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PRECLINICAL STUDY

PARP3 inhibitors ME0328 and olaparib potentiate vinorelbine sensitization in breast cancer cell lines

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Abstract

Purpose PARP-3 is member of the PARP family of poly (ADP-ribose) polymerases involved in ADPribosylation. PARPs are involved in the basic mechanisms of DNA repair. PARP3, a critical player for efficient mitotic progression, is required for the stabilization of the mitotic spindle by regulation of the mitotic components, NuMA and Tankyrase 1.

Methods The sensitization effect of vinorelbine on PARP3 inhibition-induced cytotoxicity was assessed by the SRB assay. The contribution of programed cell death and cell cycle arrest to the sensitization effect were determined by assessing changes in Annexin V, a marker of apoptosis. Alterations in cell cycle progression were assessed by cell cycle analysis. We used immunofluorescence to assess the effect of vinorelbine and/or PARP3 inhibitors on tubulin and microtubule depolarization. The PARP3 chemiluminescent assay kit was used for PARP3 activity.

Results PARP3 inhibitors sensitize breast cancer cells to vinorelbine, a vinca alkaloid used in the treatment of metastatic breast cancer. Olaparib which was originally described as a PARP1 and 2 inhibitor has recently been shown to be a potent PARP3 inhibitor while ME0328 is a more selective PARP3 inhibitor. The combination of vinorelbine with nontoxic concentrations of ME0328 or olaparib reduces vinorelbine resistance by 10 and 17 fold, respectively, potentiating vinorelbine-induced arrest at the G2/M boundary. In addition, PARP3 inhibition potentiates vinorelbine interaction with tubulin. Furthermore, olaparib or ME0328 potentiates vinorelbine-induced PARP3 inhibition, mitotic arrest, and apoptosis.

Conclusion Our results indicated this approach with PARP3 inhibitors and vinorelbine is unique and promising for breast cancer patients with metastases. This combination could significantly increase the survival of breast cancer patients with metastases.

Keywords PARP3 · Breast cancer · ME0328 · Olaparib · Vinorelbine · Drug sensitization

Introduction

Poly (ADP-ribose) polymerase (PARP) resulting poly (ADP-ribosyl)ation is an early player in modification of proteins detected at single strand breaks (SSBs) and double strand breaks (DSBs) contributing to DNA repair. The PARP superfamily member is involved in regulation of cell proliferation and differentiation by affecting transcription, chromatin status, and telomere function. PARP1 is 116 kD a nuclear protein and the most known member of PARP family whose catalytic poly(ADP-ribosyl)ation is one of the earliest responses to DNA damage such as SSBs and DSBs [1, 2]. PARP1 interacts with proteins involved in DSB repair including ATM, DNA-PKcs, Ku80, MRE11, and NSB1. PARP2 may also be involved in base excision repair (BER). PARP2 and PARP1 share many functional characteristics [3, 4]. PARP3 has two distinct functions: (a)

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participation in DSB repair pathway(s) by interaction with the classical nonhomologous endjoining (C-NHEJ) proteins, DNA-PK, KU70, KU80, and DNA ligase IV and (b) regulation of mitotic progression [3]. While PARP3 may also promote alternative NHEJ [3, 5–8], the best defined role of PARP3 is in classical NHEJ. Inhibitors of DNA-PK or PARP individually can sensitize cancer cells to DNA-damaging agents [9–11]. PARP3 is required for efficient mitotic progression and is a critical player in the stabilization of the mitotic spindle and in promoting telomere integrity by association and regulation of the mitotic components, NUMA, and Tankyrase 1. PARP3 is necessary for mitotic spindle integrity during mitosis [3]. PARP3 appears to negatively regulate G1/S cell cycle progression without interfering with centrosome duplication [5]. PARP3 interacts with PARP1 at the centrosome and inhibition of PARP1 activity increases this association [12]. Active PARP3 has the ability to stimulate Tankyrase 1 auto-ADP ribosylation and modify NUMA in a DNA-independent manner [3, 13]. PARP3 inhibition results in delayed DSB repair and delayed spindle assembly [3, 8]. Following PARP inhibition, tumour cells which are deficient in homologous repair proteins are unable to repair DNA damage, resulting in chromosomal instability, cell-cycle arrest, and subsequent apoptosis followed by cell death knowing as synthetic lethality [14–16].

In spite of the undeniable progress in terms of prevention, diagnostic, and treatment options, breast cancer remains the most common cancer and the second cause of cancer death in women.

