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Targeting Cdc42 in cancer

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Abstract

Introduction—The Rho GTPases are a family of proteins that control fundamental cellular processes in response to extracellular stimuli and internal programs. Rho GTPases function as molecular switches in which the GTP-bound proteins are active and GDP-bound proteins are inactive. This review will focus on one Rho family member, Cdc42, which is overexpressed in a number of human cancers, and which might provide new therapeutic targets in malignancies.

Areas covered—In this review the key regulators and effectors of Cdc42 and their molecular alterations are described. The complex interactions between the signaling cascades regulated by Cdc42 are also analyzed.

Expert opinion—While mutations in Cdc42 have not been reported in human cancer, aberrant expression of Cdc42 has been reported in a variety of tumor types and in some instances has been correlated with poor prognosis. Recently, it has been shown that Cdc42 activation by oncogenic Ras is crucial for Ras-mediated tumorigenesis, suggesting that targeting Cdc42 or its effectors might be useful in tumors harboring activating Ras mutations.

Keywords

transformation; small GTPase; protein kinase; signal transduction; cancer; small molecule inhibitor

1. Introduction

The Ras-related GTPase superfamily contains approximately 150 members of small monomeric guanine nucleotide binding proteins involved in a wide number of important cellular processes. These proteins can be subclassified into five major families: Rho, Ras, Rab, Arf/Sar, and Ran [1]. Aberrant regulation and/or activation of Rho GTPases has been linked to tumorigenic phenotypes in a variety of human cancers and in some instances has been correlated with poor prognosis. All aspects of cellular motility and invasion including cellular polarity, cytoskeletal re-organization, and signal transduction pathways, are affected by the interplay between the Rho-GTPases, suggesting that targeting these proteins might be useful in some types of tumors [2, 3]. This review will focus on one Rho family member, Cdc42, a ubiquitously expressed small GTPase, that is known to regulate the dynamic organization of the cytoskeleton and membrane trafficking for physiologic processes such as cell proliferation, motility, polarity, cytokinesis and cell growth [4, 5]. Like other GTPases, Cdc42 cycles between an inactive, GDP-bound state and an active, GTP-bound state. Mechanistically, Cdc42 is activated through the exchange of GDP for GTP, this reaction is mediated by guanine nucleotide exchange factors (GEFs), which catalyze the release of

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DECLARATION OF INTEREST
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GDP, allowing the loading of a GTP molecule [6]. Activated Cdc42 then mediates a signaling cascade leading to activation of more than twenty downstream effectors, including protein kinases, lipid kinases, scaffolding proteins, and cytoskeletal interacting proteins, resulting in changes in cellular processes including cell polarity, adhesion, migration, proliferation, actin cytoskeleton remodeling, membrane trafficking and transcription (Fig. 1). Conversely, inactivation of Cdc42 is achieved by hydrolysis of GTP to GDP, which is coordinated by GTPase activating proteins (GAPs), which stimulate the low intrinsic hydrolytic activity of Rho proteins. Rho guanine nucleotide dissociation inhibitors (RhoGDIs) represent another class of GTPase inhibitor, associating with cytosolic GTPases and locking them in their GDP-bounded conformation, thereby preventing any further activation. Recently, a second GDI-like mechanism for inhibiting small GTPases has been described, in which 14-3-3 proteins bind to a hybrid C-terminal phosphoserine in apposition to an adjacent farnesyl moiety [7]. However, this unusual mode of inhibition has thus far only been shown to apply to a few Rho GTPases, namely members of the Rnd family and also to the Ras GTPase Rap1 [7].

Cdc42 was originally identified in *Saccharomyces cerevisiae* as a cell-cycle mutant involved in the regulation of budding and mating projection [8]. In *Caenorhabditis elegans* and *Drosophila*, Cdc42 is required for early development, establishing and maintaining cell polarity and morphogenesis in the embryo [9, 10]. In the past two decades, extensive studies in various mammalian cell lines have been carried out by overexpressing dominant negative and/or constitutively active mutants of Cdc42. This approach has contributed greatly to the discovery of several fundamental aspects of Rho GTPase cell biology. However, this approach also has well-recognized limitations. Specifically, dominant-negative GTPases sequester upstream Rho GEFs, and, as these GEFs may activate multiple GTPases, interpreting results that rely on this approach can be problematic. Conversely, active mutants of Rho GTPases can sequester individual effectors in a manner that is no longer subject to spatiotemporal regulation, again leading to difficulties in interpretations [11]. Despite these limitations, the use of dominant-negative and constitutively active forms of small GTPases remains a valuable tool in certain circumstances. More recently, gene-targeting studies in mouse models have been employed to decipher Cdc42 signaling activities. Since *Cdc42* knockout mice have an embryonic lethal phenotype [12], tissue specific *Cdc42* knockout models have been designed to study the function of this GTPase beyond early embryogenesis [13–15]. These studies have provided powerful genetic evidence for physiological roles of Cdc42 that, in some cases, mimic disease states such as hepatic tumorigenesis, hyperproliferation of blood progenitors, impaired B cell development, and osteoporosis, among others [16–18]. This review will focus on the role of Cdc42 in cancer.

