A Novel Microtubule Inhibitor Overcomes Multidrug Resistance in Tumors

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Abstract

Microtubule inhibitors as chemotherapeutic drugs are widely used for cancer treatment. However, the development of multidrug resistance (MDR) in cancer is a major challenge for microtubule inhibitors in their clinical implementation. From a high-throughput drug screen using cells transformed by oncogenic RAS, we identify a lead heteroaryl amide compound that blocks cell proliferation. Analysis of the structure-activity relationship indicated that this series of scaffolds (exemplified by MP-HJ-1b) represents a potent inhibitor of tumor cell growth. MP-HJ-1b showed activities against a panel of more than 1,000 human cancer cell lines with a wide variety of tissue origins. This compound depolymerized microtubules and affected spindle formation. It also induced the spike-like conformation of microtubules in vitro and in vivo, which is different from typical microtubule modulators. Structural analysis revealed that this series of compounds bound the colchicine pocket at the intra-dimer interface, although mostly not overlapping with colchicine binding. MP-HJ-1b displayed favorable pharmacological properties for overcoming tumor MDR, both in vitro and in vivo. Taken together, our data reveal a novel scaffold represented by MP-HJ-1b that can be developed as a cancer therapeutic against tumors with MDR.

Significance: Paclitaxel is a widely used chemotherapeutic drug in patients with multiple types of cancer. However, resistance to paclitaxel is a challenge. This study describes a novel class of microtubule inhibitors with the ability to circumvent multidrug resistance across multiple tumor cell lines. Cancer Res; 78(20); 5949–57. ©2018 AACR.

Introduction

Microtubule is considered as one of the most important targets of anticancer drugs due to its important role in cell mitosis (1–3). Microtubule inhibitors, which inhibit cell growth by binding on microtubules and further influencing microtubules' function, have been used with great success in the treatment of variety of tumors (4, 5). Microtubule inhibitors are classified into different categories according to their binding sites: paclitaxel binding site, vinca alkaloid binding site, and colchicine binding site (6–8). Paclitaxel-binding site inhibitors disrupt mitosis by reinforcing microtubule and preventing the depolymerization of microtubule (9). Although inhibitors binding two other sites can depolymerize microtubule and block cell cycle in mitosis (10–13). These inhibitors, especially paclitaxel and vincristine, are widely used as cancer chemotherapeutics. Unfortunately, more than half cancer patients eventually would develop multidrug resistance (MDR) to chemotherapeutics (14, 15).

The most commonly characterized mechanism of MDR is mediated by the overexpression of ATP-binding cassette proteins (16–18). ABC proteins, as a kind of cell membrane transport proteins, reduce drug accumulation in cancer cells by pumping diverse compounds out. The key members of ABC transporter family include MDR1/P-gp/ABCB1 (MDR gene 1, P-glycoprotein), MRPs/ABCCs (MDR-related proteins), and BCRP/ABCG2 (breast cancer resistance protein; refs. 19, 20). With overexpression of these transport pumps, the efflux of drug increased, which results in drug resistance. As MDR becomes an inevitable obstacle for the success of chemotherapy, overcoming MDR has been a high priority for both clinical and investigation oncologists. The strategy of targeting ABC proteins has been virtually abandoned...
by the pharmaceutical industry because of the limitations of the available target-specific inhibitors, and the difficulty in designing clinical trials (21). Therefore, to seek alternative ways to circumvent cancer drug resistance is urgently needed.

In this study, we describe a small-molecule MP-HJ-1b as a novel microtubule inhibitor that binds to colchicine site. Mechanistically, MP-HJ-1b restrains microtubule polymerization and promotes microtubule depolymerization. The pharmaceutical effectiveness of MP-HJ-1b shows that it can overcome MDR in vitro and in vivo.

