Review

Cancer Research

Targeting Rac and Cdc42 GTPases in Cancer

María del Mar Maldonado and Suranganie Dharmawardhane

Abstract

Rac and Cdc42 are small GTPases that have been linked to multiple human cancers and are implicated in epithelial to mesenchymal transition, cell-cycle progression, migration/invasion, tumor growth, angiogenesis, and oncogenic transformation. With the exception of the P29S driver mutation in melanoma, Rac and Cdc42 are not generally mutated in cancer, but are overexpressed (gene amplification and mRNA upregulation) or hyperactivated. Rac and Cdc42 are hyperactivated via signaling through oncogenic cell surface receptors, such as growth factor receptors, which converge on the guanine nucleotide exchange factors that regulate their GDP/GTP exchange. Hence, targeting Rac and Cdc42 represents a promising strategy for precise cancer therapy, as well as for inhibition of bypass signaling that promotes resistance to cell surface receptor-targeted therapies. Therefore, an understanding of the regulatory mechanisms of these pivotal signaling intermediates is key for the development of effective inhibitors. In this review, we focus on the role of Rac and Cdc42 in cancer and summarize the regulatory mechanisms, inhibitory efficacy, and the anticancer potential of Rac- and Cdc42-targeting agents. *Cancer Res; 78(12); 3101–11. ©2018 AACR.*

Introduction

The homologous Rho GTPases Rac and Cdc42 play a pivotal role in cancer malignancy via regulation of cytoskeletal and microtubule dynamics, migration/invasion, metastasis, epithelial to mesenchymal transition, transcription, cell proliferation, cell-cycle progression, cell polarity, apoptosis, phagocytosis, vesicle trafficking, angiogenesis, and cell–cell and cell–extracellular matrix adhesions. Rho GTPases act as key molecular switches by alternating between their active GTP-bound form and their inactive GDP-bound form, where the exchange of GDP to GTP is catalyzed by guanine nucleotide exchange factors (GEF), whereas GTP hydrolysis is regulated by GTPase-activating proteins (GAP; Fig. 1; refs. 1, 2). A number of GEFs have been identified as oncogenes and are activated by oncogenic cell surface receptor signaling from G-protein–coupled receptors, growth factor receptors, cytokine/janus kinase/STAT receptors, and integrins. Rho GTPase activity can be further regulated by guanine nucleotide dissociation inhibitors (GDI), which prevent GEF-mediated nucleotide exchange, thereby maintaining the GTPase in an inactive state. GDIs can also bind the GTP-bound state of the GTPase and prevent nucleotide hydrolysis. The molecular mechanisms and regulatory role of GEFs, GAPs, and GDIs in Rac and Cdc42 function have been extensively reviewed (3, 4). Therefore, this review will focus on the therapeutic potential and current inhibitors available for Rac and Cdc42 targeting in cancer.

As reviewed recently in this journal, a large number of *in vitro* studies have implicated the Rac isoforms Rac1, Rac2 (in hematopoietic cells), and Rac3, and the homolog Cdc42 in human cancer, including an essential role in Ras-mediated transformation (1). Table 1 shows a survey of The Cancer Genome Atlas (TCGA) data using cBioPortal (5), where *RAC1* is upregulated in >10% of cancers with high mortality rates, including bladder, skin, esophageal, gastric, head and neck, liver, pancreatic, prostate, and uterine carcinomas, glioblastoma, mesothelioma, and sarcomas. The distribution of Rac mutations in cancer has been described, which includes the driver mutation *RAC1*(P29S) (∼5% in melanomas) and a constitutively active splice variant Rac1b (1). *CDC42* is not usually mutated but approximately 5% elevated in most cancers with the exception of cervical squamous carcinoma, pancreatic adenocarcinoma, and sarcoma, where *CDC42* is upregulated by 12%, 21%, and 14%, respectively. Therefore, targeting Cdc42 is also considered a viable option for cancer therapy (6). Although the analysis of breast invasive carcinomas demonstrated only modest percentages of elevated *RAC1* (∼5%) and *CDC42* (∼1%), a more in-depth analysis reported *RAC1* upregulation in approximately 50% of HER2-enriched and basal breast-invasive carcinoma, including association of high *RAC1* expression with poor patient survival (7).

The contribution of Rac and Cdc42 to cancer initiation and progression is considered to be high due to their central roles in oncogenic cell surface receptor and GEF signaling. Many members of the largest family of Rho GEFs, the Dbl homology (DH) family (∼70), have been identified as oncogenes (1). In addition, the dedicator of cytokinesis (DOCK) family of GEFs (11 members) that are structurally different from the DH domain Rac/Cdc42 GEFs has also been implicated in cancer (8). As has been extensively reviewed, deregulation of oncogenic GEFs such as Dbl, Vav, Trio, T-cell invasion and metastasis gene product (Tiam-1), epithelial cell transforming gene 2 (Ect2), and phosphatidylinositol-3,4,5-trisphosphate (PIP3)–dependent Rac exchange factor 1 (P-Rex-1) contributes to aberrant Rac and Cdc42 activity in cancer (1, 3, 4). An additional oncogenic effector of Rac and Cdc42 is PI3K. PI3K activates Rac and Cdc42 via activation of PIP3-regulated GEFs such as P-Rex, Vav, Sos, and SWAP70 (9). Therefore, the PI3K catalytic subunit (*PIK3CA*) mutations result in hyperactivation of Rac and Cdc42 GEFs, and hence, elevated Rac1 and Cdc42 signaling