2. Cdc42 and its regulators in cellular transformation

Considering its key role in diverse cellular functions, it is not surprising that deregulation of Cdc42, as well as its upstream regulators and downstream effectors, have been linked to a number disorders and diseases. In addition, Cdc42 has also been shown to modify the ability of other oncoproteins, including Ras and EGFR, to induce cellular transformation. Activating mutations in Ras result in the inappropriate activation of anti-apoptotic and pro-survival signaling pathways, particularly the Raf-MEK-ERK and PI3K-Akt cascades, leading to tumor initiation and progression [19]. Early studies using expression of a dominant negative Cdc42 mutant suggested that this GTPase is essential for Ras transformation of fibroblasts [20]. Subsequent studies reported that Cdc42 becomes activated upon expression of oncogenic Ras, and that it can impinge on Ras-induced signaling pathways [21, 22]. More recently, Stengel and Zheng showed that genetic deletion of Cdc42 in Ras-transformed cells results in a significant block in cell proliferation and cell cycle progression, which is not observed in non-transformed cells or cells transformed by

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the oncoprotein, c-Myc [23]. In addition to Ras-mediated transformation, Cdc42 also affects oncogenic signals from the EGFR [24]. EGFR activity is tightly regulated, not only by ligand-mediated receptor activation, but also through a course of receptor endocytosis, degradation and recycling [25]. Maintenance of normal EGFR turnover is critical for prevention of sustained signaling. One function of Cdc42 in this process is to modulate EGFR degradation. In conjunction with a GEF, Cool-1/β-Pix, Cdc42 associates with an ubiquitin ligase, c-Cbl, which is involved in the initiation of EGFR degradation. Activation of Cdc42 results in c-Cbl sequestration and prevention of ubiquitin-mediated EGFR degradation, leading to sustained EGFR signaling and, ultimately, cellular transformation [24]. The contribution of Cdc42 to EGFR-mediated transformation is also observed in human cancer models. For example, in breast cancer cells overexpressing EGFR, Cdc42 depletion has been shown to result in a c-Cbl-dependent reduction in total EGFR protein levels, and a consequent reduction in cell growth and migration [26].

Unlike Ras proteins, which are constitutively activated by point mutations in a number of human tumors, mutations in the *Cdc42* gene have not been detected in human cancers [27]. To define the potential involvement of Cdc42 in cellular transformation, early studies using dominant-negative or constitutively active mutants of this GTPase contributed greatly to the discovery of many fundamental aspects of Cdc42 biology. Microinjection of constitutively active Cdc42 in mammalian cells induces the formation of filopodia [28], whereas expression of activated forms of RhoA and Rac1 trigger the formation of stress fibers and lamellipodia, respectively [29, 30]. These observations point to the profound role of Cdc42 in regulating the actin cytoskeleton. However, it is clear that Cdc42 activity is also important for many other cellular processes such as vesicle trafficking [31], cell growth [32], regulation of cell polarity [33] and transcription [34]. Accordingly, it is not surprising that aberrant activation of Cdc42 can be oncogenic. For example, it is well documented that different constitutively active mutants of Cdc42, including Cdc42Q61L and Cdc42G12V; and Cdc42F28L, which exhibits spontaneous and accelerated cycling between the GDP- and GTP-bound states, induce foci formation and/or anchorage-independent growth in NIH-3T3 immortalized fibroblasts [35, 36]. Moreover, there is increasing evidence that deregulation of Cdc42 frequently occurs in different types of human cancers as the result of molecular alterations in the genes encoding Cdc42 regulatory proteins and/or downstream effectors, as well as for its constitutive activation mediated by mutations or amplification of cell surface receptors (Fig. 2). In addition, higher expression levels of Cdc42 are known to correlate with increased testicular cancer progression and poorer outcome [37]. Overexpression of Cdc42 has also been found in lung cancer and cutaneous melanoma and may serve as a disease marker and prognosis parameter [38]. Recently, it has been shown that Cdc42 activity is critical for transendothelial migration and lung metastasis formation *in vivo* in a β1 integrin dependent fashion [39]. This finding suggests that targeting Cdc42 in the early steps of cancer cells interaction with endothelial cells would potentially decrease cancer cell colonization and metastasis formation. Finally, SAGE (serial analysis of gene expression [40]) has revealed altered levels of Cdc42 in different types of human cancer when compared with normal tissue (Fig. 3).

