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RAC1 as a Therapeutic Target in Malignant Melanoma

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Abstract

Small GTPases of the RAS and RHO families are related signaling proteins that, when activated by growth factors or by mutation, drive oncogenic processes. While activating mutations in *KRAS*, *NRAS*, and *HRAS* genes have long been recognized and occur in many kinds of cancers, similar mutations in RHO family genes such as *RAC1* and *RHOA* have only recently been detected as the result of extensive cancer genome sequencing efforts and are linked to a restricted set of malignancies. In this review, we focus on the role of RAC1 signaling in malignant melanoma, emphasizing recent advances that describe how this oncoprotein alters melanocyte proliferation and motility and how these findings might lead to new therapeutics in *RAC1*-mutant tumors.

Keywords

malignant melanoma; driver mutations; small GTPases; fast-cycling; signal transduction; effectors; cancer therapeutics

RAC1 pathway activation in cancer

RAC1 is a well-studied and highly conserved member of the RHO family of small GTPases, a family that includes two other RAC isoforms (RAC2 and RAC2), CDC42 and RHO subgroups, and several other less-studied related proteins [1]. Like other enzymes in this class, RAC1 acts as a molecular switch, active when bound to GTP and inactive when bound to GDP. RAC1 is ubiquitously expressed and its downstream effectors are known to play key roles in numerous cellular processes such as proliferation, survival, differentiation, apical-basal polarity, actin dynamics, reactive oxygen species (ROS) production, and inflammatory responses [2–4]. The signaling activity of RAC1 is tightly regulated by activators, including guanine-nucleotide exchange factors (GEFs), and inhibitors, including GTPase activating proteins (GAPs) and guanine-nucleotide disassociation inhibitors (GDIs). In addition, RAC1 is regulated by modifications at it C-terminus, including carboxylmethylation, geranylgeranylation, and palmitoylation, as well as numerous additional posttranslational modifications that influence its localization, quantity, activity, and ability to bind effectors [5, 6]. The RAC1 protein has long been recognized as a central signaling hub that is required for transformation by many oncogenes, but the recent discovery of activating mutations in

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the *RAC1* gene in melanoma and other malignancies suggest a previously unappreciated driver role in cancer and that targeting RAC1 and/or its effectors could be beneficial in this setting. Here, we discuss the role of *RAC1* in cancer, advances in our understanding of key signaling pathways altered by activated RAC1, as revealed by new cell and animal models, and the potential therapeutic implications of these findings.

RAC GEFs in Cancer

In the absence of *RAC1* mutations, upstream elements of the RAC1 signaling pathway, in particular its GEFs, are often overexpressed and/or activated by mutation in a variety of cancers and may influence drug resistance [6–8]. For example, the phosphatidylinositol-3,4,5-triphosphate-dependent RAC exchangers PREX1 and −2 are commonly overexpressed in cancer, and promote invasion and colony formation in melanoma cells *in vitro* [9]. High levels of PREX1 protein drive a more migratory, invasive, and adhesive phenotype in ER+ breast cancer cells [10], and breast tumor tissue that expresses PREX1 has a higher recurrence and metastasis rate [11]. The related GEF PREX2 can be activated by overexpression or by missense and/or nonsense mutations that result in truncation of its C-terminal autoinhibitory element [7]. Such mutations in the *PREX2* gene result in constitutive activation of the GEF, and are seen in approximately 14% of melanomas [7]. Inducible transgenic mice containing a common cancer-associated *Prex2* mutation, when combined with a concurrent $Nras^{Q61K}$ mutation, showed accelerated melanoma development [12]. In this setting, tumor cells displayed increased phospho-AKT, which was abrogated by the RAC1 inhibitor EHT1864. Genes encoding additional RAC1 GEFs such as TIAM1, VAV, ECT2 and DOCK are also frequently altered in human cancers [13], highlighting the ubiquity of RAC pathway activation in malignant disease.

