Small-Molecule Intramimics of Formin Autoinhibition: A New Strategy to Target the Cytoskeletal Remodeling Machinery in Cancer Cells

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Abstract

Although the cancer cell cytoskeleton is a clinically validated target, few new strategies have emerged for selectively targeting cell division by modulating the cytoskeletal structure, particularly ways that could avoid the cardiotoxic and neurotoxic effects of current agents such as taxanes. We address this gap by describing a novel class of small-molecule agonists of the mammalian Diaphanous (mDia)-related formins, which act downstream of Rho GTPases to assemble actin filaments, and their organization with microfilaments to establish and maintain cell polarity during migration and asymmetric division. GTP-bound Rho activates mDia family members by disrupting the interaction between the DID and DAD autoregulatory domains, which releases the FH2 domain to modulate actin and microtubule dynamics. In screening for DID–DAD disruptors that activate mDia, we identified two molecules called intramimics (IMM-01 and -02) that were sufficient to trigger actin assembly and microtubule stabilization, serum response factor-mediated gene expression, cell-cycle arrest, and apoptosis. In vivo analysis of IMM-01 and -02 established their ability to slow tumor growth in a mouse xenograft model of colon cancer. Taken together, our work establishes the use of intramimics and mDia-related formins as a new general strategy for therapeutic targeting of the cytoskeletal remodeling machinery of cancer cells. Cancer Res; 73(22); 6793–803. © 2013 AACR.

Introduction

The mammalian Diaphanous-related (mDia) formin family of Rho-effector proteins generates linear actin filaments (F-actin) and modulates microtubule dynamics to support the establishment and maintenance of polarity in cells (1). These structural changes occur in response to demands during developmental and immunologic processes (2). Defects in formin genes are associated with an array of human diseases including inherited deafness, autism, and kidney disease (3, 4).

In cancer, tumor cells may rely upon formins to generate invasive structures during metastasis (5). However, although metastatic cells may rely upon formins for migration, there is genetic, cellular, and pharmacologic evidence that argues for a role for formins in tumor suppression (6). Consistent with such a role, knockout of the formin mDia1 in mice leads to a myelodysplastic, preleukemic phenotype (7), and suppression of the DIAPH3 gene for mDia2 is associated with prostate neoplasia (8). Expression of a dominant interfering variant of its effector mDia1 accelerates tumor growth and impairs response to the farnesyl transferase inhibitor tipifarnib (6), which relies on the small GTP-binding protein RhoB for growth inhibition and apoptosis (9). Tipifarnib has had limited clinical success, yet it remains in development for treatment of a variety of cancers (10).

Informed by genetic and molecular studies of Rho and formin signaling to the cytoskeleton and the nucleus, we hypothesized that pharmacologic activation of formins would impair malignant progression. In addition, activation of formins would stabilize microtubule (11) and actin dynamics (12) as a means to target tumor cell proliferation. Taxol binds directly to tubulin subunits to potently stabilize microtubules and is an effective therapeutic agent (13). As a result of formin activation, cell growth and metastatic processes would be impaired, interfering with the normal cytoskeletal pliancy that cancer cells rely on. To test this hypothesis, we screened for, and identified, two chemically similar molecules that block the Rho-controlled intramolecular autoinhibitory mechanism (12, 14–16). We then tested their potential as anticancer agents.

Materials and Methods

High-throughput DID–DAD binding screen

The fluorescence anisotropy assay measures changes in polarization due to the binding of fluorescein isothiocyanate (FITC)-labeled DAD peptide—T-G-V-M-D-S-L-L-E-A-L-Q-S-G-A-A-F-R-D-Y-G-R-K-K-R-Q-R-R-R—derived from the peptide sequence of mDia2 (12)—to purified recombinant mDia2 DAD protein. We developed the assay to determine how structural differences affect DAD–DID binding (17).
For high-throughput screening, the assay buffer contained 25 mmol/L HEPES, pH 7.2 (Sigma-Aldrich), NaCl 100 mmol/L, MgCl2 10 mmol/L, and CHAPS 5 mmol/L. Assays were run in black Matrical MP101, polypropylene 384-well microtiter plates. A solution of DID protein (200 nmol/L, 2× final concentration) and fluorescein-labeled DAD peptide (200 nmol/L from a 1 mmol/L stock solution) was prepared in assay buffer. Unlabeled DAD peptide was diluted from a 1 mmol/L stock solution to 50 μmol/L (10× final concentration) in assay buffer.