Most metastatic breast cancer patients have incurable disease requiring new approaches. The treatment of metastatic breast cancer has improved significantly with the use of new hormonal therapy in estrogen-receptor positive and targeted therapy in HER2 positive breast cancers [17–19]. However, chemotherapy use in metastatic ER+/HER2 negative or triple negative breast cancer (TNBC) has stagnated for the last 20 years and the 5-year survival for women with HER2-negative metastatic breast cancer is only 22%. Indeed, the median survival of patients with HER2 negative tumors is 18–22 months versus 56 months for patient with HER2 positive breast cancer. In view of the critical role of PARP3 in the function of the centrosome and the mitotic spindle, we examined the effect of compounds inhibiting PARP3 on vinorelbine cytotoxicity in human breast cancer cells lines. Vinorelbine, a vinca alkaloid analogue, is one of the most common treatments for patients with metastatic breast cancer, especially those bearing HER2 negative genotype. Vinorelbine destabilizes and prevents microtubule polymerization by inhibition of the assembly and dynamics of microtubules. This leads to a block in mitosis at the G2-M boundary and promotes apoptosis of cancer cells and subsequent cell death in interphase or at the following mitosis [20, 21]. The mitotic block of tumor cells by the vinca alkaloids is associated with fine structure changes of the mitotic spindle along with alteration of the centrosomes [22, 23]. In the context of breast cancer treatment, vinorelbine has lower neurotoxicity compared to other vinca alkaloids, making it more easily tolerated and suitable for consideration in combination therapy [20, 24]. To inhibit PARP3, we utilized ME0328 or Olaparib (AZD2281). ME0328 is a selective inhibitor of PARP3 with less activity and a weaker effect against PARP1 and PARP2. ME0328 has no inhibitory effect on the other members of PARP family [25]. Olaparib is a novel PARP1/2 and PARP3 inhibitor which induces synthetic lethality in BRCA1 and BRCA2 mutant or homologous recombination (HR) deficient cells and tumours [14, 15, 26–28]. Olaparib, a potent PARP inhibitor, was approved by the Food and Drug Administration (FDA) in 2014, and has been used as a single agent in clinical trials in breast and ovarian cancer patients with BRCA mutations [29, 30]. Based on recent results, Olaparib, alone, is a specific treatment for patients with metastatic HER2-negative breast cancer and a germ-line BRCA mutation. It also sensitizes tumor cells to DNA-damaging drugs and radiation [31–33].

Materials and methods

Cell lines, drug treatments, and reagents

Three TNBC cell lines, BT-20, MDA-436, and MDA-231, and one ER+/HER2− breast cancer cell line, MCF-7, were obtained from the American Type Culture Collection (ATCC) and maintained as monolayers at 37 °C in 5% CO_2 in RPMI supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All experiments were performed on cells in exponential growth phase. Vinorelbine, ME0328, and Olaparib were obtained from Selleckchem Company.

Sulforhodamine (SRB) cytotoxicity assay

Survival of the breast cancer cell lines was assessed 5 days after treatment using the SRB colorimetric assay as described by us [34, 35]. The IC_{50} (the concentration of drug which results in 50% of control values) of each compound was determined utilizing a range of concentrations; ME0328 (0.5–80 μM), olaparib (1–80 μM), vinorelbine (1–1000 nM). Cells were treated with vinorelbine, ME0328 or olaparib, alone or in combinations (concentrations indicated in "Results" section). 5 days after drug treatment, cells were fixed with trichloroacetic acid (10%), followed by SRB staining. Then the percentage of surviving cells was estimated using a 96-well plate reader. Plating efficiency experiments were assessed to determine the ideal plating density and ensure cells were growing exponentially at the 5-day time point. The 5-day time point was selected to permit

approximately 4 doubling times before fixing cells. The efficacy of the various drug treatments was determined by calculating sensitization values (*R*-values) using the equation described by us [36, 37]. Each experiment consisted of triplicate drug treatments and experiments were repeated at least three times. Wells treated with DMSO (vehicle), ME0328, Olaparib alone were used as controls in all experiments.

Apoptosis assay

Levels of apoptotic cell death were evaluated by monitoring drug-treated cultures for Annexin V content using flow cytometry as described [38]. Cells were treated with the stated drug combinations and concentrations for 24 h, then assayed using the PE Annexin V Apoptosis Detection Kit I (BD Pharmingen) according to the manufacturer's protocol. Briefly, treated BT-20 cells were trypsinized and washed with cold PBS followed by staining the cells resuspended in binding buffer with Annexin V-FITC and propidium iodide for 15 min at room temperature in the dark. Then, the specimens were transferred to FACS tube for reading at the FACS machine with channels FIH-2 (PI) and FIH-4 (APC).

Cell cycle assay

The breast cancer cell lines, BT-20 and MCF-7 were grown in T25 flasks, treated as indicated for 24 h, trypsinized, fixed, and permeabilized with 75% ethanol in Ca^{2+}/Mg^{2+} free PBS, and stored at − 20 °C. Cells were stained with propidium iodide (PI; Sigma-Aldrich, St. Louis, MO) which includes RNase A (Sigma-Aldrich, St. Louis, MO USA) as per our laboratory protocol and examined by flow cytometry [34, 36]. A minimum of 20,000 events was recorded for each sample.

PARP inhibition assay

T25 flasks were inoculated with 5×10^5 BT-20 cell line and grown for 24 h as previously described [34]. Cells were treated with the PARP3 inhibitors, Vinorelbine alone, or the drugs in combination for 4 h as indicated. The cells were harvested after trypsinization, centrifuging at 1500 rpm for 5 min and lysed in 25 µL of lysis buffer. The resulting supernatant was assayed for PARP activity using the PARP3 chemiluminescent assay kit (BPS bioscience).