Materials and Methods

Cell culture and proliferation assay

The HeLaR cell line was provided by Dr. Lei Huang (Shanghai Jiao Tong University School of Medicine, Shanghai, China) and cultured as described previously (22). The K562 doxorubicin-resistant cell line, A2780 paclitaxel-resistant cell line and A2780 cell line were purchased from the Center for Type Culture Collection (Chinese Academy of Sciences, China). The RL95-2 cells were grown in RPMI-1640 Medium (Gibco, catalog #12440-053) with 10% FBS, whereas NB4, MOLM-13 and SEM, MOLM-13, NOMO-1, and SHI-1 cell lines were purchased from the Cell Center of Institutes of Biomedical Sciences (Fudan University, China). The MDR and expression of ABC transporters in these cell lines were tested and described in the text. The RL95-2 and SK-NEP-1 cell lines were purchased from the Cell Center of Institutes of Biomedical Sciences (China). The RL95-2 cells were grown in DME:F12 Medium (Gibco, catalog #11330032) with 10% fetal bovine serum (Gibico, catalog #10099-141) and 0.005 mg/mL insulin. The SK-NEP-1 cells were grown in McCoy’s 5a Medium (Gibco, catalog #16600082) with 10% FBS. The NB4, SEM, MOLM-13, NOMO-1, and SHI-1 cell lines were purchased from Deutsche Sammlung von Mikroorganismen and Zellkulturen. The SEM and SHI-1 cells were grown in IMDM (Invitrogen, catalog #12440-053) with 10% FBS, whereas NB4, MOLM-13 and NOMO-1 cells were grown in RPMI-1640 Medium (Gibco, catalog #C22400500BT) with 10% FBS. The HeLa, MES-SA, A549, HCC287, H1975, MCF-7, HT-29, H9, MV-4-11, HL-60, KU812, K562, RS4;11 and SUP-B15 cell lines were purchased from ATCC. All cell lines were authenticated using short tandem repeat profiling analysis according to the American National Standard ANSI-0002-2011. The cell passages were limited to 10 generations for all experiments in the study. MDR1/ABCB1 testing was performed using MycoPlat detect MDR plaque Kit (Invitrogen, catalog #M7006) every 3 weeks.

Cellular IC_{50} value was measured by CellTitre-Glo Luminescent Cell Viability Assay (Promega, catalog #G7572). Cells were seeded in 96-well plates (5,000 cells in 100 µL complete medium per well) with different doses of compounds (MP-HJ-1b, paclitaxel or vincristine) in triplicates. After 48 hours incubation, CellTitre-Glo was added into each well. After 30 minutes incubation at room temperature, the luminescent signal was measured, collected and analyzed with EnVision Multimode Plate Reader.

Cell-cycle analysis

A total of 5 × 10^5 cells were cultured in a 10-cm dish. After 24 hours treatment with MP-HJ-1b or dimethyl sulfoxide (DMSO), cells were harvested and washed in PBS. 70% ethanol was used to fix cells at −20°C overnight. Cells were washed two times in PBS. RNase was added and cells were incubated for 30 minutes at 37°C. Propidium iodide (PI) labeling was performed for 10 minutes at room temperature prior to DNA content analysis by flow cytometry. Cell-cycle analysis was performed using FlowJo software.

Cell morphology analysis

After 12 hours treatment of MP-HJ-1b or DMSO, cells were harvested and washed with PBS for three times, and then fixed with 3% paraformaldehyde (containing 0.2% sucrose) for 30 minutes. Cells were blocked with 10% goat serum containing 0.4% TritonX-100 for 1 hour at room temperature. Three hours treatment of primary antibodies [anti-tubulin antibody (Abcam, catalog #ab6161), anti-crest antibody (Cortex Biochem, human CREST serum), anti-CEP192 antibody (Santa Cruz Biotechnology, catalog #sc-84785) or anti-pericentrin antibody (Abcam, catalog #ab4448)] was conducted at room temperature. After washing with PBS, the secondary antibodies [anti-mouse IgG (CST, catalog #4408), anti-rabbit IgG (CST, catalog #4413)] were applied for 1 hour at room temperature. Cells were washed with PBS and labeled with DAPI for 10 minutes at room temperature. Finally, anti-fluoresceinpolyvinylpyrrolidone mounting medium was used to seal coverslips.

Tubulin polymerization assay

Tubulin was re-dissolved in the cold G-PGM buffer (80 mmol/L PIPES PH6.9, 2 mmol/L MgCl2, 0.5 mmol/L EGTA, 1 mmol/L GTP) and frozen in liquid nitrogen for storage. Thawed-tubulin was centrifuged at 14,000 × g for 10 minutes at 4°C before experiments. G-PGM, paclitaxel, or MP-HJ-1b was added into tubulin homogeneous solution. Absorbance at 340 nm was continuously measured for 1 hour with Envision plate reader (PE).

