



Whole cell microtubule analysis by flow cytometry

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ABSTRACT

Perturbation of the tubulin/microtubule dynamic in cells is perhaps the single most important mode of action of anticancer drugs. Standard methods for identifying and evaluating compounds for their ability to alter tubulin polymerization are low throughput, labor intensive, expensive, or make their assessment *in vitro*. Here we report a method to rapidly quantify the extent of tubulin polymerization in whole cells using flow cytometry, and we use this technique to evaluate compounds that stabilize and destabilize microtubule formation. This facile method is useful for conveniently, quantitatively, and cost-effectively comparing small molecules that perturb tubulin polymerization.

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Microtubules serve a vital function in controlling cell dynamics and trafficking, and they are central to the process of separating DNA into daughter cells during cell division. These essential roles in critical cellular functions have made microtubules arguably the single most important anticancer target for current chemotherapeutics [1]. Over the past half-century, numerous compounds that bind to this dynamic cellular structure have been developed; these compounds induce mitotic arrest and cell death. The first classes of molecules discovered to perturb the normal state of tubulin polymerization were the Vinca alkaloids in 1958 [2] and the taxanes in 1979 [3]. These compounds respectively destabilize and stabilize the dynamic tubulin structure, resulting in growth arrest in the M phase of the cell cycle. These “antimitotics” and their derivatives have transformed cancer treatment and inspired the discovery of a number of other classes of tubulin-binding compounds [4]. Indeed, 12 microtubule-binding anticancer agents across five structural classes are now approved by the US Food and Drug Administration [4].

Despite their utility, there is an increasing prevalence of resistance in the clinic to these tubulin-binding anticancer compounds, typically due to membrane efflux pumps, alterations in the microtubule structure, or mutations in the apoptotic pathway [4,5]. In addition, many antimitotics induce significant neurotoxicity, possibly due to higher levels of tubulin in neuronal cells [4]. A number of novel classes of tubulin-binding compounds have been developed in response to these deficiencies of existing molecules; however, they are still in the early stages of development [4].

Despite the obvious importance of antimitotics as anticancer agents, standard methods to assess the effect of compounds on tubulin polymerization are laborious, low throughput, and expensive. The most common assay for assessing tubulin polymerization *in vitro* uses purified bovine tubulin, which when polymerized in the presence of GTP shows an increase in turbidity at 340 nm [6–8]. This technique allows for a direct evaluation of the tubulin/microtubule dynamic without interference from microtubule-associated proteins (MAPs)¹ or other complicating factors. However, the cost of bovine tubulin used in this assay is prohibitive to the evaluation of the thousands of compounds in typical high-throughput screening. In addition, this technique is logistically difficult to perform simultaneously on large numbers of compounds [9], although improvements using fluorescence to monitor polymerization have simplified this process [10].

In contrast to the *in vitro* method, whole cell methods enable the evaluation of compounds in the more complex cellular environment. The effects of compounds on microtubule stabilization or destabilization in cells can be measured through confocal microscopy using a fluorescently labeled antibody to α -tubulin [11]. Although this method provides a qualitative measure of the extent of tubulin polymerization, it is difficult to quantify and sample preparation can be lengthy and low throughput [11–13]. More recent developments have enabled high-content microscopy techniques allowing the quantification of polymerized tubulin in a single cell; however, this approach can only evaluate a limited

¹ Abbreviations used: MAP, microtubule-associated protein; cAMP, cyclic AMP; TCRP, time-dependent cell response profiling; STLC, S-trityl-L-cysteine; PI, propidium iodide; FITC, fluorescein isothiocyanate; RNase A, ribonuclease A; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; TX-100, Triton X-100; PBS, phosphate-buffered saline; AbDil, Antibody Diluting Solution.

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region of the cell [14–16] and generates large complex data sets that can be challenging to analyze rapidly [17]. Microscopy techniques have been further optimized to evaluate tubulin dynamics in live cells [18]. Other whole cell methods have used indirect monitoring of mitosis-related factors, including response to dibutyryl-cAMP (cyclic AMP) [19] and phosphorylation of nucleolin [20,21]. Radioactive ligand binding to cells has also been used, but the cells must be lysed to analyze the tubulin by Western blot [22]. A recently reported method uses impedance-based time-dependent cell response profiling (TCRP) to identify novel microtubule destabilizers; however, this technique responds generally to mitotic arrest averaged across many cells rather than specifically probing interactions with microtubules or MAPs [23,24].