Cdc42 is positively regulated by a large number of GEFs that are associated with transformation. For example, the Dbl protein, which was first discovered as an oncogene more than 20 years ago [41], is a guanine nucleotide exchange factor for Cdc42. Subsequently, additional Dbl related proteins were identified, many of which were likewise oncogenic when activated by truncation. The Cdc42 GEFs Vav2, Vav3, ITSN-1, P-Rex and FGD1, exemplify this phenomenon, as overexpression and/or hyperactivity has been directly linked to different types of human cancers, including leukemia, breast, prostate and brain tumors [42–44]. More recently, a structurally distinct group of RhoGEFs, the DOCK family, has been identified that activate Rho GTPases by a DH-unrelated domain named the

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DOCKER, CZH2 or DHR2 domain. The DOCK family has been implicated in a number of cellular events such as cell migration, phagocytosis of apoptotic cells, T-cell activation and neurite outgrowth [45]. Interestingly, the Cdc42-specific activator DOCK8 has been linked to a human disease, specifically to primary immune deficiency [46].

While GEFs activate Rho GTPases, GAPs act as GTPase negative regulators through the stimulation of the GTP hydrolytic activity, therefore leading to termination of the signaling event [47]. Not surprisingly, loss of function or downregulation of Rho GAPs has been observed in a variety of cancer types, suggesting that GAPs could play a tumor suppressor role by keeping the GTPase activity in check. For example, the role srGAP3, a member of the Slit-Robo sub-family of RhoGAPs has been recently reported. It has been shown that srGAP3 is absent or significantly reduced in a panel of breast cancer cell lines and that its re-expression in a subset of these cell lines inhibits anchorage-independent growth and cell invasion in a GAP-dependent manner. These effects are accompanied by an increase in phosphorylation of ezrin/radixin/moesin (ERM) proteins and myosin light chain 2 (MLC2), suggesting that srGAP3 expression leads to Rho and ROCK activation, likely through inactivation of Rac and/or Cdc42, to drive actomyosin contractility and inhibit cancer cell invasion [48]. While it is well accepted that altered GTPase function is involved in many aspects of tumorigenesis and metastasis, the role of RhoGDIs as mediators of GTPase crosstalk within the setting of cancer has been relatively under-appreciated. RhoGDIs consist of a family of three members: RhoGDI1, RhoGDI2, and RhoGDI3 [49]. RhoGDI1 is ubiquitously expressed and is the best studied member of the family. Several studies have shown that RhoGDI1 is overexpressed in colorectal and ovarian cancers, correlating with increased invasion and resistance to chemotherapy [50, 51].

In breast cancers, contradictory results have been reported, with RhoGDI1 expression reported as increased or decreased in different studies [52, 53]. Finally, RhoGDI1 is downregulated in hepatocellular carcinoma, and its expression was found to be controlled in part by the recently characterized microRNA miR-151 [54]. Alterations in RhoGDI2 expression have also been reported in different human tumor types. RhoGDI2 has been reported to have both oncogenic and tumor suppressor properties, depending on cancer tissue type. For instance, RhoGDI2 expression is reduced in bladder cancer [55], lung cancer [56], and Hodgkin lymphoma [57], but augmented in ovarian cancer [58]. In other tissues, the relationship between RhoGDI2 expression in normal versus cancer specimens is unclear. For example, RhoGDI2 expression has been reported to be increased in breast tumors and to promote invasion of breast cancer cells [59], while another report found a biphasic expression pattern of RhoGDI2 in breast cancer with decreased expression correlating with lymph node metastasis [60]. To add to this complexity, it is not entirely clear that RhoGDIs are always antagonists of Cdc42-mediated signaling. For example, RhoGDI activity was recently shown to play an essential role in Cdc42-induced cellular transformation. In this model, RhoGDI facilitates the transition of Cdc42 membrane-bound state to a cytosolic state in order to mediate Cdc42-signaling activities [61].

