ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Targeting Telomerase Enhances Cytotoxicity of Salinomycin in Cancer Cells

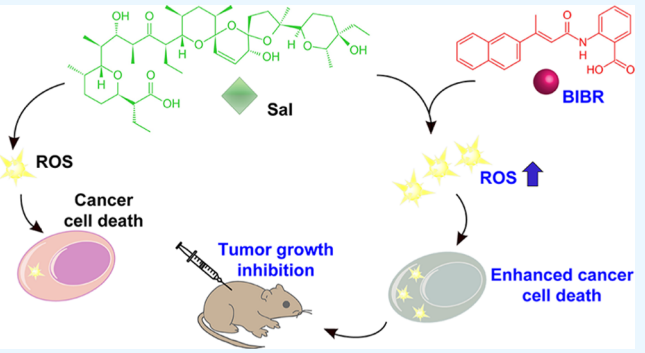
Hongshuang Qin* and Yanxiang Guo

Cite This: *ACS Omega* 2022, 7, 30565−30570

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Salinomycin exhibits significant systemic adverse reactions such as tachycardia and myoglobinuria in mammals, which hinders its application as a drug for human cancers. Although many strategies aimed at increasing salinomycin's toxicity to cancer cells have been identified to allow a lower dose of salinomycin to be used, they often cause normal cell damage by themselves. Thus, it is urgent to find more effective methods to increase salinomycin's toxicity to cancer cells with little influences on normal cells. Telomerase, which is expressed highly in most cancer cells rather than normal somatic cells, plays central roles in cancer cell fate regulation. Targeting telomerase represents a potential method for enhancing salinomycin's cytotoxicity to cancer cells with little effects on normal cells. Herein, we improve the toxicity of salinomycin against cancer cells by telomerase inhibition BIBR1532 (BIBR), which binds to the active site of telomerase reverse transcriptase. We find that a non-toxic dose of BIBR can enhance cytotoxicity of salinomycin in MCF-7 and MDA-MB-231 cells. Moreover, BIBR enhances mammosphere formation inhibition mediated by salinomycin in MCF-7 and MDA-MB-231 cells. Further studies show that BIBR enhances tumor growth inhibition induced by salinomycin in vivo. To our knowledge, this is the first example that targeting telomerase improves anti-cancer effects of salinomycin.



INTRODUCTION

Salinomycin was first extracted from the culture broth of *Streptomyces albus* in the early seventies and was identified as a monocarboxylic polyether antibiotic.[1] For a long period of time salinomycin was only used as a coccidiostat in livestock.[2] Until 2009, Weinberg group reported that salinomycin possessed anti-cancer effects, especially anti-cancer stem-like cell activities.[3] Subsequent studies that follow this lead demonstrated that salinomycin has inhibitory effects on many different types of cancers.[4−9] Unlike conventional chemotherapeutical agents, such as paclitaxel, doxorubicin, cisplatin, and temozolomide, salinomycin can eliminate not only cancer cells but also cancer stem-like cells and multidrug resistance cancer cells.[10−12] Recent studies have revealed some mechanisms of salinomycin against human cancer cells, such as interference with ATP-binding cassette transporters, inhibition of the Wnt/β-catenin signaling pathway, induction differentiation, and overproduction of reactive oxygen species (ROS).[3,8,10,13] In view of these predominant properties, salinomycin is attracting more and more attention and has been considered as a promising anti-cancer drug. However, it has been reported that salinomycin in high dose exhibits severe systemic adverse reactions in mammals, which hinders its application as a drug for human diseases.[14−17] Although many strategies, such as targeting histone deacetylase, pyruvate dehydrogenase kinase, and autophagy, have been identified to improve salinomycin's toxicity against cancer cells to allow a lower dose of salinomycin to be used, they often cause significant normal cell damage by themselves.[18−20] Therefore, it is urgent to find more effective methods for increasing salinomycin's toxicity to cancer cells with little effects on normal cells.

Telomerase, which is expressed at high levels in most types of cancer cells rather than normal cells, is a reverse transcriptase composed of two subunits: an RNA component TERC (telomerase RNA component) and a conserved catalytic subunit TERT (telomerase reverse transcriptase).[21] Telomerase can use TERC as templates for adding TTAGGG repeats to the ends of telomeres via its catalytic subunit TERT.[22] In cancer cells, telomerase maintains telomere length via its telomeric DNA synthesis activity to confer cancer cell immortality.[23] In addition to the canonical telomere elongation function, TERT has additional functions in cancer cells. The TERT in cancer cells is closely correlated with gene

Received: June 29, 2022
Accepted: August 9, 2022
Published: August 17, 2022

https://doi.org/10.1021/acsomega.2c04082
ACS Omega 2022, 7, 30565−30570

transcription, DNA damage repair, stemness maintenance, ROS regulation, and so forth.[24−28] It has been demonstrated that telomerase plays a central role in cancer cell fate regulation.[29] Thus, targeting telomerase is a promising strategy for enhancing the cytotoxicity of salinomycin in cancer cells with little influence on normal cells.

