Effect of ON 01910.Na, an Anticancer Mitotic Inhibitor, on Cell-Cycle Progression Correlates with RanGAP1 Hyperphosphorylation

Irina A. Oussenko, James F. Holland, E. Premkumar Reddy, and Takao Ohnuma

Abstract

The benzyl styryl sulfone, ON 01910.Na, is a novel anticancer agent that inhibits mitotic progression and induces apoptosis in most cancer cell lines. We examined the effect of ON 01910.Na on DNA damage–signaling molecules upstream of Cdc25C (Chk1, Chk2, and H2AX), as well as on Ran GTPase-activating protein 1 conjugated to small ubiquitin-related modifier 1 (RanGAP1-SUMO1), a mitosis coordinator. Prostate cancer, lymphoma, and leukemic cells were incubated with the drug for 4, 16, or 24 hours. Cell lysates were resolved on SDS-PAGE and analyzed by Western blot. Camptothecin and doxorubicin treatment caused activation/phosphorylation of DNA damage-responsive molecules by 4 hours, whereas ON 01910.Na did not do so. ON 01910.Na caused hyperphosphorylation of RanGAP1-SUMO1 within 4 hours that was sustained for more than 24 hours. Mild phosphorylation of Chk2 was observed only after 24-hour exposure, indicating that DNA damage response was not an initial effect of ON 01910.Na. MOLT-3 cells, synchronized by double-thymidine block, when released into a medium containing ON 01910.Na, accumulated mitotic cell number with a peak from 10 to 14 hours and remained near plateau for 20 hours, which corresponded with the time of RanGAP phosphorylation. ON 01910.Na had minimal effects on tubulin polymerization. These findings imply that ON 01910.Na neither induces DNA damage directly nor acts as a tubulin toxin. Its biological activity appears to rely on prolonged phosphorylation/hyperphosphorylation of RanGAP1-SUMO1. M-phase arrest and the consequent induction of apoptosis that follows could possibly be attributed to it. ON 01910.Na may act as an inhibitor of a RanGAP1-SUMO1 phosphatase or a stimulant of a new kinase. RanGAP1-SUMO1 appears to be a new target pathway for cancer chemotherapy. Cancer Res; 71(14):4968–76. ©2011 AACR.

Introduction

ON 01910.Na (Fig. 1) is a novel benzyl styryl sulfone, which showed cytotoxic activity against a variety of human tumor cell lines in vitro and growth-inhibitory activity in xenografts in nude mice (1). The compound is currently in phase I and II clinical trials (2–5). Although the compound was originally considered to be a novel polo-like kinase 1 (Plk1) inhibitor (1), a direct effect on Plk1 could not be confirmed in subsequent studies. The exact mechanism of action of ON 01910.Na, thus, remains unknown. Available data show that the drug produces 3 major abnormalities in tumor cells: (i) abnormal cell division including irregular chromosomal segregation and cytokinesis; (ii) G2–M arrest and apoptosis in many tumor cell lines (in contrast, normal fibroblasts were arrested in G1 phase); and (iii) decreased expression of Cdc25C (1).

In normal cells, Cdc25C is phosphorylated by Plk1. Phosphorylated Cdc25C is a phosphatase, which catalyzes Cdc2 (Cdk1) into its unphosphorylated state or active form, resulting in cell-cycle progression from G2 to mitosis. ON 01910.Na decreases Cdc25C, with the result that Cdc2 remains in a phosphorylated (inactive) state. Consequently, cells fail to traverse mitosis and become blocked in the G2–M phase of the cell cycle. Because cells cannot be arrested in mitosis indefinitely, and they are forced to activate apoptotic pathways.