The assay for high-throughput screening using the Michigan High-Throughput Screening Center (MHTSC) chemical library (18) was as follows. Assay buffer (14.5 μL) was dispensed into each well of a 384-well plate. Using the TiMo head on the Tecan Freedom EVO robot, 0.5 μL of 1 mmol/L test compounds was dispensed to each assay well and mixed. The final concentration of test compounds was 17 μmol/L and the final dimethyl sulfoxide (DMSO) concentration was 1.7%. Uninhibited control wells (44 per plate) received 0.5 μL of DMSO from compound plates. Fully inhibited control wells (four per plate) received 3 μL of 10-fold excess unlabelled DAD peptide. All wells received 15 μL of the mDia DID protein/fluorescein–DAD peptide solution. The plates were incubated at room temperature for 30 minutes and then polarization was measured using a BMG Pherastar reader.

**In silico molecular modeling**

The Internal Coordinate Mechanics (ICM) program (18) was used for protein and ligand preparation. Formin DID structures (2F31.PDB #88037; ref. 19) were retrieved from www.rcsb.org and manipulated with the ICM program. The molecular structures of IMM-01, -02, and -03 were constructed in the ICM program. The molecular modeling was implemented in the ICM program. The binding surfaces were generated using the Molecular Probes/Invitrogen.

**Cell culture and nuclear microinjection**

Cells were maintained in 0.05% fetal calf serum (FCS) 18 hours before microinjection or drug treatment. Alternatively, serum response element (SRE)-lacz or 3DA:FosHA NIH 3T3 reporter cell lines (21) were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Invitrogen) supplemented with 10% (v/v) FCS and 100 ng/mL G418. The serum response element SRE-LacZ expresses β-galactosidase under the control of three tandem SREs (21). The 3DA:Fos reporter contains the c-Fos gene under the control of three SREs (3DA) and a hemagglutinin (HA) epitope tag inserted into the fourth exon (22). SW480 colon carcinoma cells were maintained in DMEM supplemented with 10% (v/v) FCS. Drug treatments were conducted in appropriate medium with vehicle (DMSO) controls.

**Antibodies, plasmids, and other reagents**

Antibodies used in this study include anti-HA (clone 12CA5), generated at the Van Andel Research Institute Monoclonal Antibody core facility; anti-Egr1 (Cell Signaling Technology); and anti-β-tubulin (clone E7) from the Developmental Studies Hybridoma Bank. The rabbit anti-mDia2 (1358; ref. 23) was raised against the mDia2 FH2 domain generated in bacteria.

The mouse anti-mDia1 (p140mDia1) was obtained from BD Biosciences. The anti-glutubulin (glu-tub) was a generous gift from G. Gunderson (Columbia University); anti-glut-tub recognizes tubulin after posttranslational modification to expose a Glu residue at the C-terminus (24). The rabbit anti-caspase-3 (cleaved) antibody was from Cell Signaling Technology (Asp175, catalog #9664). Annexin V-FITC was from BD-Phar- mingen (catalog #556420). The anti-rabbit and anti-mouse antibodies conjugated to horseradish peroxidase were from GE Healthcare/Amersham.

**Plasmids**

Plasmids for the expression of EGFP-DAD, DAD-M1041A, and mDia1-FH2-ΔN were described previously (12, 17, 25). Cytochalasin D (cytD) and latrunculin B (LatB) were purchased from Calbiochem. Intramimics (IIM-01 and -02) were resuspended in DMSO.

**Microinjection, microscopy, Western blots, and reporter assays**

Egr1, glu-tub, and FosHA expression was monitored by indirect immunofluorescence, as described previously (11, 21). FosHA expression was quantified as the percentage of HA-positive nuclei in GFP- or yellow fluorescent protein-expressing cells. β-Galactosidase (β-gal) activity was measured using the proprietary Pierce β-Galactosidase Assay Reagent (Pierce). In brief, 1 × 10^5 cells per well were serum starved (0.05% FCS) for 14 hours in 12-well plates before stimulation with agent. Three hours after stimulation, the medium was removed and replaced with 300 μL of β-gal reagent. Plates were incubated at 37°C. β-Gal reactions were terminated after 30 minutes with 100 μL of β-Galactosidase Assay Stop Solution (Pierce). Absorbance at 405 nm was determined using an Envision 2104 plate reader (PerkinElmer). Data were exported to GraphPad (Prism) for analysis and figure composition.