Here we present a novel flow cytometry-based technique for the analysis of tubulin polymerization using α -tubulin staining. This technique allows rapid and quantitative analysis of polymerized tubulin biomass, enabling facile comparison of compounds that affect tubulin polymerization, and an evaluation of the rate at which the tubulin dynamic is perturbed. In addition, the method is amenable to the rapid assessment of small molecules and, thus, could be used to identify novel compounds that affect tubulin polymerization dynamics.

Materials and methods

General reagents and methods

Taxol, colchicine, camptothecin, doxorubicin, epothilone A, S-trityl-L-cysteine (STLC), propidium iodide (PI), monoclonal anti- α -tubulin-FITC (fluorescein isothiocyanate) antibody, and ribonuclease A (RNase A, from bovine pancreas) were purchased from Sigma-Aldrich. Vincristine was purchased from Avachem Scientific. Glutaraldehyde was purchased from Ted Pella. Flow cytometry was performed on a BD Biosciences LSR II (San Jose, CA, USA), and the data were analyzed as described using FACSDiva software.

Cell culture information

Cells (U-937, human lymphoma and HeLa, human cervical cancer) were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 μ g/ml penicillin and 100 U/ml streptomycin). Cells were incubated at 37 °C in a 5% CO₂/95% humidity atmosphere.

Whole cell analysis of tubulin polymerization

For HeLa cells, the cells were plated (100,000 cells/ml in 1 ml medium) and allowed to adhere in a 12-well plate for a minimum of 4 h. Compounds were then added as dimethyl sulfoxide (DMSO) stocks (0.1–1% DMSO final). For U-937 cells, compounds were added as DMSO stocks (1% DMSO final) to a 24-well plate, followed by the addition of 0.5 ml of a cell dilution in fresh medium (500,000 cells/ml). In both cases, the cells were incubated for 18 h. After incubation, the cells were transferred to 2-ml tubes and pelleted (600g, 3 min). In the case of treated HeLa cells, the medium was removed by aspiration 15 min prior to the indicated timepoint, and cells were trypsinized for 15 min with 0.2 ml of 0.5% trypsin with ethylenediaminetetraacetic acid (EDTA) prior to pelleting. The medium was removed via aspiration, and the cells were fixed by adding 1 ml of 0.5% glutaraldehyde in Microtubule Stabilizing Buffer (MTSB: 80 mM Pipes [pH 6.8], 1 mM MgCl₂, 5 mM EDTA, and 0.5% Triton X-100 [TX-100]). Following a 10-min incubation at room temperature, the glutaraldehyde was quenched by the addition of 0.7 ml of 1 mg/ml NaBH₄ in phos-

phate-buffered saline (PBS). The cells were pelleted (1000g, 7 min), and the supernatant was removed by gentle aspiration. The cells were resuspended in 20 μ l of 50 μ g/ml RNase A in Antibody Diluting Solution (AbDil: PBS [pH 7.4], 0.2% TX-100, 2% bovine serum albumin [BSA], and 0.1% NaN₃) and incubated overnight at 4 °C. Prior to reading, 5 μ l of a 1:50 dilution of anti- α -tubulin-FITC antibody in AbDil was added to each sample to achieve a final dilution of 1:250, and the cells were incubated in the dark for 3 h. The samples were further diluted in 200 μ l of 50 μ g/ml PI in PBS, transferred to flow tubes, and analyzed by flow cytometry. To ensure that the HeLa cells were in suspension as single cells, the samples were vortexed prior to analysis. Whole cells were identified based on their forward scatter (FSC) and side scatter (SSC) traces as well as PI staining (single cells based on pulse width and height). Cells in the G2/M phase were identified based on the 4N PI stain. Tubulin levels were based on the geometric mean of the antibody-FITC fluorescence and were normalized to a value of 100 for the vehicle control.