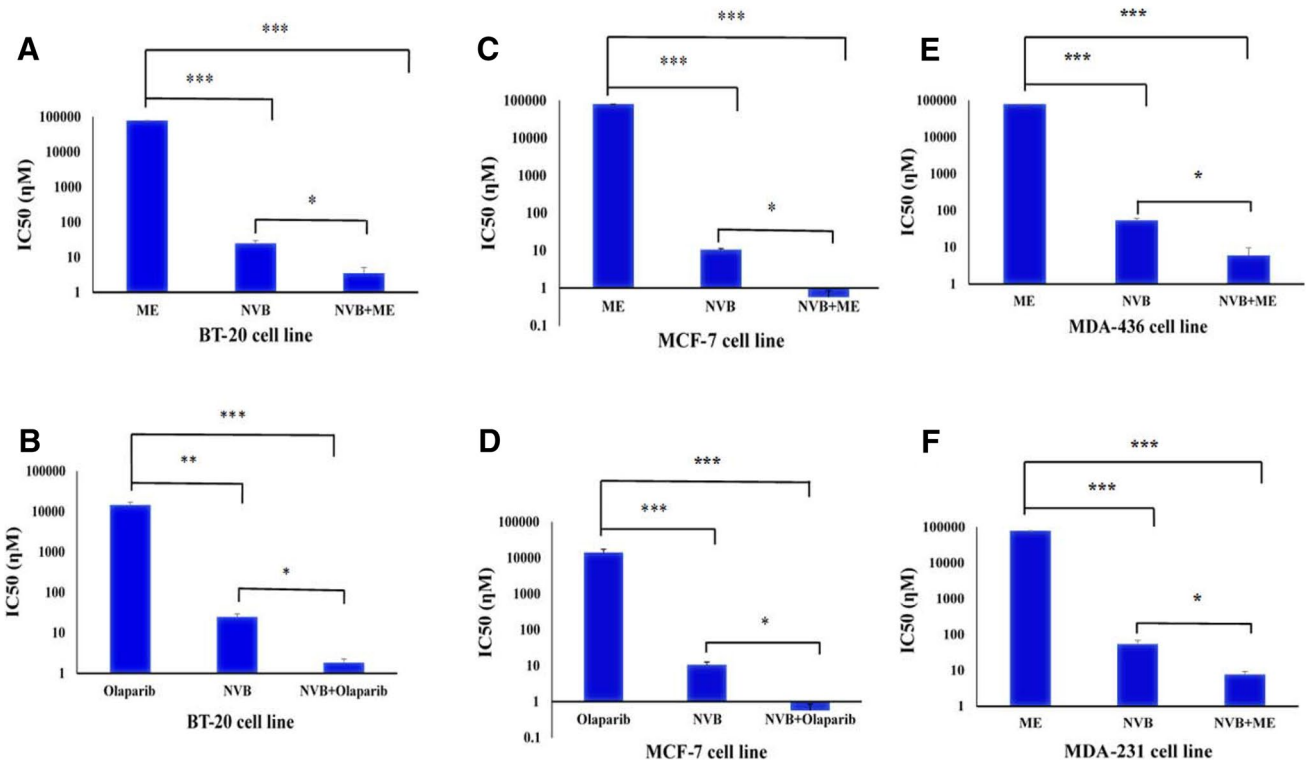

Fig. 1 Effect of combination drugs treatment on cytotoxicity and IC50 values. IC_{50} values of the PARP3 inhibitor (ME0328) alone (ME), vinorelbine alone (NVB) or vinorelbine in combination with 5 µM of ME0328 (NVB + ME) in three triple negative breast cancer cell lines **a** BT-20, **e** MDA-436, **f** MDA-231, and one ER+/HER2− breast cancer cell line **c** MCF-7. IC_{50} values of olaparib alone (Olaparib), vinorelbine alone (NVB) or vinorelbine in combination with 5 µM of olaparib (NVB + Olaparib) in **b** BT20 cells, and **d** MCF-7 cells. Data are presented as mean ± standard deviation ($n = 3$). $^*p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$

Immunofluorescent (IF) staining

To assess the effect of vinorelbine and PARP3 inhibitors on the microtubule depolarization and the mitotic spindle structure, cells were seeded in multichamber slides and then treated with vinorelbine alone or in the presence of concentrations of ME0328 or olaparib which resulted in sensitization. After 24 h of incubation, immunostaining was performed using antibody against α-tubulin. Briefly, after treatment, the cells were fixed and permeabilized using 4% formaldehyde for 15 min at room temperature followed by washing with PBS/BSA for 5 min three times followed by incubation in PBS/BSA (5%)/Triton X100 (0.1%) for 30 min at room temperature. Next, the cells were incubated overnight at 4 °C with α Tubulin (DM1A) Mouse mAb (Alexa Fluor 488 conjugate) (1:100 dilution in PBS containing 0.1% BSA). After washing the cells with PBS/BSA for 5 min three times, the cells were stained with 4,6-diamidino-2-phenylindole (DAPI; Sigma, MO, USA) (1 µg/ml final solution in PBS) for 5 min at room temperature followed by mounting in prolong gold. Fluorescence images were obtained using an (Widefield Leica DM LB2) upright fluorescence microscope with a 100X objective (green fluorescence, FITC; and blue fluorescence, DAPI). ImageJ software was used for image processing.

