In Vivo Analysis of Human Multidrug Resistance Protein 1 (MRP1) Activity Using Transient Expression of Fluorescently Tagged MRP1

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**ABSTRACT**

The multidrug resistance protein 1 (MRP1) contributes cellular resistance to a wide array of physiological toxins and chemotherapeutic agents. Its *in vivo* activity has been studied primarily in cells that have been continuously drug selected, culture conditions that might confound the effects of MRP1 expression with the effects of a cell’s detoxification machinery. Transient transfection with a MRP1-green fluorescent protein (EGFP) fusion protein allowed us to measure the activity of MRP1 in cells that had insufficient time to induce other chemoprotective proteins. Furthermore, separate transfections with MRP1-yellow fluorescent protein and a fluorescently tagged P-glycoprotein (MDR1-cyan fluorescent protein) permitted the drug-resistant properties of MRP1-expressing cells to be compared with those of MDR1-expressing cells. Our data showed that the expression of MRP1-EGFP results in significantly decreased cellular accumulation of tetrathiomolybdate and ethyl isothiouronium and decreased nuclear accumulation of doxorubicin. Additionally, MRP1-EGFP expression protected cells from the microtubule depolymerization caused by vincristine and colchicine, but not by vinblastine.

**INTRODUCTION**

MRP1 is a member of the ATP-binding cassette family of transporters (1). The protein has been implicated in the development of multidrug resistance in a variety of cancer cell lines (2). First cloned in 1992 from a daunorubicin-resistant lung cancer cell line (3), MRP1 has been associated with decreased sensitivity to a number of chemotherapeutic agents. Like the well-studied P-glycoprotein (4), MRP1, when overexpressed, can confer varying degrees of resistance to cationic and neutral hydrophobic compounds, including the anthracyclines, the Vinca alkaloids, rhodamine 123, and various acetoxymethyl esters of indicator dyes (5, 6). However, unlike P-glycoprotein, MRP1 can also transport anions such as methotrexate, calcein, anti-HIV nucleoside analogues, and heavy metals, as well as anionic conjugates of sulfate, glutathione (e.g., leukothriene C₄), and glutaric acid (e.g., 17β-estradiol 17β-d-glucuronide; 7).

Since its discovery nearly a decade ago, MRP1 has been extensively studied in drug-resistant tumor cell lines (8); in transformed cells stably transfected with the cDNA (9); in yeast (10); in microbes (11); and, more recently, in liposomes containing the purified protein (8). Some *in vivo* studies have found MRP1 to be functional in intracellular compartments (10, 12), while others have seen MRP1 active primarily at the plasma membrane (5). Some studies have suggested MRP1 to be active against mitoxantrone (13), cadmium (10), vinblastine, and colchicine, whereas others have found MRP1 to confer no increased resistance to these compounds (14, 15). Many of these differing assessments of MRP1 activity could be attributable to the properties of the cell type under investigation. However, other differences may have arisen as a result of the particular assays used to examine MRP1 activity. Whole-cell investigations have been performed either in multidrug-resistant cell lines endogenously expressing MRP1 or in stable, MRP1-transfected lines. Both of these model systems require that cells be continuously cultured in cytotoxins for the multidrug resistance phenotype to be maintained. This form of drug selection has been associated with many changes in cell physiology, including the up-regulation of DNA-repair enzymes, increased drug metabolism, and decreased sensitivity to apoptosis (16, 17). Under these selection conditions, ascertaining the effect of MRP1 expression made more complex by the presence of other resistance mechanisms.

For these reasons, we have developed a means of studying MRP1 activity in whole cells without protracted drug selection. We have created a protein with EGFP fused to the COOH terminus of MRP1, a construct that permits easy identification of MRP1 expression in cells. After transiently transfecting cells with MRP1-EGFP, we can compare cells expressing MRP1-EGFP with their nonexpressing counterparts and can thereby assess the immediate effect that MRP1 introduction has on a cell’s drug resistance properties. This fusion protein allows us to correlate degrees of MRP1 expression, assayed by EGFP fluorescence, with drug accumulation. Furthermore, separate transfections involving MRP1 fused to EYFP (MRP1-EYFP), as well as a MDR1 protein tagged with ECFP (MDR1-ECFP: Ref. 17), allow us to simultaneously compare the activities of the two proteins in the same chemotherapeutic and in the same culture conditions.