Microtubule formation in vitro

Homogeneous tubulin solution was prepared as mentioned previously. MP-HJ-1b, paclitaxel or vincristine was added into the solution and incubated for 20 minutes at room temperature. Tubulin samples were dropped onto the copper grids. The copper grids were dried after staining by 2% uranyl acetate-lead citrate. Samples were observed by transmission electron microscope (FEI Tecnai G2 Spirit TEM).

Western blot

The whole-cell proteins were obtained by using mammalian protein extraction reagent (M-PER) and centrifuged at 12,000 r.p.m. at 4°C. Proteins were mixed with loading buffer and boiled for 10 minutes. Denatured proteins were separated in 8% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to the polyvinylidene fluoride (PVDF) membrane. Membranes were incubated with antibodies [MDR1/ABCB1 rabbit antibody (CST, catalog #13342), MRPI/ABCC1 rabbit antibody (CST, catalog #14685), anti-MRP2 antibody (Abcam, catalog#ab3373), MRP3/ABCC3 rabbit antibody (CST, catalog #39909) or ABCG2 antibody (CST, catalog #4477)] at 4°C overnight. HRP-conjugated secondary antibodies were used and the specific blots were detected by ECL reagent.

Mouse model

Female nude mice were fed in the Shanghai Jiaotong University School of Medicine Experimental Animal Center with mimicking normal diet. Nude mice (4–6-weeks-old) were selected and hypodermically inoculated with 1 × 10^5 HeLa cells. Four weeks later, nude mice were grouped randomly. MP-HJ-1b, vincristine, or corn oil was administrated by intraperitoneal injection, respectively. HeLaR cells were inoculated into nude mice similarly. When tumor was large enough, the tumor tissue was surgically
extracted and transplanted to new nude mice. This process needed twice. These tumor-bearing nude mice were stochastically grouped for drug treatment after tumor formed in 2 weeks. All the animal experiments were guided and approved by The Animal Care and Welfare Committee of Shanghai Jiaotong University School of Medicine.

Protein expression and purification
The complex of two tubulins, one stathmin-like domain of RB3 (RB3-SLD) and one tubulin tyrosine ligase (TTL; the T2R–TTL complex) was produced as described with slight modifications (7,23). RB3-SLD was overexpressed in Escherichia coli BL21 (DE3), purified sequentially by anion-exchange chromatography (QFF; GE Healthcare) and gel filtration (Superox 75; GE-Healthcare). The purified protein was concentrated to 10 mg/mL and stored at −80°C until use. TTL was overexpressed in E. coli BL21 (DE3) and purified by nickel-affinity chromatography, followed by gel filtration (Superdex 200; GE-Healthcare). Purified TTL in Bis-Tris propane (pH 6.5), 200 mmol/L NaCl, 2.5 mmol/L MgCl₂, 5 mmol/L β-mercaptoethanol and 1% glycerol was concentrated to 20 mg/mL and stored at −80°C until use. Porcine brain tubulin (Cytoskeleton, catalog #T-238P) was supplied at 10 mg/mL (buffer: 80 mmol/L Pipes, pH 6.9, 2.0 mmol/L MgCl₂, 30 mmol/L CaCl₂ and 30 mmol/L MgCl₂. Seeding method was used to obtain single crystals. Rod-like crystals appeared after 2 days and grew to maximum dimensions within 1 week. For crystallization and crystals soaking, 0.1 mmol/L EGTA and 1 mmol/L GTP) and stored at −80°C until use. The T2R–TTL crystals were obtained at 20°C in a buffer consisting of 6% PEG4000, 8% glycerol, 0.1 mol/L MES (pH 6.7), 30 mmol/L CaCl₂ and 30 mmol/L MgCl₂. Seeding method was used to obtain single crystals. Rod-like crystals appeared after 2 days and grew to maximum dimensions within 1 week. For crystal soaking, 0.1 μL of MP-HJ-1c (dissolved in DMSO at 10 mM) was added to 2 μL crystal-containing drop for 18 hours at 20°C.

Data collection and structure determination
The reservoir solution supplemented with 20% (v/v) glycerol was used as the cryoprotectant. The crystals were transferred into the cryoprotectant for a few seconds, and then mounted in nylon loops and flash-cooled in liquid nitrogen. Diffraction data were collected on beamline BL19U1 of National Facility for Protein Science Shanghai (NFPS) at Shanghai Synchrotron Radiation Facility (Shanghai, China). Data were processed using HKL3000 (24). The structures were determined by molecular replacement method using the T2R-TTL structure (PDB ID: 4I55) as a search model. The refinement was performed using COOT (25) and PHENIX (26). The model quality was checked with MOLPROBITY (27). Atomic coordinates and structure factors of tubulin–MP-HJ-1c complex have been deposited in the Protein Data Bank under accession code 5YZ3.

