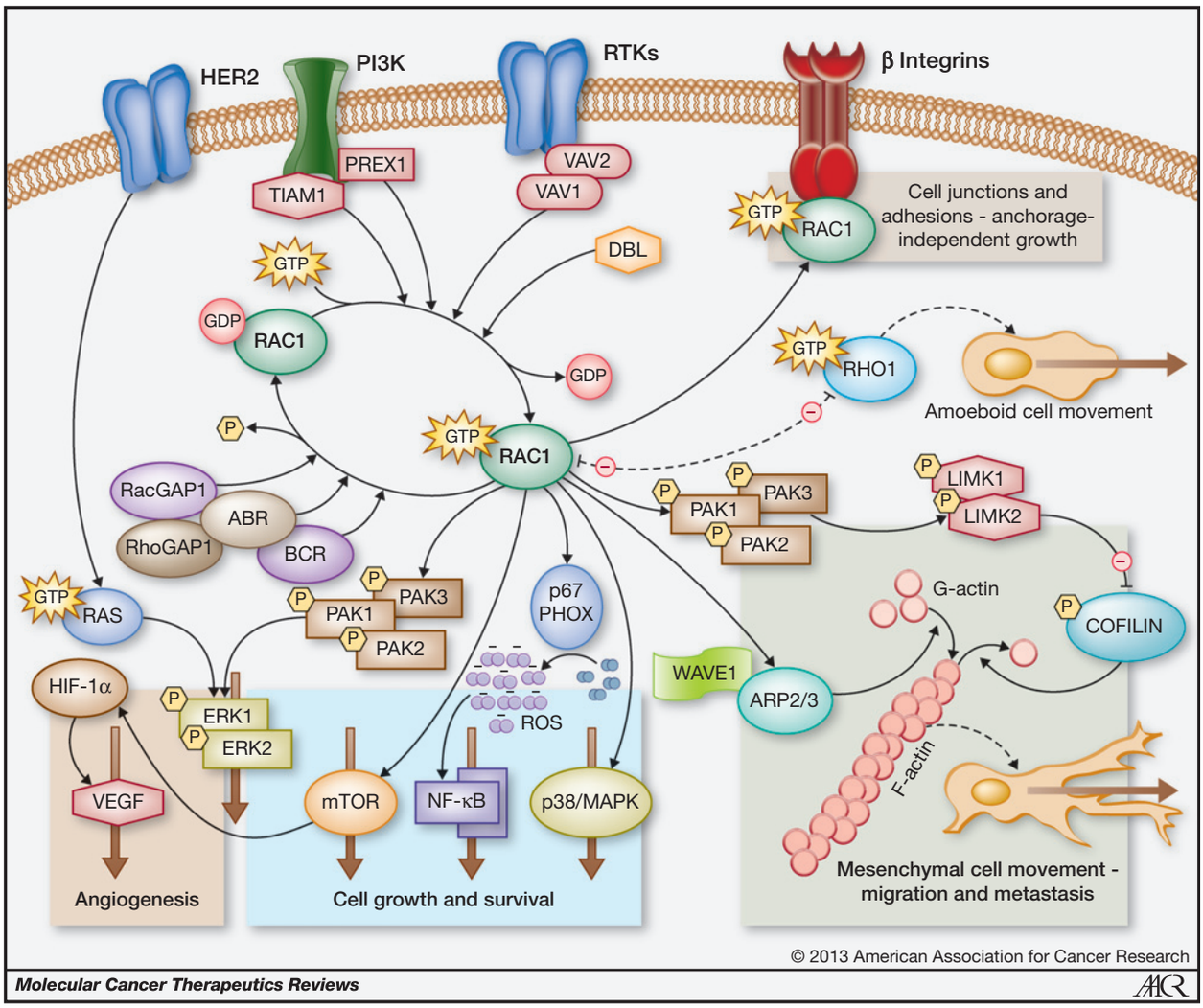

Figure 1. RAC pathways. Schematic illustration of RAC signaling pathways and effector functions. Emphasis is given to pathways known to affect tumor-related angiogenesis and metastasis.