**Materials and Methods**

**Cell Culture and Transfection.** HeLa cells were propagated in DMEM with 4.5 g/liter glucose and l-glutamine (Cellgro, Herndon, VA) in 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO). Transfections of the constructs were done with Fugene 6 as per manufacturer’s instructions (Roche Molecular Biochemicals, Brussels, Belgium).

**Construction and Expression of Vectors.** Restriction enzymes were purchased from New England Biolabs (Beverly, MA). Human MRP1 cDNA was obtained in the cloning vector pGEM-11Zf (gift of Gary Kruh, Fox Chase Cancer Center, Philadelphia, PA). To generate an expression plasmid for wild-type MRP1, designated pMRP1, MRP1 cDNA was subcloned between the SalI and XbaI sites of pEGFP-N1 (Clontech, Palo Alto, CA), a step that excised the EGFP coding sequence and created a pEGFP-N1 backbone plasmid with MRP1 placed under the control of the cytomegalovirus promoter. To generate the MRP1-EGFP fusion protein, standard mutagenesis techniques were used on pMRP1 to replace the MRP1 stop codon with an AgeI site, the site at which EGFP was introduced. pMRP1-EGFP cDNA was subcloned into the SalI and XbaI sites of pEGFP-N1 (Clontech, Palo Alto, CA), a step that excised the EGFP coding sequence and created a pEGFP-N1 backbone plasmid with MRP1 placed under the control of the cytomegalovirus promoter. To generate the MRP1-EGFP fusion protein, standard mutagenesis techniques were used on pMRP1 to replace the MRP1 stop codon with an AgeI site, the site at which EGFP was introduced. pMRP1-EGFP and pMRP1-EYFP were created by replacing EGFP with ECFP or EYFP (Clontech).

**Western Blot Analysis.** MRP1-transfected and MRP1-EGFP-transfected cells were dissociated with Cell Stripper (Cellgro) and solubilized with 1% Triton X-100. The nuclear debris was removed by a low-speed centrifugation.
and the supernatant was resolved on a 4–20% gradient gel, using SDS PAGE. After electrophoresis onto a membrane (Amersham Pharmacia Biotech, Piscataway, NJ) using a semidyve electrophoresis, proteins were immunoblotted with either the MRPI anti-MRPI monoclonal antibody (Alexis Biochemicals, San Diego, CA) and an alkaline phosphatase-conjugated antirat IgG antibody (Sigma Chemical Co.) or directly with the alkaline phosphatase conjugated Living Colors Peptide Antibody (Clontech). Protein molecular weight markers from Amersham were used to distinguish relative electrophoretic mobilities.

**Fluorescence Microscopy.** Wide-field fluorescence microscopy was performed with an IX-70 Olympus microscope using a 1.4 N.A. oil-immersion objective, and an ORCA-cooled CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan) as described previously (17, 18). Wide-field fluorescence microscopy with deconvolution was performed using a DeltaVision deconvolution microscope with a 1.4 N.A. oil-immersion ×60 objective. The following excitation and emission filters were used for wide-field fluorescent microscopy: ECFP: λex = 400–430 nm, λem = 460–500 nm; EGFP: λex = 480–490 nm, λem = 500–550 nm; TMRE: λex = 530–560 nm, λem = 570–650 nm. Confocal microscopy was performed on either an upright Axioplan 2 microscope or an inverted Axiovert 100 microscope, each with a LSM 510 confocal attachment (Carl Zeiss, Thornwood, NY), using a 1.2 N.A. water-immersion ×63 objective. The following excitation laser lines and emission filters were used: ECFP: λex = 488 nm, λem = 500–530 nm; daunorubicin and doxorubicin: λex = 488 nm, λem = 580 nm LP; and mitoxantrone: λex = 633 nm, λem = 650 nm LP.

Live cell imaging of fluorescent drugs was performed on cells 48–72 h after transfection. Cells were incubated with fluorescent drugs [TMRE (Molecular Probes, Eugene, OR), daunorubicin (Calbiochem, La Jolla, CA), doxorubicin (Calbiochem), or mitoxantrone (Sigma Chemical Co.)] in Opti-MEM with 10% FCS for 15 min. To inhibit MRPI activity, cells were incubated with 25 μM BSO for 24 h prior to imaging.

For immunofluorescence of microtubules, transfected cells were incubated in 600 nM vincristine, 600 nM vinblastine, or 2 μM colchicine for 60 min, and fixed with ice-cold methanol. Microtubules were stained using a Cy3-labeled CO2 incubator at 37°C for 15 min. To inhibit MRPI activity, cells were incubated with 25 μM BSO for 24 h prior to imaging.

