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Salinomycin sensitizes antimitotic drugs-treated cancer cells by increasing apoptosis via the prevention of G2 arrest

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ABSTRACT

Here, we investigated whether Sal could sensitize cancer cells to antimitotic drugs. We demonstrated that Sal sensitized paclitaxcel (PAC)-, docetaxcel (DOC)-, vinblastin (VIN)-, or colchicine (COL)-treated cancer cell lines, suggesting that Sal has the ability to sensitize the cells to any form of microtubule-targeting drugs. Sensitization to the antimitotic drugs could be achieved with very low concentrations of Sal, suggesting that there is a possibility to minimize Sal toxicity associated with human cancer patient treatments. Sensitization by Sal increased apoptosis, which was observed by C-PARP production. Sal sensitized the cancer cells to antimitotic drugs by preventing G2 arrest, suggesting that Sal contributes to the induction of mitotic catastrophe. Sal generally reduced cyclin D1 levels in PAC-, DOC-, and VIN-treated cells. In addition, Sal treatment increased pH2AX levels and reduced p21 levels in antimitotic drugs-treated cells. These observations suggest that the mechanisms underlying Sal sensitization to DNA-damaging compounds, radiation, and microtubule-targeting drugs are similar. Our data demonstrated that Sal sensitizes cancer cells to antimitotic drugs by increasing apoptosis through the prevention of G2 arrest via conserved Sal-sensitization mechanisms. These results may contribute to the development of Sal-based chemotherapy for cancer patients treated with antimitotic drugs.

1. Introduction

Antimitotic drugs are widely used for treating numerous types of cancers [1,2]. These compounds inhibit mitosis by targeting microtubules and preventing their polymerization or depolymerization [1]. Paclitaxcel (PAC), docetaxcel (DOC), and vinblastine (VIN) are currently the most commonly used antimitotic drugs, and depend on a diverse number of microtubule binding domains [1–3]. Since patients develop resistance to these drugs [4–7], research on increasing antimitotic-associated apoptosis has been focused on providing more effective treatments. Identifying the mechanism(s) underlying cell sensitization to antimitotic drugs would be an important step in the development of new pharmacological cancer treatments.

Salinomycin (Sal) was originally used to eliminate bacteria, fungi, and parasites [8,9]. More recently, this drug has been shown to inhibit the growth of tumor stem cells and chemoresistant cancer cells [10–15]. Sal also functions as an efflux pump p-glycoprotein (P-gp) inhibitor [16,17]. Sal is currently considered to be a potential anti-cancer drug administered for cancer chemoprevention. In addition, Sal sensitizes doxorubicin (DOX)-, etoposide (ETO)-, or radiation-treated cancer cells resulting in apoptosis by causing DNA damage and reducing p21 protein levels through increased proteasomal activity [18,19]. It is of interest to examine the effects of Sal on cancer cell sensitization when used in combination with antimitotic drugs.

In the present study, we found that the cells treated with the antimitotic drugs were not sensitized to doxorubicin (DOX) or etoposide (ETO), whereas Sal could sensitize those cancer cells. We also found that sensitization by Sal involved the prevention of G2 arrest and reduced cyclin D1 levels. Sal also increased DNA damage and reduced p21 levels in antimitotic drugs-treated cancer cells. These findings are similar to those seen DOX-, ETO-, and radiation-treated cells. Our results may contribute to the development of Sal-based therapy for cancer patients.

2. Materials and methods

2.1. Reagents and cell culture

Reagents and cell lines [18–21] in this study are provided in the Supporting information. The cell lines were cultured in RPMI1640

Abbreviations: Sal, salinomycin; PAC, paclitaxcel; DOC, docetaxcel; VIN, vinblastine; COL, colchicine; DOX, doxorubicin; ETO, etoposide; SP, SP600125; P-gp, p-glycoprotein; DMSO, dimethylsulfoxide; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; TCA, trichloroacetic acid; PBS, phosphate buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; RT, room temperature.

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containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (WelGENE, Daegu, South Korea).

2.2. Cell proliferation assay

The detailed method is described in the Supporting information.

2.3. Cell viability assay

The detailed method is described in the Supporting information.

2.4. Fluorescence-activated cell sorting (FACS) analysis

The detailed method is described in the Supporting information.

2.5. Western blot analysis

Total cellular proteins were extracted using a previously described trichloroacetic acid (TCA) method [22]. Briefly, cells grown in 60-mm dishes were washed three times with 5 mL PBS. Next, 500 μL of 20% trichloroacetic acid (TCA) were added to each plate. The cells were then dislodged by scraping and transferred to Eppendorf tubes. Proteins were pelleted by centrifugation for 5 min at 3000 rpm and resuspended in 1 M Tris–HCl (pH 8.0) buffer. The total protein concentrations were estimated. The proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and subjected to Western blot analysis as previously described [22].

3. Results

3.1. Sal sensitizes antimitotic drugs-treated cancer cells

We performed experiments to identify novel conditions for sensitization of Hs578T breast cancer cells to antimitotic drugs. We tested the responses to three well-known antimitotic cancer drugs, PAC, DOC, and VIN, by observing cellular growth and morphology using microscopy. We did not detect any sensitization effects following co-treatment with the antimitotic drugs and ETO or DOX (Fig. 1A–C and Supplementary Fig. 1A and B). In particular, DOX even antagonized sensitization effect of antimitotic drugs (Supplementary Fig. 1A and B). These results suggest that DOX and ETO, two compounds that damage DNA, do not exert synergistic or additive effects on the Hs578T cells treated with antimitotic drugs.

