Inhibition of Dynamin by Dynole 34-2 Induces Cell Death following Cytokinesis Failure in Cancer Cells

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Abstract
Inhibitors of mitotic proteins such as Aurora kinase and polo-like kinase have shown promise in preclinical or early clinical development for cancer treatment. We have reported that the MiTMAB class of dynamin small molecule inhibitors are new antimitotic agents with a novel mechanism of action, blocking cytokinesis. Here, we examined 5 of the most potent of a new series of dynamin GTPase inhibitors called dynoles. They all induced cytokinesis failure at the point of abscission, consistent with inhibition of dynamin while not affecting other cell cycle stages. All 5 dynoles inhibited cell proliferation (MTT and colony formation assays) in 11 cancer cell lines. The most potent GTPase inhibitor, dynole 34-2, also induced apoptosis, as revealed by cell blebbing, DNA fragmentation, and PARP cleavage. Cell death was induced specifically following cytokinesis failure, suggesting that dynole 34-2 selectively targets dividing cells. Dividing HeLa cells were more sensitive to the antiproliferative properties of all 5 dynoles compared with nondividing cells, and nontumorigenic fibroblasts were less sensitive to cell death induced by dynole 34-2. Thus, the dynoles are a second class of dynamin GTPase inhibitors, with dynole 34-2 as the lead compound, that are novel antimitotic compounds acting specifically at the abscission stage. Mol Cancer Ther; 10(9); 1–10. ©2011 AACR.

Introduction
Several small molecule inhibitors targeting mitotic proteins have shown promise in preclinical or early clinical development as cancer treatments (1). These mostly act at early mitotic stages, primarily disrupting metaphase–anaphase transition (and spindle assembly checkpoint; SAC) by targeting cyclin-dependent kinase, Aurora kinase, polo-like kinase (Plk), or kinesin spindle protein (1, 2). These new mitotic inhibitors prevent proliferation of most tumor cells in vitro and reduce tumor volume in vivo by inhibiting growth and/or triggering cell death following an aberrant mitotic event leading to aneuploidy (1, 2). Such compounds are expected to have a more favorable therapeutic window than currently used chemotherapeutic agents, for example, paclitaxel (1), as they would spare nondividing, nondifferentiating cells. The endocytic protein, dynamin II (dynII), may also be a novel mitotic target for development of selective anticancer drugs, because dynII exclusively functions during the abscission stage of cytokinesis and is not required for progression through any other cell cycle phase (3). Dynamin inhibitors tested to date exclusively block cytokinesis (3), a cell cycle stage not yet targeted for chemotherapeutic intervention.

We recently reported the anticancer properties of dynamin inhibitors within the long chain amines and ammonium salts series (MiTMAB; refs. 3–5). dynII is best known for its role in membrane trafficking processes, specifically in clathrin-mediated endocytosis (6–8). The MiTMABs inhibit dynamin-dependent receptor-mediated endocytosis (RME; refs. 5, 9). Consistent with a role for dynamin in cytokinesis (3, 6, 7, 10–14), the MiTMABs induce cytokinesis failure in synchronized HeLa cells, specifically blocking abscission (3). Unlike the Aurora kinase and Plk inhibitors, dynamin inhibitors tested so far do not affect progression through any other mitosis stage. Like Aurora kinases, Plk or kinesin spindle protein inhibitors (1), MiTMABs have antiproliferative and cytotoxicity properties that seem to be selective for cancer cells (3).

Several other small molecule inhibitors of dynamin have been developed by our group and others, including
dimeric tyrphostins (15), dynoles (16), iminodys (17), pthaladyns (18), and dynasore (19). These inhibit dynamin GTPase activity in vitro with a broad range of IC₅₀ values. The MiTMABs inhibit dynamin GTPase activity by targeting its pleckstrin homology domain, a module common to many proteins (4, 5). The dynoles are thought to bind an allosteric site in the GTPase domain (16). Thus, dynoles would be expected to be more efficacious and have less off-target effects than MiTMABs. We aimed to determine if the dynoles also have antiproliferative and cytotoxicity properties amenable for cancer treatment. We evaluated the 5 most potent dynoles for their effects on dividing cells. We show that the most potent inhibitor of dynamin-mediated RME (16) is also a potent inhibitor of cytokinesis in cells. Cell death follows cytokinesis failure in dynole 34-2–treated cells. The results strengthen the view that dynamin is a new therapeutic target for the possible treatment of cancer.