For immunofluorescence of microtubules, transfected cells were incubated in 600 nM vincristine, 600 nM vinblastine, or 2 μM colchicine for 60 min, and fixed with ice-cold methanol. Microtubules were stained using a Cy3-labeled anti-β tubulin antibody (Sigma Chemical Co.) according to the manufacturer’s instructions. For the immunolabeling of MRPI, cells were fixed with ice-cold methanol, incubated with the anti-MRPI-antibody MRPI at a 1:1000 dilution, washed, and subsequently incubated with an Alexa 594 conjugated antirat antibody (Molecular Probes).

**Flow Cytometry.** Flow cytometry was performed on a FACSort (Becton Dickinson, San Jose, CA). Adherent cells were nonenzymatically dissociated using Cell Stripper, resuspended in Opti-MEM with fluorescent drugs and incubated at 37°C for 30 min, and stored on ice until sample acquisition (not more than 30 min). The cells were harvested and resuspended in ice-cold PBS with a DNA stain to label dead cells [either 10 nM TOTO-3 iodide (Molecular Probes) or 1 μM propidium iodide (Sigma Chemical Co.)] immediately before data acquisition. The following wavelengths were used for excitation and emission: EGFP: λex = 488 nm, λem = 500–520 nm; daunorubicin, doxorubicin, TMRE, propidium iodide: λex = 488 nm, λem = 564–606 nm; and mitoxantrone, TOTO-3 λex = 633 nm, λem = 650 nm LP.

**RESULTS**

**MRPI-EGFP Fusion Protein Is Correctly Folded and Localized.** The short-term effects of expressing MRPI on the concentration and distribution of chemotherapeutics in cells were studied with transient transfection. To identify the expression and to study the cellular distribution of the protein, we used a MRPI-EGFP fusion protein. Two controls were used to determine whether EGFP fluorescence could be used as an indicator of the localization of MRPI. The first control was to determine whether all of the cellular EGFP was present as part of the MRPI fusion protein, a task accomplished by immunoblotting lysates of cells transfected with either wild-type MRPI or MRPI-EGFP using antibodies to either MRPI or EGFP (Fig. 1A–B). In cells transfected with MRPI, the anti-MRPI antibody recognized a doublet that migrated between Mw 160,000 and Mw 250,000, (Fig. 1A, first lane), a weight range encompassing the reported size of MRPI in both its unglycosylated and fully glycosylated states (19). In cells transfected with MRPI-EGFP, the anti-MRPI antibody recognized a slightly heavier doublet, that would be consistent with the addition of a Mw 27,000 EGFP COOH-terminal fusion (Fig. 1A, second lane). The anti-EGFP antibody recognized a similar doublet in the MRPI-1EGFP transfected cells (Fig. 1B, second lane), along with several proteins of lower molecular weight. The anti-EGFP antibody also recognized these lower molecular weight bands in cells transfected with wild-type MRPI (Fig. 1B, first lane), suggesting that these lower molecular weight bands are the result of a nonspecific cross-reaction and do not represent degraded products containing EGFP. Because both antibodies recognized identical molecular weight bands in MRPI-1EGFP transfected lanes, and because no fragment of the fusion protein was recognized by either antibody, it is likely that MRPI-1EGFP is being expressed in its entirety. EGFP expression thus correlates directly with the presence of MRPI.

The second control was to determine whether the subcellular localization of MRPI was affected by the placement of EGFP at its COOH terminus. HeLa cells were transfected with MRPI-1EGFP and observed by confocal microscopy. Within 24–48 h after transfection, cells revealed plasma membrane-localized EGFP fluorescence, with minor staining in the perinuclear region (Fig. 1C). Punctate regions of brighter fluorescence follow the cell periphery along invaginations of the membrane, demonstrating the degree to which MRPI-1EGFP localizes to the plasma membrane. Immunolabeling with an anti-MRPI antibody resulted in a distribution of immunofluorescence that was similar to the EGFP fluorescence (Fig. 1D). When the EGFP fluorescence was merged with the anti-MRPI derived fluorescence, there was nearly complete colocalization of the anti-MRPI and EGFP signals (Fig. 1E). To follow this pattern in greater detail, the relative fluorescence intensities of the two signals were plotted, using a representative line across the cell as a reference (Fig. 1, E and F). Both the anti-MRPI and the anti-EGFP signals share the same general distribution along the length of the cell, with a marked drop in intensity in the center, corresponding to the nucleus. An absence of signal in the nucleus once again indicates that EGFP has not been cleaved from MRPI, because free EGFP is small enough to diffuse through the nuclear pore. Immunolabeling of anti-MRPI in wild-type MRPI transfected cells revealed a similar pattern of fluorescence distribution (data not shown). Thus, it seems likely that the COOH-terminal addition of EGFP did not affect the localization of MRPI and that EGFP fluorescence is a good indicator of the presence of MRPI.