G-actin monomers. By inactivating cofilin, these pathways allow actin filaments to grow (7). RAC1 also causes, together with the adapter protein NCK, dissociation of WAVE1 from its regulatory complex, which stimulates activation of the ARP2/3 complex and promotes actin polymerization (8). RAC1 also appears to interact directly with pathways that regulate proliferation [through the mitogen-activated protein (MAP) kinase system, especially JNK/p38], the inflammatory response (through interactions with NF-κB), ROS-mediated cell killing (through NADPH oxidase), G_1 cell-cycle progression, and the formation of cell–cell contacts (reviewed in ref. 9).

Under normal conditions, RAC1 activity is controlled both spatially and temporally by the opposing activities of guanine nucleotide exchange factors (GEF; reviewed in ref. 10), which exchange GDP for GTP and activate RAC1, and GTPase-activating proteins (GAP), which stimulate the conversion of bound GTP to GDP and inactivate RAC1. The classical GEF activators of RAC1 include VAV1 and VAV2, DBL, and TIAM1, whereas the most common GAPs include RhoGAP1, RacGAP1, ABR, and BCR, among others. Further regulation of RAC1 activity comes from the crosstalk that exists between these related signaling pathways. For instance, RAC1 and MYC activity appear to be modulated through a negative feedback coregulatory loop involving α6β4 integrin and PAK2. Further regulatory input comes from the integration of multiple signals by downstream targets of RAC, as is seen in the modulation of STAT3 activity through LIMK1 (11).

RAC1 and Angiogenesis

Tumor angiogenesis is a requisite for supplying a tumor with the nutrients and waste exchange that are needed to grow from a microscopic collection of tumor cells to a

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macroscopic tumor organ. This highly regulated process is orchestrated by the balance of inhibitors and stimulators of endothelial cell proliferation, endothelial cell migration, and capillary formation molecules (12). Antiangiogenic agents have been developed and used for the treatment of many different types of tumors. However, clinical trials have had largely disappointing results, which has raised questions about both the strategies used in targeting angiogenesis and the ways that these agents might be used more effectively (13). The results of these trials have amplified our understanding of neoangiogenesis and of the complexity of interactions between tumor cells and the surrounding stroma. We know that sprouting angiogenesis and lymphatic blood vessel segregation require highly coordinated endothelial cell migration, an activity often associated with Rho kinases. As is discussed below, these RAC1-dependent processes help orchestrate a number of cellular responses, which include, for instance, neoangiogenesis that occurs during wound repair and tissue responses to trauma. A detailed role for RAC1 in tumor-related angiogenesis is emerging and suggests an intimate role in that process as well.

Vasculogenesis and angiogenesis are 2 distinct processes, one representing vessel formation from undifferentiated precursors whereas the other describes the process where new vessels form from the preexisting vasculature. There is evidence that RAC1 plays a role in both processes. Endothelial-specific deletion of RAC1 results in mid-gestational embryonic lethality (14). These embryos develop defects of major vessels and show a complete lack of small branched vessels within both the embryos and their yolk sacs. The effect seems to result from an inhibition of cell migration, likely mediated through an F-actin–related mechanism (15).

RAC1 regulates a diverse spectrum of cellular functions involved in vascular morphogenesis. Double disruption of CDC42 (cell division control protein 42 homolog) and RAC1 makes endothelial cells unable to form lumens or tubes and blocks endothelial cell invasion in 3-dimensional collagen matrices (16). RAC-mediated mesenchymal migration is important in the initial steps of neoangiogenesis and the separation of new vasculature into blood and lymph vessels. Endothelial-specific RAC1-knockout mice exhibit hemorrhage and edema, which was traced back to the inability of lymphatic endothelial cells to separate from established vessels and form separate lymphatic structures (17). RAC1 also plays a key role in coordinating the initial formation of cell–cell adhesions (18), which play an important role in the assembly of endothelial cells into vessel structures and the maturation of new vasculature. Microinjection of plasmids containing dominant-negative RAC constructs into human vascular endothelial cells leaves them unable to undergo the morphogenic changes that result in capillary formation, whereas dominant-negative RHO and CDC42 do not affect these same processes (19).