For SRE reporter microinjection assays, cells were maintained in 0.05% (v/v) FCS-supplemented DMEM for at least 14 hours before treatment or microinjection. In microinjection experiments, SRE-FosHA reporter cells were plated onto glass coverslips before maintenance in starvation medium. Cells nuclei were microinjected with the indicated expression plasmids (50 ng/mL). Two hours after injection, cells were exposed to drug or vehicle (DMSO) for 2 hours before formaldehyde fixation. FosHA expression was detected by indirect immunofluorescence with 12CA5 monoclonal anti-HA and donkey Texas Red anti-mouse (Jackson). Immunoblots were run on 4% to 20% Tris–glycine gels (Invitrogen), transferred to a 0.45-μm polyvinylidene difluoride membrane (PVDF; Invitrogen), and were blocked and probed in 5% BSA (Sigma-Aldrich). Rabbit anti-Egr1 was purchased from Transduction Laboratories.

**Bromodeoxyuridine incorporation**

Bromodeoxyuridine (BrdUrd) incorporation assays to monitor DNA synthesis were as described (26). In short, cells were
incubated with BrdUrd for the indicated times before fixation in 5% (v/v) acetic acid in ethanol or with 3.7% (v/v) formaldehyde in cells expressing EGFP-DAD fusion proteins (17). BrdUrd was identified by indirect immunofluorescence with monoclonal anti-BrdUrd (GE Healthcare) followed by Texas Red-conjugated donkey anti-mouse (Jackson Immunoresearch). DNA (nuclei) content was detected with Hoechst 33258 (included in the secondary step). BrdUrd positivity was determined from two to three separate experiments in which 20–30 BrdUrd-labelled nuclei were counted in three different fields.

**Flow cytometry**

Cells were treated with IMMs, DMSO (vehicle), or Taxol, detached in 10 mmol/L EDTA in PBS, and washed twice in Annexin-staining buffer (0.01 mol/L HEPES, pH 7.4, 0.14 mol/L NaCl, 2.5 mmol/L CaCl2). Cells were resuspended in Annexin-staining buffer with Annexin-V–FITC (as directed by the manufacturer). Ten thousand events were acquired on FACS-Calibur (Becton-Dickinson), and data were analyzed in the B-D CellQuest software.

**Tumor xenograft models and in vivo drug efficacy evaluation**

The Institutional Animal Care and Use Committee of the Van Andel Research Institute approved all animal studies. Athymic nude female mice 6 to 8 weeks of age were used. For xenograft tumors, subconfluent cells were harvested and resuspended in HEPES-buffered saline at 2.5 to 10 × 106 cells/mL. Each animal received a subcutaneous injection of 100 μL of resuspended cells into the right flank. Animal weight and tumor volume were monitored 3 times a week. Tumor dimensions were measured manually with calipers; tumor volume was calculated by volume (mm3) = length × width × depth.

When the tumor reached approximately 250 mm3, mice were randomly assigned into treatment groups (10 mice/group): vehicle, IMM-01 (5 mg/kg), IMM-01 (25 mg/kg), IMM-02 (5 mg/kg), or IMM-02 (25 mg/kg). Injections were intratumoral, intraperitoneal, or intravenous administered twice a week (unless otherwise indicated) until tumor volume reached 2,500 mm3. Tumors were excised, formalin-fixed, and paraffin-embedded. Sections (5 μm) were stained with hematoxylin and eosin (H&E) using the Ventana SYMPHONY (Roche) and imaged with a ×63 oil-immersion objective on a Nikon microscope.

**Results**

By exploiting our working knowledge of formin autoregulation (12, 17), we hypothesized that disrupting DID–DAD binding (shown schematically in Fig. 1A) would activate the mDia family of formins in cells. We adapted a fluorescence anisotropy assay (14) to a high-throughput format to test whether unlabeled DAD peptide competes with FITC-DAD (Fig. 1B). The resulting IC50 values (≤250 nmol/L) were consistent with those determined for both mDia1 or mDia2 DID and DAD (16, 17).