**Activity of the MRPI-1EGFP Fusion Protein.** MRPI expression is associated with the decreased intracellular accumulation of many compounds, some of which are naturally fluorescent. Therefore, it is possible to assess the effect of MRPI-1EGFP expression on the cellular accumulation and distribution of fluorescent substrates using EGFP fluorescence as a marker for MRPI-1EGFP expressing cells. The effect of MRPI-1EGFP on the accumulation of the nonfluorescent microtubule-depolymerizing agents vincristine, vinblastine, and colchicine can be measured by the extent to which these drugs are able to disrupt microtubule structure in MRPI-1EGFP expressing cells. Because transient transfection generates large numbers of both expressing and nonexpressing cells, cells that express EGFP-fluorescence can be directly compared with nonexpressing cells, both of which are side by side, in the same culture dish, serving as an internal control.

The specificity of MRPI-1EGFP activity was determined by comparing the activity of the fluorescent MRPI to that of another protein involved in multidrug resistance, P-glycoprotein (MDR1). To make direct comparisons of the two proteins, MDR1 was tagged with ECFP (17) and MRPI was tagged with EFYF. Cells were separately trans-
fected with either MRP1-EYFP or MDR1-ECFP, and then replated together, generating a mixed population of cells: some expressing neither protein, others expressing various levels of MDR1-ECFP, and still others expressing various levels of MRP1-EYFP. Because EYFP and ECFP can be spectrally distinguished, the relative activities of the two drug-resistant proteins, MRP1 and MDR1, could be directly compared.

**MRP1-EGFP Shows Activity Profiles Analogous to MRP1 with TMRE, a Positively Charged Dye.** Rhodamine 123 is a positively charged membrane-potential dye that stains the negatively charged mitochondria of living cells. Previous studies have suggested that it is substrate for MRP1 (20). However, because of the spectral overlap between rhodamine 123 and EYFP, we assayed the activity of MRP1 against the homologous dye TMRE. HeLa cells that were transfected with either MRP1-EYFP or MDR1-ECFP were subsequently examined for TMRE accumulation (Fig. 2A–D). In the field of cells seen in Fig. 2A–D, one expressed MRP1-EYFP (Fig. 2A, bottom center) and three expressed MDR1-ECFP (Fig. 2B, one on the left and two on the top center). TMRE fluorescence was observed in only three cells (Fig. 2C, right side), none of which expressed MRP1-EYFP or

![GFP](image1)

**Fig. 1.** The expression and localization of MRP1-EGFP in HeLa cells. In A–B, to determine whether all cellular EGFP was part of the MRP1-EGFP fusion protein, cell lysates of MRP1-EGFP or MRP1 transfected cells were immunoblotted with either an anti-EGFP or an anti-MRP1 antibody. Molecular masses of the bands were determined by using standard protein markers. The anti-MRP1 antibody recognized a doublet that migrated between 160 and 250 kDa in MRP1 transfected lysates (A, left lanes). In MRP1-EGFP cell lysates, both of the antibodies recognized a doublet of reduced electrophoretic mobility that was consistent with a 30-kDa addition to the wild-type protein; no proteolysis products of MRP1-EGFP were recognized by either antibody. Lower molecular-weight bands were recognized nonspecifically in both MRP1- and MRP1-EGFP-transfected cells. In C–F, to confirm the colocalization of MRP1 and EGFP in MRP1-EGFP transfected cells, cells were fixed, probed with an anti-MRP1 antibody, and examined under confocal microscopy for (C) endogenous EGFP fluorescence and (D) immunofluorescence against MRP1. In E, EGFP fluorescence was merged with the fluorescence derived from labeled MRP1 to determine the extent of the colocalization. In F, the relative fluorescence intensities of both labels were plotted for a representative line drawn along the merged image. Scale bar, 20 μm.