During the initial characterization study, we found that ON 01910.Na was not cytotoxic or inhibitory to yeast cells (Oussenko and Ohnuma, unpublished observation). In cross-resistance studies, we found that many drug-resistant cells were not cross-resistant to ON 01910.Na. Minor cross-resistance to ON 01910.Na was observed only in cisplatin- and flavopiridol-resistant cells. We attempted to develop ON 01910.Na-resistant cells by incubating tumor cells with...
Increasing concentrations of ON 01910.Na in a culture medium, but failed after 2 years of attempting to do so (6). These observations suggested that ON 01910.Na did not have direct cytocidal effects. Cell death might have occurred simply because cells cannot stay in G2–M phase indefinitely. Thus, G2–M arrest could be the primary lethal effects of ON 01910.Na.

In search of a mechanism of action, we developed a working hypothesis that ON 01910.Na acts, as do many cytotoxic anticancer agents, on DNA damage–response signal pathways (with emphasis on events upstream of Cdc25C). Nööda and colleagues (7) provided a schematic molecular organization of DNA damage checkpoints throughout the cell cycle. This scheme identified target molecules for ON 01910.Na upstream of Cdc25C. One known upstream effector molecule is Plk1. Alternatively, ON 01910.Na might have activated upstream inhibitor molecules Plk3, Chk1, or Chk2. Plk3 is inhibited by ATM. Chk1 is activated by ATR. Chk2 is activated by ATM or DNA-dependent protein kinases (DNA-PKs; ref. 8).

To our list of targets including DNA damage–response signals (e.g., Chk1, Chk2, and H2A.X), we also added Ran GTpase-activating protein 1 (RanGAP1; refs. 9–12). Ran is a Ras-related GTpase that is required for nuclear transport, cell-cycle control, mitotic spindle formation, and postmitotic nuclear reassembly. In many instances, RanGAP1 accomplishes its functions after covalently binding with SUMO1 (small ubiquitin-related modifier 1) or SUMOylation (9–12). In the present study we referred to RanGAP1 SUMO1 as a mitosis coordinator. Because many tubulin toxins are effective anticancer agents, we also examined the effects of ON 01910.Na on tubulin polymerization in vitro.

Materials and Methods

Anticancer and other agents

ON 01910.Na and its inactive analogue, ON 01911, were provided by Onconova Therapeutics, Inc. ON 01910.Na is a water-soluble white solid. The material was dissolved at 10 mmol/L in 500 mmol/L NaHCO3 buffer prepared in PBS (pH 7.4) and further diluted in distilled water to prepare a stock solution with a concentration of 1 mmol/L that was kept at −20°C. Its inactive isomer, ON 01911, was dissolved in dimethyl sulfoxide (DMSO) at 1 mmol/L concentration and was kept at −20°C. Doxorubicin, camptothecin, and nocodazole were purchased from Sigma-Aldrich.

Cell lines

The MOLT-3 acute lymphoblastic leukemia (ALL; ref. 13) was maintained in our laboratory. DU-145 prostate cancer and U937 lymphoma cells were purchased from the American Type Culture Collection (ATCC). All cell lines were maintained in RPMI-1640 medium (Gibco-Invitrogen) supplemented with 10% FBS and antibiotics (penicillin and streptomycin). These cells were tested at Johns Hopkins Genetic Resources Core Facility, Baltimore, Maryland, by obtaining short tandem repeat profiles and authenticated by comparing them with the ATCC human cell line collection (in case of DU-145 and U937) or our own reference stocks (in case of MOLT-3).