3. Cdc42 downstream effectors and cancer

Cdc42 has been found to activate more than twenty downstream effector and adaptor proteins; including actin-associated proteins, phospholipases, kinases and adaptor proteins among others [62]. For instance, Cdc42 has been shown to bind and activate PI3K [21], while one of the best characterized Cdc42 effectors, PAK1, has been shown to phosphorylate both c-Raf at S338 and MEK1 at S298, sites that are required for full activation of ERK signaling in some cell types [22]. In addition, PAK1 is frequently overexpressed and/or hyperactive in human breast, ovary, bladder, uterine, and brain cancers, due to amplification of the *PAK1* gene in an 11q13 amplicon [63]. Moreover, it has

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been shown that PAK cooperates with some other oncogenes such as K-Ras, ErbB2, β-catenin, NF1, and NF2 to promote cellular transformation [64–66]. Another PAK family member that has been implicated in several types of cancer; including breast, ovarian and cancer tumors, is PAK4 [67–70]. Furthermore, PAK4 overexpression is sufficient to lead to cancer in animal models, particularly in a mouse breast cancer model [71]. However, the mechanism by which PAK4 promotes cellular transformation is not well understood, and even though PAK4 is structurally and biochemically distinct from PAK1, most of the few currently known substrates of PAK4 are also phosphorylated by PAK1 [72].

MLK3 represents another Cdc42 downstream effector linked to cellular transformation; this kinase plays a prominent role in the regulation of neuronal cell apoptosis via JNK signaling [73]. Recent studies have demonstrated the overexpression of MLK3 in cancer cells and established its involvement in mediating various pro-oncogenic pathways [74, 75]. Notably, it has been shown that MLK3 is hyperactive in NF2 deficient cells. The mechanism by which Merlin (the product of *NF2* gene) inhibits MLK3 mediated transformation appears to be mediated by direct association with MLK3. This association impairs MLK3 activation by blocking the interaction between Cdc42 and MLK3, and consequently the activation of the Ras/Raf/MEK/ERK and JNK cascades [76]. Cdc42 also controls the activation of the classic lipid signaling enzymes phospholipase D1 and phospholipase D2 (PLD1 and PLD2), which have also been linked in a cell intrinsic manner to a prometastatic phenotype. Recently, in a mouse cancer model, ablation of the PLD1 in the tumor environment was shown to compromise the neovascularization and growth of tumors [77]. PLD1 deficiency suppressed the activation of Akt and mitogen-activated protein kinase signaling pathways by vascular endothelial growth factor in vascular endothelial cells, resulting in decreased integrin-dependent cell adhesion to, and migration on, extracellular matrices, as well as reduced tumor angiogenesis in a xenograft model. In addition, mice lacking PLD1 incurred fewer lung metastases than did wild-type mice [77].

The Cdc42-associated nonreceptor tyrosine kinase ACK1 is also linked to cellular transformation. Aberrant ACK1 signaling, promotes prostate cancer progression by activation of the androgen receptor and degradation of the tumor suppressor WWOX [78]. Moreover, ACK1 enhances oncogenic EGFR signaling and was shown to increase proliferation and invasiveness of renal and breast cancer cells [79, 80]. In a murine breast cancer metastasis model, ACK1 overexpression was associated in with increased mortality of the mice [81]. Due to these oncogenic properties, ACK1 has emerged as a potential target for cancer therapy. Other Cdc42 downstream effectors that have been linked to human cancers are IQGAP proteins, in particular, IQGAP1 and IQGAP2. IQGAP1 has been proposed to be an oncogene [82], and increased expression of IQGAP1 has been observed in several human neoplasms, including oligodendrogliomas, lung and colorectal tumors [83, 84]. The association of IQGAP1 with its binding partners Cdc42, Rac1, E-cadherin, β-catenin, components of the MAPK cascade, and others may have a role in transformation and metastasis. However, the specific interactions that directly contribute to IQGAP-mediated oncogenic activity have not been fully elucidated [85]. In contrast, there is evidence to suggest that IQGAP2 acts as a tumor suppressor. IQGAP2 expression is lost from 5/9 gastric cancer cell lines due to aberrant methylation of the IQGAP2 promoter. Abnormal methylation was also observed in 47% of primary gastric cancer tissues, and was significantly associated with tumor invasion and a poor prognosis [86]. IQGAP2 is expressed predominantly in liver. Consistent with its putative role as a tumor suppressor, targeted disruption of the IQGAP2 gene results in the development of hepatocellular carcinoma (HCC) in a mouse cancer model [87]. Although IQGAP2 has 62% identity and an overall similarity of 77% to IQGAP1 its biological role is not well understood, and even less clear are the mechanistic bases underlying the role of IQGAP1 as an oncogene and IQGAP2 as a tumor suppressor. However, it has been suggested that the tissue distribution of