Overexpression of *RAC1* and its splice variants

Many lines of evidence have established that RAC1 signaling is important in transformation by other oncogenes. Experiments in the 1990s showed that expression of a dominant negative form of RAC1 blocked transformation of NIH-3T3 cells by $HRAS^{G12V}$ [4] (possibly by preventing RAS-induced ruffling and macropinocytosis [14, 15]), and overexpression of an engineered GTPase-deficient form of RAC1 was able to transform these cells. Later experiments showed that tissue-specific deletion of *Rac1* prevented tumor formation in murine *Kras*/*Tp53*-driven models of lung cancer and pancreatic cancer, respectively [16, 17], and in a DMBA/TPA-induced (*Kras* mutant) model of skin cancer [18], indicating that *Rac1* is essential for transformation by *Kras in vivo*. These studies established RAC1 as an essential, cell-autonomous element in transformation by the KRAS oncoprotein, but did not address whether activated RAC itself is a bona-fide oncoprotein. At the time of these early studies, "canonical" *RAC1* mutations (*i.e.*, those analogous to exon 2 or 4 mutations in *RAS*) had never been encountered in human tumors or cell lines, so the clinical relevance of these experiments with GTPase-deficient forms was unclear. On the other hand, overexpression of wild-type *RAC1* has long been reported in many cancers, including melanoma, lung adenocarcinoma, glioma, pancreatic, prostate, colorectal, gastric, cervical, breast head and neck cancer as well as in various leukemias [17, 19–29]. *RAC1* overexpression is correlated with poor prognosis in nearly all cancers [30] and has been

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shown to confer resistance to both targeted therapy [23, 31] and chemotherapy [32–34]. Also, overexpression of a *RAC1* splice variant, termed *RAC1b*, has been described in breast, lung, and colorectal cancer, and is associated with chemoresistance in the latter [29, 33, 35, 36]. This variant contains an addition of 19 amino acids directly following the switch II domain. This addition impairs the intrinsic GTPase hydrolysis ability of the enzyme [36], and is associated with NF-κB activation, increased activity of matrix metalloproteinase-3, epithelial-mesenchymal transition, and genomic instability [37–40]. These reports indicate that wild-type RAC1 signaling plays a vital role in several oncogenic processes.

RAC1 mutations in cancer

When intentionally mutated and overexpressed, activated *RAC1* genes (*e.g.*, $RAC1^{G12V}$ or $RAC1^{Q61L}$) can transform cells and induce tumor formation in transgenic mice [41], and can cooperate with $HRAS^{G12V}$ in tumorigenesis in zebrafish [42], but these forms of *RAC1* have only recently been found in a few types of human tumors. Instead, when exome sequencing efforts first revealed the existence of activating mutations in *RAC1* in malignant melanoma, the vast majority of these mutations were found to affect codon 29, changing the wild-type proline to either serine or, less commonly, to leucine. Such mutations, occurring at a dipyrimidine (CCT -> TCT) site, represent a typical UV signature and occur in up to 9% of sun-exposed, cutaneous melanomas [23, 43]. Unlike common RAS oncogenic mutations that impair or abolish intrinsic GTP hydrolysis ability and render the protein constitutively active in terms of signaling, the $RAC1^{P29S}$ mutant protein retains the ability to hydrolyze GTP and GDP. Instead, this mutation activates RAC1 by conferring "fast-cycling" properties, in which GDP is exchanged more quickly for GTP [24, 44] (Box 1). The biochemical basis for the increased exchange rate is thought to be related to hydrogen bonding between the backbone carbonyl of Ser29 and the ribose hydroxyl group of GTP, favoring the GTP bound form (Fig. 1). These factors suggest that $RAC1^{P29S}$ may also retain regulation by modulators such as GEFs or GAPs, which in turn may be important when considering therapeutic strategies. This view is supported by the finding that deletion of the gene encoding the RAC-GEF DOCK1 reduces invasion and macropinocytosis in $RAC1^{P29S}$ melanoma and breast cancer cells [45].