Materials and Methods

Cell culture

HeLa and H460 cells were maintained in RPMI 1640 medium supplemented with 10% FBS and 5% penicillin/streptomycin. Other cell lines were maintained in Dulbecco’s Modified Eagle’s Media supplemented with 10% FBS and 5% penicillin/streptomycin. All cells were grown at 37°C in a humified 5% CO₂ atmosphere. HeLa, HT29, SW480, MCF-7, A2780, H460, A431, DU145, BE2-C, and SMA-560 originated from the American Type Culture Collection. SJ-G2 was gifted to us from Dr. Mary Danks (St Jude Children’s Research Hospital, Memphis, TN). All cell lines are of human origin except for SMA-560, which is of murine origin. Cell line authentication was not carried out by the authors within the last 6 months.

Cell synchronization and treatment with dynamin inhibitors

The selective cyclin-dependent kinase 1 small molecule inhibitor, RO-3306 (9 μmol/L; 20), was used to accumulate cells at the G₂–M boundary and this was followed by RO-3306 washout to allow synchronous mitotic progression, as previously described (21). Immediately following RO-3306 removal (i.e., release from the G₂–M boundary), cells were treated with a dynamin inhibitor, drug-free medium, or 0.1% dimethyl sulfoxide (DMSO) vehicle.

Time-lapse microscopy analysis

The indicated dynamin inhibitor was added to G₂–M synchronized HeLa cells immediately following release from the RO-3306 synchronization block. Cells were viewed with an Olympus IX80 inverted microscope, and a time-lapse series was acquired using a fully motorized stage, ×10 objective, and Metamorph software by using the time-lapse modules. Temperature control was achieved by using the Incubator XL, providing a humified atmosphere with 5% CO₂. Imaging was carried out for 20 hours with a lapse time of 5 minutes.

Results

The dynoles induce multinucleation

Inhibition of dynamin by the MiTMABs results in cytokinesis failure generating polyploid cells (3). We sought to determine if the dynoles also produce phenotypes consistent with dynII inhibition in cytokinesis function by testing the 5 most potent dynoles, 25, 26, 33, 34-2, and 35, on dividing HeLa cells (Supplementary Fig. S1). The in vitro IC₅₀ value of the most potent analogue, dynole 34-2, is 6.9 ± 1.0 and 14.2 ± 7.7 μmol/L for full-length dynl and dynII GTPase activity, respectively (Supplementary Table S1). The in vitro assay conditions used for dynl and dynII used the same enzyme and substrate concentrations and thus could be compared as a selectivity ratio (IC₅₀ of dynII/dynI). Selectivity ratio for all dynoles was 0.9 to 3.3. We consider these ratios to indicate that the drugs are nonselective for either isoform as only a difference in vitro that well exceeds 10-fold (ideally 100-fold) selectivity may be biologically significant in vivo. Like the MiTMABs, dynoles are pan-dynamin inhibitors. The order of potency for these dynoles is slightly different for dynl versus dynII, however dynole 34-2 is the most potent for both. To test the effect of dynoles on cytokinesis, HeLa cells were synchronized at the G₂–M boundary; then immediately upon mitotic entry, cells were treated with the indicated Dynole for 6 hours (Fig. 1A). All 5 dynoles, 25, 26, 33, 34-2, and 35, increased the percentage of multinucleated HeLa cells (Fig. 1B). As found for dynamin GTPase activity, dynole 34-2 was the most effective at inducing multinucleation (Fig. 1B). The effect was in the same order of magnitude as MiTMAB at the same concentration. The percentage of multinucleated cells induced by each dynole is likely an underestimation of their effect on cytokinesis, as not every cell would have entered mitosis during the treatment period. We conclude that the dynoles cause cytokinesis failure.