A robust line of experimental evidence supports the critical role that RAC1 plays in tumor angiogenesis. RAC1 knockdown by siRNA treatment of vascular endothelial cells inhibits VEGF-mediated tube formation as well as endothelial cell migration, invasion, and proliferation *in vitro* (20). This antiangiogenic activity translates well to *in vivo* models. Matrigel plugs embedded with the same siRNA constructs also show reduced angiogenesis. RAC1 knockdown within Neuro2a tumors almost completely inhibited the growth of those tumors in a xenograft model. Microscopic examination of those tumors showed less than half the microvascular density of control tumors. Similar antitumor activity was seen with the disruption of upstream signals using siRNAs directed at VAV2/3. Similar effects were seen when B16 melanoma and Lewis lung carcinoma cells were injected into VAV2/3-deficient host mice. Results of these studies (21) showed similarly decreased growth, increased survival, and reduced microvascular density. This effect was shown to result, at least partially, from the impaired function of the VAV2/3-deficient endothelial cells.

There does appear to be some redundancy of the RAC1-mediated functions in some tumor models. D'Amico and colleagues (22) were surprised to find that endothelial-specific knockout of RAC1 did not alter tumor growth or angiogenesis. While the absence of β3 integrin alone caused tumors to grow more rapidly, the same endothelial-specific RAC1 depletion in β3-deficient mice retarded the growth of melanotic tumors to rates similar to FLK-1 knockout animals, which lack VEGF receptor 1. These studies suggest redundant pathways for stimulating angiogenesis: one activated by VEGF that is RAC1-dependent and another activated by β3 integrin that uses pathways which are RAC1-independent. The extent to which this apparent redundancy is relevant to other tumor or host types remains to be seen, although studies summarized above suggest that it is not universal.

This collective literature suggests a pivotal role for RAC1 with its upstream and downstream signaling network in the process of tumor angiogenesis. RAC1 and its related pathways seem to be obvious potential targets for therapies intended for the treatment of numerous human diseases that involve abnormal neovascularization, most notably solid tumors.

RAC1 and Metastasis

Metastasis is a complex multistep process. It involves invasion of local tissues, intravasation of cancer cells into blood and lymphatic vessels, transit of cancer cells through these vascular trees, survival in foreign environments, extravasation within distant organs, transformation to micrometastasis-forming small cancer nodules, and finally an invasion within the distant tissues, transforming micrometastases into macrometastases (23). Metastasis has a major impact on the morbidity and mortality of patients with cancer. Despite this clinical relevance, metastasis remains the most poorly elucidated aspect of carcinogenesis. Understanding the mechanisms

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of metastasis will require further clarification of the underlying cellular and molecular events that control the metastatic cascade from onset to colonization (24). The cellular processes that control motility and adhesion are critical to multiple steps in the metastatic cascade. As illustrated above, RAC1 plays an integral role in both of these cellular processes. The interactions of cancer cells with the endothelium, stroma, and extracellular matrix largely determine patterns of metastatic spread (25). RAC1 participates intimately in the formation of cell–cell adhesions and so likely plays a role in determining these patterns as well. The epithelial-to-mesenchymal transition that heralds the acquisition of an invasive phenotype involves intracellular machinery that facilitates particular types of cell migration. Where RHO typically coordinates cellular activities associated with amoeboid cell motility, RAC regulates actin cytoskeleton reorganization to form cell surface extensions (lamellipodia) typical of mesenchymal movements. This activity is required for cell migration/invasion during cancer metastasis (26).

Members of the RAC family of small GTPases are key regulators of actin cytoskeletal structures and play integral roles in integrin-mediated adhesion and migration. A number of correlative studies have lent support for a central role of this pathway in a number of the mechanisms of metastasis. Baugher and colleagues found a direct correlation between metastatic potential and endogenous RAC activity in a panel of metastatic human breast cancer cells (27). RAC1 activity and expression levels of PAK1 were associated with poorly differentiated tumors, local invasion, and lymph node metastasis in urothelial carcinoma of the upper urinary tract (28). Most squamous cancers of the head and neck exhibit high levels of GTP-bound RAC1 due to EGFR-based autocrine activation of VAV2. Abrogation of VAV2 activity by RNA interference (RNAi) dramatically reduces the migratory and invasive phenotypes that these cells exhibit (29). Glioblastomas and breast cancer cells express high levels of the GEFs TRIO, ECT2, and VAV3, which act through RAC1 to promote growth and metastasis (30). Depletion of these GEFs suppresses cell migration, invasion, and proliferation.