Next, we tested whether Sal can sensitize the Hs578T cancer cells to the antimitotic drugs. We found that Sal reduced proliferation of the PAC-, DOC-, and VIN-treated cells (Fig. 1A–C), suggesting that Sal can sensitize the antimitotic drug-treated cells. We also found that the cells were sensitized with 0.1 μM of Sal (Fig. 1D), suggesting that Sal toxicity can be minimized when used in combination with antimitotic drugs.

Antimitotic drugs target specific domains in microtubules. Both PAC and DOC target the taxane site, VIN targets the vinca domain, and COL targets the colchicine domain [1]. Although COL is not used as an anti-cancer drug, we also determined whether Sal sensitizes cells to COL. Sal sensitized COL-treated cancer cells, whereas DOX or ETO did not (Supplementary Fig. 1C and D). This finding indicated that Sal can sensitize the cells to most microtubule-targeting drugs independent of the targeted microtubule domains.

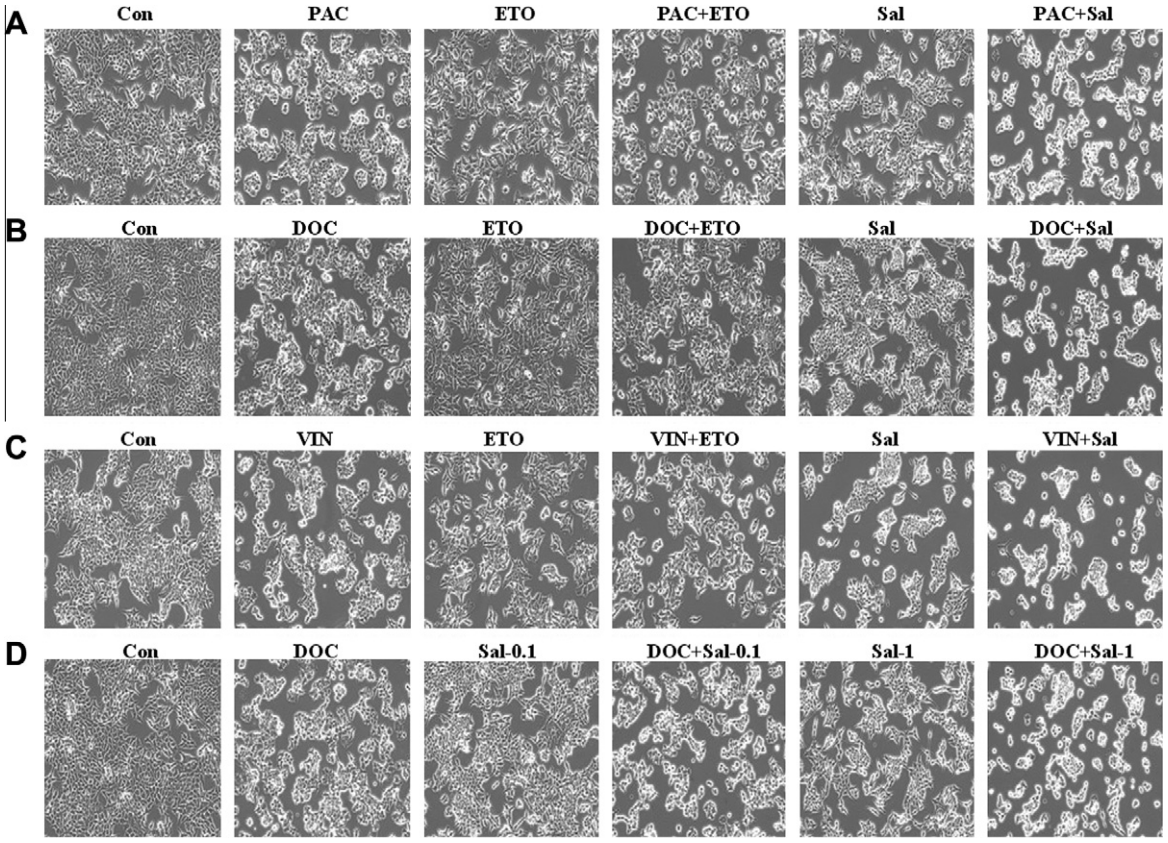

Fig. 1. Sal sensitizes cancer cells to antimitotic drugs, but ETO does not. (A–C) Hs578T were grown on 6-well plates and treated with 10 nM PAC, 10 ng/mL DOC, 3 nM VIN, 25 μM ETO, 1 μM Sal, 25 μM ETO with 10 nM PAC (PAC + ETO), 25 μM ETO with 10 ng/mL DOC (DOC + ETO), 25 μM ETO with 3 nM VIN (VIN + ETO), 1 μM Sal with 10 nM PAC (PAC + Sal), 1 μM Sal with 10 ng/mL DOC (DOC + Sal), 1 μM Sal with 3 nM VIN (VIN + Sal), or DMSO (Con). After 24 h, all cells were observed using an inverted microscope with a 10× objective lens. (D) Hs578T were grown on 6-well plate and treated with 10 ng/mL DOC, 0.1 μM Sal (Sal-0.1), 1 μM Sal (Sal-1), 0.1 μM Sal with 10 ng/mL DOC (DOC + Sal-0.1), 1 μM Sal with 10 ng/mL DOC (DOC + Sal-1), or DMSO (Con). After 24 h, cells were observed using an inverted microscope with a 10× objective lens.