Time course for whole cell analysis of tubulin polymerization

To adapt this procedure to a time course format, U-937 cells were plated and treated as described above. Cells were treated for 1–8 h (colchicine and STLC), 0–8 h (vincristine), or 0–60 min and 7 h (taxol and epothilone A). The initial timepoint at 0 min corresponds to pelleting of the cells immediately after compound addition, followed by fixation, which corresponds to a 3-min incubation time. For all other timepoints, following the appropriate incubation, cells were transferred to 2-ml tubes and pelleted (800g, 2 min). Following medium removal by aspiration, the samples were processed as described above.

High-throughput screening using whole cell analysis of tubulin polymerization

To adapt this procedure to a 96-well plate format, U-937 cells were plated and treated as described above with two modifications. Following fixation, the cells were resuspended in 25 μ l of 250 μ g/ml RNase A in AbDil with a 1:250 dilution of anti- α -tubulin-FITC antibody and incubated at room temperature in the dark for 3.5 h. The samples were further diluted in 200 μ l of 50 μ g/ml PI in PBS and then transferred to a 96-well plate. The plate was analyzed by flow cytometry on a BD Biosciences LSR II, which was equipped with an autosampler.

Statistical analysis

P values were calculated relative to the vehicle control using a two-tailed Student's *t* test. A *P* value of less than 0.05 or 0.01 was considered as significant. IC₅₀ values are reported as averages \pm standard errors.

Results

Whole cell assay for assessment of tubulin polymerization: overview

The dual-stain flow cytometry method to rapidly assay tubulin polymerization in whole cells is shown schematically in Fig. 1. Cells are incubated with compound for 18 h, tubulin monomers are washed out of the cells using a mild lysis buffer [25,26], and cells are treated with glutaraldehyde, a fixative known to preserve the microtubule network in cells [27]. After quenching the glutaraldehyde, samples are incubated overnight in the presence of ribonuclease A (to cleave cellular RNA) and sequentially exposed to a FITC-labeled tubulin antibody and PI (a nuclear stain); samples

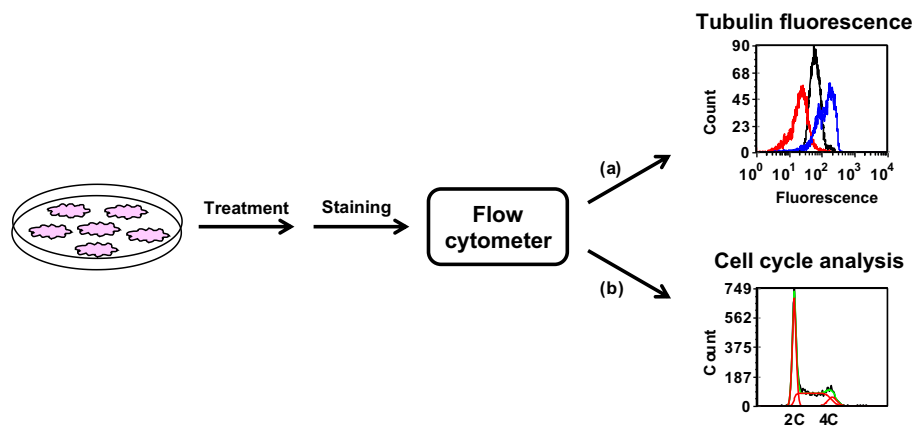


Fig. 1. Schematic for assessing tubulin polymerization by flow cytometry. Following compound treatment, cells are stained and analyzed by flow cytometry to simultaneously detect changes in tubulin fluorescence with a destabilizer (red) or a stabilizer (blue) relative to the control (black) (a) and cell cycle progression (b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

are then analyzed by flow cytometry. Compounds that destabilize tubulin polymerization are expected to show a decrease in fluorescence relative to the control, whereas microtubule stabilizers should show an increase in fluorescence, consistent with the change in concentration of tubulin biomass in the polymerized microtubules.