The effects of the proteins in this regulatory network may not simply result from promoting or inhibiting RAC1 activity. For instance, silencing of the D4-GDI (RHO GDP dissociation inhibitor) with RNAi in human breast cancer cell lines that overexpress this inhibitor actually abrogates tumor growth and lung metastasis (30). D4-GDI associates with RAC1 and RAC3 in these cell lines, but not with other RHO GTPases. The exact mechanism that mediates this phenomenon has not been elucidated, but the loss of D4-GDI inhibition appears to block anchorage-independent growth, restore anoikis, and induce apoptosis (31).

Hepatocellular carcinomas (HCC) often harbor activating mutations in LMCD1, a downstream target of RAC, that increase lamellipodial protrusion and augment the metastatic virulence of the tumor cells carrying the mutation (31). In other HCC patient samples and cell lines, microRNA-142-3p (miR-142-3p) targets RAC1 expression by binding to the 3′-untranslated region (UTR) of RAC1 (32). Overexpression of miR-142-3p reduces RAC1 mRNA and protein levels and inhibits colony formation, migration, and invasion in the HCC cell lines. As miR-142 is frequently downregulated in HCCs (33), this shows yet another mechanism by which these cells augment RAC activity to promote malignant transformation.

RAC activity mediates some tissue tropism. For instance, prostate cancers overexpress PREX1, which acts as a phosphoinositide 3-kinase (PI3K)-dependent GEF to activate RAC1 (34). The activity of this GEF mediates spontaneous prostate cancer metastasis *in vivo* (30). This process may occur through RAC1-mediated tight binding to bone marrow endothelial cells, which promotes retraction of endothelial cells and diapedesis/extravasation of the tumors in these capillary beds (35). This results from RAC1 activation of β1 integrin, which allows tight binding to occur.

The interplay between the RAC and RHO systems regulates the dynamic reorganization of the actin cytoskeleton. Some tumor types acquire aberrant regulation within these systems, which allows tumor cells to switch back and forth between amoeboid and mesenchymal-type movement (27, 28). Mesenchymal-type migration in melanoma cells is driven by *NEDD9* (a metastasis-related gene) and *DOCK3* (a RAC GEF) through the modulation of RAC1 activity (35). PTHrP (parathyroid hormone–related peptide)-stimulated cell migration and invasion in colorectal adenocarcinomas is dependent on RAC1 activity, which is augmented by the increased expression of α6β4 integrin and TIAM1 these cells exhibit (36).

RAC1 and both its upstream activators and downstream effectors therefore appear to modulate numerous critical elements of invasion and metastasis. This has been shown repeatedly over multiple tumor types and at multiple unique points in the RAC1 pathways. The consistency of these effects strengthens the validity of RAC1 as a potentially viable target of efforts to disrupt invasion and metastasis.

Alternative Roles for RAC1 in Tumorigenesis

RAC1 also helps regulate the activity of the mTORC1 and mTORC2 complexes by anchoring those complexes to particular locations within the actin cytoskeleton in a GTP-independent manner (3). Modulation of cell growth and proliferation has also been seen through RAC1-mediated activation of NF-κB, especially in melanoma cells (37). NF2-knockout mouse embryonic fibroblasts (MEF) exhibit increased RAC1 activity (RAC1 activity is inversely regulated by NF2), loss of contact inhibition, and significantly increased canonical Wnt signaling (5). Transfection of these cells with a dominant-negative RAC1 construct abrogates the increased activity of the Wnt

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pathway and restores contact inhibition. This suggests a central role of the RAC1–NF2 interplay in regulating Wnt signaling and in the process of contact inhibition. It has been shown that RAC1 plays a key role in the initial formation and subsequent strengthening of cell–cell adhesion and the formation of cellular junctions (4). Colorectal cancer cells express an alternatively spliced RAC1b and depend on RAC1b signaling for survival (38).

One consequence of abnormal HER2/neu activity is the generation of autocrine and paracrine growth factors, including TGF-β, which can stimulate the growth not only of the malignant cells which contain amplified HER2 but also of nearby stromal cells that do not. RAC1 mediates the expression of many of these signals (39). Abrogation of this activity by overexpression of a dominant-negative RAC1 construct suggests that therapeutic targeting of this pathway may affect not only the malignant cells themselves but may also have effects on the microenvironment that could impede tumor growth and deter malignant transformation.