$RAC1^{P29S}$ is the third most common driver mutation in sun-exposed melanoma behind $BRAF^{V600E}$ (~50%) and $NRAS^{Q61K/R/L}$ (~30%), [23, 31, 43]. In melanoma, $RAC1^{P29S}$ is often found in combination with additional gain-of-function mutations in other oncogenes (*e.g.*, *BRAF* or *NRAS*) and/or loss of function mutations in tumor suppressor genes (*e.g.*, *NF1*, *TP53*, or *PTEN*), suggesting that $RAC1^{P29S}$ is not generally sufficient on its own to drive tumor formation. Mouse models are consistent with this view, as conditional expression of $Rac1^{P29S}$ in melanocytes did not result in the development of nevi or melanoma [46]. However, when combined with $Braf^{V600E}$ and loss of *Tp53* or *Nf1*, the *Rac1* mutation caused an acceleration in melanoma growth and reduced survival [46]. In humans, the co-occurrence of *BRAF* and *RAC1* mutations confer a poor prognosis and resistance to BRAF inhibitor targeted therapy, underscoring the clinical significance of these combined mutations [31, 47]. In addition, an analysis of TCGA data suggests that *RAC1* mutations are linked to elevated PD-L1 expression in melanoma cells, and thus might confer resistance to immunotherapy [48].

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Additional mutations in *RAC1* have been found that alter A159 (analogous to A146 in KRAS, which is associated with fast-cycling properties [49]), predominantly in head and neck cancers; N92, in melanoma, myeloma, and sarcoma [50, 51]; C157, and also at the canonical G12, mainly in prostate cancer; and Q61 sites, mainly in testicular germ cell cancer [52], respectively, as often found in *HRAS* and *NRAS* (Fig. 2). Other, less common mutations have been noted at additional sites, and these also appear to result in a gain of function. Some of these same gain-of-function mutations (*e.g.*, N92T) have also been found in *RAC2* (a gene expressed only in hematopoietic cells) and are associated with myeloid and lymphoid immunodeficiencies [53]. While extensive comparisons of the various RAC1 mutant proteins have not been reported, pull-down assays from HEK293 cells have shown that the Rac1^{A159V} mutant GTPase is more active than RAC1^{P29S}, but less active than RAC1^{Q61R} [22]. However, whether the degree of RAC1 biochemical activation generally correlates with its *in vivo* effects is not known. As with the *RAC1$^{P29S/L}$* mutation in melanoma, the *RAC1^{A159V}* mutation found in head and neck squamous cell cancers (HNSCC) is associated with poor prognosis. While the relationship between this *RAC1* mutation and clinical outcomes is not yet understood in detail, gene set enrichment analyses indicate that active RAC1 is associated with dysfunction of immune-related gene sets [54].

The role of Rac1 effectors in oncogenesis

Like other small GTPases, RAC1 propagates its downstream biological effects by binding to and activating various effector proteins. Presumably, it is by activating one or more of these effectors that mutant RAC1 contributes to transformation. Among the most studied effectors are the Group A p21-activated protein kinases PAK1, −2, and −3, the lipid kinase phosphatidyl inositol-3 kinase (PI3K)-β, and actin assembly factors such as formins (*e.g.*, mDIA2) and WASP-family verprolin-homologous protein 2 (WAVE2) [55] (Fig. 3). When activated by RAC1, Group A PAKs promote cell proliferation and survival primarily through phosphorylation of c-RAF at S338, MEK1 at S298, β-catenin at S675, and Merlin at S518 [56]. Other validated PAK substrates that are likely relevant to these cellular phenomena include Aurora kinase A and Polo-like kinase 1, which promote cell cycle progression, and BAD, which, when phosphorylated by PAK, dissociates from the pro-apoptotic protein BCL2-X_L, thus promoting survival [56]. PAK1 also orchestrates cell invasion and metastasis by phosphorylating LIMK, which in turn phosphorylates cofilin and inhibits its ability to sever actin filaments. For these reasons, PAK likely represents an important effector for mutationally activated RAC1 in promoting transformation. In support of this view, genetic or pharmacological blockade of PAK function reduces the oncogenic effects of RAC1^{P29S} in both cell-based models and animal models of melanoma [46, 57].