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IQGAP2 mRNA is distinct from that of IQGAP1 [88], as is the subcellular location of these proteins (xx). In rabbit gastric parietal cells, IQGAP1 and IQGAP2 are localized to the basolateral and apical membranes, respectively, and have different functions [89]. Similarly, the localization of *Xenopus* IQGAP2 in cells differs to that of IQGAP1 [90]. *Xenopus* IQGAP1 accumulates at adherens junctions, whereas IQGAP2 exhibits significant nuclear localization, which suggests that the two IQGAPs differ in the way they regulate the cytoskeleton. Collectively, these data suggest that IQGAP1 and IQGAP2 have distinct, yet partially overlapping functions that may account for their proposed roles as oncogenic or tumor suppressor proteins.

While the studies discussed above suggest a role for activated Cdc42 in tumorigenesis, recent studies indicate that in other cellular contexts Cdc42 may also act as a tumor suppressor. For example, it has been shown that liver specific deletion of Cdc42 results in chronic liver damage, hepatomegaly and development of HCC by 8 months of age [16]. While the underlying mechanism of Cdc42-mediated tumor suppression remains largely unknown, tissue specific gene targeting studies indicate that the role of Cdc42 in cell cycle progression, survival or proliferation varies between cell types. There is evidence suggesting that maintenance of cell polarity could be one way in which Cdc42 contributes to tumor suppression. In a 3D culture system of MDCK cells, Cdc42 is important for the establishment of polarized epithelial cysts by regulating the vesicular transport of proteins to the apical surface of the cell. This Cdc42-mediated protein trafficking occurred in a Par6-aPKC-dependent manner and is required for lumen formation [91]. In addition, the Cdc42-Par6-aPKC complex regulates the formation of normal 3D polarized epithelial cysts by controlling mitotic spindle orientation and the direction of cell division. In particular, Cdc42, or Par6/aPKC deficient cells, form aberrant cysts with multiple lumens [92]. The involvement of Cdc42 in the maintenance of planar cell polarity has been further corroborated in a mouse for pancreas development. Deletion of Cdc42 in the developing pancreas revealed that Cdc42 is required for apical polarization and is responsible for microlumen formation during the early stages of pancreatic development as well as for maintaining cell polarity [14]. However, while the role of Cdc42 in the establishment and maintenance of epithelial polarization is clearly important in development, whether Cdc42 exerts such a role in tumor initiation and/or progression remains largely unknown. In the future, it will be important to determine the activity and expression levels of Cdc42 during tumor initiation, tumor maintenance, or tumor metastasis. Given the important role of Cdc42 in establishing and maintaining tissue/cell, it is fundamental to keep in mind that suppression of mitogenic signals that flow through the Cdc42 signaling axis may come at the price of disrupting cell polarity machinery, at least transiently. The considerations of these effects may impact our assessment of the feasibility, risk/benefit, and potential efficacy related to targeting Cdc42 in specific cancer types/stages. On the other hand, it will also be interesting to explore the possibility that targeting Cdc42 could synergize with existing chemotherapy for improved outcomes.

4. Inhibitors targeting Cdc42 signaling

The involvement of Rho GTPases signaling in various human diseases makes them strong candidates for pharmaceutical intervention [93]. While Rho GTPases have proven to be formidably difficult to target directly, small molecule inhibitors of Rho GTPase effectors have facilitated the understanding of the biology of this family of proteins and to block oncogenic effects. In the case of Cdc42 effectors, the small molecules PF-3758309 and FRAX597 have been used to block the oncogenic activity of PAK kinases in a variety of cancer models, including colon, breast and skin tumors [64, 65, 94–97]. Cdc42 activation has also been targeted by small molecules, though there are currently few compounds that are selective for this enzyme (Table 1). Secramine was the first small-molecule inhibitor for