Statistical analysis

The experiments were performed in triplicate and statistical significance analyzed using two-tailed Students *t*-test. Data were considered statistically significant if $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Results

ME0328 or olaparib sensitize breast cancer cells to vinorelbine associated with enhanced apoptosis and cell cycle arrest

We showed that a nontoxic concentration of ME0328, a specific inhibitor of PARP3, highly sensitize three TNBC cell lines (BT-20, MDA-436, and MDA-231) and one ER+/HER2− breast cancer cell line, MCF-7 to vinorelbine. In a similar fashion, olaparib (a PARP1/2 inhibitor which is also a potent PARP3 inhibitor) at a nontoxic concentration, greatly sensitized these cell lines to vinorelbine. The IC_{50} (the concentration of drug which results in 50% of control values) of each compound was determined utilizing a range of concentrations; ME0328 (0.5–80 µM), olaparib (1–80 µM). Based on the IC_{50} values obtained for ME0328 and Olaparib, nontoxic concentration of these compounds were selected for combination drug treatment with vinorelbine. At selected nontoxic concentrations of ME0328 and olaparib, there was no evidence of cell cytotoxicity, no decreased cell growth or no increased cell death (Fig. 1a–f). Vinorelbine resulted in cytotoxicity in all breast cancer cell lines albeit at different IC_{50}s. Interestingly, the combination of 5 µM of ME0328 and vinorelbine reduced vinorelbine IC_{50}s significantly in all treated breast cancer cell lines. We demonstrated an approximate ten fold IC_{50} reduction of vinorelbine in all TNBC cell lines (BT-20, 24.6–3.53 ηM, MDA-436, 53.6–5.9 ηM and MDA-231, 54.7–7.69 ηM) (Fig. 1a, e, f). Furthermore, there was approximately a 16-fold reduction in the IC_{50} of vinorelbine in the MCF-7 cell line (MCF-7, 9.4–0.51 ηM) (Fig. 1c). When tested, at a clinically achievable concentration, olaparib sensitized BT-20 and MCF-7 cell lines approximately 17 and 14 folds respectively, to vinorelbine (Fig. 1b, d). Interestingly, experiments with a tubulin stabilizing agent such as paclitaxel with ME0328 in the BT-20 cell line resulted in much less sensitization (data not shown) suggesting that the interaction of PARP3 with tubulin is favored by tubulin depolarization.

To assess the contribution of programed cell death to the sensitization effect observed, we monitored Annexin V expression 24 h after drug treatment. We found that Annexin V staining was increased twofold in BT-20 cells treated with ME0328 in combination with vinorelbine compared to

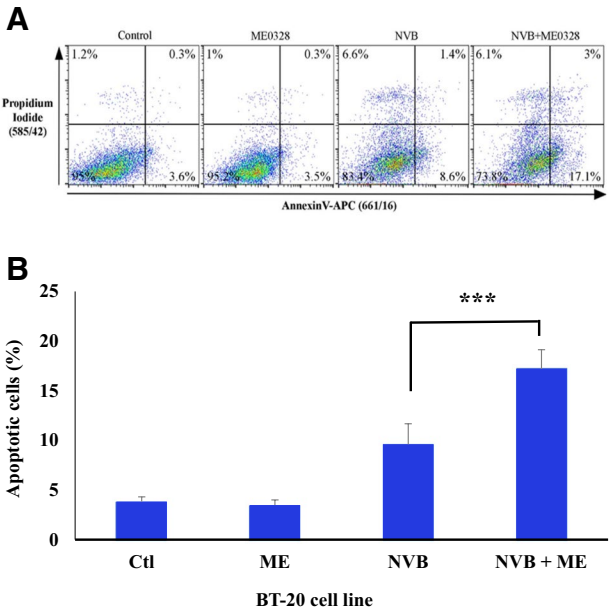

Fig. 2 Effect of combination drugs' treatment on apoptosis. Annexin V analysis of BT20 cells treated with ME0328 alone (ME), vinorelbine alone (NVB), or vinorelbine at IC_{50} concentration in combination with 5 µM of ME0328 (NVB + ME) for 24 h. **a** Representative analysis of Annexin V detection. **b** Quantification of three independent experiments. Data are presented as mean ± standard deviation ($n = 3$). $***p \leq 0.001$

vinorelbine alone in three independent experiments (Fig. 2a, b). Likewise, there was no significant evidence of cytotoxicity or increased apoptotic cells with nontoxic concentration of ME0328.