The WAVE complex and formin proteins represent additional effectors by which RAC1 controls actin polymerization. Active RAC1 activates WAVE2 by binding directly to the amino terminus of the small substrate for insulin receptor, IRSp53, which in turn binds to WAVE2 forming a trimolecular active complex [58, 59]. When activated, WAVE2 binds the ARP2/3 complex which leads to polymerization of monomeric (G-) actin to filamentous (F-) actin, forming lamellipodia. These changes in cytoskeletal structure alter the cellular distribution of the transcription factor Serum Response Factor (SRF) and its coactivator, Myocardin-Related Transcription Factor (MRTF) [60], because SRF/MRTF signaling is

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highly sensitive to the concentration of G-actin in cells. Under basal cellular conditions, G-actin binds SRF/MRTF and sequesters it in the cytoplasm, and when G-actin is depleted through polymerization SRF/MRTF translocates into the nucleus where it functions as a transcription factor, resulting in the expression of genes associated with motility. Interestingly, while SRF/MRTF had long ago been identified as a potential RAC1 effector [61, 62], it is far better known as an effector of a different small GTPase, RHOA [63]. It was therefore somewhat of a surprise when transcriptional analyses of *RAC1*^{P29S} melanocytes revealed *SRF* and many of its targets as among the most upregulated genes compared to wild-type cells [46]. PAK and SRF likely represent independent effectors in RAC1 signaling, as previous mutational analysis of the RAC1 switch one region showed that mutants that selectively uncouple RAC1 from PAK nevertheless maintain lamellipodia formation and SRF activation [61]. The activation of SRF by RAC1 is likely to be independent of RHOA, as treatment of epithelial cells with a potent RHOA inhibitor did not RAC1-mediated activation of this transcription factor [62]. In melanoma, Lionarons *et al.* further showed that activated SRF/MRTF promotes transcription of epithelial-mesenchymal transition (EMT) genes such as *CDH2*, *VIM*, *FN1*, and *COL15A1* [46]. In melanomas bearing mutations in both *BRAF* and *RAC1*, the mesenchymal phenotype of transformed melanocytes may contribute to resistance to BRAF inhibitors. In this setting, combining a BRAF inhibitor and an SRF/MRTF inhibitor augmented drug sensitivity [46].

A similar signaling module may also underlie the recent findings of Mohan *et al.*, who described RAC1^{P29S} signaling within melanocyte lamellipodia [13]. In this schema, RAC1, likely acting via Group A PAKs, stimulates the phosphorylation and inactivation of Merlin, a tumor suppressor protein that, among other activities, regulates actin cytoskeletal remodeling. They found that the presence of these biochemically active, F-actin enriched lamellipodia drove proliferation in the absence of growth factors, independent of MAPK activity. This pathway also mediates resistance to BRAF inhibitors in *BRAF/RAC1* double mutant melanoma cells, and such drug resistance can be reversed when actin polymerization is blocked by inhibitors of formins or ARP2/3. It is possible that these effects are mediated by a common mechanism, such as the release of SRF/MRTF to the nucleus, as suggested by Lionarons *et al.* In both cases, these results emphasize how the effects of Rac1 on the cytoskeleton are intimately linked to its effects on the cell cycle, and how RAC1-mediated changes in actin dynamics can influence tumor cell behavior.

Towards potential therapeutics

The discovery of *RAC1* mutations in melanoma and other cancers may provide additional therapeutic targets in such tumors. Like other small GTPases, the mutant RAC1 protein is itself difficult to target directly, as it binds GTP with nM affinity and presents no obvious pockets for small molecule docking, and to date, no FDA-approved drugs are known to be effective in melanomas or other cancers bearing the *RAC1*^{P29S} mutation. Nevertheless, tumors harboring this mutation might be sensitive to small molecules that impede membrane RAC1 localization, GEF/ RAC1 interactions, nucleotide binding, and/or the binding of effectors proteins.

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Interfering with RAC1 localization

The signaling activities of Rac1 require membrane localization and this association is mediated by the addition of isoprenoid moieties to the C-terminus of the protein [64]. Small molecules targeting geranylgeranyl transferases type I (GGTI), the enzyme responsible for this prenylation, have been described [65], and some of these compounds have shown promising cell-based and preclinical results [66–68]. Unfortunately, the only GGTI that was evaluated in a clinical trial did not show efficacy [69, 70]. It should also be noted that, at least in some cell types, loss of GGTI may paradoxically activate RAC1 [71]. Intervening at earlier steps in isoprenoid synthesis, such as blocking HMG-CoA reductase with statins, also reduces RAC1 membrane association and activity [72]. However, this approach suffers from lack of specificity, as many proteins in addition to RAC1 depend on the synthesis of isoprenoid precursors [73, 74].