RAC1 as a Therapeutic Target

Several attempts have been made at targeting RAC1 and its regulatory network. While RAC1 and its primary regulators (GEFs and GAPs) are not classically druggable targets, there has been some success in designing drugs that can target their activity. When the activity of a naturally occurring antibiotic (brefeldin A, Fig. 2A) was shown to target the complex formed by the interaction of a G-protein (ARF) with its GEF (40, 41), many took note of the unique way that this small molecule interacted with this transient intermediate complex (Fig. 2B and C) and began a search for synthetic compounds that might similarly show interfacial inhibition with other G-protein–GEF complexes (42).

NSC23766 is a synthetic compound identified using computational screens to identify small molecules that interact with the surface of RAC1 that mediates GEF activation (43) and fits nicely into a surface groove of RAC1 known to be critical for GEF specification (Fig. 3A). It effectively inhibits RAC1 binding and activation by the Rac-specific GEFs TRIO or TIAM1 in a concentration-dependent manner without interfering CDC42 or RHOA binding *in vitro*. It also potently blocks serum- or platelet-derived growth factor (PDGF)-induced RAC1 activation and lamellipodia formation without compromising cellular movement mediated by CDC42 or RHOA. Treatment of cells in culture with NSC23766 blocks invasion and metastasis of multiple different tumor types (44, 45). It has also been shown to block neoangiogenesis in different disease models (44, 46, 47). While *in vivo* data remain sparse on these agents, NSC23766 has been used in several small studies to block the dissemination of lymphomas (48, 49). Others showed that pharmacologic blockade of RAC1 activity by NSC23766 can induce cell-cycle arrest or apoptosis of different breast cancer cell lines without affecting the growth of normal mammary epithelial cells (50), and preliminary studies suggest that RAC inhibition might reverse some trastuzumab-resistant phenotypes (45). Small studies have suggested that other RAC1 inhibitors, such as EHT 1864 (ref. 51; Fig. 3A), can downregulate estrogen receptor (ER) expression in ER+ breast tumors (52).

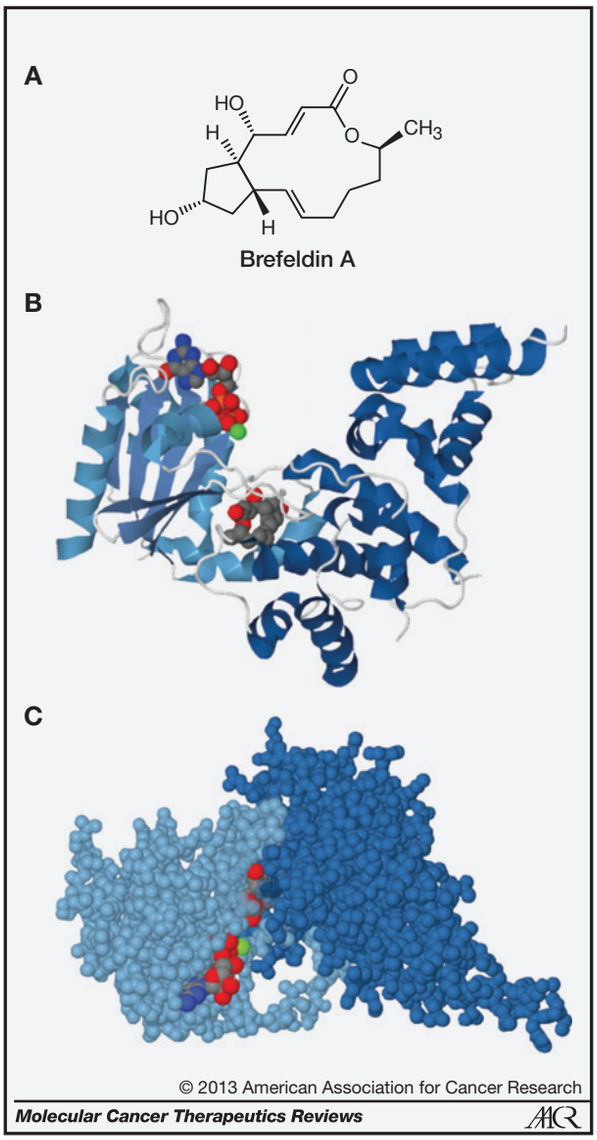

Figure 2. Brefeldin A, a model for small-molecule inhibition of GTPases. A, molecular structure of brefeldin A. B and C, crystal structure of brefeldin A (middle) in complex with its target GTPase (ARF, light blue) and GEF (Sec7, dark blue). Images are shown in both ribbon diagram (B) and a space-filling model (C). The hydrolyzed GDP is seen at front with the catalytic magnesium ion in green. Brefeldin binds the surface of ARF that interacts with its GEFs and prevents the conformational changes that result in GDP for GTP exchange. Images were generated using Protein Data Bank (PDB) data published by Mossessova and colleagues, manipulated with Jmol software, and rendered using POV-ray.