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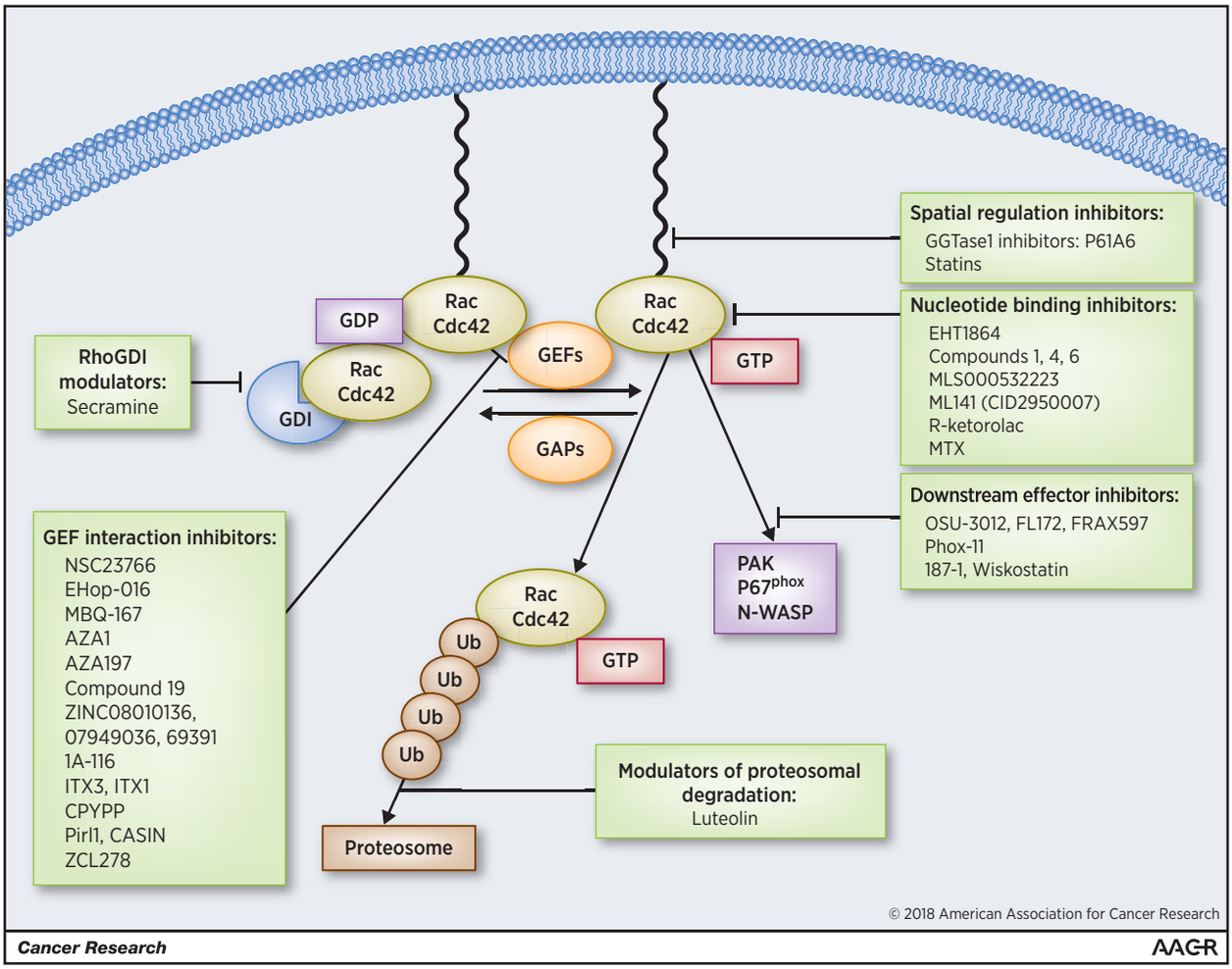


Figure 1.

Targeting Rac and Cdc42. Currently available inhibitors target Rac and Cdc42 activation by disrupting GEF interactions, inhibiting nucleotide binding, blocking lipid modifications, and modulating Rho GDIs and proteasomal degradation, as well as by inhibiting downstream effector activity.

(9). In addition, Cdc42 has been shown to regulate cancer via activated Cdc42-associated tyrosine kinase (Ack1), Ras, and EGFR. The oncogene Ack1, which is activated by Cdc42 via EGFR/HER2, regulates Akt-mediated survival signaling to result in tumor recurrence and therapy resistance (10). Accordingly, inhibition of Cdc42 in Ras-transformed cells was shown to decrease oncogenic signaling via Akt, thus contributing to reduced cancer malignancy (11). Studies have also shown that Cdc42 prevents EGFR degradation by blocking receptor ubiquitination (12). Therefore, Cdc42 inhibitors have the potential to chemosensitize cancer therapies targeting Ras and EGFR.