Inhibiting GEF/RAC1 interactions

Unlike most RAS mutants, RAC1 mutant proteins are usually fast-cycling rather than GTPase deficient. Given these enzymatic properties, it is attractive to target its upstream signaling elements, in particular RAC1 GEFs. In support of this idea, genetic or pharmacologic disruption of the RAC1 GEF DOCK1 has been shown to suppress Rac1^{P29S} GDP/GTP exchange and to reduce matrix invasion and macropinocytosis in *RAC1*-mutant mouse embryonic fibroblasts, human melanoma IGR-1, and breast cancer MDA-MB-157 cells [45]. Thus, the general strategies for selecting treatment for tumors bearing fast-cycling RAC1 mutants might differ from those developed for RAS mutants, which most often result in near-total loss of GTPase activity and lack of response to GEFs.

A number of compounds have been described that interfere with GEF/ RAC1 interactions. Among these are NSC23766 and its derivatives such as EHop-016 and MBQ-167 [75]. NSC23766 inhibits TIAM1- and TRIO-mediated cell growth and transformation, reversing tumor cell phenotypes in prostate cancer cells without affecting CDC42 and RHOA activation [76]. However, NSC23766 has been shown to inhibit agonist signaling in *Rac1*$^{-/-}$ platelets [77] and to interfere with muscarinic acetylcholine receptors (mAChRs) in cardiac myocytes [78], raising serious questions as to its specificity and mechanism(s) of action. In addition, this compound has very low efficacy, making it poorly suited for clinical application [79]. A derivative compound, EHop-016, blocks RAC1/VAV2 rather than RAC1/ TRIO binding, decreases RAC1-mediated activation of PAK1, and suppresses RAC1-driven directed cell migration of metastatic cancer cells. A drawback of this compound, however, is that, when used at high concentrations, it also targets CDC42 [80]. The latest molecule in this series, MBQ-167, is the first to display sub-micromolar IC50 values for RAC1 inhibition, although it too inhibits CDC42 activation [81]. MBQ-167 was shown to display profound effects on the growth of xenografted GFP-HER2-BM breast cancer cells in nude mice without causing undue toxicity, indicating that this compound has promise for further development. As this compound has only been tested in a limited number of cell types, it will be important to determine if its beneficial effects are related to RAC pathway activity in cancer cells. Other candidate molecules designed to interfere with GEF/RAC1 interactions include ZINC69391and its derivative 1A-116, which block the interaction of P-REX1, and

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perhaps other GEFs, with RAC1, and which have shown anti-metastatic effects in breast cancer models [82].

Impairing nucleotide binding

As small GTPases such as RAC1 have very high affinity for GTP, it seems unlikely that a small molecule could be designed to outcompete it. Nevertheless, a molecule that causes displacement of bound GTP from RAC, but not CDC42 or RHO, has been described [83]. This compound, EHT1864, binds to all RAC isoforms, displacing bound nucleotides and preventing GEF-mediated nucleotide exchange, as well as impeding RAC binding to downstream effectors. Unfortunately, like the GEF/GTPase blocker NSC23766, EHT1864 also has notable off-target effects in wild-type cells, including inhibition of mAChRs [77]. Despite these issues, EHT1864 remains in wide use in preclinical studies and, given its unique proposed mechanism of action that directly targets RAC1, might provide a basis for further development.

Targeting Rac effectors

Despite efforts to target Rac1 activators or RAC1 itself, as detailed above, to date the most effective small molecule approach for blocking RAC1 signaling has been to target its effectors and further downstream elements, in particular readily druggable enzymes such as protein or lipid kinases. For RAC1, this means considering protein kinases such as the aforementioned group A PAKs (PAK1, −2, and −3), MEK kinases (MEKKs), mixed lineage kinases (MLKs), and $p70^{S6K}$; lipid modifying enzymes such as PI3Kβ, PI45K, DAG kinase, PLC, and PLD; proteins that promote ROS generation such as $p47^{phox}$ and $p67^{phox}$, and actin-modifying proteins such as WAVE2 (via IRSp53) leading to activation of SRF/MRTF, formins, and IQGAP [84]. Of these effectors, PAK is the best characterized druggable target downstream of RAC1. PAKs contribute to the ERK, β-catenin, Aurora A, and Merlin activation, and melanoma cells and xenografts bearing the *RAC1*^{P29S} mutation have been shown to be sensitive to PAK inhibitors [31, 57]. In addition, the Rasopathy-like developmental phenotypes conferred by *RAC1*^{P29S} expression in zebrafish embryos can be reversed by PAK inhibitors [57]. Unfortunately, despite these promising data, a clinical path forward remains uncertain due to the essential role of PAK2 in cardiac function in adult organisms [85]. It remains possible that selective PAK1 inhibitors, such as that described by a group from Novartis [86], could be further developed for use in *RAC1* mutant or -dependent tumors.