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Cdc42 to be identified. This compound blocks membrane recruitment of prenylated Cdc42 in a RhoGDI1 dependent fashion [98]. However, the use of this inhibitor to interrogate Cdc42 signaling has some limitations. In terms of selectivity, sequestering RhoGDIs in a complex with Cdc42 by secramine could also affect the activation status of Rho and Rac, inducing off-target effects, and thus complicating our understanding about the specific roles of Cdc42 in complex cellular pathways. More recently, two novel small-molecule inhibitors of Cdc42 were identified in high throughput screens. ZCL278 is a chemical compound that targets the interaction of Cdc42 with the GEF Intersectin, and perhaps other Cdc42 GEFs. This molecule was reported to suppress Cdc42 actin-based cellular functions in Swiss 3T3 cells and to inhibit the migratory ability of PC-3 cells in a concentration-dependent manner with no cytotoxic effects [99]. Mechanistically, this molecule binds into the surface groove of Cdc42, which is critical for GEF binding. The structural analysis suggests that ZCL278 disrupts the Cdc42-GEF interaction as well as GTP/GDP binding, thereby preventing any further Cdc42 activation. A second Cdc42 small-molecule inhibitor, termed CID2950007, is a non-competitive allosteric inhibitor that selectively binds the guanine nucleotide associated Cdc42 and induces ligand dissociation. This compound binds to an allosteric pocket adjacent to the nucleotide binding site to promote nucleotide dissociation and thus would not be expected to interfere with GEF binding to Cdc42 switch regions [100]. These findings suggest that Cdc42 inhibitors might be clinically useful. However, we remain in the early stages of recognizing and exploiting the pharmacological and therapeutic potential of Cdc42 inhibitors. Whatever their ultimate clinical value, the development of Cdc42-selective small-molecule inhibitors would certainly accelerate our understanding of Cdc42 functions, and, when combined with other loss-of-function methods, will help us to further delineate and distinguish the unique functions of this GTPase.

5. Conclusion

Cdc42 signaling plays an important role in controlling cell proliferation, cell polarity, survival and invasion. Constitutive activation of Cdc42 regulated pathways is a common event in human cancer, usually resulting from molecular alterations in key signaling components of the Cdc42 cascade as opposed to mutations in Cdc42 itself. There is accumulating evidence indicating that the function and signaling pathways regulated by Cdc42 are tissue- and cell type-specific, and that the general principles of Cdc42 function defined by *in vitro* methods or from one tissue cell type may not apply to another cell type *in vivo*. Future mouse genetic studies of Cdc42, combined with specific regulator and effector knock-out studies, will be useful to better understand specific signaling pathways regulated by Cdc42.

6. Expert opinion

It is clear that Cdc42 signaling plays an important role in the pathogenesis and progression of a wide array of human cancers. Efforts to block Cdc42 activity directly are ongoing, based on structural considerations and on a better understanding of Cdc42 processing and cellular localization. However, there has been limited success in GTPase inhibitor discovery. Prior Cdc42 inhibitors either lack selectivity and act on multiple GTPases or only target particular protein-protein interactions. The function of Cdc42 is controlled by multiple upstream regulators and mediated by multiple downstream effectors. Blocking one set of interactions may be useful in some scenarios; however, the protein could still remain active by interacting with other partners, allowing bypass of inhibition.

For the present, however, it has proven simpler to target downstream effectors of Cdc42, as a rich pipeline of drugs that act on proteins in the MAPK and PI3K pathways are already in clinical development. In parallel, new ways of identifying critical effectors of Cdc42 for

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malignant transformation are being evaluated. While Raf-MEK-ERK and PI3K represent the best-characterized pathways used during tumor development, several additional Cdc42 effectors have been implicated in different types of human cancer. For example, in xenograft studies, the administration of a PLD1 small-molecule inhibitor was shown to prevent *in situ* vascularization and tumor growth, phenocopying the lack of tumor growth observed in *Pld* $^{-/-}$ mice [77]. Similarly, the administration of PAK kinases small-molecule inhibitors in a variety of cancer models has also been shown to suppress tumor growth and oncogenic signaling [64, 65, 95, 97]. Finally, an ACK1 small-molecule inhibitor has been shown to suppress ACK1 activation and abrogate transcriptional activity of the androgen receptor in prostate cancer cells [78]. Taken together, each of these effectors represents a potential drug target for Cdc42-driven tumors, given that deletion and/or pharmacological inhibition of each leads to impaired tumorigenesis in different cancer models. The true promise of each of these effectors, however, will have to be further explored using additional cancer models to define the extent to which generalizations can be made about the usage and requirements of these effector pathways.