In this study, we propose to improve toxicity of salinomycin (see structure in Figure 1a) in cancer cells by targeting

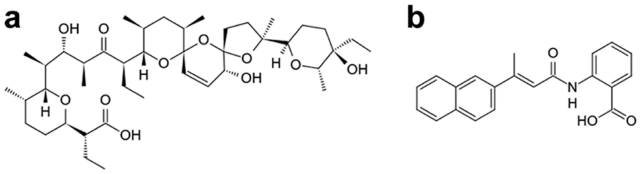


Figure 1. (a) Structure of salinomycin. (b) Structure of BIBR1532.

telomerase via BIBR1532 (BIBR, Figure 1b), which is a specific telomerase inhibitor that binds to the active site of TERT.[30] We find that a non-toxic dose of BIBR can enhance cytotoxicity of salinomycin in MCF-7 and MDA-MB-231 (M231) cells. Furthermore, BIBR reinforces mammosphere formation inhibition mediated by salinomycin in MCF-7 and M231 cells. Mechanism studies show that BIBR improves salinomycin's toxicity partially through enhancing ROS generation. More importantly, BIBR enhances tumor growth inhibition induced by salinomycin. This is the first example that targeting telomerase increases anti-cancer effects of salinomycin. Our studies will shed light on salinomycin application in anti-cancer treatment.

RESULTS

Cytotoxicity of BIBR. For assessing the effects of BIBR on the anti-cancer activities of salinomycin without interference, the cytotoxicity of BIBR was detected first. After treatment with BIBR, the cell viability of MCF-7 and M231 cells was tested by the Cell Counting Kit-8 (CCK-8) assay. As shown in Table 1, BIBR at the concentrations of 1, 5, 10, and 15 μM had slight effects on the cell viability of MCF-7 and M231 cells, whereas the cell viability inhibition induced by BIBR at the concentrations $\geq$ 20 μM reached a significant level ($P < 0.05$). Therefore, the concentration of 15 μM was selected to use in the subsequent experiments.

Table 1. Effects of BIBR on the Cell Viability of MCF-7 and M231 Cells[a]

	MCF-7 cell		M231 cell	
BIBR (μM)	cell viability (%) ± SD	P value	cell viability (%) ± SD	P value
0	100.0 ± 3.10	—	100.0 ± 3.07	—
1	99.75 ± 3.27	0.914	99.78 ± 3.01	0.880
5	99.28 ± 3.10	0.749	99.25 ± 2.54	0.584
10	98.18 ± 2.96	0.425	97.67 ± 2.89	0.152
15	96.75 ± 2.33	0.154	97.22 ± 2.51	0.088
20	94.91 ± 2.25	0.049	96.52 ± 2.03	0.039
25	93.61 ± 2.98	0.036	94.89 ± 4.05	0.032
30	90.01 ± 4.63	0.019	91.53 ± 3.04	0.003

[a]MCF-7 and M231 cells were incubated with different concentrations of BIBR for 72 h, and the cell viability was tested by the CCK-8 assay. BIBR, BIBR1532. M231, MDA-MB-231. SD, standard deviation. — indicates not done.

BIBR Enhances the Cytotoxicity of Salinomycin in MCF-7 and M231 Cells. Next, we tested the effects of non-toxic dose of BIBR on salinomycin's anti-cancer activities. MCF-7 and M231 cells were incubated with BIBR (15 μM) and different concentrations (1, 2, 4, 8, and 16 μM) of salinomycin. As shown in Figure 2, the inhibitory effects of