With respect to PI3K as a potential therapeutic target, RAC1 selectively engages the PI3Kβ isoform to activate AKT [87], and therefore isoform-specific inhibitors of this enzyme might be effective in *RAC1*-driven tumors. To date, the data are mixed on this point, with one study showing that selective PI3Kβ inhibitors impeded the growth and migration of melanoma cell lines driven by mutant *RAC1* but not by mutant *BRAF*, while selective PI3Kα inhibitors had the opposite profile [88], and another study showing limited activity for any PI3K inhibitor in *RAC1*^{P29S} melanocytes. In this latter study, however, an inhibitor of AKT3 was effective, implying some role for the PI3K/AKT signaling axis in such tumor cells [46].

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RAC1^{P29S} activates the SRF/MRTF transcriptional pathway and the WAVE2/ARP2/3 actin complex, which results in a melanocytic to mesenchymal phenotypic switch and the formation of filamentous actin that increases mesenchymal cell movement, migration and metastasis. Depleting MRTF using RNAi or using the specific SRF/MRTF inhibitors CCG-1423 and CCG-203971, respectively, suppressed the melanocytic to mesenchymal transition in melanoma cell lines. In mice, co-treatment with the SRF/MRTF inhibitor CCG-257081 and the BRAF inhibitor PLX4720 suppressed tumor growth [46], and in zebrafish embryos, an SRF/MRTF inhibitor (CCG-203971) reduced the abnormalities induced by the injection of RAC1^{P29S} mRNA [88]. It should be noted that the exact molecular target of this series of SRF/MRTF pathway inhibitors is uncertain. Rather than affecting SRF or MRTF directly, a recent report showed that these compounds bind to Pirin, an iron-binding co-transcription factor not previously linked to SRF/MRTF [89]. Given these findings, whether Pirin also plays a role in oncogenic Rac1 signaling is a question well worth asking, especially as Pirin has previously been implicated in melanoma cell senescence, migration, and progression [90–92]. Of note, degrader versions of a Pirin inhibitor has already been described that effectively abolish Pirin expression in cells when used at nM concentrations [93]. Other small molecule inhibitors of actin nucleation and/or polymerization, such as those that target Arp2/3 or formins, might also be of use in *RAC1* mutant tumors, as suggested by Mohan *et al.* [13].

Concluding remarks

In the short time since *RAC1* activating mutations were first identified in malignant melanoma, the presence of such mutations has also been recognized in other human cancers and useful cell-based and animal models constructed. Importantly, the outline of the oncogenic signaling mechanisms employed by RAC1 has become clearer, emphasizing the role of some expected effectors such as the PAKs, while also revealing some surprising new candidates, in particular SRF/MRTF, which highlights an important but often ignored link between actin-induced cytoskeletal changes and oncogenic transcriptional events. These findings in turn suggest new therapeutic strategies that could be of use in cancers driven by this mutation. However, as neither PAK nor SRF/MRTF inhibitors are currently in clinical use or being tested in human trials, there is still much work to be done in translating these findings into practice. Future work will need to address several critical issues, such as the biology underlying the unusual spectrum of mutations encountered in *RAC1*-mutant cancers, their role in therapeutic resistance, and the possibility of blocking oncogenic RAC1 signaling via inhibiting specific GEFs, direct effectors such as PAKs and/or PI3K isoforms, and/or proteins that regulate actin polymerization. Finally, given the recent rapid progress in RAS-targeting inhibitors, it is also possible that direct inhibitors of mutant RAC1 might be developed. Such therapeutics might help address the drug-resistant phenotype associated with *RAC1* mutations, thus providing a new weapon in our armamentarium against cancer.