Drug treatment, sample collection, and preparation of total cell lysates

Exponentially growing cells were seeded at cell densities of 200,000 cells/mL (U937, MOLT-3) or 20,000 cells/mL (DU145) and cultured for 24 hours. Then, drugs and control vehicles were added appropriately, and cells were further cultured for designated time periods. Afterward, suspension cells were collected by centrifugation, washed with ice-cold 1× PBS and spun again to obtain cell pellets. In case of cells in a monolayer culture, the medium was aspirated and cells were carefully rinsed with ice-cold 1× PBS and spun again to obtain cell pellets. Cells were then resuspended in 1 mL of 1× PBS into Eppendorf tubes and cell pellets were obtained by centrifugation at 600 × g for 4 minutes at 4°C. Total cell lysates were prepared either by resuspending cells directly in reducing/denaturing SDS-PAGE sample buffer ("1 × SB" method) or by lysing cells under nondenaturing conditions ("Lys A" method). Further details of the "1 × SB" method...
are as follows: cell pellets were resuspended in proportion to their wet weight in 0.6 to 0.9 mL of gel sample buffer [1× SB] according to the method described by Laemmli (14): 62.5 mmol/L Tris–HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mmol/L dithiothreitol (DTT), 0.01% w/v bromophenol blue supplemented with inhibitors [complete protease inhibitors (Roche), 1 mmol/L NaF, 1 mmol/L Na3VO4, and 0.5 mmol/L phenylmethylsulfonylfluoride] and RNase-free DNase I (Roche; 1500 units per mL of the sample buffer); then, chromosomal DNA was sheared by passing samples through a 25-G needle 10 times up and down, and samples were stored at −80°C. Further details for the “Lys A” method were as follows: cells were resuspended in 200 to 400 μL of lysis buffer A (10 mmol/L Tris–HCl pH 8.0, 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L EDTA, 1 mmol/L EGTA, and 0.5% NP-40, supplemented with complete protease inhibitors), in a method adapted from Agarwal and colleagues (15), kept on ice for 30 to 40 minutes, during which time samples were vortexed gently every 10 minutes, and then centrifuged to remove cell debris at 13,500 × g for 30 minutes at 4°C; the supernatants (“lysates”) were then transferred to fresh tubes containing 10% FBS and 0.01% w/v phenylmethylsulfonylfluoride] and RNase-A (DNase-free) for 30 minutes at 37°C. After the incubation, thymidine-containing medium was removed, and cells and the flasks were rinsed with prewarmed RPMI-1640 medium. Cells were re-plated in RPMI-1640 containing 10% FBS and incubated for 16 hours, after which cells were washed with RPMI-1640 and replated in RPMI-1640 containing 10% FBS and 2 mmol/L thymidine and incubated for 12 hours at 37°C. After double-thymidine exposure, cells were washed free of thymidine, divided into 3 portions, and seeded in 100-mm plates for continued incubation in either regular medium (i.e., “release medium”), release medium supplemented with 1 μmol/L of ON 01910Na (final concentration), or release medium supplemented with 1 μmol/L of nocodazole (final concentration). At timed intervals, plates of each type were withdrawn, and cells were subjected to: (i) cell-cycle distribution analysis, and (ii) Western blot analysis for expression of RanGAP1, phospho-RanGAP1, and Lamin B.

**Western blot analysis and antibodies used**

Western Blot analyses were carried out in a standard manner (16). Shortly before being loaded on a gel, cell lysates of the type “1× SB” were simply defrosted, whereas protein samples from cell lysates of the type “Lys A” were prepared by mixing 20 μL of cell lysate containing 20 μg of protein with 10 μL of 3× SB Laemmli Sample Buffer; then, both sample types were boiled for 5 minutes in water bath, cooled on ice, and microcentrifuged for 5 minutes. Samples were resolved on 7% (unless otherwise stated) SDS-PAGE alongside prestained protein standards (SeaBluePlus; Invitrogen). Using Mini-Genie Electroblotter (Idea Scientific), proteins were transferred from the gel onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad) for 1.5 to 2 hours at 12 V in Towbin buffer supplemented with 0.2% SDS. The PVDF membrane was prewetted in methanol, soaked in water for 5 minutes, and equilibrated in the transfer buffer for 20 minutes before the electro-blotting procedure. After the transfer, membranes were blocked in 1× TTBS/5% dry milk for 1 hour at room temperature, then probed overnight at 4°C with a selected primary antibody: anti-RanGAP1, anti-Lamin B, anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA); anti-SUMO-1, anti-phospho-Chk1 (S296), anti-phospho-Chk2 (T68), anti-Chk1, anti-Chk2, anti-phospho-histone H2AX (S139), anti-ct-tubulin [Cell Signaling Technology]; or anti-phospho-RanGAP1 (S428) antibody (Sigma-Aldrich); this was followed by routine washing and then coupling to a corresponding horseradish peroxidase–conjugated secondary antibody (anti-mouse and anti-goat antibodies were from Santa Cruz Biotechnology, and the anti-rabbit antibody was from Alpha Diagnostic International). Probed proteins were visualized using ECL Plus Western Blotting Detection System (GE Healthcare) and the images were developed on Blue X-ray film CL-XPosure (Pierce; Thermo Fisher).