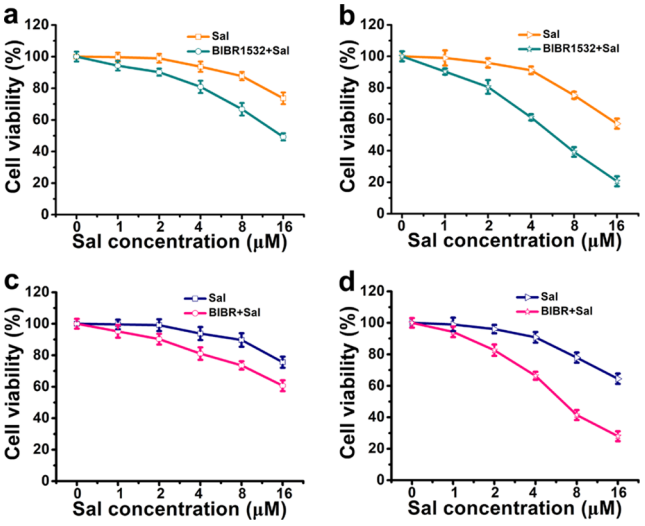


Figure 2. Effects of BIBR on the cell viability inhibition induced by salinomycin in MCF-7 and M231 cells. (a,b) MCF-7 cells were exposed to BIBR (15 μM) and different concentrations (1, 2, 4, 8, and 16 μM) of salinomycin for 48 h (a) and 72 h (b). (c,d) M231 cells were exposed to BIBR (15 μM) and different concentrations (1, 2, 4, 8, and 16 μM) of salinomycin for 48 h (c) and 72 h (d). The cell viability was detected by the CCK-8 assay. The results are shown as the mean ± SD (n = 3). BIBR, BIBR1532. Sal, salinomycin.

different concentrations (1, 2, 4, 8, and 16 μM) of salinomycin on MCF-7 and M231 cell viability were improved by BIBR. Similar effects were found in A549 cells (Figure S1). BIBR also enhanced cytotoxicity of salinomycin in MCF-10A cells (Figure S2). Moreover, simultaneous and sequential combined treatments of BIBR and salinomycin contributed to synergistic inhibitory effects on MCF-7 and M231 cells (Figure S3).

BIBR Improves Mammosphere Formation Inhibition Induced by Salinomycin. It is well known that MCF-7 and M231 cells contain cancer stem-like cells, which can form mammospheres in serum-free and anchorage-independent culture condition.[10,31] We thus detected the effects of BIBR on mammosphere formation inhibition mediated by salinomycin. MCF-7 and M231 cells were exposed to BIBR (15 μM) and salinomycin (4 μM) for 72 h. The cells were cultured in serum-free medium in ultralow adherence plates for 7 d. Then, the mammosphere formation was examined. As shown in Figures 3 and 4, BIBR enhanced mammosphere formation inhibition induced by salinomycin in MCF-7 and M231 cells, suggesting that BIBR increased the inhibitory function of salinomycin on cancer stem-like cells. It has been reported that cancer stem-like cells are more sensitive to BIBR or salinomycin.[3,32] Therefore, the enhanced effects of BIBR on salinomycin's cytotoxicity were compared between mammospheres and MCF-7 cells. MCF-7 secondary mammospheres (Figure S4) and MCF-7 monolayer cells were exposed to BIBR (15 μM) and salinomycin (4 μM) for 72 h. As shown in Figure S5, the cell viability inhibition in mammospheres treated with