While many of the early studies of these agents seem promising, NSC23766 does not have sufficient efficacy to be useful clinically. Using data gained from studies on

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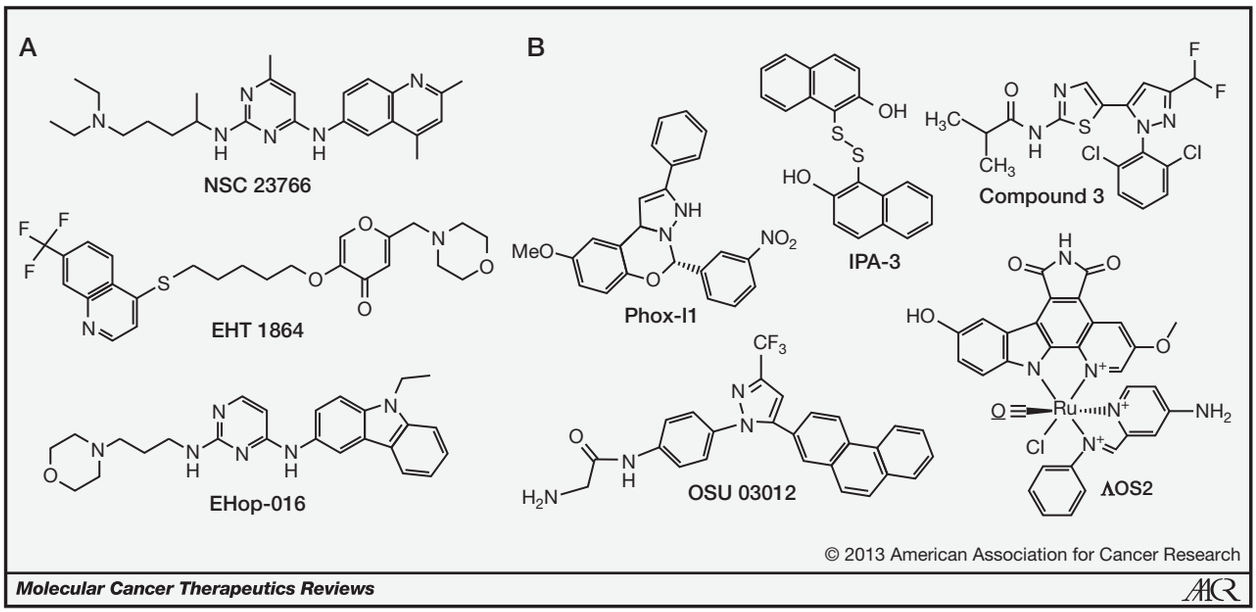

Figure 3. RAC inhibitors and RAC pathway inhibitors. Chemical structures for RAC inhibitors (A) and inhibitors of RAC effector molecules (B) discussed in the text.

the complex formed by NSC23766 and RAC1, several new structurally unrelated compounds were identified in computational screens that also had potential to specifically block RAC-GEF interactions (53), although wet studies on these compounds remain forthcoming. Similarly, Montalvo-Ortiz and colleagues used NSC23766 as a starting point to synthesize a number of related compounds, searching for molecules with greater potency and lower IC_{50}s that would make them more plausible agents for therapeutic use. They developed EHop-016 (Fig. 3A), which blocks the interaction of the RAC-GEF Vav2 with a nucleotide-free RAC1 (G15A), which has a 100-fold higher affinity for activated GEFs, without affecting the association of the RAC-GEF TIAM-1 with RAC1 at micromolar concentrations. EHop-016 decreases RAC downstream effects of PAK1 (p21-activated kinase 1) activity and directed migration of metastatic cancer cells (54). EHop-016 and other compounds under development may hold potential as a targeted therapeutics for the treatment of metastatic cancers with high RAC1 activity.