![DIAGRAM](image2)

**Fig. 2.** The activity of MRP1-EYFP is analogous to wild-type MRP1. HeLa cells were separately transfected with MRP1-EYFP and MDR1-ECFP, plated together, and then visualized 48 h after transfection under wide-field fluorescence microscopy. All of the cells were incubated in 50 μM TMRE 15 min prior to visualization. In A and F, cells expressing MRP1 were visualized using EYFP fluorescence; in B and G, cells expressing MDR1 were visualized using ECFP fluorescence; in C and H, cells accumulating TMRE were visualized using rhodamine fluorescence. In D, cells expressing either MRP1 (green) or MDR1 (blue) excluded TMRE (red) as is evident in the merged image. In E, a line plot of the fluorescence intensities of a representative region in the merged image demonstrates the exclusion of TMRE (red line) from the cells expressing MRP1 (green line) or MDR1 (blue line). In F–J, cells were incubated with the MRP1-inhibitor BSO (25 μM) for 24 h. In J, MRP1-EYFP cells accumulated TMRE, whereas MDR1-ECFP cells did not. In J, a line scan quantifies the degree to which TMRE was excluded from MDR1-ECFP cells but not from MRP1-EYFP cells. Scale bar, 20 μm.
MDR1-EGFP, as evident in the merged image (Fig. 2D). Because cells expressed either drug-resistance protein or accumulated TMRE, the expression of MDR1 or MRP1 was responsible for lowering the accumulation of TMRE. The line profile of ECFP, EYFP, and TMRE fluorescence intensities also indicates that TMRE fluorescence was diminished to background levels in cells expressing either MDR1-ECFP or MRP1-EYFP (Fig. 2E).

BSO is reported to be an inhibitor of MRP1 activity because it depletes the cell of glutathione, a cofactor thought to be necessary for MRP1 function (7). On the administration of BSO, cells expressing MRP1-EYFP accumulated TMRE (Fig. 2, F and H), but cells expressing MDR1-ECFP did not (Fig. 2, G and H). Both the merged image (Fig. 2D) and the line scan (Fig. 2F) make this pattern evident: BSO specifically reversed the activity of the fluorescently tagged MRP1 protein. Because MRP1-EYFP is active against a previously characterized substrate, and because its activity can be distinguished from that of MDR1 by an MRP1-specific inhibitor, it is likely that the fluorescent tag has not significantly affected the activity of MRP1.

**Anthracyclines.** We next assayed the activity of MRP1-EGFP against the anthracyclines daunorubicin and doxorubicin, both thought to be MRP1 substrates (15). When transiently transfected cells were incubated in 10 μM daunorubicin, cells expressing the protein (Fig. 3A) showed no detectable drug accumulation (Fig. 3B), whereas neighboring, nonexpressing cells accumulated daunorubicin in nuclear and perinuclear regions (Fig. 3, B and C). To obtain a quantitative relationship between MRP1-EGFP expression and daunorubicin accumulation, total cellular EGFP and daunorubicin fluorescence were measured for a large number of cells using flow cytometry. Cells exhibiting higher MRP1-EGFP fluorescence showed lower daunorubicin fluorescence, with the highest expressing cells showing 10-fold decreased daunorubicin fluorescence compared with nonexpressing cells (Fig. 3D). Cells transfected with MDR1-EGFP showed a similar relationship between expression and daunorubicin accumulation (Fig. 3E).

Although MRP1 was able to reduce the daunorubicin levels inside a cell, it exhibited only a marginal effect on the total cellular accumulation of doxorubicin, an anthracycline closely related in structure to daunorubicin. Cells with the highest levels of MRP1-EGFP expression showed only a 2-fold reduction in total doxorubicin fluorescence, as assayed by flow cytometry (Fig. 3I). Despite this attenuated activity on the total cellular accumulation of doxorubicin, cells expressing MRP1-EGFP revealed an altered intracellular distribution of the chemotherapeutic. As seen under confocal microscopy, MRP1-EGFP expressing cells had significantly reduced doxorubicin fluorescence inside the nucleus but had levels of doxorubicin fluorescence in the cytoplasm similar to that of nonexpressing cells (Fig. 3, F and G). In contrast, the nucleus of nonexpressing cells was brightly fluorescent with the drug, as was the perinuclear region (Fig. 3H). Because the cellular targets of doxorubicin are located primarily inside the nucleus, this altered distribution away from the nucleus may account for MRP1-mediated doxorubicin resistance. Expression of MDR1-EGFP had similar effects on doxorubicin distribution; cells expressing MDR1-EGFP had diminished drug accumulation in the nucleus (data not shown), whereas the total intracellular drug fluorescence remained unchanged (Fig. 3J). Altered patterns of
doxorubicin accumulation have been previously associated with multidrug-resistant cells (21).