https://doi.org/10.1021/acsomega.2c04082
ACS Omega 2022, 7, 30565−30570

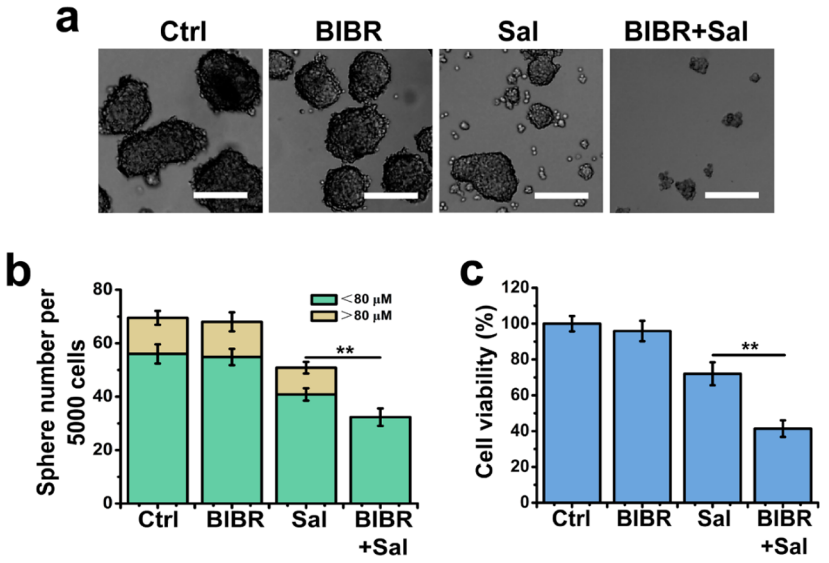


Figure 3. Effects of BIBR on mammosphere formation inhibition induced by salinomycin in MCF-7 cells. (a) Representative images of mammospheres treated with BIBR and salinomycin. MCF-7 cells were incubated with BIBR (15 μM) and salinomycin (4 μM) for 72 h. Then, 5000 cells were seeded into serum-free medium and cultured for 7 d to form mammospheres. Scale bar = 50 μm. (b) Mammospheres were quantitated. The results are shown as the mean ± SD (n = 6). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). (c) Cell viability of the mammospheres was tested. Results are shown as the mean ± SD (n = 6). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). Ctrl, control. Sal, salinomycin. BIBR, BIBR1532.

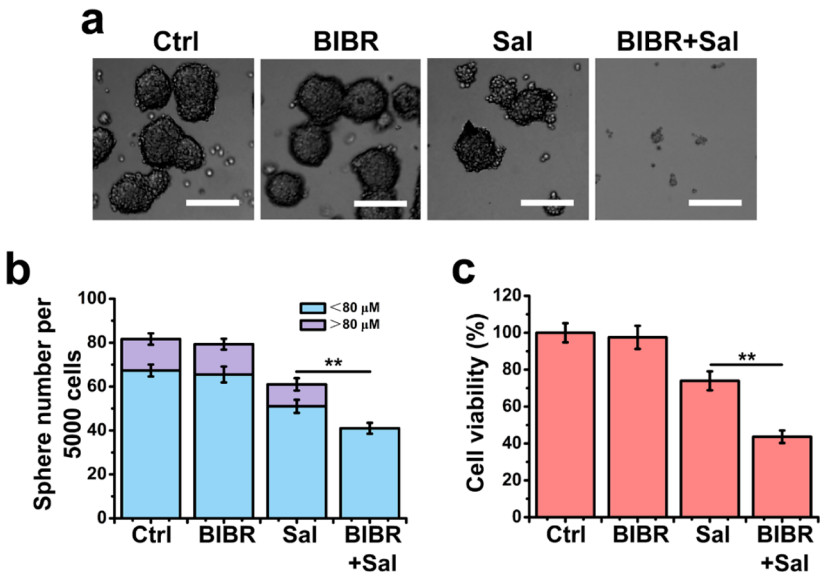


Figure 4. Effects of BIBR on mammosphere formation inhibition induced by salinomycin in M231 cells. (a) Representative images of mammospheres treated with BIBR and salinomycin. M231 cells were treated with BIBR (15 μM) and salinomycin (4 μM) for 72 h. Then, 5000 cells were cultured in serum-free medium for 7 d to form mammospheres. Scale bar = 50 μm. (b) Mammospheres were quantitated. The data are presented as the mean ± SD (n = 6). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). (c) Cell viability of the mammospheres was tested. The data are presented as the mean ± SD (n = 6). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). Ctrl, control. Sal, salinomycin. BIBR, BIBR1532.

both BIBR and salinomycin was higher than that in MCF-7 cells treated with both BIBR and salinomycin.

BIBR Increases Salinomycin's Cytotoxicity Partially via Enhancing ROS Generation. Recent studies show that ROS production is one of the primary mechanisms by which salinomycin mediates toxicity to cancer cells.[5,10] We thus measured the ROS levels in MCF-7 cells after the treatments of BIBR and salinomycin by staining with dichlorofluorescein diacetate. As shown in Figure 5a, the ROS level in the group treated with BIBR and salinomycin was higher than that in the group treated with salinomycin, suggesting that BIBR enhanced ROS generation induced by salinomycin. *N*-Acetyl-L-cysteine (NAC), a ROS scavenger,[10] partially prevented cell growth arrest (Figure 5b), indicating that BIBR improved salinomycin's cytotoxicity in part by enhancing ROS generation.