Cell cycle analysis with the various cell lines confirmed that G2/M arrest is increased by the combination of ME0328 or olaparib and vinorelbine. We assessed for changes in cell cycle progression after treatment of MCF-7, BT-20, and MDA-231 cells (Fig. 3a–e) with vinorelbine alone and the combination of either ME0328 or olaparib plus vinorelbine. Vinorelbine alone increased G2/M arrest but this was further increased by either ME0328 or olaparib combined with vinorelbine following a 24 h drug treatment. No significant change in cell cycle progression was observed for all these cell lines treated with either ME0328 or olaparib alone (Fig. 3a–e). Representative analyses of G2/M cell cycle arrest for BT-20 and MDA-231 cell lines are shown in Fig. 3f, g, respectively.

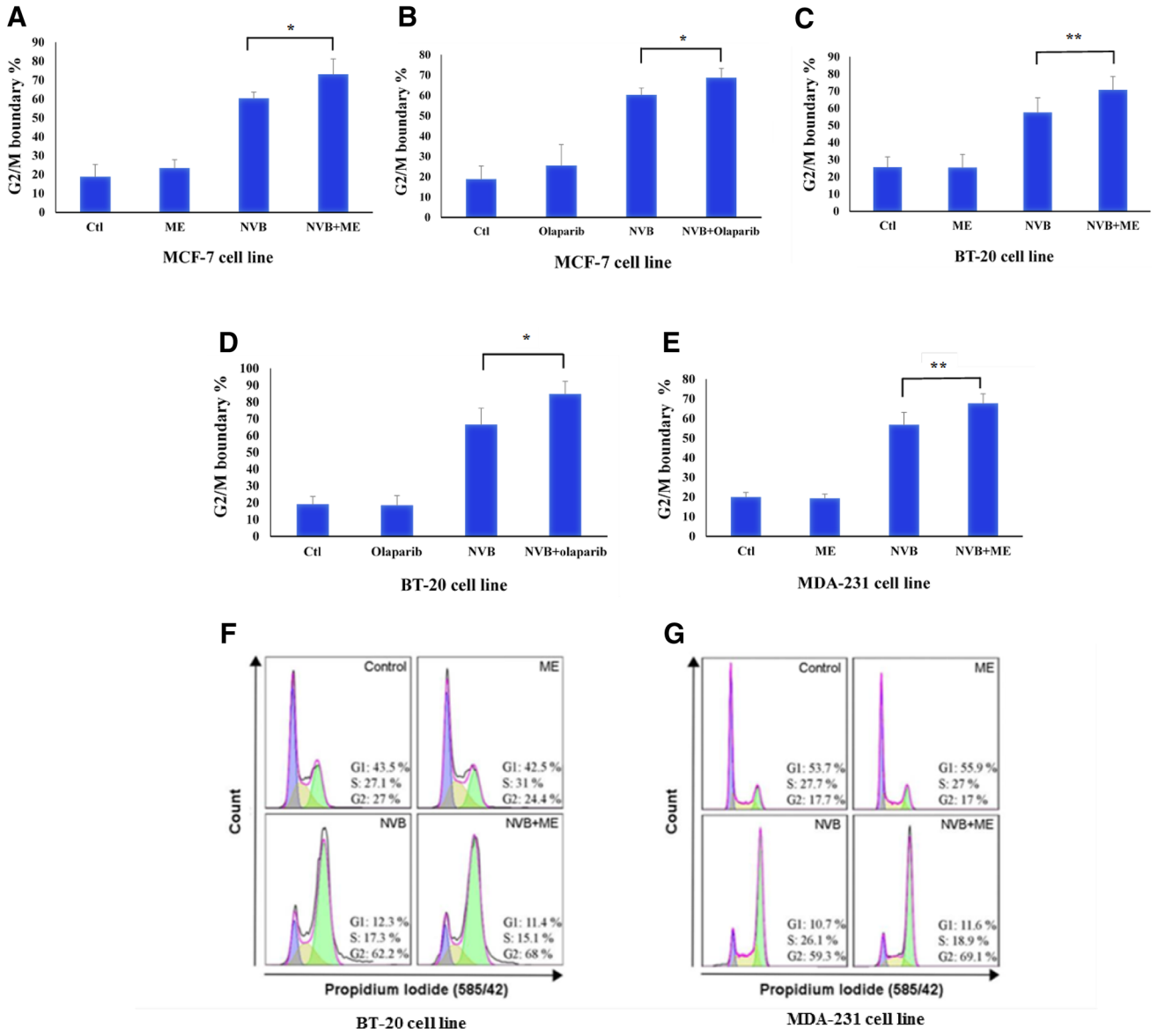

Fig. 3 Effect of combination drugs treatment on G2/M phase of cell cycle. Bar graphs of G2/M cell cycle boundary of MCF-7 (**a, b**), BT-20 (**c, d**), and MDA-231 (**e**) cell lines 24 h after treatment with either ME0328 alone (ME) or olaparib alone and vinorelbine alone (NVB) or vinorelbine at its IC_{50} concentration in combination with 5 μM of either ME0328 (NVB + ME) or Olaparib (NVB + Olaparib). Representative analysis of G2/M cell cycle arrest in **f** BT-20 and **g** MDA-231 cell lines. Accumulation of G2/M boundary was further increased by the combination of NVB with either ME0328 or olaparib. Data are presented as mean ± standard deviation ($n = 3$). $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$