Mitoxantrone. Previously published reports have differed in their assessment of MRP1 activity against the anthracenedione mitoxantrone. Some have suggested that MRP1 expression alone confers resistance to the chemotherapeutic (13, 22), whereas others have seen no effect of MRP1 expression on mitoxantrone resistance (15). When transiently transfected HeLa cells were incubated in 2 μM mitoxantrone, cells with elevated MRP1-EGFP levels (Fig. 3K, cell in the upper center with an arrow), showed diminished levels of mitoxantrone accumulation (Fig. 3L). However, cells with lower levels of MRP1 (Fig. 3K, bottom center), had levels of mitoxantrone that were similar to nonexpressing cells (Fig. 3L). In the merged image (Fig. 3M), we can clearly distinguish a spectrum of drug accumulation that corresponds to the degree of MRP1-EGFP expressed in the cell. Flow cytometry reveals that MRP1 activity against mitoxantrone (Fig. 3N) is considerably diminished when compared with daunorubicin (Fig. 3D). Cells expressing the highest level of MRP1-EGFP showed less than a 10-fold reduction in drug accumulation (Fig. 3N), a reduction comparable with MDR1-EGFP expressing cells (Fig. 3O). This weak activity may account for the differing assessments of mitoxantrone as a substrate of MRP1.

Microtubule-depolymerizing Agents. Because the microtubule-depolymerizing chemotherapeutics vincristine, vinblastine, and colchicine are not fluorescent, the transport functions of MRP1 were assayed by the relative degrees of microtubule depolymerization in cells expressing and not expressing the protein. After an incubation in 600 nM vincristine, HeLa cells, transiently transfected with either MDR1-ECFP or MRP1-EYFP, were fixed and then stained with a fluorescent antitubulin antibody. The field of cells in Fig. 4A–E includes one cell strongly expressing MRP1-EYFP (Fig. 4A), two cells strongly expressing MDR1-ECFP (Fig. 4B), and a number of surrounding cells expressing neither protein (Fig. 4D). The microtubules of nonexpressing cells were severely disrupted by vincristine, so much so that they had altered cell morphology (Fig. 4D). These cells were round and considerably smaller than their MRP1-EYFP- or MDR1-ECFP-tagged counterparts. However, the cell expressing MRP1-EYFP and the two cells expressing MDR1-ECFP showed relatively little microtubule depolymerization; each cell retained an intricate microtubule network. Moreover, whereas the MTOC was intact in the MDR1-ECFP expressing cells, it was no longer apparent in the MRP1-EYFP expressing cells. Therefore, MRP1 provides some protection against the depolymerizing effects of vincristine, if not as much as does MDR1.

Next, we assayed the effect of MRP1-EYFP on colchicine activity. Cells incubated in 2 μM colchicine were unable to maintain microtubule integrity in the absence of a MRP1 or MDR1 (Fig. 4I). These cells were diffusely stained with the antitubulin antibody, but they showed no discernable microtubule structure. Expression of MDR1-ECFP, however, rescued this loss; the ECFP-tagged cell (Fig. 4G) had relatively undamaged microtubules, with the MTOC still intact (Fig. 4H). Similarly, the two MRP1-EYFP-expressing cells (Fig. 4F) had a complex network of microtubules with the MTOC quite evident (Fig. 4J).
Finally, we examined the effect of expressing MRP1-EYFP on vinblastine activity. Cells lacking both multidrug resistance proteins were clearly affected by vinblastine; they had no MTOC and considerably disrupted microtubules (Fig. 4N). All four of the cells that express MDR1-ECFP showed relatively undisrupted microtubule structures, with a MTOC somewhat visible in each cell (Fig. 4M). In contrast, the cell expressing MRP1-EYFP could not be distinguished from the nonexpressing cells (Fig. 4O). As we see in the detail of the antitubulin-stained field (Fig. 4O), the center cell, with clearly disrupted microtubule structure, was the one that expressed MRP1-EYFP. The adjacent cell with a largely intact MTOC was expressing MDR1-ECFP. Surrounding cells, like the cell expressing MRP1, had microtubules that had been severely damaged by vinblastine. It seems, therefore, that MRP1, unlike MDR1, provides little to no protection against the effects of vinblastine. A differential effect of MRP1 expression on vincristine and vinblastine has been suggested previously (15).

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