BIBR Enhances Tumor Growth Inhibition Induced by Salinomycin. To evaluate the effect of BIBR on tumor growth inhibition induced by salinomycin in vivo, MCF-7 cells were subcutaneously injected into nude mice and treated with BIBR,

https://doi.org/10.1021/acsomega.2c04082
ACS Omega 2022, 7, 30565−30570

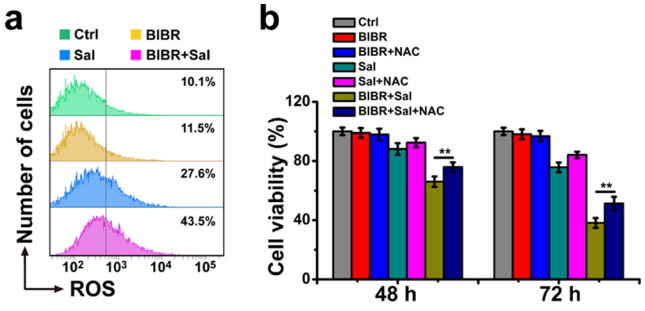


Figure 5. Effects of BIBR on ROS generation induced by salinomycin in MCF-7 cells. (a) ROS production in MCF-7 cells incubated with BIBR (15 μM) and salinomycin (8 μM) for 72 h was tested using dichlorofluorescein diacetate (10 μM) as a probe by flow cytometry. (b) Cell viability of MCF-7 cells exposed to BIBR (15 μM), salinomycin (8 μM), and NAC (10 mM) for 48 and 72 h. The results are shown as the mean ± SD (n = 3). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). Ctrl, control. Sal, salinomycin. BIBR, BIBR1532.

salinomycin, or both for 36 days. As shown in Figure 6a–c, the tumor size and weight in the combined treatment group were less than those in the groups treated with single BIBR or salinomycin. Furthermore, compared with the BIBR or salinomycin group, tumor tissues were looser in the group treated with both BIBR and salinomycin (Figure 6d). These results showed that the combined treatment of BIBR and salinomycin exhibited enhanced inhibitory effects on tumor growth compared to single treatments of BIBR or salinomycin. Figure S6 shows the body weight change of the mice.

DISCUSSION

In this study, two breast cancer cell lines MCF-7 and M231 cells were selected to explore strategies for enhancing salinomycin's anti-cancer effects. Breast cancer is one of the three most common cancers worldwide.[33] Moreover, MCF-7 and M231 cells are known to contain cancer stem-like cells, which are beneficial to anti-cancer stem-like cell studies.[10,31] Therefore, selecting MCF-7 and M231 cells for assessing salinomycin's anti-cancer activities has important significance.

High doses of BIBR also have cytotoxicity. To avoid the interferences, we tested the cytotoxicity of BIBR first and selected a non-toxic dose of BIBR to assess the effects of BIBR on salinomycin's anti-cancer activities. Furthermore, low doses of BIBR will have little effects on normal cells.

Telomerase inhibition has long-term and short-term effects on cancer cells. Cell death due to telomere shortening is the long-term effect, which needs a long lag period. The short-term effects are concerned with the non-canonical functions of TERT.[34−36] One of the primary non-canonical functions of TERT is that TERT can attenuate ROS to prevent cell damage in cancer cells in the stress state.[27] Our results showed that BIBR increased salinomycin's cytotoxicity and improved ROS generation within 72 h. Moreover, the binding site of BIBR in telomerase is the active site of TERT.[30] Therefore, we reasoned that the enhanced effects of BIBR on salinomycin's cytotoxicity in cancer cells were associated with the interference of the non-canonical function of TERT, but not telomere length-dependent function.

CONCLUSIONS

In summary, our data highlight the roles of BIBR in enhancing the cytotoxicity of salinomycin in MCF-7 and M231 cells. Furthermore, BIBR can reinforce the inhibitory effects of salinomycin on mammosphere formation in MCF-7 and M231 cells. In addition, we find that BIBR increases salinomycin's cytotoxicity in part by enhancing ROS generation. More importantly, BIBR can enhance tumor growth inhibition