Dynoles cause cytokinesis failure at abscission

The role of dynII in cytokinesis seems to be restricted to the abscission phase (3, 12). Depletion of dynII by short interfering RNA and MiTMAB treatment results in cells spending a prolonged period of time connected via an intracellular bridge (ICB) before completion of mitosis or formation of a multinucleated cell (3). To characterize the point of mitotic failure induced by the dynoles, we calculated the time cells took to undergo (i) prophase (Pro) to metaphase (Met), (ii) Met to anaphase (Ana), (iii) Ana to full membrane ingression (Ing), and (iv) Ing to either generation of 2 daughter cells (Comp) or a multinucleated cell (Multi). Majority of DMSO-treated cells that entered mitosis completed it within 90 to 150 minutes (Fig. 1C). Cells treated with dynole 25, 26, and 33 spent a significantly longer period of time in mitosis (dynole
Dynoles cause cytokinesis failure by blocking completion of abscission. A, schematic of experimental design. G2–M synchronized cells (RO-3306) were released into either drug-free medium or medium containing dynamin inhibitors and analyzed at 6 hours for multinucleation or over 20-hour period by using time-lapse microscopy. B, synchronized HeLa cells were treated with each dynole (10 μmol/L) or MiTMAB (10 μmol/L) as described in A. At 6 hours, cells were fixed, stained for α-tubulin, and the percentage of cells that were multinucleated were scored by using immunofluorescence microscopy. Bar graph is mean ± SEM, n = 3. C–F, synchronized HeLa cells were treated with vehicle (0.1% DMSO) or each dynole (10 μmol/L) as described in A, then visualized by time-lapse microscopy. Cells entering mitosis were scored for the time taken to progress through mitosis from prophase (Pro) to completion of mitosis (Comp) or generation of a binucleated cell (Multi; G). Cells were also scored for the time taken to progress through the following critical mitotic phases: metaphase (Met) to anaphase (Ana; D), Ana to full membrane ingression (Ing; E), and Ing to Comp or Multi (F). *, P < 0.05, Student’s t test.

G and H, representative time-lapse images of HeLa cells treated with DMSO and 10 μmol/L Dynole 34-2 undergoing mitosis. DMSO-treated cells (G) completed abscission generating 2 independent cells. Dynole 34-2–treated cells (H) failed cytokinesis by failing to abscise the ICB. The cleavage furrow regressed, forming a binucleated cell. Percentage of cells that entered mitosis and failed cytokinesis are shown below.

treatment median time >135 minutes vs. 102.5 minutes in DMSO-treated cells; Fig. 1C). Dynole-treated cells rounded up upon mitotic entry, aligned and segregated their chromosomes (Fig. 1D), and produced a cleavage furrow that generated an ICB (Fig. 1E) with similar kinetics to vehicle-treated control cells. The point of delay was identified as the abscission stage (Ing-Comp or Multi), with dynole 25-, 26-, and 33-treated cells spending approximately twice as long in this phase (median 32.5 minutes in DMSO vs. median of more than 60 minutes in dynole-treated cells; Fig. 1F). This is consistent with dynII inhibition (3). In contrast, treatment with dynole 34-2 or 35 completed each mitotic transition with similar kinetics to control cells (Fig. 1C–H). Despite this, time-lapse microscopy indicated that the latter 2 dynoles also caused cytokinesis failure at the point of abscission, resulting in binucleation (Fig. 1H). Dynole 34-2 was the most potent for cytokinesis failure, with 81.8% of cells that entered...
mitosis becoming multinucleated (Fig. 1H). Unlike treatment with the other dynoles, dynole 34-2- and dynole 35-treated cells did not stay connected via an ICB for a prolonged period of time. Instead the membrane rapidly regressed, generating a binucleated cell (Fig. 1H). We conclude that the dynoles cause cytokinesis failure by blocking abscission, which is consistent with inhibition of dynII.