Whole cell analysis of tubulin polymerization

A panel of six well-characterized cytotoxic compounds was evaluated in the whole cell tubulin polymerization assay described above: compounds that destabilize microtubules (colchicine and vincristine), compounds that stabilize microtubules (taxol and epothilone A [28]), and compounds that do not affect tubulin but cause cell cycle arrest (camptothecin, S-phase arrest [29]; and doxorubicin, G2-phase arrest [30]). Analysis of these compounds in HeLa cells (human cervical cancer cell line) using the flow cytometry method depicted in Fig. 1 allows a rapid and quantitative analysis of tubulin polymerization levels, with concurrent cell cycle analysis (to identify compounds that cause mitotic arrest in addition to perturbing tubulin dynamics). As shown in Fig. 2A, all compounds arrested HeLa cells in the expected phase of the cell cycle. The results of the whole cell tubulin polymerization assay are shown in Fig. 2B. The microtubule destabilizers colchicine and vincristine induced a significant reduction in the fluorescence signal in this assay, whereas the known microtubule stabilizers taxol and epothilone A showed a substantial increase in signal; these differences are observed both when examining all cells (left side of Fig. 2B) and when analyzing just those cells in the G2/M phase of the cell cycle that typically have higher amounts of polymerized tubulin (right side of Fig. 2B). In contrast, compounds that induce cell death through other modes of action show no effect in this assay; camptothecin induced no change in fluorescence signal, nor did doxorubicin (Fig. 2B).

There are some compounds that induce mitotic arrest without directly affecting tubulin dynamics. STLC and monastrol bind to the Eg5 motor kinase, resulting in the formation of monoasters during cell division [20,31]. After verifying that STLC induced the expected G2/M-phase arrest (Fig. 3A), HeLa cells treated with this compound were evaluated in the whole cell tubulin polymerization assay. In this assay, STLC induced a smaller but statistically significant increase in the overall fluorescence, a result still seen after accounting for the induced M-phase arrest (Fig. 3B). This result demonstrates the potential of this assay to identify com-

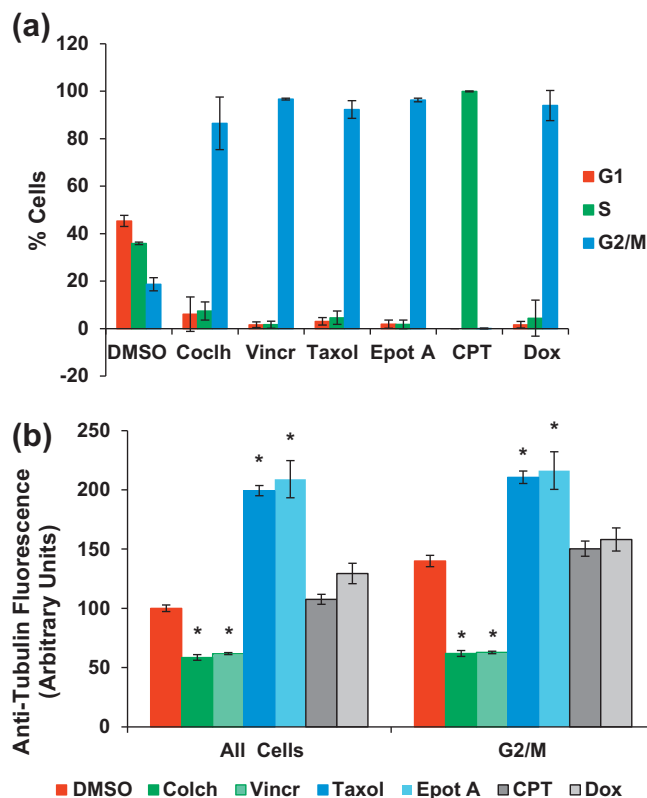


Fig. 2. Representative data for whole cell analysis of tubulin polymerization after treatment with various anticancer agents. HeLa (human cervical cancer) adherent cells were treated with colchicine (50 nM), vincristine (200 nM), taxol (50 nM), epothilone A (50 nM), camptothecin (50 nM), or doxorubicin (200 nM) for 18 h. (A) Cell cycle arrest in HeLa cells following 18 h of treatment, as assessed by PI staining and flow cytometry. (B) Tubulin fluorescence in HeLa cells following 18 h of treatment, as assessed by anti- α -tubulin staining and flow cytometry and described in text. Colch, colchicine; Vincr, vincristine; Epot A, epothilone A; CPT, camptothecin; Dox, doxorubicin. Error bars represent standard deviation of the mean ($n = 3$). Asterisk (*) indicates $P < 0.01$ relative to DMSO control based on a two-tailed Student's t test.

pounds that affect tubulin structure without directly binding microtubules.