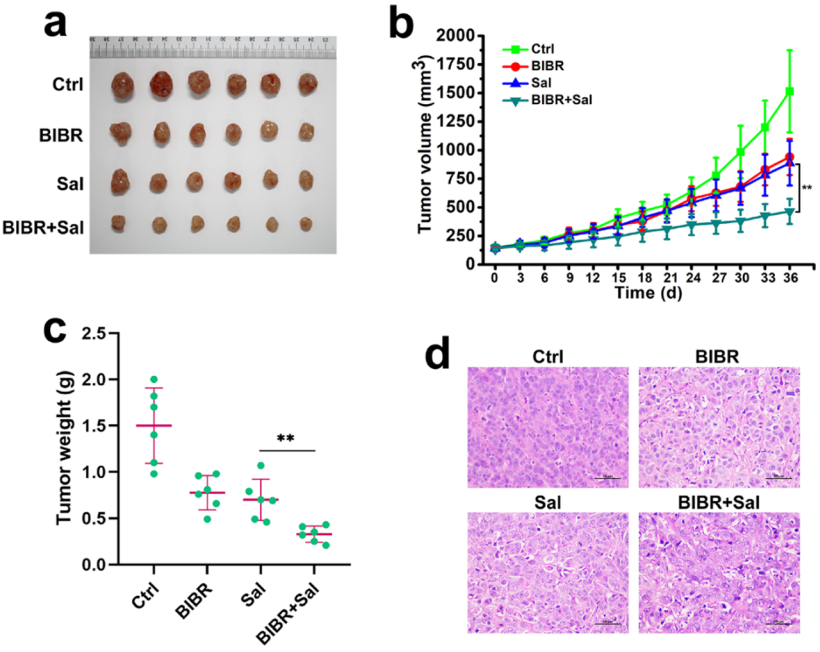


Figure 6. Effect of combined treatments of BIBR and salinomycin on tumor growth in vivo. (a) Photographs of the dissected tumors. (b) Tumor growth curves were plotted. The results are shown as the mean ± SD (n = 6). $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). (c) Tumor weight of the dissected tumors. $^{**}P < 0.01$ (two-way ANOVA, Tukey's *post hoc* test). (d) Representative micrographs of H&E staining of tumor tissues with different treatments. Scale bar = 50 μm. Ctrl, control. Sal, salinomycin. BIBR, BIBR1532.

https://doi.org/10.1021/acsomega.2c04082
ACS Omega 2022, 7, 30565−30570

induced by salinomycin. Our work suggests that targeting telomerase is an efficient way of improving salinomycin's anticancer effects.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04082.

Materials and methods in detail, effects of BIBR on the cell viability inhibition induced by salinomycin in A549 cells, cell viability of MCF-10A cells treated with BIBR and salinomycin, effects of simultaneous and sequential combined treatments of BIBR and salinomycin on MCF-7 cells, proportion of $CD44^{+}/CD24^{-/low}$ cells in the secondary mammospheres of MCF-7 and M231 cells, expression of ALDH1 in the secondary mammospheres of MCF-7 and M231 cells, effects of combined treatment with BIBR and salinomycin on the cell viability of mammospheres and MCF-7 cells, and body weights of mice (PDF).

■ AUTHOR INFORMATION

Corresponding Author

Hongshuang Qin − *Department of Life Science, Lvliang University, Lvliang, Shanxi 033001, China;* orcid.org/0000-0001-6221-079X; Phone: 86-358-2274324; Email: hsqin@llu.edu.cn; Fax: 86-358-2274324

Author

Yanxiang Guo − *Department of Life Science, Lvliang University, Lvliang, Shanxi 033001, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c04082

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (2021L565), the Key Research Program of Lvliang City (Rc2020215), and the National Natural Science Foundation of China (21807096).

■ REFERENCES

(1) Miyazaki, Y.; Shibuya, M.; Sugawara, H.; Kawaguchi, O.; Hirose, C.; Nagatsu, J.; Esumi, S. Salinomycin, a new polyether antibiotic. *J. Antibiot.* **1974**, *27*, 814−821.

(2) Danforth, H. D.; Ruff, M. D.; Reid, W. M.; Miller, R. L. Anticoccidial activity of salinomycin in battery raised broiler chickens. *Poult. Sci.* **1977**, *56*, 926−932.

(3) Gupta, P. B.; Onder, T. T.; Jiang, G.; Tao, K.; Kuperwasser, C.; Weinberg, R. A.; Lander, E. S. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* **2009**, *138*, 645−659.

(4) Jangamreddy, J. R.; Ghavami, S.; Grabarek, J.; Kratz, G.; Wiechec, E.; Fredriksson, B. A.; Rao Pariti, R. K.; Cieślar-Pobuda, A.; Panigrahi, S.; Łos, M. J. Salinomycin induces activation of autophagy, mitophagy and affects mitochondrial polarity: differences between primary and cancer cells. *Biochim. Biophys. Acta* **2013**, *1833*, 2057−2069.

(5) Verdoodt, B.; Vogt, M.; Schmitz, I.; Liffers, S. T.; Tannapfel, A.; Mirmohammadsadegh, A. Salinomycin induces autophagy in colon and breast cancer cells with concomitant generation of reactive oxygen species. *PLoS One* **2012**, *7*, No. e44132.