Statistical analysis
All data are expressed as the mean ± standard error (SEM) of three independent experiments unless stated otherwise. The data were analyzed with Student t tests. P values <0.05 were considered significant.

Results
Identification of MP-HJ-1b
In an effort to conquer the seemingly impregnable fortress of oncogenic RAS, we constructed a phenotypic screening to search for compounds that are able to inhibit the proliferation of NRAS-G12D-transformed cells (28). In brief, by comparing IL-3–dependent BaF3 cell proliferation with that of mutant RAS-driven BaF3 cell, this cell-based screening system could provide an unbiased search for cytotoxic compounds that preferentially target RAS signaling. We screened an in-house compound library comprised more than 30,000 chemical entities. The compound library was made of small molecules with drug-like properties, which were generated by combinatorial chemistry specific designed for high-throughput screening (29). One

Table 1. Antiproliferative IC₅₀ values of MP-HJ-1b against cancer cell lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>Tumor types</th>
<th>Ras mutant</th>
<th>IC₅₀(µmol/L)</th>
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<tr>
<td>HNLa</td>
<td>Cervical cancer</td>
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</tr>
<tr>
<td>MES-5A</td>
<td>Uterine sarcoma</td>
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<td>KRAS-G12S</td>
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<td>Lung cancer</td>
<td>—</td>
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<tr>
<td>H9975</td>
<td>Lung cancer</td>
<td>—</td>
<td>0.151</td>
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<td>Ovarian cancer</td>
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<td>0.014</td>
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<td>Acute lymphoblastic leukemia</td>
<td>—</td>
<td>0.075</td>
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Figure 1. Compound structures. A, Chemical structure of MP-HJ-1a. B, Chemical structure of MP-HJ-1b.
compound MP-HJ-1a (Fig. 1A) was identified as the top lead for its inhibitory effects on the proliferation of NRASG12D-driven BaF3 cells. On the basis of the core structure of MP-HJ-1a, we put intensive medicinal chemistry efforts to generate a series of analogues. Compound MP-HJ-1b (Fig. 1B) was eventually chosen as the leading compound for following pharmacological characterization.

To investigate whether MP-HJ-1b is specific effective to RAS-mutant cells, 22 human cell lines, from different types of tumors, were initially tested with MP-HJ-1b and the viability was detected by CellTiter-Glo Luminescent Cell Viability Assay (Table 1). To our surprise, MP-HJ-1b showed strong anti-proliferation effects on all tumor cell lines. These data suggested that MP-HJ-1b has an interesting property of general cytotoxicity instead of RAS-mutant-specific killing. In addition, the anti-proliferative effect of MP-HJ-1b was also observed with elevated IC₅₀ values on other non-oncogenic cells (Supplementary Table S1; Supplementary Fig. S1). This result has been further verified over more than 1,000 tumor cell lines (Supplementary Excel file), which lead us to hypothesize that MP-HJ-1b might have interrupted essential components of cancer cells growth and survival.

**Figure 2.** MP-HJ-1b blocks mitosis by inhibiting microtubule polymerization

To understand the deleterious effect of MP-HJ-1b on cells viability, we examined the influence of MP-HJ-1b on different cellular events by monitoring cell cycle with flow cytometer. Typically, MP-HJ-1b treated HeLa cells showed cell cycle arrest at G₂-M phase (Fig. 2A). The mitotic blocking function of
MP-HJ-1b was further verified from other cell lines (Supplementary Fig. S2). Consistently, MP-HJ-1b promoted cell apoptosis and increased cell death (Supplementary Fig. S3).

To clarify the specific period of cell-cycle blockage, we performed immunofluorescence-based imaging analysis to MP-HJ-1b–treated cells. As shown in Fig. 2B, the metaphase cell displays a bipolar spindle apparatus and chromosomes lined up along the equatorial plate. After MP-HJ-1b treatment, the normal spindle was disappeared. Interestingly, several tubulin-spots, scattered chromosomes and centromeres were present in the cell (Fig. 2B; Supplementary Fig. S4). This phenomenon suggests that the cell was arrested in pro-metaphase according to the disappearance of nuclear membrane and characterization of chromosomes and centromeres.