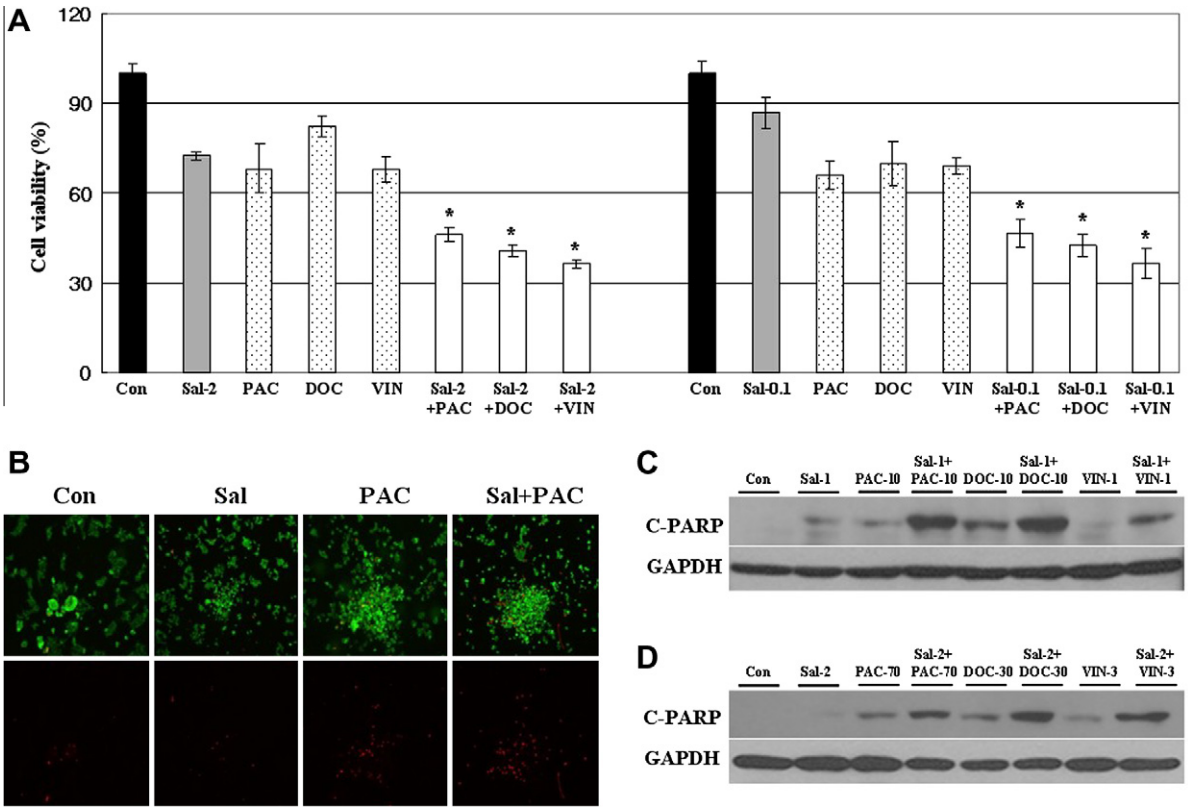

Fig. 2. Co-treatment with Sal and antimitotic drugs increases cellular death and apoptosis. (A) Hs578T cells were plated on 96-well plates and grown to 30–40% confluence. The cells were then stimulated for 48 h with 2 μM Sal (Sal-2), 0.1 μM Sal (Sal-0.1),10 nM PAC, 10 ng/mL DOC, 3 nM VIN, 2 μM Sal with 10 nM PAC (Sal-2 + PAC), 2 μM Sal with 10 ng/mL DOC (Sal-2 + DOC), 2 μM Sal with 3 nM VIN (Sal-2 + VIN), 0.1 μM Sal with 10 nM PAC (Sal-0.1 + PAC), 0.1 μM Sal with 10 ng/mL DOC (Sal-0.1 + DOC), 0.1 μM Sal with 3 nM VIN (Sal-0.1 + VIN), or DMSO (Con). A cell proliferation assay was performed as described in "Section 2". The data represent the mean ± SD of at least three independent experiments; $^{*}P < 0.05$ compared to the corresponding control. (B) Hs578T cells were plated on 96-well plates and grown to 30–40% confluence. The cells were then stimulated for 48 h with 10 nM PAC, 2 μM Sal, 2 μM Sal with 10 nM PAC (Sal + PAC), or DMSO (Con). Cell viability (live/dead assay) was then measured as described in "Section 2". (C and D) Hs578T cells were grown on 60-mm dishes and then treated with 10 nM PAC (PAC-10), 70 nM PAC (PAC-70), 10 ng/mL DOC (DOC-10), 30 ng/mL DOC (DOC-30), 1 nM VIN (VIN-1), 3 nM VIN (VIN-3), 1 μM Sal (Sal-1), 2 μM Sal (Sal-2), 1 μM Sal with 10 nM PAC (Sal-1 + PAC-10), 1 μM Sal with 10 ng/mL DOC (Sal-1 + DOC-10), 1 μM Sal with 1 nM VIN (Sal-1 + VIN-1), 2 μM Sal with 70 nM PAC (Sal-2 + PAC-70), 2 μM Sal with 30 ng/mL DOC (Sal-2 + DOC-30), 2 μM Sal with 3 nM VIN (Sal-2 + VIN-3), or DMSO (Con). After 24 h, Western blot analysis was performed using antibodies against C-PARP and GAPDH.