**Dynoles disrupt midbody localization of calcineurin but not phospho-dynII and γ-tubulin**

The abscission phase involves formation of a central γ-tubulin midbody ring (MR) within the ICB before abscission. Two flanking midbody rings (FMR) reside on either side of the centrally located MR and contain calcineurin (CaN) and dynII phosphorylated at S764 (10). Ca\(^{2+}\) influx at the midbody activates CaN to dephosphorylate dynII, subsequently triggering abscission (10, 22). CaN inhibition results in abscission failure and multinucleation (10). Dynamin inhibitor treatment did not affect MR localization of γ-tubulin (Supplementary Fig. S2A and shown in red in Supplementary Fig. S2B row 1; ref. 3). All 5 dynoles and MiTMAB disrupted FMR localization of CaN with less than 30% of cells displaying the correct ring localization (Supplementary Fig. S2 and shown in green in Supplementary Fig. S2B row 3). Treatment with MiTMAB, but not the dynoles, caused disruption of phospho-dynII at the FMRs (Supplementary Fig. S2 and shown in green in Supplementary Fig. S2B row 2). Thus, dynoles selectively disrupt correct subcellular localization of CaN but not that of phospho-dynII or γ-tubulin at the midbody.

**Dynoles inhibit growth in a range of cancer cell lines**

We next assessed whether the dynoles can inhibit cell proliferation and induce cell death like MiTMABs (3). Cell growth was assayed using the MTT assay in 10 cancer cell lines derived from different tissues: SMA-560 (mouse glioma) and SJ-G2 (glial) HeLa (cervical), MCF-7 (breast), A2780 (ovary), H460 (lung), A431 (skin), DU145 (prostate), BE(2)-C (neuronal), and HT29 and SW480 (colon). Following 72 hours of continuous exposure to the dynoles, all cell lines showed a dose-dependent decline in cell growth (dynole 34-2 dose-response curve shown in Fig. 2A). The sensitivity to the Dynoles varied among the cell lines, with GI\(_{50}\) values ranging from 4.0 to 36.7 μmol/L (Table 1). In 11 of the 12 cell lines, Dynole 34-2 showed the lowest mean GI\(_{50}\) compared with the other 4 dynole compounds, whereas in A431 cells an equivalent level of inhibition to dynole 35 was observed (Table 1). A comparison of the GI\(_{50}\) values among the cell lines revealed that SMA-560, SJ-G2, SW480, MCF-7, and A2780 cells were as sensitive to dynole 34-2 as HeLa cells, whereas the other 6 cell lines were significantly less sensitive to dynole 34-2 (Table 1 and Fig. 2A). In SMA-560, SW480, and HeLa cells, at concentrations of 10 μmol/L or more, dynole 34-2 induced cell death as the response curves fell below zero, indicating that the viable cell number at the end of the experiment was less than at the start. At concentrations less than 10 μmol/L, growth inhibition is likely a combination of growth arrest and cell death. Prostate (DU-145) and skin (A431) cancer cells were least sensitive to dynole-mediated growth inhibition (Table 1 and Fig. 2A). A colony formation assay confirmed the antiproliferative effect of all 5 dynoles in SJ-G2 and HeLa cells, with a 50% reduction in colony formation at approximately 2 and 0.1 μmol/L of dynole 34-2, respectively (Fig. 2B and C). Thus, the dynoles prevent cell proliferation and reduce viability in a range of cancer cell lines.

**Dividing cancer cells are more sensitive to the dynoles**

The therapeutic potential of an antimitotic agent is because of its ability to inhibit growth or cause cell death following mitotic insult. We sought to confirm if the antiproliferative effect of the dynoles occurs following a failed mitotic division by scoring the total viable cell number by using trypan blue exclusion assay. Only 20 hours after release from G2-M block, the total viable HeLa and SMA-560 cells treated with the dynoles was markedly reduced in a dose-dependent manner (Fig. 2D). The total number of viable HeLa cells treated with 10 μmol/L dynole 33, 34-2, and 35 was less than the number of cells seeded (indicated by dashed line, Fig. 2D), indicating that these compounds not only blocked proliferation but also caused cell death. Dynole 34-2 was the most potent with no viable HeLa cells remaining at 10 μmol/L. Similar findings were observed in SMA-560 glioblastoma cells (Fig. 2E). These cells were slightly less sensitive than HeLa cells, and dynole 34-2 was also the most potent in these cells, blocking SMA-560 cell growth and inducing cell death at 10 μmol/L. Almost all cells treated with dynole 34-2 failed cytokinesis under the same treatment conditions (Fig. 1H).