**Cell synchronization**

Double-thymidine exposure technique was used (17, 18). Exponentially growing MOLT-3 cells were washed and seeded at a density of 500,000 cells/mL in RPMI-1640 medium containing 10% FBS and 2 mmol/L thymidine (Sigma-Aldrich) and incubated for 12 hours at 37°C. After the incubation, thymidine-containing medium was removed, and cells and the flasks were rinsed with prewarmed RPMI-1640 medium. Cells were re-plated in RPMI-1640 containing 10% FBS and incubated for 16 hours, after which cells were washed with RPMI-1640 and replated in RPMI-1640 containing 10% FBS and 2 mmol/L thymidine and incubated for 12 hours at 37°C. After double-thymidine exposure, cells were washed free of thymidine, divided into 3 portions, and seeded in 100-mm plates for continued incubation in either regular medium (i.e., “release medium”), release medium supplemented with 1 μmol/L of ON 01910Na (final concentration), or release medium supplemented with 1 μmol/L of nocodazole (final concentration). At timed intervals, plates of each type were withdrawn, and cells were subjected to: (i) cell-cycle distribution analysis, and (ii) Western blot analysis for expression of RanGAP1, phospho-RanGAP1, and Lamin B.

**Cell-cycle distribution analysis by flow cytometry**

MOLT-3 cells obtained from the above-described synchronization experiment were collected by centrifugation, fixed in 70% ethanol, washed, resuspended in 1× PBS, treated with RNase-A (DNase-free) for 30 minutes at 37°C, mixed in a 1:5 proportion with 25 μg/mL propidium iodide (PI) solution in 1× PBS, filtered through Falcon mesh caps to remove cell aggregates, and analyzed by a FACScan cytometer (Becton Dickinson). Data for 20,000 events per sample were collected and analyzed using “CellQuest” software (Becton Dickinson). To calculate the percentage of cells in each cell phase on the fluorescence histograms obtained, we selected data from 1 sample in each treatment series where picks corresponding to 2N and 4N DNA content, were clearly defined, and then we positioned the gates over the sub-G1, G1, S, and G2–M areas of the histogram. Thus selected, statistical gates were applied to the rest of samples in the corresponding series.

In a replication of the synchronization experiment, the percent of mitotic cells was determined simultaneously with cell-cycle distribution analysis by double-staining fixed cells for phospho(Ser10)-histone H3 (marker of mitosis) as well as for DNA content with PI using a method adapted from Xu and colleagues (19). In short, after fixation the cells were resuspended in 1 mL of 1× PBS containing 0.25% Triton X-100 and incubated on ice for 15 minutes. After centrifugation, the cell pellet was resuspended in 100 μL of 1× PBS/1% bovine serum albumin (BSA) containing anti-pS10H3 antibody at 1:30 dilution and incubated for 2 hours at room temperature. Then cells were centrifuged, the unbound antibody was washed off with 1 mL of 1× PBS/1% BSA, and cells were incubated in 100 μL of 1× PBS/1% BSA containing fluorescein isothiocyanate (FITC)-conjugated secondary antibody at 1:30 dilution for 30
minutes in the dark. Subsequently, cells were washed with 1 mL of 1× PBS/1% BSA to remove the unbound antibody, resuspended in 1 mL of 1× PBS containing 25 μg/mL PI and 250 μg/mL RNase-A, and incubated for at least 30 minutes at room temperature in the dark. Samples were filtered through Falcon mesh caps just before running them on a FACScan cytometer. Proper gating and fluorescence compensation were done on samples stained singly with either antibodies or PI. Anti-pS443/447 antibody (primary antibody) was obtained from Millipore and the goat anti-rabbit IgG FITC conjugate (secondary antibody) was from Jackson ImmunoResearch Laboratories.