On the basis of this observation, we hypothesized that MP-HJ-1b might have interfered cell cytoskeleton system. We were specifically interested in exploring which composition of these tubulin-spots was abrupted by MP-HJ-1b, because all three common subtypes of tubulin: α-tubulin, β-tubulin and γ-tubulin can constitute microtubule, spindle, centrosome and microtubule organizing center (MTOC) in mitosis, respectively. The existence of two centrosomes in each cell after the compound treatment indicated that the spike-like spots were not centrosomes (Fig. 2C and D). MTOC, as the beginning of microtubule, is centered on the γ-tubulin and mediates tubulin nucleation phenomenon and replaces centrosome in cells without centriole. Next, we labeled γ-tubulin and α-tubulin at the same time (Fig. 2E). As the results showed in Fig. 2E, the spike-like spots are not MTOC. These data suggest that MP-HJ-1b blocks mitosis by influencing microtubule.

To investigate whether compound MP-HJ-1b affects the tubulin polymerization, a cell-free in vitro tubulin polymerization assay was performed. Dissolved tubulin polymerizes gently in vitro at 37°C with supply of GTP. This process was accelerated when paclitaxel (a microtubule stabilizing agent) presented (Fig. 2F and G). On the contrast, vincristine inhibited polymerization reaction and depolymerized microtubule dramatically (Fig. 2F). Interestingly, MP-HJ-1b showed the same depolymerization effect as that of vincristine in a dose-dependent manner (Fig. 2G). These data lead us to hypothesize that MP-HJ-1b suppresses microtubule polymerization via direct binding to the tubulin heterodimer.

**MP-HJ-1b alters microtubule in a distinct mechanism**

To further examine the biophysical impact of MP-HJ-1b on microtubule, we compared it with vinca alkaloids and paclitaxel, in which both are the most widely used microtubule-related antitumor drugs. Paclitaxel is a microtubule stabilizing agent and promotes microtubule polymerization. Although vinca alkaloids inhibit microtubule polymerization and promote microtubule depolymerization. Both MP-HJ-1b and vincristine blocked mitosis and arrested cells in pro-metaphase (Fig. 3A). Disordered chromosomes and dissembled nuclear envelope surrounded scattered chromosomes were observed in cell treated with both compounds. A close examination revealed that MP-HJ-1b induced a spike-like structure in the compounds treated cells.

We further verified the inhibitory effect of MP-HJ-1b on the polymerization of microtubules in vitro by transmission electron microscope (TEM). The α-tubulin and β-tubulin built a hollow tubular structure at room temperature in the present of guanosine triphosphate (GTP; Fig. 3B), and paclitaxel could stabilize this structure (Fig. 3C). In contrast, there was no visible microtubule structure when vincristine was added at the beginning of the reaction (Fig. 3D). Although these globular proteins, α-tubulin and β-tubulin, were aggregated smooth and irregular pellets when

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**Figure 3.** Alteration of microtubules in cells and in vitro caused by tubulin inhibitors. A, Compound MP-HJ-1b (200 nmol/L), vincristine (50 nmol/L), and colchicines (250 nmol/L) depolymerize microtubules in HeLa cells. B–E, Appearance of tubulin formation was monitored by TEM. 1 μmol/L paclitaxel, 8 μmol/L vincristine, or 20 μmol/L compound MP-HJ-1b.
MP-HJ-1b induced formation of a new conformation of microtubules although the efficiency was lower than vincristine (Fig. 2F). These results suggested that MP-HJ-1b uses a different mechanism to block microtubule’s normal function from that of vincristine.

Crystal structure of the tubulin–MP-HJ-1c complex

To understand the molecular mechanism of MP-HJ-1b on inhibition of microtubule polymerization, we performed a structural analysis. Compound MP-HJ-1c, an analog of MP-HJ-1b with the comparable pharmacological efficacy (Supplementary Fig. S5) and better solubility was used. The crystal structure of a protein complex composed of α/β-tubulin, the stathmin-like protein RB3 and tubulin tyrosine ligase (T2R–TTL), complexed with MP-HJ-1c, was solved at 2.55 Å resolution (Fig. 4A). Details of the data collection and refinement statistics are summarized in Supplementary Table S2.