Although the results presented in Figs. 2 and 3 were generated from HeLa cells (grown as adherent cultures), suspension cell lines can also be conveniently evaluated using this technique. As shown

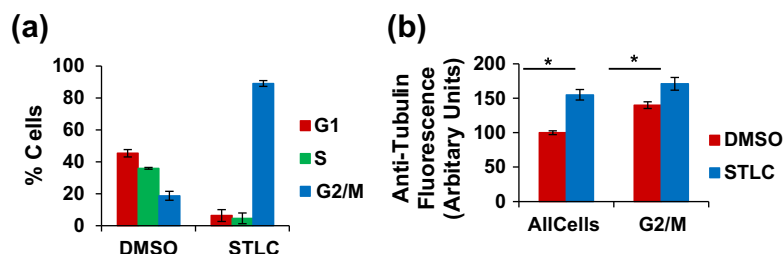


Fig. 3. Representative data for HeLa cells treated with 5 μM STLC for 18 h. (A) Cell cycle arrest in the G2/M phase following compound treatment, as assessed by PI staining. (B) Enhancement in tubulin fluorescence was observed following analysis of the whole population of cells as well as those cells specifically in the G2/M phase. Error bars represent standard deviation of the mean ($n = 3$). Asterisk (*) indicates $P < 0.05$ relative to DMSO control based on a two-tailed Student's t test.

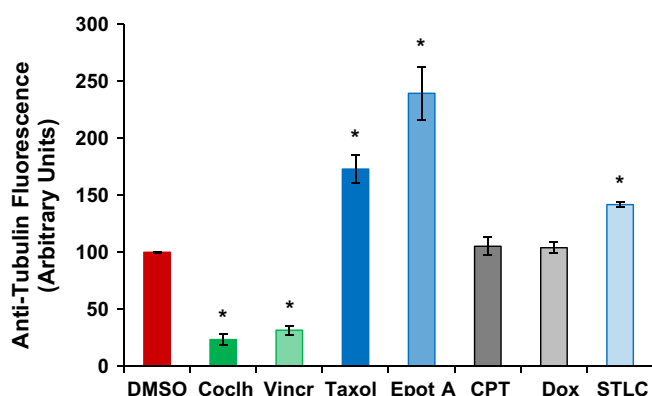


Fig. 4. Representative data for whole cell analysis of tubulin polymerization following 18-h compound treatment in U-937 (human lymphoma) suspension cells. Cells in the G2/M phase of the cell cycle were analyzed via the whole cell tubulin quantitation method described in the text after treatment with colchicine (100 nM), vincristine (100 nM), taxol (50 nM), epothilone A (50 nM), camptothecin (50 nM), doxorubicin (200 nM), or STLC (5 μM). Colch, colchicine; Vincr, vincristine; Epot A, epothilone A; CPT, camptothecin; Dox, doxorubicin. Error bars represent standard deviation of the mean ($n = 3$). Asterisk (*) indicates $P < 0.05$ relative to DMSO control based on a two-tailed Student's t test.

in Fig. 4, treatment of U-937 cells (human lymphoma cancer cell line) with the same panel of cytotoxins produces a result similar to that with HeLa cells.

When comparing compounds that affect tubulin polymerization, it is useful to determine IC_{50} values from dose–response curves generated from an *in vitro* assay. However, this *in vitro* experiment requires a large amount of tubulin and is inconvenient when assessing many compounds simultaneously, and the IC_{50} values obtained do not match the cellular cytotoxicity IC_{50} values [9,32–35]. Alternately, stabilized cytoskeletons can be isolated and analyzed by enzyme-linked immunosorbent assay (ELISA), but this method is low throughput and requires the isolation of tubulin polymers in cell lysate [36]. In contrast, multiple compounds can be conveniently compared using this whole cell tubulin polymerization assay. For this experiment, cells were treated for 18 h with a range of concentrations of taxol and colchicine as well as recently described compounds 8H and 22, small molecules known to destabilize microtubule formation much less potently than colchicine and vincristine [37]. As shown in Fig. 5, an obvious dose response is apparent for all compounds using the whole cell tubulin assay, with the following IC_{50} values: taxol = 9.6 ± 2.8 nM, colchicine = 21.6 ± 0.8 nM, compound 8H = 9.8 ± 3.6 μM , and compound 22 = 8.0 ± 2.6 μM . These values are generally 10–100 times lower (more potent) than their observed effect in the *in vitro* tubulin polymerization assay (taxol = 400 nM [38], colchicine = 1.4 μM [32], and compound 8H > 25 μM [37]) and more faithfully reflect the cell culture efficacy and toxicity of these compounds; the 72–