Targeting Cdc42 itself or specific Cdc42 effector pathways might be useful in tumors bearing mutations in *Ras* genes, as mouse models suggest a role of Cdc42 in Ras-driven tumors *in vivo*. Deletion of Cdc42 in H-Ras transformed cells demonstrated a role for this GTPase in the development of H-Ras driven cancers based on an observed decrease in hyperproliferation in cells lacking Cdc42 [20, 23]. Additionally, in this system, loss of Cdc42 in non-transformed cells has a very modest effect in terms of proliferation but is required in the context of activated Ras, defining a synthetic lethal interaction in these cells. While Cdc42 may be important for the growth of Ras-driven tumors, the exact role and mechanism of activation in these cells remain to be fully elucidated, as several pathways can lead to Cdc42 activation, which in turn can drive a number of downstream cellular behaviors. These data support a critical role for Cdc42 in Ras-driven tumorigenesis, providing another member of a growing list of potential sites for therapeutic intervention and warranting further investigation of this pathway in Ras mutant cells.

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* = of importance, ** = of considerable importance

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ARTICLE HIGHLIGHTS

- Cdc42 regulates several cellular processes like cell adhesion, migration, polarity and proliferation through the activation of downstream effectors.
- Deregulation of Cdc42 frequently occurs in different types of human cancers as the result of molecular alterations in the genes encoding Cdc42 regulatory proteins and/or downstream effectors, as well as for its constitutive activation mediated by mutations or amplification of cell surface receptors.
- Depending of the cellular context, Cdc42 is capable of functioning as either an oncogene or a tumor suppressor.
- Targeting Cdc42 might result beneficial in specific cancer types/stages where conventional treatment is not effective.

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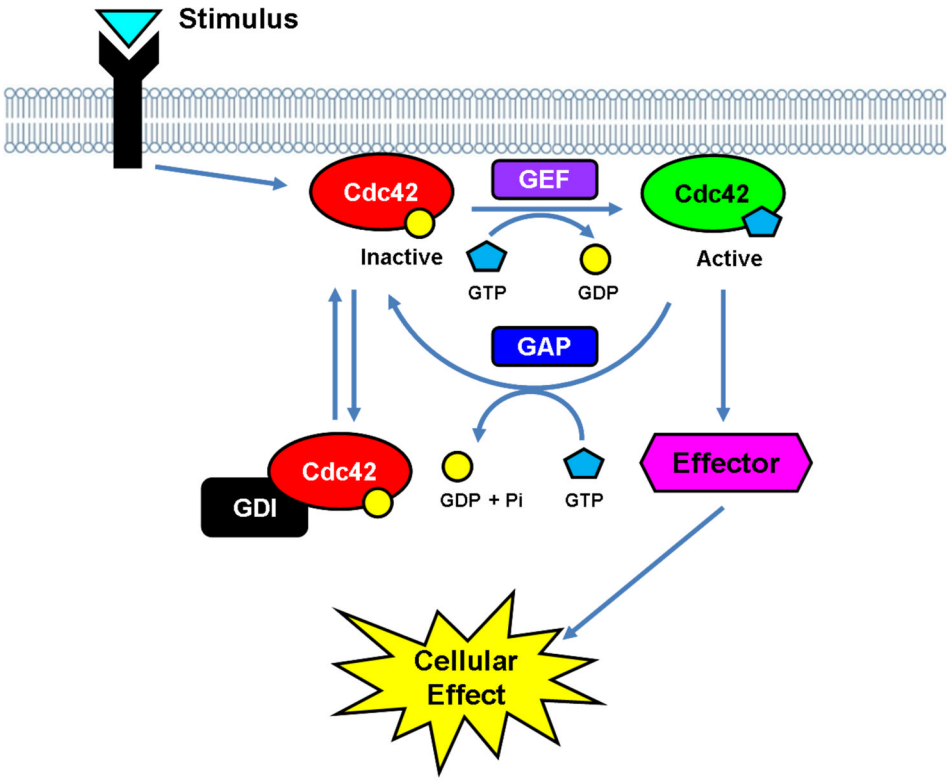

Figure 1. Regulation of Cdc42

Cdc42 cycles between the GDP-bound inactive state and the GTP-bound active state. The GTP binding and GTP hydrolysis cycle is tightly regulated at specific intracellular locations by GEFs, GAPs, and GDIs. Upon activation by various stimuli, activated Cdc42 can transiently interact with multiple effector proteins to transduce signals that impact on cell functions.