The high-resolution and clear density map enabled us to determine the position and orientation of the small-molecule inhibitor unambiguously (Fig. 4B), thus revealing the detailed interactions between MP-HJ-1c and tubulin (Fig. 4C). As seen in the crystal structure, MP-HJ-1c bound to the colchicine-binding site, which is a big pocket surrounded by a super β-sheet and two α-helices, and is capped by two loops (Fig. 4C). The agent MP-HJ-1c made hydrogen bonds with the side chains of P[N165, E198 and Y200, and the main-chain oxygen atom of Y236 (Fig. 4C). MP-HJ-1c also made extensive hydrophobic interactions with β-tubulin (Fig. 4C). A structure-based pharmacophore model for CBIs has been proposed recently (30), which consists of three hydrophobic centers (I, II, and III) and two hydrogen bond centers (IV and V). MP-HJ-1c occupied two hydrophobic centers I and II, and one hydrogen bond center IV (Fig. 4C).

Compared with colchicine, MP-HJ-1c was located much deeper in the β subunit and made no interaction with the α subunit (Fig. 4D). Very little overlap with colchicine was observed. Comparison between tubulin–MP-HJ-1c and tubulin–colchicine complex structures showed that the binding of the different colchicine-site ligands did not affect the global conformation of tubulin nor the T2R complex. The root mean square deviation (RMSD) for 2,146 Cα atoms between tubulin–MP-HJ-1c and tubulin–colchicine complexes is 0.33 Å. The major conformational changes concern the αT5 loop of the colchicine domain (Fig. 4D).

MP-HJ-1b overcomes MDR in vitro and in vivo

As MP-HJ-1b could efficiently block cell migration and colony formation (Supplementary Fig. S6; Supplementary Fig. S7), we next investigated whether MP-HJ-1b is able to overcome MDR in cancer, as MDR has posed a major challenge for cancer chemotherapy agents in clinical treatment. Three pairs of cell lines, including HeLa (cervical cancer) and HeLaR (paclitaxel resistance), RB3 (ovarian cancer), and A2780R (paclitaxel resistance), were randomly assigned to three groups that were intraperitoneal oil, respectively. For tumors derived from HeLa and K562 cells, the tumor volume reached approximately 100 mm³, the nude mice were randomly assigned to three groups that were intraperitoneal administered 25 mg/kg MP-HJ-1b, 0.2 mg/kg vincristine, or corn oil, respectively. For tumors derived from HeLa and K562 cells, both compounds inhibited tumor growth and showed excellent anticancer activity (Fig. 5C, Supplementary Tables S4–S5, Supplementary Figs. S10–S11). Consistent with cellular proliferation shown on Fig. 5B; Supplementary Table S3. MP-HJ-1b clearly showed a distinct pharmacological efficacy from these two compounds by sufficiently suppressing MDR in different type of tumor cell lines.

As MP-HJ-1b showed promising pharmacological properties with half-life of more than 9 hours (Supplementary Fig. S9), we further performed in vivo experiments to examine the relevant pharmacological antitumor efficacy of MP-HJ-1b using vincristine as a reference. We built three subcutaneous xenograft models by transplanted HeLa, HeLaR or K562 cells into nude mice. When the tumor volume reached approximately 100 mm³, the nude mice were randomly assigned to three groups that were intraperitoneal administered 25 mg/kg MP-HJ-1b, 0.2 mg/kg vincristine, or corn oil, respectively. For tumors derived from HeLa and K562 cells, both compounds inhibited tumor growth and showed excellent anticancer activity (Fig. 5C, Supplementary Tables S4–S5, Supplementary Figs. S10–S11). Consistent with cellular proliferation shown on Fig. 5B; Supplementary Table S3. MP-HJ-1b clearly showed a distinct pharmacological efficacy from these two compounds by sufficiently suppressing MDR in different type of tumor cell lines.

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results, the tumor growth was also inhibited by MP-HJ-1b in the HeLaR xenograft model but not by vincristine (Fig. 5C; Supplementary Table S4). In addition, there were no body weight lost and observed side effects during the drug treatment (Fig. 5D; Supplementary Fig. S10), suggesting the tolerability of MP-HJ-1b. The results show that MP-HJ-1b overcomes MDR both in vivo and in vitro.