Rac and Cdc42 may be activated by shared GEFs such as Vav and Ect2, Rac-specific GEFs (e.g., Tiam-1, p-Rex1), or Cdc42-specific GEFs [e.g., Intersectin, FYVE, RhoGEF, and PH domain containing protein1 (FGD1)] to activate a number of oncogenic signaling pathways. Direct downstream effectors of Rac and Cdc42 include p21-activated kinases (PAK), IQ motif containing GTPase-activating protein, Wiskott Aldrich Syndrome protein (WASP), WASP family verprolin-homologous protein and mammalian enabled (Mena)/vasodilator-stimulated phosphoprotein, and p67phox complexes. These effectors regulate migration/invasion via *de novo* actin polymerization, cell polarization, and matrix metalloproteinase secretion (13). PAK signaling via Rac and Cdc42 has been extensively studied in cancer and shown to regulate Src, focal adhesion kinase (FAK), PI3-K/Akt/mTOR, MAPKs [ERK, jun kinase (JNK), and p38 MAPK], protein kinase C, and STATs (14). Activated Rac has also been shown to affect cell proliferation via signaling to the oncogenes c-Myc and Cyclin D, as well as mTOR complex1 (mTORC1) and mTORC2 activation (15). Recent studies also suggest that nuclear Rho GTPases may have an additional role in regulating DNA damage response (16). Therefore, through these diverse downstream effectors, Rac and Cdc42 regulate tumor formation, growth, and metastasis, and are poised as new therapeutic targets for multiple aggressive cancers.

Rac- and Cdc42-Targeting Approaches

Rho GTPases have been previously considered "undruggable" due to their globular structure with limited small-molecule

Table 1. *RAC* and *CDC42* alterations in cancer

Cancer type	*RAC*	*CDC42*	Source
Bladder urothelial carcinoma	9%	4%	TCGA, Provisional
	12%	7%	(103)
Breast invasive carcinoma	4.5%	1.7%	TCGA, Provisional
	4%	0.1%	(104)
Cervical squamous cell carcinoma	9%	12%	TCGA, Provisional
Colorectal adenocarcinoma	5%	0%	TCGA, Provisional
Cutaneous melanoma	30% (3.6% P29 Mut)	6.3%	TCGA, Provisional
Esophageal carcinoma	15%	4%	TCGA, Provisional
Glioblastoma	24%		(105)
Stomach adenocarcinoma	3.3%	1%	TCGA, Provisional
	17%	4%	(106)
Head and neck squamous cell carcinoma	11.7% (0.7% Mut)	4%	TCGA, Provisional
	9% (1% Mut)	5%	(107)
Hepatocellular carcinoma	10%	4%	TCGA, Provisional
Lung adenocarcinoma	6%	1%	TCGA, Provisional
Mesothelioma	14%	4%	TCGA, Provisional
Pancreatic adenocarcinoma	6%	2.7%	TCGA, Provisional
	21%	21%	(108)
Prostate adenocarcinoma	9%	4%	TCGA, Provisional
	10%	5%	(109)
Sarcoma	14.5%	14%	TCGA, Provisional
Thyroid carcinoma	5%	3%	TCGA, Provisional
Uterine carcinosarcoma	9%	17.5%	TCGA, Provisional

NOTE: Percentage amplifications, mRNA upregulations, and driver mutations (as computed from cBioPortal; ref. 102).

binding pockets, high affinity for GTP or GDP binding, and the micromolar levels of GTP available in cells. The complexity of Rac and Cdc42 downstream signaling pathways also confounds the challenge of targeting a particular cellular process. Nonetheless, several rational strategies have emerged to inhibit the activation of Rac and Cdc42 (summarized in Fig. 1). Of these current approaches, blocking interaction with GEFs (Table 2) or nucleotide binding (Table 3) has emerged as principal strategies for Rac and Cdc42 inhibition in cancer cells and mouse models.

GEF interaction inhibitors

Rho GTPases are activated through exchange of GDP for GTP via GEFs, which not only facilitates interaction with downstream effectors, but also dictates the specificity of the signaling cascades that are activated (1, 17). Using structure-based mutagenesis studies, the Trp56 residue located on the groove formed by the switch I, switch II, and the β1/β2/β3 regions of Rac was identified as a critical determinant for GEF binding (18). The small-molecule NSC23766 was identified from a structure-based virtual screen of compounds that fitted into the surface groove of Rac1 critical for the Tiam1 and Trio GEF interaction (19). Confirming the oncogenic role of Tiam1 and Trio in cancer (3), NSC23766 reduced growth and invasion in several cancer types, including prostate, breast, gastric, chronic myelogenous leukemia, anaplastic large cell lymphoma, and glioblastoma (19–24). However, off-target effects in mouse platelets, such as receptor downregulation (25), as well as the high IC_{50} (∼50 μmol/L) of NSC23766 make it ineffective for pharmacologic use.

Our group used NSC23766 as a template for the design of derivatives, maintaining the central pyrimidine core of NSC23766 that binds the critical Tryp56 in Rac, and identified the Rac inhibitor EHop-016 (26). In metastatic cancer cells, EHop-016 blocks the interaction of the oncogene Vav with Rac and inhibits Rac activation with an IC_{50} of approximately 1 μmol/L, and Cdc42 at ≥10 μmol/L, without affecting Rho (14, 27). Moreover, EHop-016 reduced mammary tumor growth by approximately 80% in nude mice and inhibited angiogenesis and metastasis (28). The efficacy of EHop-016 as a Rac inhibitor has been subsequently validated in breast and prostate cancer cell lines, leukemia, melanoma, T lymphocytes, and fibrosarcoma (27, 29–32).