We also tested ability of Sal to sensitize two other breast cancer cell lines (MCF7 and MDA-MB231) and ES2 ovarian cancer cells. We found that ETO or DOX did not sensitize DOC- or COL-treated MCF7, MDA-MB231, or ES2 cells. However, all of these cells were sensitized by co-treatment with Sal (Supplementary Fig. 2A–C). These results suggest that Sal can generally sensitize any type of cancer cell to antimitotic drugs.

3.2. Co-treatment with Sal and antimitotic drugs increases cellular death and apoptosis

We measured cell proliferation using an MTS-based assay to determine whether Sal reduces the viability of Hs578T cells treated with the antimitotic drugs. As seen in Fig. 2A, both high (2 μM) and low (0.1 μM) concentrations of Sal reduced the viability of PAC-, DOC-, and VIN-treated cells. When we performed tests to determine whether Sal increases cell death among antimitotic drug-treated cells, we found that Sal increased the number of dead cells in PAC-treated cells (Fig. 2B). These results support the hypothesis that Sal sensitizes antimitotic drug-treated cancer cells by reducing cell viability and increasing cellular death. We confirmed the results from this experiment in ES2 cells (Supplementary Fig. 3A–C).

We also determined whether Sal increases the levels of apoptosis in cells treated with the antimitotic drugs. As seen in Fig. 2C, we found that Sal increased C-PARP production in PAC-, DOC-, or VIN-treated cells. When we increased the concentration of the antimitotic drugs, we also observed increased C-PARP levels following co-treatment with Sal (Fig. 2D). Our observation indicated that Sal contributes to increased apoptosis in cancer cells treated with antimitotic drugs. When we compared the levels of C-PARP expression among cells co-treated with Sal and PAC, DOC, or VIN, we found that all cells had similar levels, suggesting that Sal exerts similar effects when used in combination with different antimitotic drugs. These findings demonstrate that sensitization of the cancer cells to antimitotic drugs by Sal is mediated by increased apoptosis.

3.3. Sal prevents G2 arrest by antimitotic drugs

Next, we examined the mechanism underlying the sensitization and apoptosis mediated by Sal in antimitotic drug-treated cells. Since antimitotic drugs increase the number of cells in G2 arrest [1,23], we investigated the ability of Sal to affect G2 arrest in antimitotic drug-treated cells. FACS analysis demonstrated that Sal reduced the number of cells in the G2 phase among PAC-, DOC-, VIN-, or COL treated-cells (Fig. 3A–D and Supplementary Fig. 4A and B). Sal even reduced the increased number of cells in the G2 phase among cells treated with much higher concentrations of PAC or VIN (Fig. 3B and Supplementary Fig. 4B).

Sal also reduced the overall number of antimitotic drug-treated cells in the above G2 phase (Fig. 3B–D and Supplementary Fig. 4B), indicating that the number of aneuploid cells were reduced by Sal.