RAC1 Inhibition for Drug-Resistant Tumors

Acquired resistance to targeted therapeutics remains a fundamental cause of relapse and failure. The treatment of resistant tumors has proved a major challenge to the field of cancer therapeutics. Efforts to elucidate mechanisms of resistance continue to identify targets that might prove useful in overcoming resistance. RAC1 and its closely related partners continue to emerge as critical mediators of resistance in diverse situations.

Perhaps the most well-characterized model for RAC-mediated resistance is that described in HER-2–positive breast tumors. RAC1 expression increases with inactivation of PTEN and with overexpression of insulin-like growth facor-1 receptor (IGF-1R; ref. 55), 2 mechanisms that underlie acquired resistance to treatment with anti-HER2/neu therapies. Reducing the activity of this pathway using either the small-molecule RAC1 inhibitor NSC23766 or by siRNA knockdown of TIAM1 resensitizes trastuzumab-resistant breast tumors to that same agent by preventing the endocytic downregulation of HER2 receptors (44). High-throughput screens using RNAi to targeting genes commonly amplified in breast tumors with acquired resistance to HER2-targeted therapies identified RAC1 amplification as one of the most biologically relevant mechanisms of resistance. The subsequent inhibition of RAC1 restored sensitivity to lapatinib-resistant tumors (56).

Other studies remain underway to explore the ability of RAC1 inhibition to resensitize resistant ovarian cancers and leukemic cells to chemotherapy (cisplatin, methotrexate), with promising initial results presented at recent meetings (57). With the study of resistance mechanisms maturing and expanding, it will be interesting to see the extent of involvement that RAC has in mediating resistance to both targeted therapy and to traditional chemotherapy.

Targeting Other Members of the RAC Pathway

Some studies indicate that greater specificity might be achieved by targeting the downstream effectors of RAC activity. One important effector of RAC activity is $p67^{phox}$, which combines with other components of the NADPH oxidase system when activated to generate a fully functional complex for producing ROS. This complex has been

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a target for investigators who aim to moderate the consequences of inflammation. Bosco and colleagues have shown proof of principle in targeting this pathway with the development of Phox-I1 (Fig. 3B), which targets a pocket formed when RAC1 complexes with $p67^{phox}$ (58). Phox-I1 and several derivative compounds seem to be effective at inhibiting the RAC-dependent generation of ROS in a number of *in vitro* models of inflammation.

Other downstream targets are classical kinases and more readily targeted with traditional pharmacologic approaches. Bristol-Myers Squibb has developed a series of compounds that inhibit LIM kinases (59). Several of the compounds designed to target the LIM kinases, however, have had cytotoxic off-target effects on microtubule formation whereas a few do not (such as compound 3, Fig. 3B). These less-cytotoxic compounds appear in early studies to block some of the invasive phenotypes seen in breast cancer and squamous cell carcinoma cell lines (60).

Much recent effort has also gone into the development of different types of PAK inhibitors. One small-molecule inhibitor, OSU 03012 (Fig. 3B), is a derivative of the COX inhibitor celecoxib which was originally developed to target PDK1 (61), but was subsequently shown to inhibit PAK1 activity at even lower concentrations (within the 1 μmol/L range; ref. 62). This compound also appears to inhibit JAK/STAT and MAPK pathways as well. Limited studies with this compound in preclinical *in vitro* tumor models have shown that it inhibits cell proliferation in thyroid cancer cell lines and decreases the motility of those same cells (62). This compound also reverses resistance to imatinib mesylate in some cell lines (63) and sensitizes transformed astrocytes to killing by both radiation and by PI3K/AKT or MAPKK1/2 inhibitors. In small studies, it has been shown to restore tamoxifen sensitivity in xenograft models of breast cancer (64).

IPA-3 has been developed as a non–ATP-competitive PAK1 inhibitor (ref. 65; Fig. 3B). It is highly selective and potent ($IC_{50} \sim 2.5$ μmol/L) but has shown some chemical characteristics, such as covalent binding to the target, that make it less appealing for *in vivo* or therapeutic use. Higher concentrations of IPA-3 are necessary to inhibit PAKs as its activity requires a large transferable pool of the compound in the tumor microenvironment. A few small studies have published preclinical data showing that IPA-3 can induce apoptosis in a number of cancer cell lines (66), decrease cell spreading and adhesion in Scwannoma cell lines (67), and overcome resistance to PI3K pathway inhibitors in some lymphomas (65). This same drug will block the transformation of breast tumor cells to form a multi-acinar phenotype in a three-dimensional (3D) culture (68).