We screened 10,000 drug-like compounds from a representative chemical library (see Materials and Methods). The library was developed using methods of chemotypic coverage to select a chemically diverse compound set (18). The compounds were screened at a single concentration, and then active compounds were retested at multiple concentrations to identify dose-dependent inhibitors. Two structurally related carbothioamides with IC50 values less than 150 nmol/L were identified (Fig. 1C, inset): N1-[(tert-butyl)-2-(2,4-dihydroxybenzylidene)hydrazine-1-carbothioamide (IMM-01) and N1-[(tert-butyl)-2-[1-(3,5-difluoro-2-hydroxyphenyl)ethyliden]hydrazine-1-carbothioamide (IMM-02). IMM-01 and -02 inhibited DID–DAD binding with IC50 values of 140 and 99 nmol/L (Hill slope values of 0.75 and 0.76, respectively (Fig. 1C). A third structurally related molecule (Fig. 1C, inset), N1-[(tert-pentyl)-2-[2-(tert-butylthio)benzylidene]hydrazine-1-carbothioamide), did not have activity in the assay. Other carbothioamides are currently in clinical trials as anticancer agents (27), suggesting the IMMs might be bioavailable and, therefore, could be developed for use in the clinic.

In *silico* docking (16) of IMM-01 and -02 into the mDia1 DID structure (28, 19) suggested that these molecules occupy the space within the armadillo-repeat region (ARR; ref. 29) normally occupied by DAD (Fig. 1D and E). The two intramimics bind in similar but distinct orientations that could contribute to differential binding affinity and physiologic activity. The inactive IMM-03 (Fig. 1D, shown in red) did not align into the DAD pocket.

To examine IMM effects on cytoskeletal dynamics, NIH 3T3 cells previously maintained in low serum were used. Using fluorescent phalloidin to monitor F-actin and antibodies specific for stabilized, detyrosinated tubulin (24), we observed that IMM-01 induced filopodia-like structures similar to those observed in cells expressing constitutively active mDia1 or mDia2 (17, 30). Likewise, we observed microtubule stabilization consistent with formin activation in NIH 3T3 cells (Fig. 2A–C; refs. 11, 31, 32). Microtubule stabilization was confirmed by immunoblotting of NIH 3T3 whole-cell lysates (Fig. 2D). To test the impact on a Taxol-sensitive transformed tumor cell line, SW480 colon carcinoma cells were exposed to increasing concentrations of IMM-01 or -02. Exposure to 100 μmol/L IMM-01 for 1 hour induced microtubule stabilization at levels similar to those induced by Taxol (Fig. 2E); IMM-02 showed an equivocal effect. The difference in induction of stabilization between treated and untreated NIH 3T3 and SW480 cells is unclear; it may reflect fundamental differences in dynamic instability of transformed and nontransformed cells (31, 33), or alternatively, the nature of the affected target formin expressed in these two different cell types (34).

Rho GTPase/formin signaling is known to induce new gene expression via the MAL–SRF transcription factor axis that relates changes in actin dynamics (35). To test for SRF activation, we used untransformed NIH 3T3 reporter cell lines stably expressing SRF-sensitive SRE–controlled reporter genes (SRE-LacZ or SRE-FosHA reporter; ref. 21). Cells were exposed to agents for 2 hours before fixation and assay (Fig. 3A–C). FCS stimulation (15%) and 1 μmol/L cytoD were used as positive controls for sensitivity to actin dynamics; (cytoD impairs actin monomer binding to MAL, releasing...
MAL to the nucleus to activate SRF (36). Treatment with 10 µmol/L IMM-01 or -02 significantly induced LacZ expression, producing β-gal activity comparable with that from cytD treatment (Fig. 3A and B). LatB blocks actin monomer incorporation into filaments (37). Cotreatment of NIH 3T3-SRE-LacZ cells with 10 µmol/L of either IMM-01 or -02 plus 1 µmol/L LatB inhibited SRF induction, confirming that IMM-mediated SRF activation depended on F-actin assembly.