(6) Ketola, K.; Hilvo, M.; Hyötyläinen, T.; Vuoristo, A.; Ruskeepää, A. L.; Orešič, M.; Kallioniemi, O.; Iljin, K. Salinomycin inhibits prostate cancer growth and migration via induction of oxidative stress. *Br. J. Cancer* **2012**, *106*, 99−106.

(7) Calzolari, A.; Saulle, E.; De Angelis, M. L.; Boe, L.; Pelacchi, A.; Ricci-Vitiani, F.; Baiocchi, L.; Testa, M.; Testa, U. Salinomycin potentiates the cytotoxic effects of TRAIL on glioblastoma cell lines. *PLoS One* **2014**, *9*, No. e94438.

(8) Fuchs, D.; Daniel, V.; Sadeghi, M.; Opelz, G.; Naujokat, C. Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells. *Biochem. Biophys. Res. Commun.* **2010**, *394*, 1098−1104.

(9) Qi, D.; Liu, Y.; Li, J.; Huang, J. H.; Hu, X.; Wu, E. Salinomycin as a potent anticancer stem cell agent: State of the art and future directions. *Med. Res. Rev.* **2022**, *42*, 1037−1063.

(10) Mai, T. T.; Hamaï, A.; Hienzsch, A.; Cañeque, T.; Müller, S.; Wicinski, J.; Cabaud, O.; Leroy, C.; David, A.; Acevedo, V.; Ryo, A.; Ginestier, C.; Birnbaum, D.; Charafe-Jauffret, E.; Codogno, P.; Mehrpour, M.; Rodriguez, R. Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat. Chem.* **2017**, *9*, 1025−1033.

(11) Koo, K. H.; Kim, H.; Bae, Y. K.; Kim, K.; Park, B. K.; Lee, C. H.; Kim, Y. N. Salinomycin induces cell death via inactivation of Stat3 and downregulation of Skp2. *Cell Death Dis.* **2013**, *4*, No. e693.

(12) Naujokat, C.; Steinhart, R. Salinomycin as a drug for targeting human cancer stem cells. *J. Biomed. Biotechnol.* **2012**, *2012*, 950658.

(13) Lu, D.; Choi, M. Y.; Yu, J.; Castro, J. E.; Kipps, T. J.; Carson, D. A. Salinomycin inhibits Wnt signaling and selectively induces apoptosis in chronic lymphocytic leukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 13253−13257.

(14) Dorne, J. L.; Fernández-Cruz, M. L.; Bertelsen, U.; Renshaw, D. W.; Peltonen, K.; Anadon, A.; Feil, A.; Sanders, P.; Wester, P.; Fink-Gremmels, J. Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. *Toxicol. Appl. Pharmacol.* **2013**, *270*, 196−208.

(15) Plumlee, K. H.; Johnson, B.; Galey, F. D. Acute salinomycin toxicosis of pigs. *J. Vet. Diagn. Invest.* **1995**, *7*, 419−420.

(16) Aleman, M.; Magdesian, K. G.; Peterson, T. S.; Galey, F. D. Salinomycin toxicosis in horses. *J. Am. Vet. Med. Assoc.* **2007**, *230*, 1822−1826.

(17) Van Der Linde-Sipman, J. S.; Van Den Ingh, T. S.; Van Nes, J. J.; Verhagen, H.; Kersten, J. G.; Beynen, A. C.; Plekkringa, R. Salinomycin-induced polyneuropathy in cats: morphologic and epidemiologic data. *Vet. Pathol.* **1999**, *36*, 152−156.

(18) Booth, L.; Roberts, J. L.; Conley, A.; Cruickshanks, N.; Ridder, T.; Grant, S.; Poklepovic, A.; Dent, P. HDAC inhibitors enhance the lethality of low dose salinomycin in parental and stem-like GBM cells. *Cancer Biol. Ther.* **2014**, *15*, 305−316.

(19) Skeberdytė, A.; Sarapinienė, I.; Krasko, J. A.; Barakauskienė, A.; Žilionytė, K.; Prokarenkaitė, R.; Sužiedėlis, K.; Bukelskienė, V.; Jarmalaitė, S. Salinomycin and dichloroacetate synergistically inhibit Lewis lung carcinoma cell proliferation, tumor growth and metastasis. *Biochem. Biophys. Res. Commun.* **2020**, *523*, 874−879.