Tubulin polymerization assay
We used fluorescence-based Tubulin Polymerization Assay Kit (Cytoskeleton; catalogue # BK011P) according to the manufacturer's instructions. The kit-based assay is an adaptation of an assay originally described by Bonne and colleagues (20), where polymerization is followed by fluorescence enhancement due to the incorporation of a fluorescent reporter into microtubules as polymerization occurs. Briefly, test compounds and their corresponding vehicles were pipetted into a 96-well microtiter plate, then the tubulin reaction mix was quickly added to the wells, and the tubulin polymerization was initiated by transferring the plate to a 37°C chamber of a plate reader. Fluorescence emission was measured at 425 to 445 nm (excitation wavelength was 340 to 360 nm) using the Safire 2 Plate Reader (Tecan). Kinetics of changes in fluorescence was measured every minute for 60 minutes. The tubulin polymerization was monitored at this point, and new bands were tentatively named by 2 methods: "1× SB" and "Lys A". Method "1× SB" achieved better preservation of SUMOylated RanGAP1 (both, phosphorylated and nonphosphorylated), whereas "Lys A" resulted in substantial deSUMOylation yielding larger amounts of free RanGAP1 and otherwise undetectable phospho-RanGAP1. The deSUMOylation observed could be attributed to the presence of inadequate protease inhibitors in the nondenaturing lysis buffer in "Lys A" method. We routinely included a standard protease inhibitor cocktail, which, according to the manufacturer (Roche), protects against Ser-, cysteine-, and metalloproteases; however, SUMO proteases represent a distinct class of proteases and require specific inhibitors such as iodoacetamide or N-ethyl maleimide (21). In method "1× SB," cell lysis takes place under denaturing conditions, thus preventing action from any type of proteases. We applied the "1× SB" method of preparing cell lysates throughout the rest of the experiments. However, the aforementioned deSUMOylation of samples allowed us to distinguish at least 2 phosphorylation states of RanGAP1-SUMO1. Thus, samples in 3A that were prepared by "Lys A" method, resolved on 6% SDS-PAGE gel, and probed identically (Fig 3B, the inset oval) showed separation of 3 bands: RanGAP1-SUMO1, phosphorylated RanGAP1-SUMO1, and hyperphosphorylated RanGAP1-SUMO1.

Phosphorylation and hyperphosphorylation of RanGAP1-SUMO1, and not of free RanGAP1, are revealed when adequate lysis method is used
We compared 2 procedures for the preparation of total cell lysates (Fig. 3). In Figure 3A, DU-145 cells were treated with ON 01910.Na for 16 hours and processed for total cell lysates by 2 methods: "1× SB" and "Lys A." Method "1× SB" achieved better preservation of SUMOylated RanGAP1 (both, phosphorylated and nonphosphorylated), whereas "Lys A" resulted in substantial deSUMOylation yielding larger amounts of free RanGAP1 and otherwise undetectable phospho-RanGAP1. The deSUMOylation observed could be attributed to the presence of inadequate protease inhibitors in the non-denaturing lysis buffer in "Lys A" method. We routinely included a standard protease inhibitor cocktail, which, according to the manufacturer (Roche), protects against Ser-, cysteine- and metalloproteases; however, SUMO proteases represent a distinct class of proteases and require specific inhibitors such as iodoacetamide or N-ethyl maleimide (21). In method "1× SB," cell lysis takes place under denaturing conditions, thus preventing action from any type of proteases. We applied the "1× SB" method of preparing cell lysates throughout the rest of the experiments. However, the aforementioned deSUMOylation of samples allowed us to distinguish at least 2 phosphorylation states of RanGAP1-SUMO1. Thus, samples in 3A that were prepared by "Lys A" method, resolved on 6% SDS-PAGE gel, and probed identically (Fig 3B, the inset oval) showed separation of 3 bands: RanGAP1-SUMO1, phosphorylated RanGAP1-SUMO1, and hyperphosphorylated RanGAP1-SUMO1.