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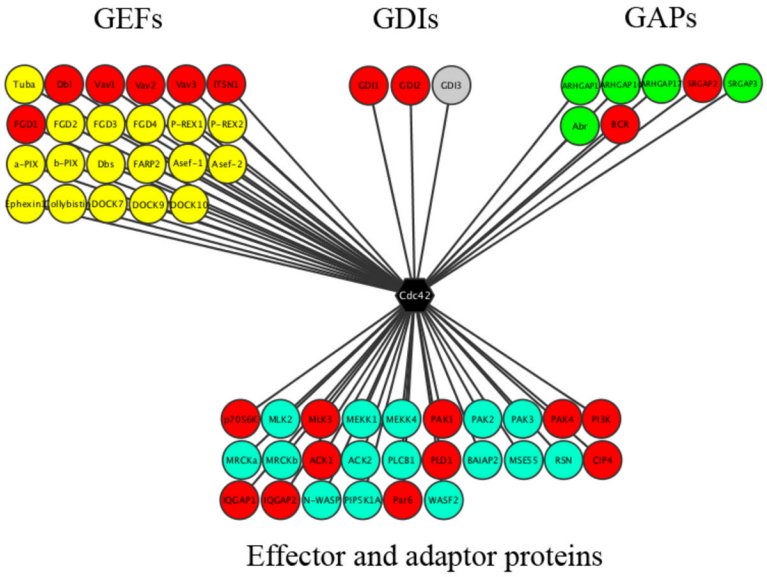

Figure 2. Cdc42 Regulators and Effectors

Specific Cdc42 GEFs, GAPs, GDIs and downstream effectors are represented in yellow, green, gray and blue respectively. All the Cdc42 interactors involved in cellular transformation are highlighted in red.

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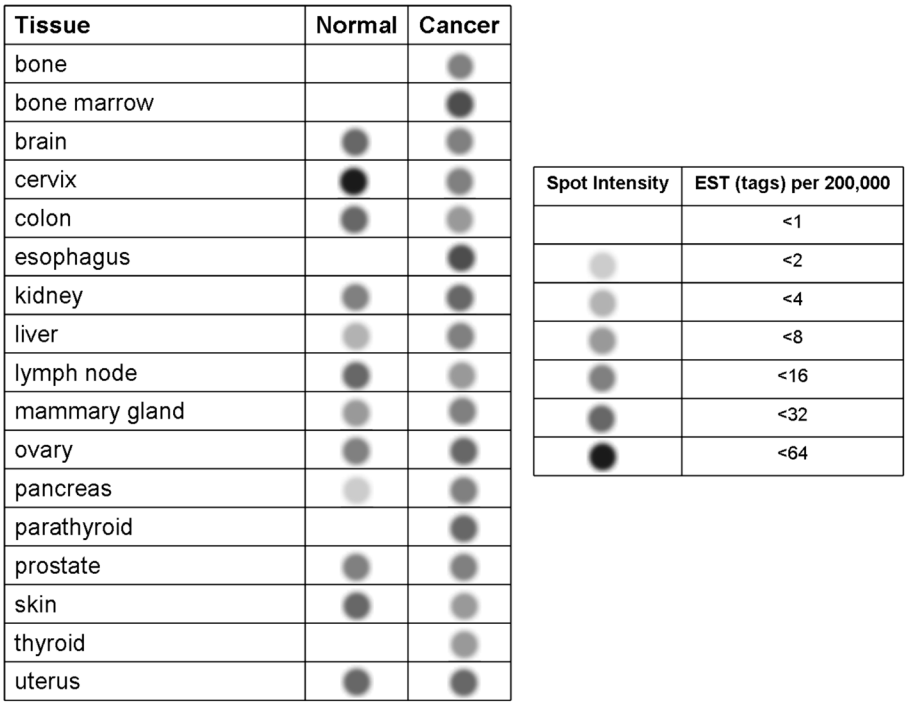

Figure 3. Cdc42 Expression in Normal and Cancerous Tissues

The expression of Cdc42 was analyzed by SAGE (serial analysis of gene expression). These data illustrate the distribution of Cdc42 in a wide variety of normal and cancerous human tissues. Raw data was obtained from the SAGEmap website [40]. EST, expressed sequence tag.

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Table 1

Cdc42-Selective Small Molecule Inhibitors.

Inhibitor	Mode of Action	Reference
Secramine A	Blocks membrane recruitment of prenylated Cdc42 in a RhoGDI1 dependent fashion	[98]
ZCL278	Targets Cdc42–GEF interactions and inhibits Cdc42-mediated cellular processes	[99]
CID29950007	Allosteric inhibitor that binds the guanine nucleotide associated Cdc42 and induces ligand dissociation	[100]