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Box 1.

Fast Cycling GTPase Mutants

Mutations that alter the intrinsic GTP on-off rate, as opposed to the hydrolysis rate, were first encountered during structure-function studies of HRAS [94]. Such mutants were found to be more potent transforming agents than classical GTPase deficient mutants, leading to the idea that GTPase cycling itself may be required for or may contribute to oncogenic signaling. Alternatively, it is possible that completely GTPase deficient versions are too toxic to most cells, as has been proposed for the fast cycling RHO^{Y42C} mutant found in diffuse gastric cancer [95]. Mutants such as $RAC1^{P29S}$ may hit a “sweet-spot” of activity: enough to support transformation, but not so active as to induce senescence or apoptosis.

Figure 1

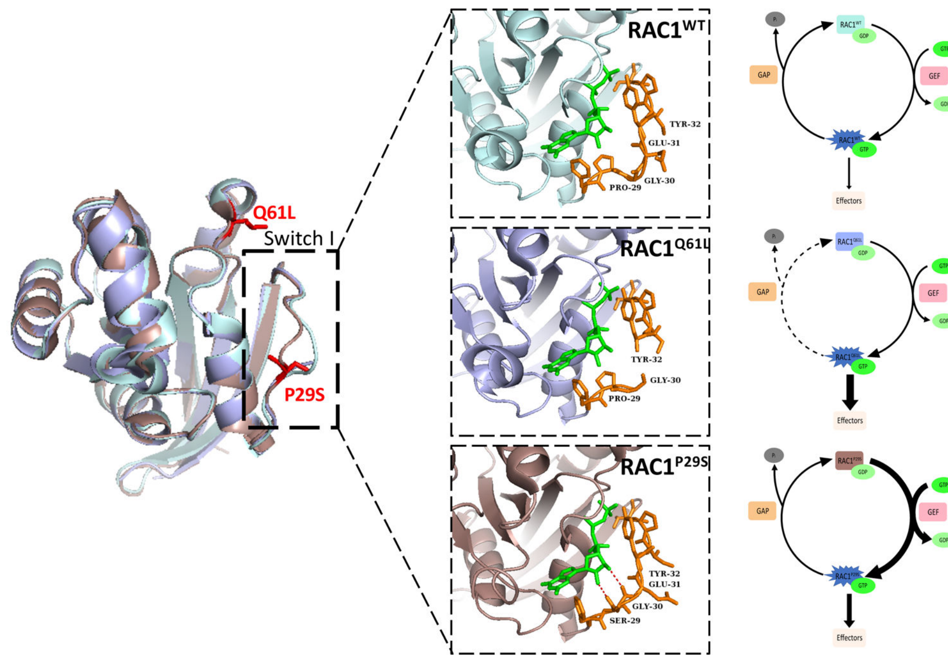

Figure 1. Structure and Biochemistry of Wild-type RAC1, the Constitutively Active RAC1^{Q61L} mutant, and the Fast Cycling RAC1^{P29S} Mutant.

Comparison of crystal structures of RAC1^{WT} (light blue, PDB 3TH5) with RAC1^{Q61L} (light purple, PDB 4GZL), and RAC1^{P29S} (dark pink, PDB 3SBE) in complex with the slow hydrolyzing GTP analog GMPPNP (green). RAC1^{P29S} possesses a more flexible switch I domain than RAC^{WT} and RAC1^{Q61L}. The proline to serine substitution enables hydrogen bonding between Ser-29 and Gly-30 of switch I and GTP and constitutes the difference between fast cycling mutations such as P29S and GTP-hydrolysis deficient mutations such as Q61L. Residue 29 and surrounding residues are displayed in orange stick format to illustrate hydrogen bonding (red).