ME0328 or olaparib enhances vinorelbine-induced microtubule destabilizing effect

The effect on tubulin was assessed 24 h after treatment with vinorelbine alone or in combination with either ME0328 or olaparib. At selected nontoxic concentrations of ME0328 and olaparib (5 μM), there was no evidence of altered microtubule polymerization and dynamics. As we expected for microtubule destabilizing agents, cells treated with vinorelbine demonstrated microtubule dynamic alteration due to interference with tubulin polymerization. Vinorelbine induces mitotic arrest via tubulin depolymerization and microtubule dynamics changes which lead to highly condensed chromosomes and mitotic spindles disorientation. These effects were potentiated by the combination of either ME0328 or olaparib with vinorelbine (Fig. 4a–c). Drug combinations enhanced spindle microtubule disorientation and increased splayed, defective microtubules, and multipolar mitotic spindles. Furthermore, there was abundant multinucleated giant cells resulting from enhanced tubulin depolymerization in cells treated with drug combinations compared to the cells treated with vinorelbine alone (Fig. 5). Collectively, the combination of ME0328 or olaparib with vinorelbine showed greater mitotic arrest via chromosome misalignment and splayed microtubule spindle.

ME0328 or olaparib reduce PARP-3 activity more than vinorelbine alone

The effect of the various agents on PARP3 activity in cell lysates was monitored after treatment of the cells with vinorelbine, ME0328 or olaparib alone, and vinorelbine in combination with either 5 μM of ME0328 or olaparib. After 4 h treatment the cells treated with vinorelbine, ME0328, or olaparib alone demonstrated a reduction of PARP3 activity. Vinorelbine alone decreased PARP3 activity significantly and the effect of ME0328 or olaparib on vinorelbine was associated with a further significant decrease of PARP3 activity (Fig. 6). The cells treated with combination of

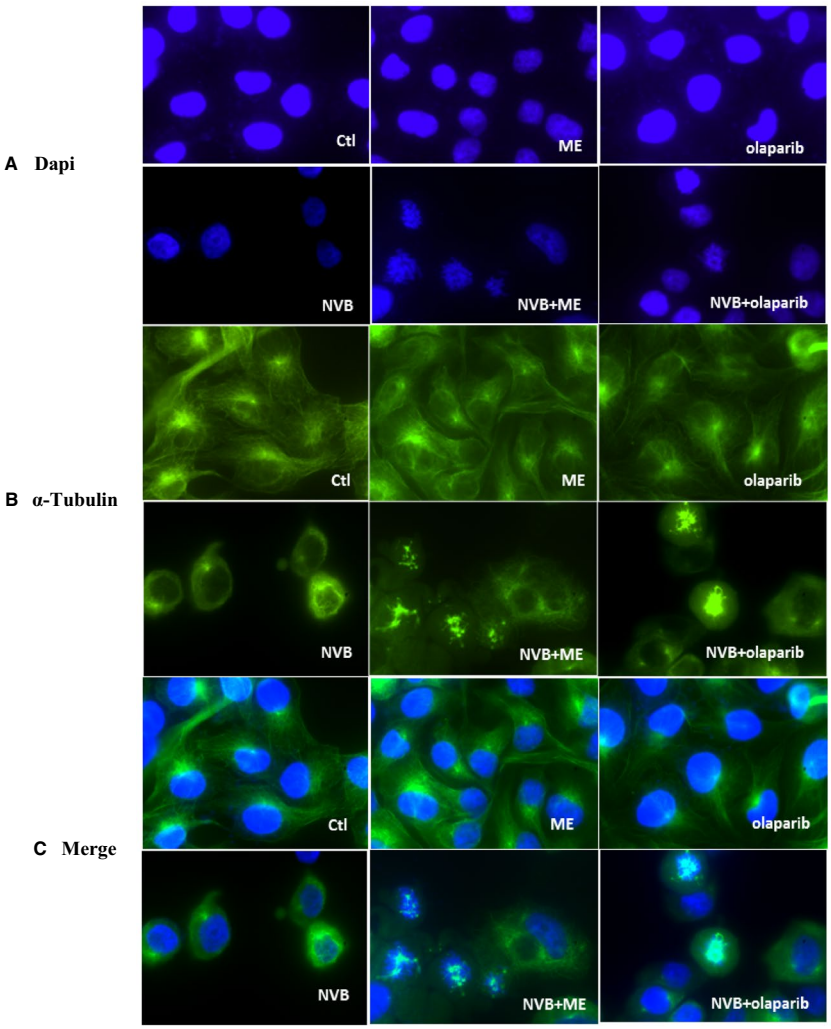

Fig. 4 Combination drug treatment increased spindle tubulin dynamic changes and mitotic arrest. Immunofluorescent staining of BT-20 cell line after treated with vinorelbine (NVB), ME0328 (ME), and olaparib alone or vinorelbine in combination with 5 μM of either ME0328 (NVB + ME) or olaparib (NVB + Olaparib) for 24 h. **a** Cells were stained with Dapi for DNA (blue). **b** α-tubulin for microtubules (green). **c** The merge with a 100× objective

vinorelbine and olaparib showed the lowest level of PARP3 activity.