(20) Endo, S.; Nakata, K.; Sagara, A.; Koikawa, K.; Ando, Y.; Kibe, S.; Takesue, S.; Nakayama, H.; Abe, T.; Okumura, T.; Moriyama, T.; Miyasaka, Y.; Ohuchida, K.; Ohtsuka, T.; Mizumoto, K.; Nakamura, M. Autophagy inhibition enhances antiproliferative effect of salinomycin in pancreatic cancer cells. *Pancreatology* **2017**, *17*, 990−996.

(21) Harley, C. B. Telomerase and cancer therapeutics. *Nat. Rev. Cancer* **2008**, *8*, 167−179.

(22) Jiang, J.; Wang, Y.; Sušac, L.; Chan, H.; Basu, R.; Zhou, Z. H.; Feigon, J. Structure of Telomerase with Telomeric DNA. *Cell* **2018**, *173*, 1179−1190.

(23) Roake, C. M.; Artandi, S. E. Regulation of human telomerase in homeostasis and disease. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 384−397.

(24) Ghosh, A.; Saginc, G.; Leow, S. C.; Khattar, E.; Shin, E. M.; Yan, T. D.; Wong, M.; Zhang, Z.; Li, G.; Sung, W. K.; Zhou, J.; Chng,

W. J.; Li, S.; Liu, E.; Tergaonkar, V. Telomerase directly regulates NF-κB-dependent transcription. *Nat. Cell Biol.* **2012**, *14*, 1270−1281.
(25) Mukherjee, S.; Firpo, E. J.; Wang, Y.; Roberts, J. M. Separation of telomerase functions by reverse genetics. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, E1363−E1371.
(26) Hannen, R.; Bartsch, J. W. Essential roles of telomerase reverse transcriptase hTERT in cancer stemness and metastasis. *FEBS Lett.* **2018**, *592*, 2023−2031.
(27) Indran, I. R.; Hande, M. P.; Pervaiz, S. hTERT overexpression alleviates intracellular ROS production, improves mitochondrial function, and inhibits ROS-mediated apoptosis in cancer cells. *Cancer Res.* **2011**, *71*, 266−276.
(28) Li, Y.; Tergaonkar, V. Noncanonical functions of telomerase: implications in telomerase-targeted cancer therapies. *Cancer Res.* **2014**, *74*, 1639−1644.
(29) Low, K. C.; Tergaonkar, V. Telomerase: central regulator of all of the hallmarks of cancer. *Trends Biochem. Sci.* **2013**, *38*, 426−434.
(30) Bryan, C.; Rice, C.; Hoffman, H.; Harkisheimer, M.; Sweeney, M.; Skordalakes, E. Structural Basis of Telomerase Inhibition by the Highly Specific BIBR1532. *Structure* **2015**, *23*, 1934−1942.
(31) Sheridan, C.; Kishimoto, H.; Fuchs, R. K.; Mehrotra, S.; Bhat-Nakshatri, P.; Turner, C. H.; Goulet, R., Jr.; Badve, S.; Nakshatri, H. CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. *Breast Cancer Res.* **2006**, *8*, R59.
(32) Doğan, F.; Özateş, N. P.; Bağca, B. G.; Abbaszadeh, Z.; Söğütlü, F.; Gasımlı, R.; Gündüz, C.; Biray Avcı, Ç. Investigation of the effect of telomerase inhibitor BIBR1532 on breast cancer and breast cancer stem cells. *J. Cell. Biochem.* **2019**, *120*, 1282−1293.
(33) Harbeck, N.; Gnant, M. Breast cancer. *Lancet* **2017**, *389*, 1134−1150.
(34) Wang, J.; Chen, Y.; Ren, J.; Zhao, C.; Qu, X. G-Quadruplex binding enantiomers show chiral selective interactions with human telomere. *Nucleic Acids Res.* **2014**, *42*, 3792−3802.
(35) Asai, A.; Oshima, Y.; Yamamoto, Y.; Uochi, T. A.; Kusaka, H.; Akinaga, S.; Yamashita, Y.; Pongracz, K.; Pruzan, R.; Wunder, E.; Piatyszek, M.; Li, S.; Chin, A. C.; Harley, C. B.; Gryaznov, S. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res.* **2003**, *63*, 3931−9.
(36) Celeghin, A.; Giunco, S.; Freguja, R.; Zangrossi, M.; Nalio, S.; Dolcetti, R.; De Rossi, A. Short-term inhibition of TERT induces telomere length-independent cell cycle arrest and apoptotic response in EBV-immortalized and transformed B cells. *Cell Death Dis.* **2016**, *7*, No. e2562.

https://doi.org/10.1021/acsomega.2c04082
ACS Omega 2022, 7, 30565−30570