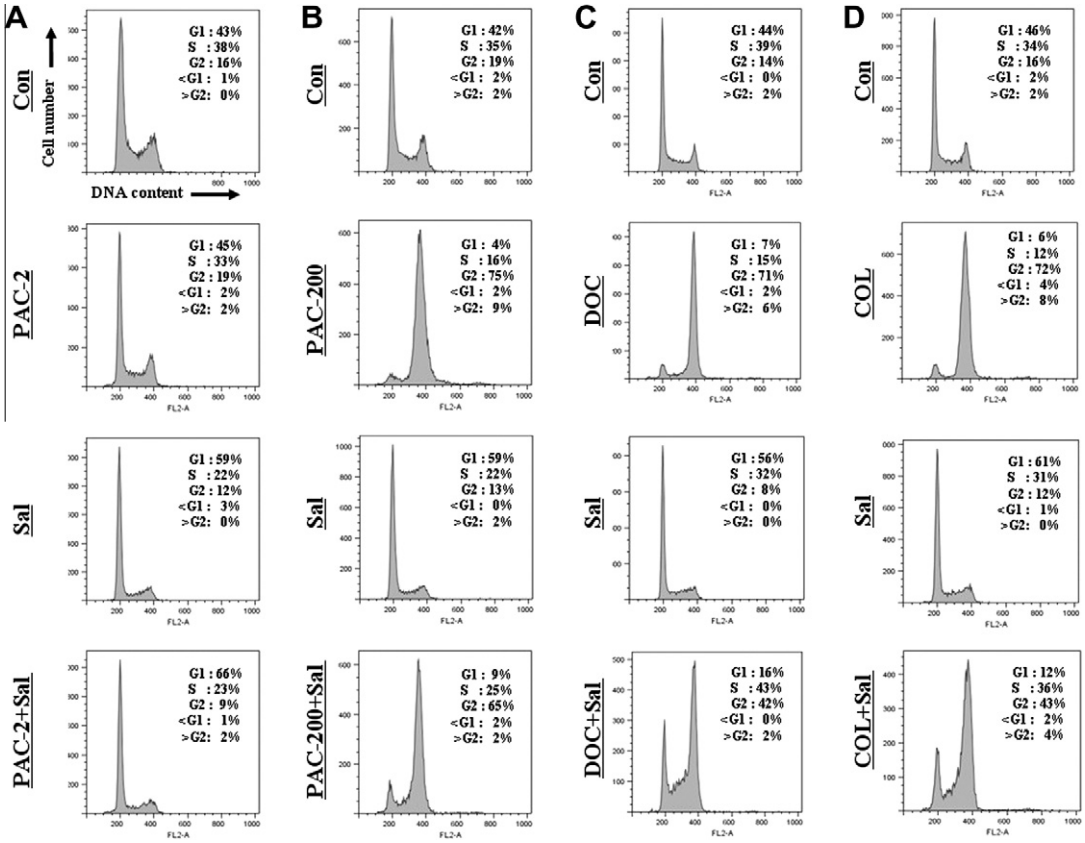

Fig. 3. Sal prevents G2 arrest induced by antimitotic drugs. (A–D) Hs578T cells were plated on 60-mm dishes. The cells were then incubated for 24 h with 2 nM PAC (PAC-2), 200 nM PAC (PAC-200), 30 ng/mL DOC, 5 μM COL, 2 μM Sal, 2 μM Sal with 2 nM PAC (PAC-2 + Sal), 2 μM Sal with 200 nM PAC (PAC-200 + Sal), 2 μM Sal with 30 ng/mL DOC (DOC + Sal), 2 μM Sal with 5 μM COL (COL + Sal), or DMSO (Con). After 24 h, FACS analysis was performed as described in "Section 2".

Previously, SP600125 (SP) was shown to increase G2 arrest and the number of aneuploid cells [24–26]. We observed that SP promoted G2 arrest in a dose-dependent manner (Supplementary Fig. 4C). We also determined that the 20 μM SP increased the aneuploid cell numbers. We then performed tests to determine whether Sal also prevents G2 arrest and increased number of aneuploid cells promoted by SP. We found that co-treatment with Sal prevented G2 arrest and the formation of aneuploid cells (Supplementary Fig. 4C). Considering that increased G2 phase and aneuploid cell formation following treatment with antimitotic drugs contributes to the resistance of cancer cells to these drugs [1,23], our results suggest that Sal reduces cancer cell survival by delaying mitosis.

3.4. Sal reduces cyclin D1 levels in antimitotic drug-treated cells

We next assessed whether co-treatment with antimitotic drugs and Sal altered the levels of cell cycle proteins. These proteins are important for the response to microtubule targeting drugs [27–30]. This experiment was based on the assumption that the proteins affected by the co-treatment could be potential targets for reducing cell viability.

We tried to identify proteins that may be commonly down- or up-regulated by Sal in all of cells co-treated with the three antimitotic drugs. In order to identify proteins that were involved in the long-term effects of Sal, we examined protein expression at early (6 h) and late (20 h) periods. We found that treatment with Sal reduced cyclin D1, Cdk2, and Cdk4 levels in PAC-, DOC-, and VIN-treated cells at both early and late periods (Fig. 4A and B). We confirmed a slight reduction in both Cdk4 and Cdk2 levels by densitometric evaluation (data not shown). Cyclin D1 levels were down-regulated the most among these three proteins, suggesting that cyclin D1 might be a major and common target necessary for sensitization by Sal.

3.5. Sensitization by Sal is associated with increased DNA damage and reduced p21 levels in antimitotic drug-treated cells

Previously, DNA damage and p21 levels were found to be involved in sensitization by Sal in DOX-, ETO-, and radiation-treated cancer cells [18,19]. We therefore performed tests to see whether Sal also increases DNA damage and reduces p21 levels in antimitotic drug-treated cells. We found that pH2AX levels were increased and p21 levels were decreased by the co-treatments (Supplementary Fig. 5A and B). These results indicate that Sal sensitizes cancer cells to antimitotic drugs by increasing DNA damage and reducing p21 levels, similar to co-treatment with DOX-, ETO-, or radiation.

Collectively, our results indicated that Sal sensitizes antimitotic drugs-treated cancer cells by increasing apoptosis. We also observed that Sal prevented G2 arrest that was promoted by treatment with the antimitotic drugs. The effects of Sal were accomplished by reducing cyclin D1 expression, increasing pH2AX levels, and down-regulating p21.

4. Discussion

Sal sensitizes cancer cells to anticancer agents that damage DNA including DOX, ETO, and radiation [18,19]. The present study was conducted to determine whether Sal may be useful for sensitizing cells to non-DNA damaging agents such as antimitotic drugs. Our results demonstrated that Sal sensitizes the cancer cells to antimitotic drugs via several novel mechanisms.