Discussion

The PARP3 inhibitors, ME0328, or olaparib combined with vinorelbine are a unique and promising approach for treatment of breast cancer patients with metastases. Several inhibitors of PARP1 and PARP2 have been developed and are currently in clinical trials. Recently, specific inhibitors of PARP3 have been synthesized including ME0328 [25]. Furthermore, olaparib, a small molecule inhibitor, which was first described as a PARP1 and PARP2 specific inhibitor, also inhibits PARP3 to a similar extent [14]. In phase 3 clinical trial, olaparib monotherapy provided a significant benefit in patients with HER2 negative BRCA-mutated metastatic breast cancer [33]. In view of the critical role of PARP3 in the function of the centrosome and the mitotic spindle, we examined the effect of ME0328 and olaparib on vinorelbine cytotoxicity. Our findings point to a new approach of utilization of PARP3 inhibitors in targeted anti-cancer strategies to suppress mitotic spindle formation and increase mitotic arrest. Our results demonstrated that the combination of vinorelbine with a PARP3 inhibitor either ME0328 or olaparib showed significant decreases in the IC_{50}s across all examined breast cancer cell lines (from 7 to 18.5 fold). Vinorelbine-induced cytotoxicity was greatly enhanced upon combination of vinorelbine with either PARP3 inhibitor. Furthermore, the sensitization effect of ME0328 on vinorelbine was confirmed with an apoptosis assay. Vinorelbine-induced microtubule depolarization binds with unpolymerized tubulin and interferes with proper microtubule growing preventing mitotic spindle formation during mitosis. Disruption of spindle microtubule networks leads to cell cycle arrest and apoptosis [22, 39]. PARP3 is required for efficient mitotic progression and is necessary for mitotic spindle integrity during mitosis and mitotic spindle dynamics [7, 12, 13]. PARP3 inhibitors enhance the mitotic block of tumor cells by vinorelbine which is associated with fine structural changes of the mitotic spindle along with alteration of centrosomes plus apoptosis.

In spite of a report suggesting that vinca alkaloids cause DNA damage (i.e., γH2AX) [40], in our settings changes in γH2AX were not associated with the synergy seen with PARP3 inhibitors and vinorelbine (data not shown). Vinorelbine alone or either PARP3 inhibitor alone inhibited PARP3 activity. The decrease of PARP3 activity was potentiated in drug combination treatments. Vinorelbine alone is known to be an anti-microtubule agent inducing apoptosis and cell death due to G2/M phase arrest [21, 41]. Our cell cycle results demonstrated that G2/M arrest is increased by the combination of either PARP3 inhibitor with vinorelbine as compared to vinorelbine alone. The largest increase of G2/M boundary observed in BT-20 cells was seen when these cells were treated with the combination of vinorelbine and ME0328.

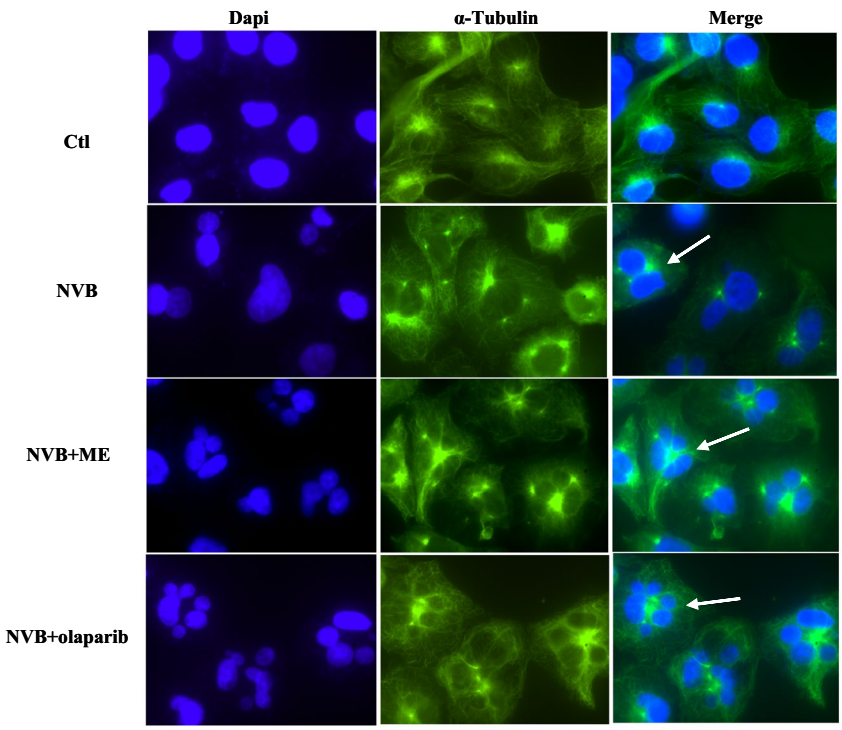

Fig. 5 Combination drug treatment increased multinucleated giant cells via spindle microtubule alteration and cell cycle arrest. Immunofluorescent staining of BT-20 cell line after treatment with vinorelbine (NVB) or vinorelbine in combination with 5 μM of either ME0328 (NVB + ME) or olaparib (NVB + Olaparib) for 24 h. Cells were stained with Dapi for DNA (blue). α-tubulin for microtubules (green) and the merge with a 100× objective. Abundant multinucleated giant cells observed in cells treated with NVB + ME and NVB + Olaparib comparing with NVB alone