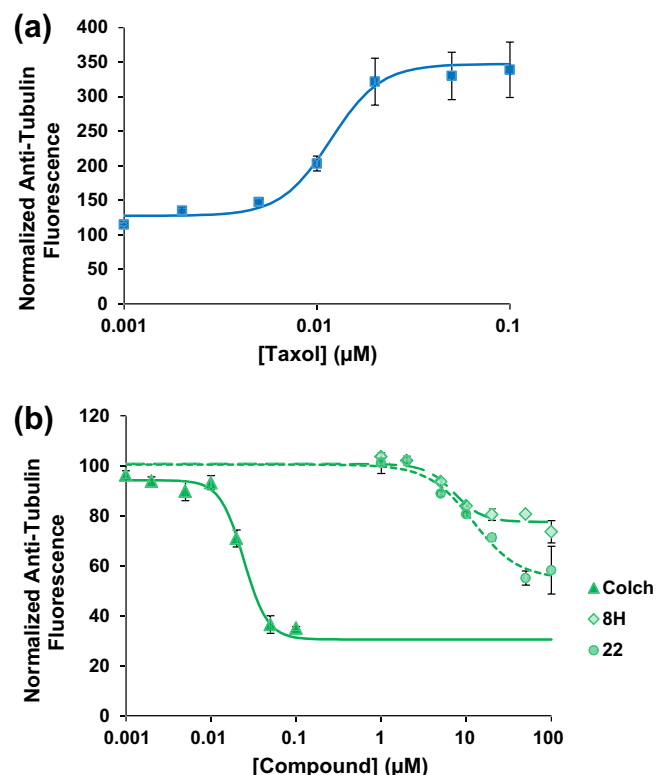


Fig. 5. Representative data of dose–response curves for U-937 cells treated with increasing concentrations of the microtubule stabilizer taxol (A) and the microtubule destabilizers colchicine, compound 8H, and compound 22 (B). IC_{50} values were calculated using the average and standard deviation of three independent experiments fit with a logistic dose–response curve. Colch, colchicine. Error bars represent standard deviation of the mean ($n = 3$).

h IC_{50} values for cell death induction are as follows: taxol = 10 nM [32], colchicine = 20–70 nM [32], compound 8H ~ 3.0 μM [37], and compound 22 = 1.8–5.6 μM [37].

Kinetics of microtubule perturbation

Microtubule polymerization kinetics is extremely difficult to follow in whole cells; in general, the timing of tubulin polymerization/depolymerization in response to compound is monitored *in vitro* [6,7,9,32–35]. There are limited examples in which the dynamics of microtubules are measured following compound exposure in mammalian cells; however, these reports focus on a single compound [39,40], analyze tubulin levels in cell lysate [41], or fail to demonstrate consistent responses to a range of tubulin binders [42]. As such, direct head-to-head comparisons of the