In parallel, SRE-FosHA reporter cells (38) were microinjected with a plasmid expressing a dominant interfering variant of mDia1 (FH2ΔN) (25) 2 hours before IMM exposure. EGFP-DAD and an inactive mutant (DAD-M1041A) were the
Figure 2. Intramimic exposure disrupts cytoskeleton organization. A–C, microtubule stabilization and actin rearrangements consistent with formin activation in NIH 3T3 fibroblasts exposed to 1 or 100 μmol/L of IMM-01 for 4 hours before fixation and staining with Alexafluor-phalloidin (blue), mouse anti-β-tubulin (green), and rabbit anti-glu (detyrosinated tubulin) tub antibodies (red). D, immunoblots of 100 μmol/L IMM- and 100 nmol/L Taxol-induced stabilization of microtubules in NIH 3T3 cells maintained in 0.05% FCS over time (1–4 hours). E, both intramimics and Taxol (at indicated concentrations) induced microtubule stabilization as detected by rabbit anti-glu-tubulin antibodies in SW480 cells growing in 10% FCS.
positive and negative controls, respectively, for activation of formins (Fig. 3C; ref. 12). FH2ΔN blocked IMM- and DAD-induced activation of SRF, showing that SRF activation by IMM-01 relied on activation of cellular formins.

We interrogated expression of a cellular SRF target, Egr1 (39), in serum-deprived SW480 cells exposed to IMM-01 or -02. Egr1 expression was monitored by indirect immunofluorescence. Consistent with SRF activation, both IMM-01 and -02 induced F-actin architecture relative to treatment with vehicle, serum, or cytoD. Treatment with 10 μmol/L IMM modestly increased F-actin content. F-actin accumulated at cell–cell junctions in cells treated with IMM-02, suggesting that formin activation reinforced those junctions. Whether this is due to direct induction of formin-mediated actin assembly or due to indirect induction of cell-structure genes is a topic of further investigation.

To study effects on cell-cycle progression, NIH 3T3 fibroblasts were exposed to IMM-01 or -02. IMM-01 inhibited induction of SRF activity by blocking F-actin assembly. IMM-02, shown in A using ONPG as a substrate instead of X-gal (see Materials and Methods). C, quantification of the percentage of FosHA-positive cells.

Figure 3. Intramimics activate SRF-regulated gene expression. A, 10 μmol/L IMM treatment significantly \( (P < 0.05) \) induced β-galactosidase activity from a stably transformed SRE-LacZ reporter gene; blue, precipitating X-Gal as a substrate in formaldehyde-fixed cells. Cotreatment with 1 μmol/L LatB inhibited induction of SRF activity by blocking F-actin assembly. B, quantification of β-galactosidase activity from the SRE-lacZ reporter gene from assays shown in A using ONPG as a substrate instead of X-gal (see Materials and Methods). C, quantification of the percentage of FosHA-positive cells.
had no effect. To ensure that IMMs were not affecting the cellular uptake of BrdUrd or inhibiting DNA synthesis per se, IMMs were added 10 hours after serum stimulation, and the cells were fixed and analyzed 16 hours poststimulation for BrdUrd-positive nuclei (Fig. 5A, gray bars). These data showed that, in nontransformed NIH 3T3 cells, the ability to affect progression into DNA synthesis occurred in the G1 phase.

To compare these results with those from tumor cells, SW480 colon carcinoma cells maintained in serum were exposed to IMM-01 for 22 hours; these cells do not uniformly arrest in G0 in low concentration serum. Unlike the nontransformed NIH 3T3 cells, there was little or no effect on BrdUrd incorporation (Fig. 5B). However, most cells became binucleate or multinucleate compared with cells exposed to vehicle. This result was consistent with persistent activation of formins by EGFP-DAD during later stages of the cell cycle prior to cytokinesis in NIH 3T3 cells (40).

We then returned to nontransformed NIH 3T3 fibroblasts and tested whether exposure to IMMs 16 hours after serum stimulation affected progression through G2 into mitosis and cytokinesis. Neither IMM (30 μmol/L) induced the expected binucleation in cells fixed and examined 24 hours after serum stimulation; the cells failed to divide, remained mononucleate, and took on the appearance shown in Fig. 2. The defect is likely from the loss of cytoskeletal dynamics due to inappropriate formin activation in the G2 phase before breakdown of the nuclear envelope. The specific impact on progression through G2 and mitosis is a topic of ongoing study. We then evaluated the impact of IMMs on cell survival by monitoring markers of programmed cell death.