Phosphorylation of RanGAP1-SUMO1 was the initial event in the presence of ON 01910.Na, whereas phosphorylation of DNA damage-response–signaling molecules was the initial event with doxorubicin and camptothecin
The different kinetics of phosphorylation of DNA damage-response molecules and the RanGAP1-SUMO1 mitosis coordinator are illustrated in Figure 4. Within 4 hours, camptothecin and doxorubicin activated Chk1, Chk2, and histone H2AX, whereas ON 01910.Na and nocodazole did not. In contrast, ON 01910.Na and nocodazole produced phosphorylation of RanGAP1-SUMO1 by 4 hours. At 24 hours, RanGAP1-SUMO1 continued being activated. Camptothecin- or doxorubicin-induced activation of Chk1 and Chk2 had subsided by then.
with a concomitant reduction of histone H2A.X phosphorylation. ON 01910.Na induced delayed mild activation of Chk2, suppression of Chk1 and Chk1 phosphate, and strong activation of histone H2A.X. Phosphorylation of Ser139 residue of the histone variant H2A.X, forming gammaH2A.X, is an early cellular response to the induction of DNA double-strand breaks. Detection of this phosphorylation event has emerged as a highly specific and sensitive molecular marker for monitoring DNA damage initiation and resolution (22).

Phosphorylated histone H3 at Ser10, specific for mitotic cells, was detected in the presence of ON 01910.Na or nocodazole at 4 and 24 hours, which is indicative of mitotic cell arrest. In contrast, doxorubicin and camptothecin reduced mitotic cell fraction, consistent with known S-phase cell kill.

These data show that early and prolonged phosphorylation of RanGAP1 SUMO1 occurred after treatment with ON 01910.Na or nocodazole as an initial event, whereas Chk2 and histone H2A.X phosphorylation was a secondary phenomenon.

Camptothecin and doxorubicin produced no phosphorylation of RanGAP1 SUMO1.

**Synchronization of cell cycle confirmed ON 01910.Na-induced phosphorylation of RanGAP1 SUMO1 and mitotic cell arrest**

Synchronization studies are illustrated in Figure 5. MOLT-3 cells that were released into drug-free medium reached mitotic cell peak after 9 to 10 hours and continued to cycle with synchronized transition into G1 at 15 to 16 hours (Fig. 5B).
During this period, RanGAP1/C1 SUMO1 was not phosphorylated, and no cleaved Lamin B was detected (Fig. 5C). MOLT-3 cells, which were synchronized by double-thymidine block and released into medium supplemented with ON01910.Na, resulted in peak accumulation of mitotic cells at 10 to 14 hours. The G2–M cell fraction remained in a near-plateau state for more than 20 hours (Fig. 5B). This time period correlated with the hyperphosphorylation of RanGAP1/C1 SUMO1 (Fig. 5D). Cleaved Lamin B, detected at 16 hours and thereafter in ON01910.Na-containing release medium, confirmed an active apoptotic process during this period.

ON 01910.Na had no effects on tubulin polymerization
Tubulin polymerization assay (Fig. 6) revealed that both ON 01910.Na and control ON 1911 had only a minor inhibition or no effects, whereas nocodazole inhibited the process. Lack of effects of ON 01910.Na on tubulin polymerization is confirmatory of an earlier report (1).