An organometallic compound, which uses a bulky ruthenium core to create a selective allosteric PAK1 inhibitor, was recently reported (69). This compound, Λ-OS2 (Fig. 3b), shows high selectivity for PAK1 and an IC_{50} around 350 nmol/L (70). Several studies have been reported using similar compounds targeting other kinases, but further characterization of the effects of Λ-OS2 in living systems remains forthcoming.

RAC1 and the Therapeutic Window

Studies conducted using inhibitors of RAC1 *in vivo* are limited, making inferences as to the likely therapeutic window speculative, but worth discussion. We can begin to make some educated guesses as to what toxicities might occur based on observations made in knockout mice. While RAC1 knockout is embryonic lethal, causing defects in germ layer formation, a number of tissue-specific RAC1 knockouts have been viable (2). Many phenotypes observed in these mice resulted from effects on embryonic development and would not likely be of consequence in mature organisms. Other phenotypes offer insights into the potential adverse effects of treatment with RAC1 inhibitors. These include: hair loss, cardiac hypertrophy, ineffective immune cell chemotaxis, and impaired neoangiogenesis. One should be careful in assuming a likely toxicity from such studies, however, as (i) gene knockout does not necessarily equate with pharmacologic inhibition and (ii) the effects of inhibition on different RAC functions are likely dose-dependent. RAC conducts functions both as a scaffolding protein and as a specific regulator of downstream targets. Thus, loss of the entire protein through genetic deletion may have quite a different effect from that of inhibiting a particular function.

Other inferences may be made from described roles of RAC1 in normal physiology. Given the well-described role for RAC1 in insulin sensitivity and glucose uptake in skeletal muscle (71), one might speculate that RAC inhibition would induce a state of relative insulin resistance and prevent contraction-related glucose uptake in skeletal muscle (72). It will be interesting to see which of these effects have true biological relevance and which will be of no specific consequence when investigators begin to report *in vivo* studies.

Conclusions

RAC1 has recently emerged as a critical regulator of tumor angiogenesis and metastasis and a promising therapeutic target for cancer drug discovery. From a mechanistic perspective, several important questions require further elucidation, for example, the mechanisms underlying the reduced tumor vascular perfusion induced by RAC1 inhibition remain unclear. Collective studies serve to illustrate the pivotal role that RAC1 plays in the development and progression of diverse tumor types. The data are especially strong with respect to the role of RAC1 in angiogenic and invasive behaviors. Targeting RAC1 and its associated pathways is an interesting strategy that could have marked effects on these malignant behaviors. Interestingly, such strategies may also help to reverse certain mechanisms of resistance to other targeted therapeutics, such as aberrant downregulation of surface receptor expression in some models and the

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aberrant overexpression of hormone receptors in others. Few of the current pharmacologic candidates show properties that would make them acceptable for clinical use, but they have been improving with each iteration, both in potency and in specificity. The preclinical data to date consistently support the idea that modulating these pathways may have very desirable effects on tumor progression and continues to warrant ongoing development of newer and more specific agents. From a therapeutic perspective, it will be instrumental to classify the tumor types that will benefit most from anti-RAC1 therapy and to further validate combination approaches with existing antiangiogenic and chemotherapeutic regimens. As with any biological system, effective manipulation of the RAC1 network to affect the desired outcomes may not be simply a matter of modulating overall RAC activity. This is illustrated by the studies of D4-GDI, in which lifting this RAC-1 inhibition yields the seemingly paradoxical effect of blocking tumor growth and spread. The manipulation of individual RAC-mediated processes through the inhibition of different members of its regulatory network may have a divergence of consequences. Further studies into the precise function of RAC1 in the tumor microenvironment, both on the cancer cells themselves as well as on the surrounding stromal and endothelial cells, should help us understand the consequences of modulating these pathways. These studies will also provide further insights to fuel novel approaches for combating these malignancy-associated cellular processes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H.K. Bid, R.D. Roberts, P.K. Manchanda
Development of methodology: H.K. Bid, P.K. Manchanda
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.K. Bid
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.K. Bid, R.D. Roberts, P.K. Manchanda
Writing, review, and/or revision of the manuscript: H.K. Bid, R.D. Roberts, P.K. Manchanda, P.J. Houghton
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.K. Bid, R.D. Roberts, P.K. Manchanda, P.J. Houghton

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