First, we examined the effect of either IMM-01 or -02 on caspase-3 cleavage during induction of apoptosis in NIH 3T3 cells and SW480 cells, relative to Taxol or doxorubicin as positive controls (Fig. 6). In NIH 3T3 cells, 100 μmol/L of either IMM-01 or -02 induced caspase-3 cleavage at levels similar to, if not greater than, the effects of Taxol (Fig. 6A, top row). SW480 cells have high intrinsic or steady-state levels of caspase activity (Fig. 6A, bottom row, DMSO). At the most effective (100 μmol/L) concentration, it was difficult to detect cleaved caspase-3 because the cells detached from the tissue culture dish as they underwent apoptosis. To confirm apoptosis in all SW480 cells, including the nonadherent cells, all cells were harvested and stained with fluorescent Annexin-V to measure the exposure of membrane phosphatidylserine that occurs during the apoptotic process (Fig. 6B). The percentage of Annexin-V–positive cells for both IMMs at 100 μmol/L was similar to, if not greater than, the effects of Taxol (Fig. 6B, inset). Taken together, the impact of IMMs

Figure 4. Egr1 expression is induced in colon carcinoma cells by IMM treatment. SW480 cells were treated with vehicle, 15% FCS, 1 μmol/L cytoD, or increasing concentrations (1–100 μmol/L) of IMM-01 and -02 for up to 2 hours. A–C, increasing concentrations (1–100 μmol/L) of IMMs induced Egr1 expression as detected by indirect immunofluorescence (A) and Western blot analysis (B and C).
on growth and survival in nontransformed and transformed cells was clearly different. Future studies will address the mechanism of the cell-cycle block and determine tumor cell types sensitive to IMMs.

To test IMMs for antitumor effects in vivo, athymic nude mice were implanted with SW480 cells and treated with IMM or vehicle. IMMs slowed tumor growth in a dose-dependent manner when administered intravenously via the tail vein (Fig. 7). Intravenous IMM-01 at 5 mg/kg slowed the growth of three of 11 (27%) tumors and, at 25 mg/kg, of three of four (75%) tumors. H&E staining suggested that IMM-01 treatment disrupted the growth and organization of viable tumor cells (Fig. 7B). Results varied with IMM-01 administered via intratumoral or intraperitoneal injection. Interestingly, IMM-01 was more effective than IMM-02 in vivo, despite similar efficacies in cell-based assays.

Discussion

In this study, IMMs slowed tumor growth in an SW480 xenograft model, completely eliminating the tumor burden in a small number of animals. We suggest that relative to Taxol, IMMs would produce fewer deleterious side effects in the treatment of solid tumors and myeloid-derived cancers because they do not directly bind tubulin. Instead, they affect a component of the machinery, the formins, responsible for coordinating actin and microtubule dynamics (1). Further, it is...
Intramimics Target Diaphanous-Related Formin Autoregulation

Figure 7. Intramimics slow tumor growth in a subset of SW480 tumor xenografts. A, SW480 tumors were treated 2 (or 5) times a week via intravenous tail vein, intraperitoneal, or intratumoral injections of IMMs (5 or 25 mg/kg). Treatment began when tumors reached 100 mm³ (time = 0 week). Tumors that decreased in volume or slowed growth to more than 2 times the SD of the average size of vehicle-treated tumors were designated as responders. Tumor growth was normalized to pretreatment sizes. Tumor size and growth exhibited a high degree of variability. Intravenous injections of the IMMs slowed tumor growth in a subset of tumors (data not shown for intraperitoneal or intratumoral injections). IMM-01 at a dose of 25 mg/kg modestly suppressed tumor growth relative to vehicle treatment. Inset box-whisker plot shows normalized tumor volume for all tumors after 3 weeks of treatment; P-values were derived from a paired t test. B, H&E staining of sections from formaldehyde-fixed paraffin-embedded tumors excised from mice treated by tail-vein injection (3 weeks of treatment).

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Intramimics Target Diaphanous-Related Formin Autoregulation

Intramimics represent a novel therapeutic strategy, redirecting formin-directed cytoskeletal remodeling in disease affected by formin dysfunction (3). They will serve as leads for further therapeutic development. Recently, two independent studies (48, 49) identified and characterized small-molecule inhibitors of formins useful for studying formin function in various cellular contexts. Likewise, intramimics may be used as pharmacologic probes for studying Rho-formation signaling in other fundamental biologic processes that rely upon formins to guide the construction of cellular infrastructure (50). These studies provide the foundation for interrogating the role of the cytoskeleton in maintaining regulated cell growth and differentiation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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