However, the relatively high effective concentrations and the moderate bioavailability (∼30% with $t_{1/2}$ of 4.5 hours) of EHop-016 (33) needed improvement. From screening EHop-016 derivatives, we found that this class of compounds was most active when the carbazole fragment was connected to a central core of 1,5,-disubstituted 1,2,3-triazoles (14) and therefore, synthesized MBQ-167 to retain this carbazole core (34). Although EHop-016 adopts a U-shaped conformation into a binding pocket adjacent to the Trp56 of Rac, it is predicted to interact more closely with Asn39 and Asp38 in the switch I region of Rac (27). Similarly, *in silico* modeling predicted that MBQ-167 binds deeper into the putative binding pocket of Rac and Cdc42, forming H bonds with the side-chain of Asn39. As predicted by studies where substitution of Asn39 of both Rac and Cdc42 results in the loss of GEF binding (18), MBQ-167 inhibits both Rac and Cdc42 activation. MBQ-167 inhibits Rac 1/2/3 activity with an IC_{50} of 103 nmol/L and Cdc42 activity with an IC_{50} of 78 nmol/L, thus making MBQ-167 one of the most potent Rac and Cdc42 inhibitors currently described in the literature. In immunocompromised mouse models, MBQ-167 inhibited metastatic breast cancer growth by approximately 90% saturating at 1 mg/kg body weight (34). Our studies with EHop-016 and MBQ-167 have validated the hypothesis that inhibition of Rac and Cdc42 activation leads to reduced metastatic cancer cell viability, migration, tumor growth, metastasis, and angiogenesis (28, 34). Moreover, in addition to reducing tumor growth, these drugs also prevent the infiltration of tumor-associated macrophages and neutrophils as well as cytokine release (unpublished data), which signifies additional beneficial effects of Rac and Cdc42 inhibition in the tumor microenvironment.

Table 2. GEF-interacting inhibitors of Rac and Cdc42 in cancer: compounds tested in cancer models

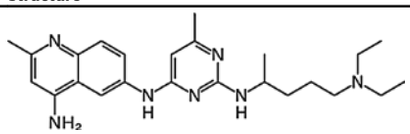
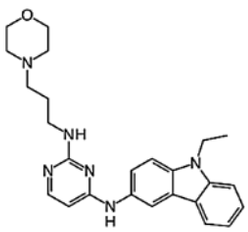
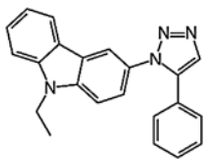
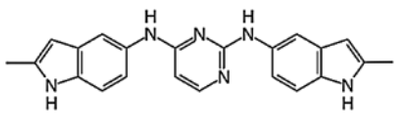
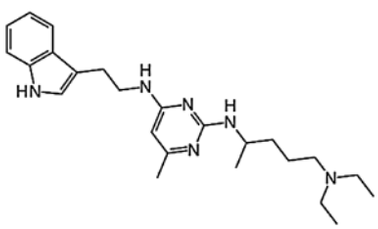
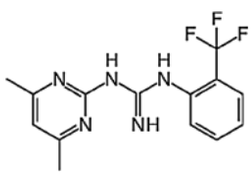
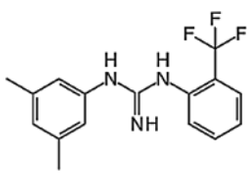
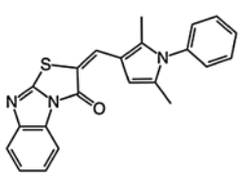
Inhibitor	Target	IC_{50}	Structure	*In vitro* studies	*In vivo* studies
NSC23766	Tiam-1, Trio/Rac	50–100 μmol/L (19)		(17, 20, 21, 23, 24, 30, 110, 111)	(21, 22, 24)
EHop-016	Vav/ Rac	1 μmol/L (27)		(27, 29–31)	(28, 30)
MBQ-167	Rac, Cdc42	0.1 μmol/L, 0.08 μmol/L (34)		(34)	(34)
AZA1	Rac1 and Cdc42	2–20 μmol/L (effective range) (35)		(35)	(35)
AZA197	Dbs/Cdc42	1–10 μmol/L (36)		(36)	(36)
ZINC69391	Tiam1/Rac1	31–61 μmol/L (43)		(43–45, 112)	
1A-116	p-REX/Rac1	4–21 μmol/L (43)		(43–45, 112)	
ITX3	TrioN/ Rac1, Rho	76 μmol/L (47)		(47)	

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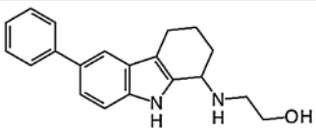
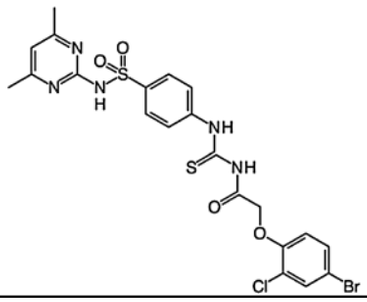
Table 2. GEF-interacting inhibitors of Rac and Cdc42 in cancer: compounds tested in cancer models (Cont'd)