To determine if cytotoxicity of the dynoles is selective for dividing cells, we carried out a lactate hydrogenase release assay to assess membrane integrity, indicative of cell viability. An acute treatment of 6 hours was used because only a small percentage of asynchronously growing HeLa cells would enter mitosis in this window, because their doubling rate is approximately 10 to 11 hours. In contrast, more than 60% of HeLa cells would progress through mitosis during the 6-hour treatment period following synchronization at G2-M boundary. All 5 dynoles were cytotoxic in G2-M synchronized HeLa cells (Fig. 2F) and evident at concentrations as low as 1 μmol/L with a dramatic increase in cytotoxicity observed at concentrations of 30 μmol/L or more. Dynole 34-2 was again the most potent compound causing cytotoxicity in more than 50% of cells at 30 μmol/L compared with less than 20% of cells treated with 30 μmol/L dynole 25 and 33. All dynoles were equally potent in asynchronous cells at concentrations of 30 μmol/L or more (Fig. 2G). However, at concentrations of 10 μmol/L or more; in contrast...
Figure 2. Dividing cancer cells are more sensitive to antiproliferative and cytotoxic effects of dynoles. A, cell proliferation was assessed by the MTT assay with increasing concentrations of Dynole 34-2 after 72 hours in 11 cancer cell lines. B and C, line graphs show the relative percentage of colonies present in HeLa (B) and SJ-G2 (C) cancer cell lines at day 7, after treatment with increasing concentrations of the dynoles. Values normalized to untreated controls. D and E, G2-M synchronized HeLa (D) and SMA-560 (E) cancer cells were treated with increasing concentrations of dynoles. Graphs (mean ± SD, n = 2) display the total number of viable cells present after 20 hours dynole exposure. F and G, lactate hydrogenase assay measured cytotoxicity in G2-M synchronized (F) and asynchronously (G) growing HeLa cells exposed to increasing concentrations of the dynoles and MITMAB for 6 hours. Graphs show mean ± SD, n = 2.
Table 1. Growth inhibition induced by the Dynoles in a range of human cancer cell lines

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<th>BE2-C</th>
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NOTE: Cells were treated with Dynoles for 72 hours followed by MTT assay. The table shows GI50 (half maximal growth inhibitory concentration) values (mean ± SEM) of 5 Dynoles, calculated from the MTT dose–response curves. n = 3. Comparison of each dynamin inhibitor with Dynole 34-2 within an individual cell line: aP < 0.05; bP < 0.01. Comparison of an individual dynamin inhibitor in different cell lines with HeLa cells: cP < 0.05, dP < 0.01.

Dynole 34-2 induces apoptosis following cytokinesis failure

The dynoles cause growth arrest/cell death and cytokinesis failure. We next tested the hypothesis that the dynoles induce growth arrest and/or cell death specifically following cytokinesis failure by using time-lapse microscopy to follow individual HeLa cells. Only dynole 34-2 (10 μmol/L) induced cell death specifically following cytokinesis failure within the 20-hour treatment period (Fig. 3A and B). Of those dynole 34-2 treated cells that failed cytokinesis, 97.3% ± 7.0% resulted in cell death (Fig. 3A). MiTMAB was also very effective at causing cell death (83.4% ± 1.6%), specifically following binucleation (3). In contrast, cell death occurred in less than 7% of HeLa cells that failed cytokinesis treated with the other dynoles, 25, 26, 33, and 35 (Fig. 3A). Instead, the resulting multinucleated cells remained viable for the duration of the experiment (up to 20 hours). HeLa cells have a doubling rate of approximately 10 to 11 hours, thus cells would be expected to enter a second mitotic division during the 20-hour experimental period if their growth was not arrested. Cells treated with dynoles 25, 26, 33, and 35 did not undergo a subsequent division and thus were considered growth arrested. DMSO-treated cells did not undergo cell death and were often observed entering a second cell division within the 20-hour treatment period.