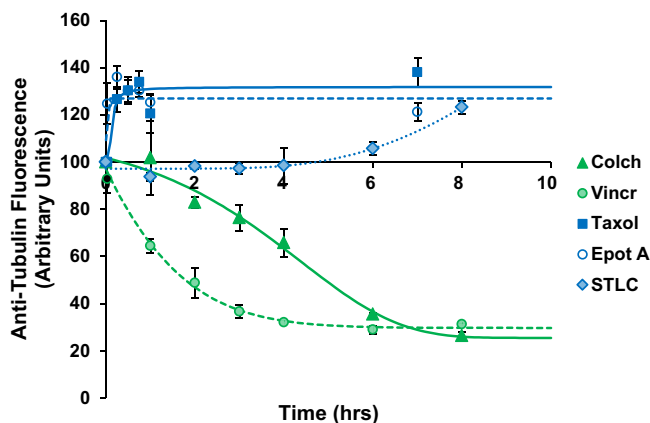


Fig. 6. Representative data of time-dependent response of tubulin polymerization and cell cycle arrest in U-937 cells following compound treatment with colchicine (100 nM), vincristine (200 nM), taxol (50 nM), epothilone A (50 nM), and STLC (5 μ M). The data were normalized to the DMSO control at each timepoint. To control for cell cycle arrest, only cells in the G2/M phase were analyzed. Colch, colchicine; Vincr, vincristine; Epot A, epothilone A. Error bars represent standard deviation of the mean ($n = 3$).

effect of various anticancer drugs on the kinetics of microtubule polymerization have not been made. Given the simplicity and throughput of this new whole cell method for evaluation of microtubule polymerization, it is ideally suited to this type of kinetic analysis. To assess the kinetics of microtubule perturbation by small molecules, cells were fixed after varying periods of compound exposure and their tubulin levels were monitored (Fig. 6). The tubulin destabilizers colchicine and vincristine show tubulin destabilization that is complete in 4–6 h; this timing is approximately consistent with the observed cell cycle arrest (see Supplementary Fig. 1 in Supplementary material). The tubulin stabilizers taxol and epothilone A evoke a significantly more rapid response in cells. A stabilization phenotype is observed within 15 min of treatment for both compounds, and a maximum response is reached within 1 h (Fig. 6); these cells show G2/M-phase cell cycle arrest at 3–4 h (Supplementary Fig. 1). This rapid response aligns with taxol's ability to suppress plus-end dynamics within 5 min of compound exposure [39]. Finally, treatment with STLC shows a much slower response (>6 h) that lags behind G2/M-phase arrest; this is consistent with the indirect nature of STLC modification of tubulin dynamics.

Medium-throughput whole cell analysis of tubulin polymerization

Given the importance of tubulin polymerization modifiers as anticancer agents, there is significant interest in finding new compounds that have this property. This could be either completely de novo, from an unbiased screen, or through assessment of a library of compounds designed to perturb tubulin dynamics. Neither the in vitro tubulin polymerization assay nor the microscopy method is well-suited for the direct comparison of antimetotics due to cost, low correlation to in-cell efficacy, low signal-to-noise, and the inability to generate IC_{50} values [17]. This new flow cytometry method, when paired with an autosampler, provides a medium-throughput, cell-based, high signal-to-noise method for the characterization of small libraries of tubulin binders that addresses the limitations of in vitro tubulin polymerization and microscopy. To demonstrate the utility of this method in a medium-throughput format, a “needle in a haystack” assay was performed whereby known tubulin stabilizers and destabilizers were spiked into a plate containing test compounds from an in-house library and vehicle controls. The treated cells (U-937) were then analyzed to

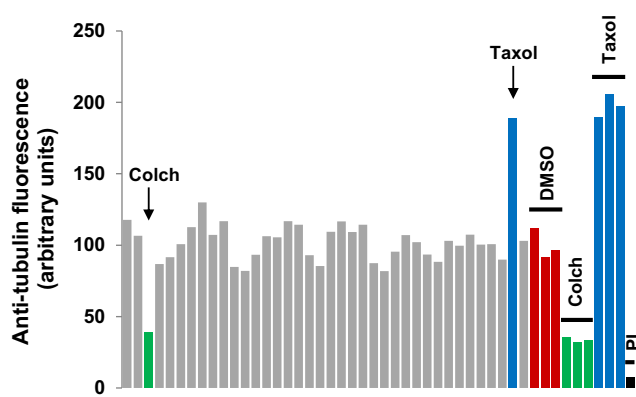


Fig. 7. Tubulin stabilization and destabilization is detectable in a medium-throughput readout using the whole cell tubulin polymerization assay and an autosampler. DMSO vehicle control wells are shown in triplicate (red bars), as are taxol (50 nM) and colchicine (100 nM) control wells (blue and green bars, respectively). The PI control well (black bar) contains no α -tubulin antibody. All other wells contain 1 μ M of compound from an in-house compound collection, with spiked wells containing taxol or colchicine at control concentrations. The colchicine and taxol “hits” are more than 3 standard deviations from the DMSO mean. Colch, colchicine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

evaluate the extent of tubulin polymerization in whole cells. As shown by the data in Fig. 7, the compounds known to affect tubulin polymerization are easily identified as hits in such an experiment.