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Figure 2

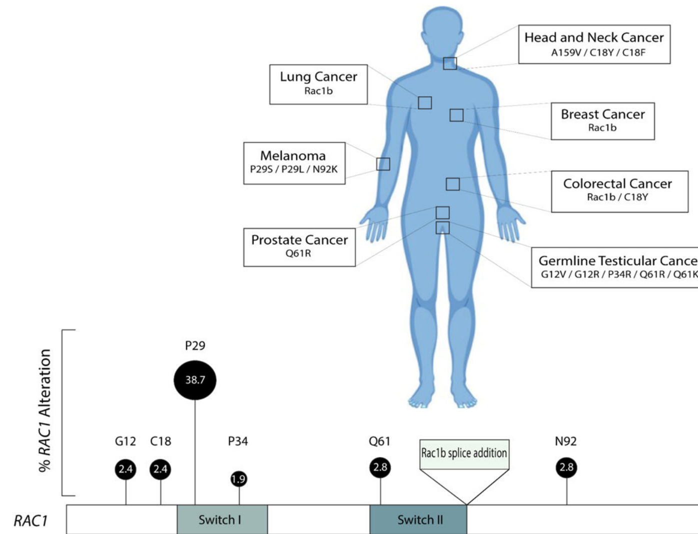

Figure 2. RAC1 Variations and Frequency in Cancer.

Representation of RAC1 hotspots and associated cancers. *RAC1* point mutations associated with various types of cancer are depicted. Some of the most common cancer-associated mutations affect either the switch 1 (residues 26–45) or switch 2 (residues 59–74) region of RAC1. These regions represent key structural elements in the GTPase, mediated interactions with effectors and GEFs, respectively, and contain conserved residues important for nucleotide and Mg^{2+}-ion coordination. The percentage of *RAC1* hotspot mutations in *RAC1*-altered cancer is represented by height and size of locus marker. Frequencies are derived from cbioportal curated set of nonredundant studies.

Figure 3

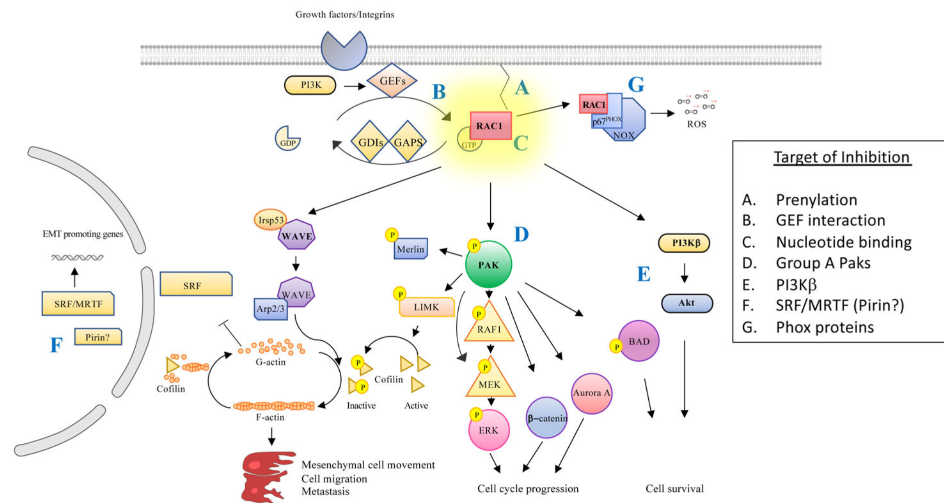

Figure 3. Signaling from RAC1 and Potential Vulnerabilities to Targeted Therapy.

(A) RAC activation requires membrane localization mediated by prenyl modifications at its C-terminus, creating an opportunity for small inhibitors. (B) Fast-cycling mutants such as RAC1^{P29S}, which rapidly exchange GDP for GTP, require stimulation by GEFs, and inhibitors of GEF/RAC interactions have been described, as have (C) molecules that displace bound GTP. (D) Effectors such as PAK (which regulates the activation of ERK, β-catenin, Aurora A, and BAD), (E) PI3Kβ, which regulates cell survival via AKT, and (F) WAVE2, which regulates actin polymerization and cytoskeletal structure, via IRSP53 and ARP2/3, as well as gene transcription via SRF/MRTF and, possibly, Pirin, are also potentially druggable. (G) ROS, generated by the RAC1 effectors p40^{Phox} and p67^{Phox}, may also play a role in oncogenic signaling from this small GTPase and are potentially targetable by small molecule inhibitors.

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