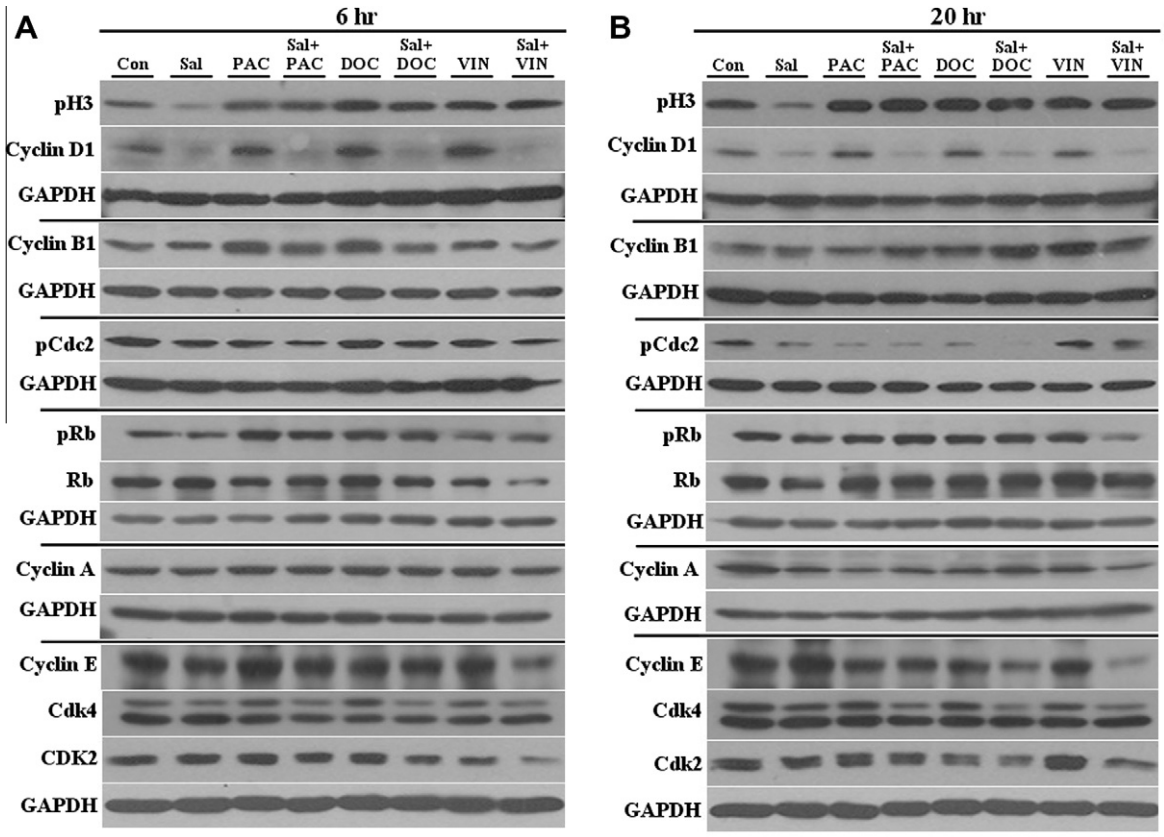

Fig. 4. Sal reduces cyclin D1 levels in antimitotic drugs-treated cells. (A and B) Hs578T cells were grown on 60-mm dishes and then incubated with 10 nM PAC, 10 ng/mL DOC, 1 nM VIN, 1 μM Sal, 1 μM Sal with 10 nM PAC (Sal + PAC), 1 μM Sal with 10 ng/mL DOC (Sal + DOC), 1 μM Sal with 1 nM VIN (Sal + VIN), or DMSO (Con). After 6 or 20 h, Western blot analysis was performed using antibodies against pH3, pCdc2, cyclin D1, cyclin A, cyclin E, cyclin B1, Cdk2, Cdk4, pRb, Rb, and GAPDH.

Most importantly, we demonstrated that Sal sensitized the cancer cells to three different antimitotic drugs (PAC, DOC, and VIN). Sal also sensitized COL-treated cells, indicating that Sal generally promotes sensitization to most microtubule-targeting drugs. Both PAC and DOC target the taxane site in microtubules, COL targets the coclchine domain, and VIN targets the vinca domain [1]. Thus, Sal induces sensitization to common microtubule-targeting drugs independent of their specific targeting domains.

We also found that Sal increased the number of dead cells following PAC treatment. In addition, we detected increased C-PARP levels after co-treatment with Sal and antimitotic drugs. These results indicated that increased apoptosis contributes to sensitization to antimitotic drugs by Sal. We also observed that the degree of sensitization was similar in the PAC-, DOC-, and VIN-treated cells, as determined by microscopy and by measuring C-PARP production. Our findings support the hypothesis that sensitization to antimitotic drugs by Sal is similar for all types of microtubule-targeting drugs. Furthermore, our results were not limited to the Hs578T breast cancer cell line. We demonstrated that sensitization by Sal occurred in various cancer cell lines including other breast cells (MCF7 and MDA-MB231) and ovarian ES2 cells.

Interestingly, we observed that DOX or ETO did not sensitize the cells to antimitotic drugs. In particular, DOX antagonized the sensitization effect of the antimitotic drugs. These findings suggest that the clinical use of a combination therapy that includes compounds that induce DNA damage (DOX or ETO) and antimitotic drugs should be limited.

The effects of Sal were facilitated by a number of sensitization mechanisms including inhibition of ionophores, increased DNA damage, or the prevention of the P-gp pumping [10–19]. It is possible that the more various mechanisms of Sal compared to DOX or ETO contributed to increased sensitization to the antimitotic drugs. A previous report indicated that toxicity associated with relative high concentrations of Sal may be harmful to normal cells [31]. Based on our proliferation assay and microscopy observations, a low concentration of Sal (0.1 μM) effectively sensitized the cancer cells to antimitotic drugs, indicating that Sal toxicity can be minimal when used in combination with antimitotic drugs. These results suggest that the sensitization effects of Sal for clinical applications can be achieved with relatively low concentrations of Sal that avoid toxicity and prevent harm to normal cells.

While seeking a definitive mechanism of Sal action, we observed that Sal reduced the number of cells in G2 arrest and aneuploid cell formation following treatment with antimitotic drugs. G2 arrest and aneuploidy are known to have anti-apoptotic functions, thereby enabling cancers cells to resist treatment with antimitotic agents [1,23]. Our findings indicate that Sal co-treatment prevented delayed mitosis for survival in antimitotic drug-treated cells. Sal then increased the rate of mitotic catastrophe.