Inhibitor	Target	IC_{50}	Structure	*In vitro* studies	*In vivo* studies
CASIN	Intersetin1/ Cdc42	2 μmol/L (53)		(53)	(53)
ZCL278	Intersetin1/ Cdc42	Kd = 11.4 μmol/L (55)		(55)	

Other NSC23766 derivatives, which have been developed to retain the central pyrimidine core, such as AZA1 and AZA197, are not as effective as EHop-016 or MBQ-167. AZA1 inhibits Cdc42 and Rac1 activation at concentrations ranging from 5 to 20 μmol/L and reduces migration and downstream signaling through PAK in prostate cancer cells and decreases tumor growth in mice (35). AZA197, a Cdc42-selective small-molecule inhibitor, is also active at an IC_{50} of 1 to 10 μmol/L and inhibits the Cdc42-GEF Dbs activity. Accordingly, AZA-197 treatment decreased PAK and ERK activities, cyclin D expression, and colon cancer cell invasion and proliferation, as well as tumor growth in mice (36, 37). Another molecule that inhibits Cdc42–Dbs interaction with an IC_{50} of 67 μmol/L is Compound 19 (38). However, this compound has not been analyzed in cell or animal models.

Ferri and colleagues screened a ZINC database for Rac inhibitors and identified a range of compounds with IC_{50}s from 12 to 57 μmol/L. From this screening, compounds 4 and 5, ZINC08010136 (IC_{50}, 12.2 μmol/L), and ZINC07949036 (IC_{50}, 24.1 μmol/L) were selected for further testing and shown to interfere with the Tiam-1/Rac1 interaction (39, 40). The same research group subsequently published a series of small molecules also targeting Tiam-1/Rac activation with IC_{50}s ranging from 2.5 to 27.9 μmol/L (41, 42). Although these compounds have yet to be tested in cancer cells, they are expected to be active in cancer models.

Another docking-based screening approach identified ZINC69391 as a structurally distinct compound from NSC23766 that still interferes with the Rac1–Tiam1 interaction with an IC_{50} of 61 μmol/L. This compound was tested in metastatic breast cancer cell lines and mouse models and inhibited cell proliferation, cell-cycle progression, migration, and metastasis (43). A more potent analog of ZINC69391, 1A-116, was also described by the same group and blocks the P-Rex1/Rac1 interaction with an IC_{50} of 4.0 μmol/L. Both ZINC69391 and 1A-116 have also been analyzed in breast cancer, glioma, and leukemia cells yielding antiproliferative and anti-invasive effects, indicating the therapeutic potential of inhibiting Rac activation in multiple aggressive cancers (43–45). The 1A-116 was also recently shown to chemosensitize tamoxifen-resistant breast cancer cells, thus demonstrating the utility of Rac inhibition to overcoming therapy resistance (46).

Although these compounds inhibited the Tiam-1/Rac interaction, the small-molecule compound ITX3 was shown to interfere selectively with the related GEF TrioN binding to Rac at concentrations ranging from 50 to 100 μmol/L. ITX3 is an analog of ITX1 (IC_{50} 110 μmol/L), identified through a chemical library screen in yeast cells using GEF activity assays. Even though it showed higher potency than ITX1, the relatively high IC_{50} of ITX3 still limits its clinical applicability (47).

As expected from the structural similarity to GTP, 6-Thio-GTP, a metabolite of Azathioprine that is used as an immunosuppressive agent for the treatment of inflammatory diseases, competitively blocks Rho-GEF binding. 6-Thio-GTP has been shown to inhibit Rac1 activation by Vav in lymphocytes and breast cancer cells (48, 49).

Most current inhibitors of the GEF/Rac/Cdc42 interaction focus on preventing the Dbl family GEFs. However, the small-molecule inhibitor CPYPP interferes with the DOCK family GEF DOCK2, which is predominantly expressed in hematopoietic cells (50). A DOCK2-selective inhibitory peptide has also been shown to block B-cell migration and is considered a viable drug target in leukemia (51). In addition, the DOCK1 selective inhibitor TBOPP was shown to specifically inhibit DOCK1-regulated invasion in Ras-transformed cells and tumor growth and metastasis in mice with tumors from Ras-mutant lung carcinoma cells (52), thus illustrating the utility of DOCK-targeted anticancer drugs.

Structural studies with Cdc42 have revealed that similar to the critical Trp 56 in Rac1, Phe56 in Cdc42 is a significant determinant for GEF binding. Hence, Pirl1 was discovered through a biochemical suppression approach from cytoplasmic extracts of *Xenopus laevis* eggs. Pirl1 targets Cdc42 by specifically blocking PIP_2-mediated GEF activity on the Cdc42/RhoGDI complex with an IC_{50} of 3 μmol/L (53). Cdc42 activity–specific inhibitor (CASIN), a structural derivative of pirl1, also disrupts intersectin (ArhGEF4)-mediated Cdc42 activation with a similar IC_{50} of 2 μmol/L. Using CASIN, Cdc42 was shown to regulate colon cancer initiation by interacting with the pivotal tumor suppressor adenomatous polyposis coli in incipient

Table 3. Nucleotide binding inhibitors of Rac and Cdc42 in cancer: compounds tested in cancer models