Discussion

The importance of tubulin-binding compounds in anticancer therapy is unquestioned given the clinical success of taxol, vinblastine, and other drugs that disrupt proper tubulin polymerization/depolymerization dynamics. In addition, there are emerging roles for tubulin binders in the treatment of diseases other than cancer [43,44]. Although the current classes of tubulin-binding agents are life-saving drugs, many of these compounds suffer from poor oral bioavailability, neurotoxicity, and loss of efficacy over time due to resistance. There is a clear and pressing need for the continual development of next-generation tubulin-binding small molecules.

The two standard methods for assessing the influence of a compound on tubulin polymerization, a turbidity-based in vitro assay and fluorescence microscopy, both suffer from certain limitations. Assessment of tubulin polymerization in vitro requires large quantities of precious tubulin and does not directly correlate to the efficacy of compounds in the more complex cellular environment. In addition, this method typically uses bovine tubulin when the effect of a compound on human tubulin is what is of actual interest. Although fluorescence microscopy does provide a whole cell approach for the analysis of tubulin polymerization, it has a slower analysis speed, it is limited to adherent cells, and the readouts are difficult to quantify, complicating head-to-head comparison of compounds. In contrast, flow cytometry serves as a powerful tool to rapidly and quantitatively analyze a variety of cellular phenotypes based on staining cells with an appropriate fluorescently labeled antibody. The combination of a tubulin antibody with a DNA dye allows the detection of compounds that cause mitotic arrest and that also affect microtubules.

When compared with existing methods for the identification and assessment of tubulin-binding compounds, the flow cytometry technique described here has several advantages. First, because it is a whole cell technique, the potency of compounds in this assay is more likely to reflect their potency in cytotoxicity experiments.

For example, it is well documented that the in vitro tubulin polymerization assay returns IC₅₀ values for compounds that are often two to three orders of magnitude higher (less potent) than the cell death IC₅₀ [32,33,45,46]. As demonstrated in Fig. 5 and mentioned in the text, the tubulin polymerization IC₅₀ values for colchicine, taxol, compound 8H, and compound 22 in this new flow cytometry assay closely mimic their cell death IC₅₀ values. Second, the kinetic response of whole cells to tubulin-binding compounds is easily monitored and can be directly linked to cell cycle arrest. This key feature allows the direct comparison of the kinetic effect of compounds on microtubule dynamics in cells. Third, because there is no need for purified tubulin, this flow cytometry-based assay is extremely cost-effective because the primary cost is based only on a commercially available fluorescently labeled tubulin antibody. Finally, the ability to interface with existing high-throughput platforms should allow this flow cytometry method to be used in a high-throughput fashion to identify novel compounds that perturb tubulin polymerization. The cost of the in vitro turbidity method makes high-throughput screening prohibitive, and higher throughput methods such as TCRP, dibutylryl-cAMP, and nucleolin staining probe general mitotic arrest rather than specific interactions with tubulin.

There are some caveats with this new method. As a laser-based system, compound autofluorescence can artificially enhance the observed signal. This can be easily accounted for through the use of tubulin antibodies with red fluorescent probes. Compounds that induce alternate aberrant tubulin phenotypes through binding of MAPs, such as monoaster formation following disruption of Eg5, are identified in this assay. Given that most tubulin-binding compounds act by interrupting tubulin dynamics, the discovery of compounds that generate aberrant tubulin phenotypes by binding to alternate proteins could identify novel anticancer targets. For example, compounds that inhibit Eg5 are currently in phase I or II trials.

The method described here offers a new means by which to discover and assess, in a cellular milieu, compounds that perturb microtubule dynamics. Given the importance of tubulin binders as anticancer agents and the laborious nature of standard tubulin polymerization assays, this method should be useful in the discovery and characterization of novel antineoplastic compounds that act through the disruption of the tubulin/microtubule dynamic.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ab.2011.08.020](https://doi.org/10.1016/j.ab.2011.08.020).

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