Therefore, we concluded that the prevention of both G2 arrest and aneuploidy is a major mechanism of sensitization by Sal that promotes apoptosis in antimitotic drug-treated cancer cells. In our Western blot analysis of cell cycle proteins, we found that treatment with Sal largely reduced cyclin D1 levels in cancer cells treated with the three different antimitotic drugs (PAC, DOC, and VIN) at both early and late periods, indicating that cyclin D1 may be a common target of the co-treatments. Again, we found that cells co-treated with Sal had similarly reduced levels of cyclin D1 in the three different antimitotic drugs.

Since sensitization by Sal in DOX-, ETO-, or radiation-treated cells increases DNA damage [18,19], we tested whether Sal increases DNA damage in antimitotic drug-treated cells. The levels

of pH2AX protein were increased following co-treatment with the antimitotic agents and Sal, suggesting that increased DNA damage contributes to the sensitization. Previously, the p21 tumor suppressor protein was shown to exert an anti-apoptotic effect, thereby promoting cell survival following exposure to anticancer drugs [32,33]. Since it has been reported that DOX-, ETO-, or radiation-mediated increases in p21 expression is reduced by co-treatment with Sal [18,19], we also performed experiments to determine whether Sal could reduce increases in p21 levels resulting from antimitotic drugs. We found that Sal also reduced p21 levels in cells exposed to antimitotic drugs, suggesting that the mechanism of sensitization by Sal is conserved for co-treatment with DOX, ETO, radiation, or antimitotic drugs. Conservation of a sensitization mechanism assumes that Sal targets the same molecules, even when this agent is used in different combinations with various anti-cancer compounds.

In summary, the results from our study demonstrated that Sal can sensitize cancer cells to antimitotic drugs by increasing apoptosis, preventing both G2 arrest and aneuploidy, and reducing cyclin D1 protein levels. Increasing DNA damage and reducing p21 levels also contributes to this sensitization, which represents a conserved mechanism of sensitization by Sal in DOX-, ETO-, and radiation-treated cells. Our findings may contribute to the development of Sal-based combination therapies for patients treated with anti-cancer agents that target microtubules.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.12.141.