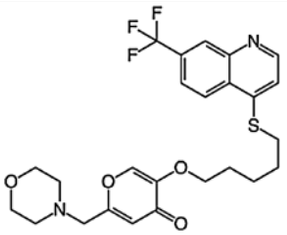
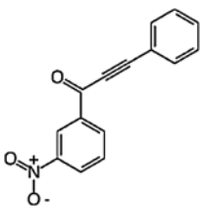
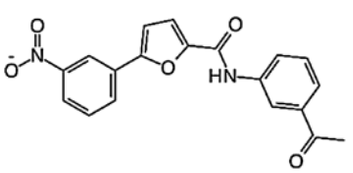
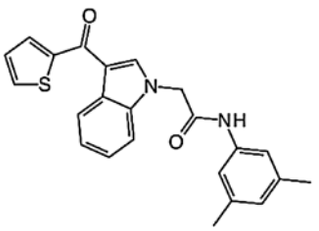
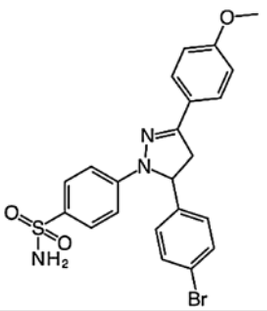
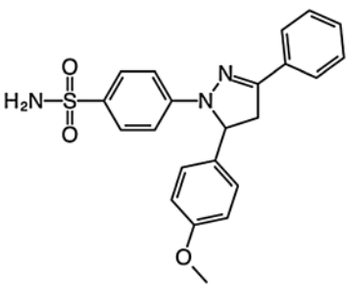
Inhibitor	Target	IC_{50}	Structure	*In vitro* studies	*In vivo* studies
EHT-1864	Rac1	1–5 μmol/L (56)		(58, 59, 61, 113, 114)	(61)
MLS000532223	Rho, Rac, Cdc42	∼10 μmol/L (62)		(62)	
Compounds 1,6	Rac1	Compound 1 EC_{50}: 8.3 μmol/L Compound 6 EC_{50}: 22.4 μmol/L (63)	 	(63)	
CID44216842	Cdc42	∼3–5 μmol/L (68)		(68)	
ML141 (CID2950007)	Cdc42	∼200 nmol/L–5.4 μmol/L (69)		(29, 68, 69, 101, 115, 116)	

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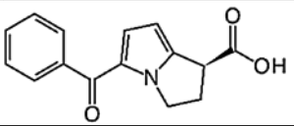
Table 3. Nucleotide binding inhibitors of Rac and Cdc42 in cancer: compounds tested in cancer models (Cont'd)

Inhibitor	Target	IC_{50}	Structure	*In vitro* studies	*In vivo* studies
R-ketorolac	Rac1, Cdc42	Rac1, 0.574 μmol/L; Cdc42, 1.07 μmol/L (70)		(70, 71)	(72)

intestinal tumor cells. Accordingly, Cdc42 inhibition via CASIN treatment reduced *in vivo* tumorigenicity in colorectal cancer xenograft models and abrogated progression of mouse and human tumor organoids (54). Similarly, the small-molecule ZCL278, discovered through a high-throughput *in silico* screening, binds close to Phe56 in the surface groove of Cdc42 and disrupts the interaction between Intersectin and Cdc42. ZCL278 also inhibited migration in metastatic prostate cancer cells (55). Therefore, the screening for Cdc42-specific inhibitors using the unique Phe56 in its GEF-binding groove has led to the elucidation of Cdc42-specific functions driving cancer initiation and progression.

Nucleotide binding inhibitors

Unlike the GEF-binding inhibitors, which usually block specific classes of GEFs or individual GEFs from binding to the GEF-interacting domains of Rac and (or) Cdc42, a viable alternative is to prevent nucleotide binding. This strategy was used to develop EHT 1864, which inactivates Rac isoforms (1,2,3, and the Rac1b splice variant) by a tight interaction that displaces guanine nucleotides. Therefore, EHT 1864 inhibits upstream GEFs as well as downstream effector binding at concentrations ranging from 10 to 50 μmol/L (56). Studies have used EHT 1864 to elucidate the central role of Rac in transformation (via Tiam-1 or Ras), downregulation of estrogen receptor expression in breast tumors, migration in fibrosarcoma and melanoma cells, and breast cancer invasion and tumor growth (56–61). However, off-targets effects of this compound have been observed in mouse platelets, such as triggering of platelet apoptosis (25), thus raising concerns about its safety.

MLS000532223 was characterized using a flow cytometry bead-based multiplex assay for molecules that inhibit Rho family activation by noncompetitive inhibition of GTP binding. MLS0005322233 inhibits all Rho GTPases tested (Rho, Rac, and Cdc42), whereas the related compound MLS000573151 is specific for Cdc42 and affects the cytoskeletal dynamics of mast cells and leukemia cells. However, these compounds are active at approximately 10 μmol/L, thus limiting their pharmacologic use (62).

Other compounds that interfere with Rac1 nucleotide binding are compounds 1 and 6. These compounds disrupt the binding between Rac1 and its effector PAK1 and reduce cell proliferation and migration in various pancreatic cancer cell lines with EC_{50}s of 8.3 and 22.4 μmol/L, respectively (63). The reported IC_{50}s for these compounds are in the nanomolar range as determined by an *in vitro* assay using purified recombinant proteins. Hence, further studies are needed to confirm their efficacy in cell-based systems.