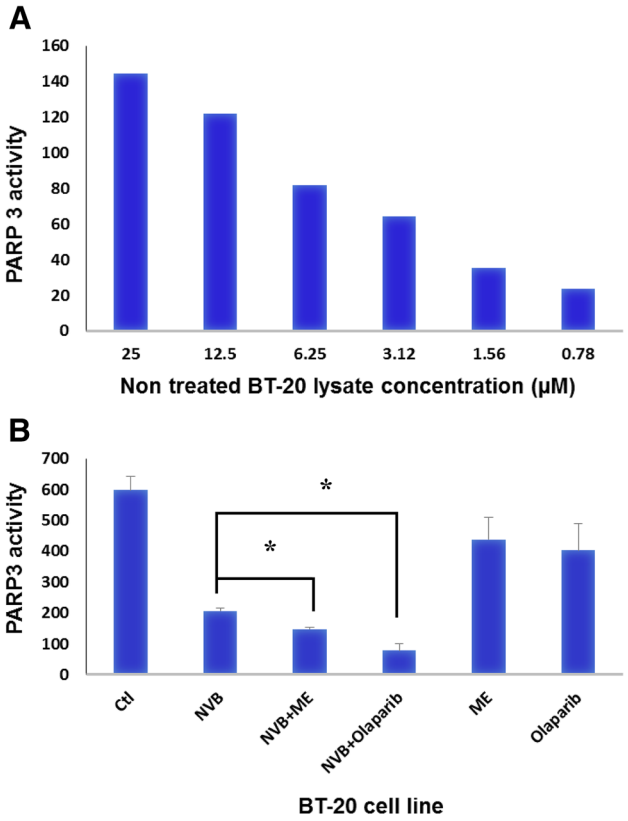

Fig. 6 Effect of combination drugs treatment on PARP3 activity in breast cancer cell line. **a** PARP3 activity in non-treated BT-20 cell lysate. **b** BT-20 cell line treated with vinorelbine (NVB), ME0328 (ME), and olaparib alone or vinorelbine at IC50 concentration in combination with 5 µM of either ME0328 (NVB + ME) or olaparib (NVB + Olaparib). Data are presented as mean ± standard deviation ($n = 3$). *$p \leq 0.05$

Vinorelbine promotes G2/M boundary cell cycle arrest accompanied by an increase in apoptotic cell death in breast cancer cells due to microtubules depolarization [20, 21]. Apoptotic cell death occurrence after G2/M phase cell cycle arrest has been previously demonstrated [39, 41]. PARP3 as a DNA binding enzyme preferentially locates at daughter centrioles and is involved in cell cycle progression and cell division during mitosis [12]. Our flow cytometry results showed that both PARP3 inhibitors enhanced G2/M arrest induced by vinorelbine in breast cancer cells.

Vinorelbine stimulates microtubule depolarization followed by suppression of spindle microtubule dynamics [20, 21, 42]. Vinorelbine attaches to unpolymerized tubulin and block microtubule formation. Microtubule disassembly increases splayed and defective tubulin which leads to mitotic catastrophe and G2/M boundary arrest which is the main cause of cell death [20–22]. In dividing cells, the centrosome duplicates once per cell cycle and its number and structure are highly regulated during each cell cycle to organize an effective bipolar spindle in the mitotic phase [43]. We showed that there are more multinucleated giant cells and abnormal mitosis in combination drug treatments compared to vinorelbine alone. Abnormal mitosis and cell cycle alterations due to inhibition of the assembly of microtubules are expected upon vinorelbine-induced cytotoxicity [21]. In view of the critical role of PARP3 in the function of the centrosome and the mitotic spindle [12], PARP3 inhibitors appear to be altering the function of the centrosome and the mitotic spindle dynamics which lead to enhancement of vinorelbine induced mitotic catastrophe and cell death.

Importantly, human PARP3 depleted cells and PARP3−/− mice are viable and phenotypically largely normal [7]. In addition, olaparib which is a highly potent PARP1/2 and PARP3 inhibitor in combination with temozolomide in rat athymic models was as well tolerated as a compound which is mostly a PARP1/2 inhibitor suggesting that inhibiting PARP3 will not add to the bone marrow toxicity of anticancer drugs [44]. This suggests that combination of PARP3 inhibitors with vinorelbine may be well tolerated in humans.

In conclusion, our findings strongly support the clinical assessment of PARP3 inhibitors in combination with vinorelbine in metastatic breast cancer. This could greatly improve the therapy of breast cancer especially triple negative breast cancer which represents 15–20% of all breast cancer diagnosed every year and these patients are not suitable for either hormone therapy or HER2 immunotherapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments comply with the current laws of Canada in which they were performed.

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