Analysis of dynole-treated cells that successfully completed mitosis revealed that the 2 daughter cells remained viable during the 20-hour treatment period (Fig. 3A and B). In contrast, cell death was induced in approximately 40% of MiTMAB-treated cells that completed mitosis successfully (Fig. 3A). This is still significantly lower than the percentage of MiTMAB-treated cells that underwent cell death following cytokinesis failure. Dynole 34-2–induced cell death was confirmed by an increase in less than 2N DNA content by using fluorescence-activated cell sorting (FACS) analysis (Fig. 3C). A corresponding increase in 4N cells, indicative of multinucleated cells, was observed following 24-hour exposure to dynole 34-2 (Fig. 3D). Dynole 25 also caused an increase in the percentage of 4N cells (Fig. 3D). Consistent with the time-lapse data, no corresponding increase in less than 2N cells was observed following treatment with dynole 25 (Fig. 3C). Dynole 34-2–induced cell death was characterized by cell shrinkage, membrane blebbing (time-lapse microscopy), and DNA fragmentation (FACS analysis)—characteristics of apoptosis (Fig. 3B and C). Apoptosis was evident by an increase in PARP cleavage following dynole 34-2 and MiTMAB treatment and exposure to ultraviolet irradiation compared with treatment to the effect in G2–M synchronized cells, no cytotoxicity was evident in asynchronous cells (Fig. 2G). These findings indicate that the dynoles have antiproliferative properties that are relatively selective for dividing cancer cells at low concentrations, which are in line with their IC50 value for dynamin GTPase activity.
with DMSO (Fig. 3E and F). Collectively, these findings suggest that immediately following cytokinesis failure, dynole 25, 26, 33, and 35 inhibit cell proliferation, whereas dynole 34-2 causes cell death.

**Nontumorigenic fibroblasts are less sensitive to dynole 34-2**

The antiproliferative properties of the MiTMABs are relatively selective for cancer cells. We sought to determine the ability of dynole 34-2 to induce cell death and inhibit proliferation in nontumorigenic fibroblasts. NIH3T3 fibroblasts were compared with HeLa carcinoma cells. NIH3T3 cells do not synchronize with RO-3306 treatment (G2–M boundary) as effectively as HeLa cells and therefore experiments were carried out in asynchronously growing cells. Nevertheless, both cell lines have a similar doubling rate. Thus, a similar number of cells in both populations would be expected to proceed through mitosis within the same treatment period. An acute 6-hour treatment with dynole 34-2 resulted in cytotoxicity in more than 50% of HeLa cells and less than 5% of NIH3T3 cells (Fig. 4A). Assessment of cell proliferation by scoring for total viable cell number revealed that growth was inhibited in both cell types following a 20-hour exposure. The effect was reduced in the nontumorigenic fibroblasts (Fig. 4B and C). Cell death as indicated...
by a total loss in viable cells was evident after 24-hours exposure to 10 μmol/L dynole 34-2 in HeLa cells (Fig. 4B), but a significant number of NIH3T3 cells were viable following exposure up to 30 μmol/L (Fig. 4C). This difference was still evident following a prolonged 7 day exposure to dynole 34-2 (Fig. 4D and E). The difference between cancer and nontumorigenic cells was consistently observed among a panel of 3 cancer cell lines (HeLa, HT29, and SJ-G2) and 2 nontumorigenic fibroblasts (NIH3T3 and F1). Specifically, dynole 34-2 caused a concentration-dependent inhibition in cell proliferation in all cell lines (Fig. 4D), but in contrast to the cancer cells, a significant number of viable cells were present at the end of the 7-day treatment in both fibroblasts (Fig. 4E). Thus, nontumorigenic fibroblasts seem to be less sensitive to dynole 34-2 than cancer cells.