A series of isoquinolines and phenanthridine derivatives were also developed to block nucleotide binding with Rac1, Cdc42, and Rac1b. Efficient Rac1 and Cdc42 inhibition was induced by compound 4 with IC_{50}s of 8 and 6 μmol/L for Rac1 and Cdc42, respectively (64). Intriguingly, this compound was more effective at inhibiting the constitutively active splice variant Rac1b (IC_{50} = 2.7 μmol/L), and thus hold utility as therapy for lung, thyroid, breast, and colorectal cancers with upregulated Rac1b (65–67).

There are several nucleotide-binding inhibitors that specifically block Cdc42 activity. The structural analogs CID2950007 (ML141) and CID44216842 inhibit Cdc42 activation with EC_{50}s of approximately 2 and 1 μmol/L, respectively, and were discovered through a bead-based multiplex flow cytometry assay (68). CID2950007 reduced bradykinin-induced filopodia formation in fibroblasts, thus indicating specific inhibition of Cdc42 activation (69). These compounds inhibited ovarian cancer cell migration, without being cytotoxic in multiple cell lines (68). Even though CID44216842 is more potent, its low solubility limits its further development as an anticancer agent (68).

Recent studies have also demonstrated that the R-enantiomers of nonsteroidal anti-inflammatory drugs function as allosteric inhibitors of Rac1 and Cdc42. R-ketorolac and R-naproxen inhibited Rac1 and Cdc42 at micromolar IC_{50}s (70) with R-ketorolac inhibiting Rac with an EC_{50} of 0.574 μmol/L and Cdc42 at 1 μmol/L, and consequently reducing ovarian cancer cell migration, invasion, and adhesion (71). Moreover, R-ketorolac reduced tumor development in a spontaneous breast cancer transgenic mouse model (72). This FDA-approved drug is the first Rac and Cdc42 inhibitor to be used in a P0 clinical trial, which demonstrated improved ovarian cancer patient survival (71).

Another FDA-approved drug is mitoxantrone (MTX), a topoisomerase II inhibitor, which is currently used as a cancer therapeutic. A GTPase activity AlphaScreen assay identified MTX to interfere with GTP binding with broad selectivity, targeting RhoA, Rac1, and Cdc42, and thus reduced F-actin reorganization and cell migration (73). Because this drug is known to have adverse side effects, inhibition of Rho GTPases may add to its overall off-target effects.

Spatial regulation inhibitors

Rho GTPases require to be localized to cell membranes for activation by receptor-regulated GEFs. Therefore, posttranslational modifications that target them to the plasma membrane, such as addition of isoprenoid moieties (prenylation) at the C-terminal "CAAX box," are essential for their biological functions (74). Hence, similar to the strategy of inhibiting Ras activation with farnesyltransferase inhibitors, geranylgeranyl transferase inhibitors, such as PAIA6, have been used to alter Rho GTPase function and induce anticancer properties in various model systems (75, 76). Although these inhibitors are not specific to Rac and (or) Cdc42, testing of prenylation inhibitors such as GGTI-2418 in preclinical and clinical trials has shown multiple anticancer effects.

Similarly, because statins lower lipid synthesis by inhibiting HMG-CoA reductase, they can also be used to prevent isoprenoid synthesis and thus prenylation of Rho GTPases; however, this

strategy is also limited by its nonselectivity. Moreover, alternate mechanisms have also been associated with statins, such as increased nuclear Rac1 degradation (77). Although statins have been shown to have antitumor properties (78–80), further studies are needed to determine the specificity of such inhibition.

RhoGDI modulators

Another potential strategy for Rac and Cdc42 inhibition is targeting of RhoGDIs. RhoGDIs sequester inactive GDP-bound Rho GTPases in the cytosol and inhibit their activation. Hence, the design of an interfacial interactor to prevent the release of a RhoGDI from a Rho GTPase is a potential inhibitory strategy (17). Thus, Secramine was developed to block membrane recruitment of prenylated Cdc42 by sequestering the Cdc42. RhoGDI complex, and thus inhibit actin polymerization (81). However, this strategy may not be selective, because RhoGDI also binds Rac and Rho. Moreover, GDIs play a complex role in cancer where both up- and downregulation of Rho GDIs have been shown to result in increased malignancy (17).

GAP modulators

Because GAPs catalyze the hydrolysis of GTP to GDP, increasing Rho GAP activity is a potential mechanism for Rho GTPase inactivation. As expected, GAPs have been shown to act as tumor suppressors; however, some GAPs are also overexpressed in certain cancers, thus confounding the development of GAP activators as anticancer agents (17). Nevertheless, a number of Rac and Cdc42 GAPs, such as β2-chimaerin, ARHGAP24, and Binder of Arl Two (BART), have been shown to exert a negative effect on cancer progression (1, 4, 82). However, so far, no mimics of GAPs have been developed for use as Rac and Cdc42 inhibitors.