References

[1] M.A. Jordan, L. Wilson, Microtubules as a target for anticancer drugs, Nat. Rev. Cancer 4 (2004) 253–265.
[2] H. Yang, Q.P. Dou, Targeting apoptosis pathway with natural terpenoids: implications for treatment of breast and prostate cancer, Curr. Drug Targets 11 (2010) 733–744.
[3] P. Araque Arroyo, R. Ubago Perez, B. Cancela Diez, M.A. Fernandez Feijoo, J. Hernandez Magdalena, M.A. Calleja Hernandez, Controversies in the management of adjuvant breast cancer with taxanes: review of the current literature, Cancer Treat. Rev. 37 (2011) 105–110.
[4] E. Rivera, Management of metastatic breast cancer: monotherapy options for patients resistant to anthracyclines and taxanes, Am. J. Clin. Oncol. 33 (2010) 176–185.
[5] K. Matsuo, V.K. Bond, D.D. Im, N.B. Rosenshein, Prediction of chemotherapy response with platinum and taxane in the advanced stage of ovarian and uterine carcinosarcoma: a clinical implication of in vitro drug resistance assay, Am. J. Clin. Oncol. 33 (2010) 358–363.
[6] B.T. McGrogan, B. Gilmartin, D.N. Carney, A. McCann, Taxanes, microtubules and chemoresistant breast cancer, Biochim. Biophys. Acta 1785 (2008) 96–132.
[7] D. Pectasides, E. Pectasides, G. Papaxoinis, N. Xiros, C. Sykiotis, A. Papachristodoulou, N. Tountas, J. Panayiotides, T. Economopoulos, Combination chemotherapy with carboplatin, paclitaxel and pegylated liposomal doxorubicin for advanced or recurrent carcinosarcoma of the uterus: clinical experience of a single institution, Gynecol. Oncol. 110 (2008) 299–303.
[8] Y. Miyazaki, M. Shibuya, H. Sugawara, O. Kawaguchi, C. Hirsoe, Salinomycin, a new polyether antibiotic, J. Antibiot. (Tokyo) 27 (1974) 814–821.
[9] N. Mahmoudi, J.V De Julian-Ortiz, L. Ciceron, J. Galvez, D. Mazier, M. Danis, F. Derouin, R. Garcia-Domenech, Identification of new antimalarial drugs by linear discriminant analysis and topological virtual screening, J. Antimicrob. Chemother. 57 (2006) 489–497.
[10] P.B. Gupta, T.T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R.A. Weinberg, E.S. Lander, Identification of selective inhibitors of cancer stem cells by high-throughput screening, Cell 138 (2009) 645–659.
[11] D. Fuchs, A. Heinold, G. Opelz, V. Daniel, C. Naujokat, Salinomycin induces apoptosis and overcomes apoptosis resistance in human cancer cells, Biochem. Biophys. Res. Commun. 390 (2009) 743–749.
[12] T.T. Dong, H.M. Zhou, L.L. Wang, B. Feng, B. Lv, M.H. Zheng, Salinomycin selectively targets 'CD133+' cell subpopulations and decreases malignant traits in colorectal cancer lines, Ann. Surg. Oncol. 18 (2011) 1797–1804.
[13] C. Gong, H. Yao, Q. Liu, J. Chen, J. Shi, F. Su, E. Song, Markers of tumor-initiating cells predict chemoresistance in breast cancer, PLoS ONE 5 (2010) e15630.
[14] M.R. Bardsley, V.J. Horvath, D.T. Asuzu, A. Lorincz, D. Redelman, Y. Hayashi, L.N. Popko, D.L. Young, G.A. Lomberk, R.A. Urrutia, G. Farrugia, B.P. Rubin, T. Ordog, Kitlow stem cells cause resistance to kit/platelet-derived growth factor alpha inhibitors in murine gastrointestinal stromal tumors, Gastroenterology 139 (2010) 942–952.
[15] Y. Wang, Effects of salinomycin on cancer stem cell in human lung adenocarcinoma A549 cells, Med. Chem. 7 (2011) 106–111.
[16] R. Riccioni, M.L. Dupuis, M. Bernabei, E. Petrucci, L. Pasquini, G. Mariani, M. Cianfriglia, U. Testa, The cancer stem cell selective inhibitor salinomycin is a p-glycoprotein inhibitor, Blood Cells Mol. Dis. 45 (2010) 86–92.
[17] D. Fuchs, V. Daniel, M. Sadeghi, G. Opelz, C. Naujokat, Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells, Biochem. Biophys. Res. Commun. 394 (2010) 1098–1104.
[18] J.H. Kim, M. Chae, W.K. Kim, Y.J. Kim, H.S. Kang, H.S. Kim, S. Yoon, Salinomycin sensitizes cancer cells to the effects of doxorubicin and etoposide treatment by increasing DNA damage and reducing p21 protein, Br. J. Pharmacol. 162 (2011) 773–784.
[19] W.K. Kim, J.H. Kim, K. Yoon, S. Kim, J. Ro, H.S. Kang, S. Yoon, Salinomycin, a p-glycoprotein inhibitor, sensitizes radiation-treated cancer cells by increasing DNA damage and inducing G2 arrest, Invest New Drugs (2011), in press.
[20] J.H. Kim, T.H. Kim, H.S. Kang, J. Ro, H.S. Kim, S. Yoon, SP600125, an inhibitor of Jnk pathway, reduces viability of relatively resistant cancer cells to doxorubicin, Biochem. Biophys. Res. Commun. 387 (2009) 450–455.
[21] J.H. Kim, S.C. Lee, J. Ro, H.S. Kang, H.S. Kim, S. Yoon, Jnk signaling pathway-mediated regulation of Stat3 activation is linked to the development of doxorubicin resistance in cancer cell lines, Biochem. Pharmacol. 79 (2010) 373–380.
[22] K.H. Lee, K.J. Moon, H.S. Kim, B.C. Yoo, S. Park, H. Lee, S. Kwon, E.S. Lee, S. Yoon, Increased cytoplasmic levels of CIS, SOCS1, SOCS2, or SOCS3 are required for nuclear translocation, FEBS Lett. 582 (2008) 2319–2324.
[23] M. Kavallaris, Microtubules and resistance to tubulin-binding agents, Nat. Rev. Cancer 10 (2010) 194–204.
[24] J.J. Lee, J.H. Lee, Y.G. Ko, S.I. Hong, J.S. Lee, Prevention of premature senescence requires JNK regulation of Bcl-2 and reactive oxygen species, Oncogene 29 (2010) 561–575.
[25] A.M. Mingo-Sion, P.M. Marietta, E. Koller, D.M. Wolf, C.L. Van Den Berg, Inhibition of JNK reduces G2/M transit independent of p53, leading to endoreduplication, decreased proliferation, and apoptosis in breast cancer cells, Oncogene 23 (2004) 596–604.
[26] D.O. Moon, M.O. Kim, C.H. Kang, J.D. Lee, Y.H. Choi, G.Y. Kim, JNK inhibitor SP600125 promotes the formation of polymerized tubulin, leading to G2/M phase arrest, endoreduplication, and delayed apoptosis, Exp. Mol. Med. 41 (2009) 665–677.
[27] S.B. Rho, B.R. Kim, S. Kang, A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cells, Gynecol. Oncol. 120 (2011) 121–127.
[28] D.O. Moon, M.O. Kim, S.H. Kang, K.J. Lee, M.S. Heo, K.S. Choi, Y.H. Choi, G.Y. Kim, Induction of G2/M arrest, endoreduplication, and apoptosis by actin depolymerization agent pextenotoxin-2 in human leukemia cells, Involving activation of ERK and JNK, Biochem. Pharmacol. 76 (2008) 312–321.
[29] R. Wilken, M.S. Veena, M.B. Wang, E.S. Srivatsan, Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma, Mol. Cancer 10 (2011) 12.
[30] S.N. Kim, N.H. Kim, Y.S. Park, H. Kim, S. Lee, Q. Wang, Y.K. Kim, 7-Diethylamino-3(2'-benzoxazolyl)-coumarin is a novel microtubule inhibitor with antimitotic activity in multidrug resistant cancer cells, Biochem. Pharmacol. 77 (2009) 1773–1779.
[31] W. Boehmerle, M. Endres, Salinomycin induces calpain and cytochrome c-mediated neuronal cell death, Cell Death Dis. 2 (2011) e168.
[32] R.H. Weiss, P21Waf1/Cip1 as a therapeutic target in breast and other cancers, Cancer Cell 4 (2003) 425–429.
[33] M. Idogawa, Y. Sasaki, H. Suzuki, H. Mita, K. Imai, Y. Shinomura, T. Tokino, A single recombinant adenovirus expressing p53 and p21-targeting artificial microRNAs efficiently induces apoptosis in human cancer cells, Clin. Cancer Res. 15 (2009) 3725–3732.