Discussion
ON 01910.Na is a novel anticancer agent that inhibits mitotic progression and induces apoptosis in most cancer cell lines. We
Figure 5. Synchronization of MOLT-3 cells by double-thymidine block followed by release in drug-free medium or medium supplemented with either ON 01910.Na or nocodazole (NOC; both at 1 μmol/L), ND, no drug. Samples were collected at designated time points after release from the block and analyzed by flow cytometry for the cell-cycle distribution (A and B) and by Western blot analysis (C and D). Percentage of mitotic cells was separately obtained by fixing cells for phospho(Ser10)-histone H3 (5B). Abbreviations used (in C and D): FL, full length; CL, cleaved; pRS, phospho-RanGAP1-SUMO1; RS, RanGAP1-SUMO1; R, RanGAP1; β-A, β-actin.
examined whether ON 01910.Na activated DNA damage-response proteins, and found that ON 01910.Na induced no consistent activation or only delayed activation of proteins traditionally associated with this response. In contrast, both doxorubicin and camptothecin, used as controls, provided quick activation of Chk1, Chk2, and histone H2AX. We interpreted the delayed phosphorylation of Chk2 and histone H2AX and suppression of Chk1 by ON 01910.Na and nocodazole as a secondary response. In contrast, we found that ON 01910.Na-induced hyperphosphorylation of RanGAP1-SUMO1 was correlated with accumulation of mitotic cells, prolonged M-phase arrest, and apoptotic cell death. The inactive analogue ON 01911 was unable to induce phosphorylation of RanGAP1-SUMO1.

On the basis of these results, we conclude that ON 01910.Na is neither a DNA damage-response inducer nor a tubulin toxin, both of which are common mechanisms associated with the action of many anticancer agents. ON 01910.Na's biological activity may, at least in part, rely on prolonged hyperphosphorylation of RanGAP1-SUMO1, which correlates with M-phase arrest, leading physiologically to induction of apoptosis. Our observation is consistent with a report by Swaminathan and colleagues (11), who showed that RanGAP1-SUMO1 was phosphorylated at the onset of mitosis and remained associated with RanBP2 (Ran-binding protein2) upon nuclear pore-complex disassembly. Nocodazole-induced cell-cycle arrest led to quantitative conversion of RanGAP1-SUMO1 into phosphorylated RanGAP1-SUMO1. We find that these characteristics are shared with ON 01910.Na, although we also found a striking difference between ON 01910.Na and nocodazole: nocodazole produced inhibition of tubulin polymerization, whereas ON 01910.Na did not. Although ON 01910.Na did not have a direct effect on microtubule polymerization in vitro, this does not exclude the possibility that it might indirectly affect polymerization in vivo.

It may be concluded that RanGAP1 hyperphosphorylation is an effect of ON 01910.Na that correlates with its effect on cell-cycle progression. Whether there are any direct threads underlying the correlation of the effects of ON 01910.Na on cell-cycle progression and hyperphosphorylation of RanGAP1-SUMO1 is unknown. It is possible that either a kinase or a phosphatase may be the target of the drug; however, further studies are required to determine this. If the drug does target a kinase or a phosphatase, it is likely that RanGAP1 represents just one affected target. Further studies will also be required to determine whether RanGAP1 and/or other targets are functionally relevant to the observed cell-cycle arrest.

Furthermore, whether other agents that correlate with (hyper)phosphorylation of RanGAP1-SUMO1, per se, can be useful anticancer agents remains to be established. The impressive activity of ON 01910.Na in human cancer cell lines in vitro (1) as well as in human tumor xenografts (23,24) and some clinical cancers (2, 4, 5) suggests that inhibition of dephosphorylation of RanGAP1-SUMO1-phosphate is a new molecular target for cancer chemotherapy.

Disclosure of Potential Conflicts of Interest

J.F. Holland is a consultant to Onconova Therapeutics, Inc. E. Premkumar Reddy is a consultant and stock-holder of Onconova Therapeutics, Inc. The other authors disclosed no potential conflicts of interest.

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References


Correction: Effect of ON 01910.Na, an Anticancer Mitotic Inhibitor, on Cell-Cycle Progression Correlates with RanGAP1 Hyperphosphorylation

In this article (Cancer Res 2011;71:4968–76), which was published in the July 15, 2011, issue of Cancer Research (1), an incorrect version of Fig. 1 was published in the print version of the journal. The correct version of this figure is provided below.

Reference


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