Modulators of protein stability

Rac and Cdc42 stability can be regulated by SUMOYlation, which increases stability of the GTP-bound form, and ubiquitination, which targets them for proteasomal degradation (83). Therefore, inhibition of SUMOylation or increased ubiquitination is rational strategy to inhibit Rac and Cdc42. Luteolin is a flavonoid that promotes proteasomal degradation of Cdc42 and thus reduces invasion and migration of glioblastoma cells (84). Luteolin has also been tested *in vitro* and *in vivo* in various cancers, such as leukemia and prostate cancer, and was effective in decreasing cell growth and angiogenesis, and increasing apoptosis (85). Nonetheless, no further advancements have been made in the development of this compound as an anticancer therapeutic.

Downstream effector inhibitors

Much attention has been devoted to the prevention of downstream signaling from Rac and Cdc42 by targeting oncoproteins such as PAKs. The status of the current PAK inhibitors, such as OSU-03012 (86), FL172 (87), and FRAX597 (88), has been extensively reviewed (89). Unfortunately, the PAK4 inhibitor PF-3758309 failed clinical trials, due to its undesirable pharmacokinetics and dose–response relationship, and a PAK inhibitor has yet to receive FDA approval.

Other downstream effector inhibitors, such as Phox-I1 and 8-hydroxy-2-deoxyguanine, which target the binding between Rac1 and its downstream effector $p67^{phox}$, a structural component of the NADPH oxidase complex, have been shown to inhibit cancer progression (90). Further studies are needed to validate the clinical applicability of these compounds.

Efforts are also underway to target the interaction of Cdc42 with downstream effector N-WASP, thus inhibiting the activation of the actin-related protein (Arp)2/3 complex and ensuing actin polymerization. Potential of inhibitors, such as the 14-aminoacid cyclic peptide 187-1 and wiskostatin, as anticancer compounds remains to be recognized (91, 92).

Concluding Remarks

Rac and Cdc42 GTPases are key signaling intermediates whose dysregulation has been distinctly associated with cancer initiation and progression. Rational design of small-molecule inhibitors to Rac and Cdc42 has shown promising preclinical outcomes. Nonetheless, no clinically effective drugs targeting these Rho GTPases have been approved for cancer therapy, and nor are they currently in clinical trials. Testing these inhibitors, in patient-derived orthotopic xenograft mouse models of different cancer types, is also essential for demonstrating drug efficacy in a physiologically relevant setting (93–96). Furthermore, studies regarding the toxicity and pharmacokinetic properties of these inhibitors are scarce in the literature. Additional factors such as feedback mechanisms and impact on the tumor microenvironment, as well as effects on the immune and cardiovascular systems, need to be considered in further development of Rac and Cdc42 inhibitors as anticancer agents.

The central role of Rac and Cdc42 in multiple oncogenic signaling pathways also needs to be recognized regarding the therapeutic utility of Rac and Cdc42 inhibition in cancer. Rac and Cdc42 are pivotal downstream regulators of a myriad of cell signaling receptors, as well as Ras/MAPK and PI3-K/Akt pathways, for which a number of therapies exist or are currently undergoing clinical trials. However, acquired resistance to such targeted therapies is frequently a cause of failure in cancer treatment. Therefore, inhibition of Rac and Cdc42 may be a sound strategy to potentiate receptor- and PI3-K–targeted therapies.

Our studies with the Rac/Cdc42 inhibitors EHop-016 and MBQ-167 in breast cancer models (HER2 and triple-negative breast cancer) have shown that consistent with their role in suppression of cell growth and migration/invasion, Rac and Cdc42 inhibition does not reduce the size of the primary tumor but prevents its further growth and metastasis. Therefore, as illustrated by reports where Rac1 inhibition sensitized pancreatic and breast cancer cells to radiotherapy (97, 98), Rac and Cdc42 inhibitors may be useful in combination therapy with classic chemo- and radiotherapies.

Moreover, Rac/Cdc42/PAK signaling is specifically implicated with therapy resistance of HER2-type breast cancers. Accordingly, studies by our group and others have shown that Rac/Cdc42 inhibition decreases the viability of breast cancers resistant to EGFR/HER2 therapy (unpublished data and refs. 14, 24, 99, 100). Moreover, a recent study demonstrated the utility of using EHop-016 as combined therapy with Akt/mTOR inhibitors to treat Integrin-mediated high-grade myxofibrosarcoma (30). In addition, Rac/Cdc42 inhibition as a strategy to overcome therapy resistance has been validated using 1A-116, EHT 1864, and ML141 (46, 57, 101).

In conclusion, future therapeutic strategies should focus on the utility of Rac/Cdc42 inhibitors in combination therapy with existing cancer therapeutics, as well as to diminish cancer therapy resistance. Such combination therapies with novel targeting strategies, such as immunoliposomes to target overexpressed receptors, will facilitate the administration of lower concentrations of Rac and Cdc42 inhibitors, thus circumventing potential immune and cardiovascular toxicities. Considering their enormous potential for preventing not only tumor growth and metastasis, but also the tumor-promoting effects of the immune and stromal cells in the tumor microenvironment, Rac and Cdc42 inhibitors are poised for clinical testing to assess the risks and benefits of targeting Rac/Cdc42 in human cancer.

Disclosure of Potential Conflicts of Interest

S. Dharmawardhane has ownership interest (including patents) in US patents US 08884006 B2 and US 9278956 B1. No potential conflicts of interest were disclosed by the other author.

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