Discussion

The endocytic protein dynamin has been suggested to be a novel antimitotic drug target for the treatment of cancer because the MiTMAB dynamin inhibitors exclusively block the abscission phase of cytokinesis, inhibit cell proliferation, and reduce viability (3). The MiTMABs are a series of phospholipid-competitive inhibitors that target the pleckstrin homology domain of dynamin and potentially of other proteins (4, 5). Thus, they are subject to anticipated actions in addition to dynamin inhibition. We now show that the dynoles also have potent antimitotic effects that phenocopy the MiTMABs, causing cytokinesis failure at the abscission step, blocking cell proliferation, and reducing cell viability in multiple tumor cell lines from different tissue types. Dynoles block dynamin GTPase activity via a distinct mechanism to the MiTMABs, being uncompetitive with respect to GTP, thus they are proposed to bind an allosteric site within the GTPase domain (16). Among the dynoles tested here, the most potent compound, dynole 34-2, induced cell death exclusively following cytokinesis failure. Both non-dividing differentiated cells and nontumorigenic fibroblasts were less sensitive to cell death mediated by dynole 34-2. Thus, the dynoles have antiproliferative and cytotoxic properties that reduce cancer cell viability, while having reduced activity in noncancer cells. Our new findings with a second class of mechanistically distinct dynamin inhibitors strengthen the evidence that the
antimitotic action of dynamin inhibitors may be primarily because of targeting dynamin in cytokinesis. The MiTMABs and dynole dynamin inhibitors represent a new class of antimitotic compounds, specifically blocking abscission. The dynoles disrupt FMR localization of CaN, but not phospho-dynII. Such an action might conceivably prevent CaN-mediated dynII dephosphorylation at Ser-764, which is known to trigger abscission (10, 22). MiTMABs also disrupt FMR localization of CaN, and consistent with their ability to block dynamin’s phospholipid interaction (5, 23), FMR localization of phospho-dynII is also disrupted by these compounds. These phenotypes are quite distinct from that induced by other antimitotic compounds, which disrupt earlier stages of mitosis, causing chromosome misalignment leading to prolonged activation of the SAC (1, 24, 25), monopolar spindles (24–26), or inhibition of the SAC (27). Regardless of their mode of action, all of these antimitotic compounds, including dynamin inhibitors have anticancer properties.

The ability of antimitotic compounds to induce apoptosis exclusively in dividing cells is a highly desirable property for a potential cancer treating drug (27–30). MiTMAB induced cell death in dividing and nondividing cells, albeit to a lesser extent in nondividing cells. Thus, MiTMABs may have an additional off-target action, resulting in nonspecific cytotoxicity. In contrast, cell death was only observed following cell division in dynole 34-2–treated cells. Dynole 34-2–induced cell death was also selective for cancer cells as it had minimal cytotoxic effects on nontumorigenic proliferating cells, indicating that it has a favorable therapeutic window. The other four dynoles, 25, 26, 33, and 35, also induced cell death. However, their ability to do so was significantly reduced compared with dynole 34-2, only observed in assays that monitored cell death after several days, for example, MTT assay. This suggests that following cytokinesis failure, dynole 25, 26, 33, and 35 induce cell cycle arrest followed by cell death several days later, whereas dynole 34-2–treated cells immediately undergo cell death. Consistent with dynole 34-2 being the most effective at inducing cell death, it is also the most potent inhibitor of dynamin GTPase activity in vitro and RME in cells (16). However, the ability of dynole 34-2 to inhibit dynamin’s GTPase activity is not vastly different from the other four dynoles. It is possible that although all dynoles analyzed share the same cellular target, thus inducing cytokinesis failure and cell death, the dramatic increase in cytotoxicity and specific induction of cell death exclusively following cytokinesis failure caused by dynole 34-2 suggests that it has 2 mechanisms of action, one of which is shared with the other dynole compounds. The cellular response induced by other targeted antimitotic compounds is also varied and in part depends on cell line and/or inhibitor and/or assay (31, 32). Time spent blocked in mitosis (33), p53 status (34–36), and expression level of prosurvival Bcl-2 family members (37, 38) is also thought to contribute. It will be important to understand what drives a particular cellular response to a specific antimitotic agent to predict tumor response and patient outcome.

Our observations provide strong evidence that inhibition of dynamin is a new approach to cancer therapy, potentially reducing tumor volume in patients. The cytotoxicity and antiproliferative properties of MiTMABs (3) and dynoles in a broad array of cancer cell lines also suggest that dynamin inhibitors may be effective for the treatment of many tumor types. Dynole 34-2 is the lead compound in this class, because it possesses desirable anticancer properties and leads to cell death after failed cytokinesis. It is amenable to further drug development for the treatment of cancer. Our findings show that a second mechanistically distinct class of dynamin inhibitor exclusively blocks the abscission stage of cytokinesis and do not act on other points in mitosis, strengthening the idea that they primarily act by targeting dynamin GTPase activity. The dynoles show encouraging antimitotic properties and suggest that other dynamin inhibitors under development may also prove to be antimitotic.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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