Discussion

Targeting microtubule remains the first line ammunition for many clinicians, even with more selective approaches available for cancer treatment. Because of the essential role of microtubule plays in cancer cells and existence of several difficulty-to-treat malignancies, such as pancreatic cancer, microtubule inhibitors, paclitaxel and vincristine are commonly used in the treatment of varieties cancers, including breast carcinoma, ovarian cancer, acute leukemia, malignant lymphoma, lung cancer (31–33). Specifically, the results of recent clinical studies indicated that microtubule inhibitors nab-paclitaxel combining with gemcitabine prolongs survival of pancreatic ductal adenocarcinoma patients. Authors have proposed that nab-paclitaxel may play a role for targeting KRAS by disrupting its intracellular trafficking (34). Even though the primary observation indicated that...
MP-HJ-1b might not be a RAS-mutant–specific compound, the potential role of MP-HJ-1b in RAS protein trafficking is worth to explore in the future. Second, the development of MDR in cancer is an unavoidable challenge for microtubule inhibitors for their clinical implementation. ABC transporters play the crucial role in the development of MDR. Even though MDR1/ABCB1 is considered as one of the most common and critical pumps for drug efflux and reducing intracellular drug accumulation, it is extremely difficult to target ABC transporters in overriding cancer MDR. Because the complexity of drug pumps expression pattern and functional redundancy greatly limit the utility of those ABC inhibitors in cancer therapy (35–37). Thus to develop a mechanistic different microtubule inhibitors could be clinically beneficiary to circumvent cancer drug resistance. Our data reveal that MP-HJ-1b binds to microtubule colchicine-binding site, which has no drug being approved to target this site so far. It is interesting to observe that colchicine-binding site microtubule inhibitors can act as vascular-targeting agents. Therefore, besides used as antimitotic agents, colchicine-binding site compounds can rapidly depolymerize microtubules of newly formed vasculature to shut down the blood supply to tumors (38, 39). The potent in vivo pharmacological efficacy of MP-HJ-1b need to be further explored on its inhibition on vascular-targeting function.

As we know that currently most microtubule targeted compounds have been discovered from natural products. It is difficult and expensive to make and optimize those natural products for intracellular drug accumulation, it is extremely difficult to target ABC transporters in overriding cancer MDR. Because the complexity of drug pumps expression pattern and functional redundancy greatly limit the utility of those ABC inhibitors in cancer therapy (35–37). Thus to develop a mechanistic different microtubule inhibitors could be clinically beneficiary to circumvent cancer drug resistance. Our data reveal that MP-HJ-1b binds to microtubule colchicine-binding site, which has no drug being approved to target this site so far. It is interesting to observe that colchicine-binding site microtubule inhibitors can act as vascular-targeting agents. Therefore, besides used as antimitotic agents, colchicine-binding site compounds can rapidly depolymerize microtubules of newly formed vasculature to shut down the blood supply to tumors (38, 39). The potent in vivo pharmacological efficacy of MP-HJ-1b need to be further explored on its inhibition on vascular-targeting function.

It is still unclear that different tubulin targeting agents, such as taxanes and vincas show separate antitumor effects. However, this tissue-specific inhibitory mechanisms provides a rationale to develop novel approaches aim to improve on existing compounds by profiling tumor sensitivities, that could reduce side effects such as peripheral neuropathy toxicity (1). With more available compounds targeting the numerous other components of the tubulin–microtubule complex certainly would be an important aspect of fully exploiting its anticancer potential in that the drug synergistic effect can be achieved by combining two or more targets in the same system.

Nevertheless, the feedback from bedside clearly indicated an urgent need of search new strategy of targeting microtubule and overcoming MDR in human cancer treatment. The series of small molecules represented by MP-HJ-1b showed promising pharmacological efficacy on overriding MDR both in vitro and in vivo. The molecular mechanism of MP-HJ-1b’s inhibitory function is different from previous reported anti-MDR drugs by binding to the colchicine-binding site of tubulin, consequently depolymerizes microtubules and affects formation of the spindle. Our data reveal a novel scaffold that could be further developed as cancer therapeutics, especially for MDR cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: N. Ning, M. Wu, C.-H. Yun, X. Deng, Q. Chen, R. Ren


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Acknowledgments

This work was supported by the National Key Research and Development Program (2016YFC0902800 to R. Ren), the Key Project of National Science Foundation of China (81230055 to R. Ren), the Samuel Waxman Cancer Research Foundation CoP Program, the National Natural Science Foundation of China (No. U1405223 to X. Deng), the Fundamental Research Funds for the Central Universities of China (No. 20720160064 to X. Deng), and the Key Project of Natural Science Foundation of China (81672722 to Q. Chen).

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Received February 10, 2018; revised July 12, 2018; accepted August 16, 2018; published first August 22, 2018.

References


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