Combination of PRL-3 Inhibitor with Sorafenib Synergistically Promotes AML Apoptosis

Xiaomin Chen

Sun Yat-sen University First Affiliated Hospital

Dade Rong

Sun Yat-sen University Zhongshan School of Medicine

Wanhua Cai

Sun Yat-sen University Zhongshan School of Medicine

Xiuzhen Tong (✉ tongxzh@mail.sysu.edu.cn)

Sun Yat-sen University First Affiliated Hospital

Haihe Wang

Sun Yat-sen University Zhongshan School of Medicine

Research Article

Keywords: PRL-3 inhibitor, Sorafenib, Acute myeloid leukemia, Apoptosis, Therapy

Posted Date: February 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-191026/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Phosphatase of regenerating liver-3 (PRL-3) is recognized as a novel independent crucial driver for AML progression. Thus, the specific inhibitor of PRL-3 would be a potential therapeutic agent to AML in clinic, but there is no such preclinical application reported yet. Here we evaluated the cytotoxicity of PRL-3 inhibitor, BR-1, against AML cells ML-1 and MOLM-13. Meanwhile, the effect of BR-1 on the biological characteristics of AML cells and the underlying mechanism were investigated along with the combination of BR-1 and sorafenib on AML cell viability. Our results show that BR-1 promotes apoptosis by inactivation of JAK/STAT5 and PI3K/AKT pathways, while inhibits cell proliferation through arresting cell cycle in the S phase. In addition, combination of BR-1 with a FLT3 inhibitor, sorafenib can further improve the therapeutic effect on AML. Thus, our results demonstrated that BR-1 would be a novel and potent therapeutic agent to AML, and its combination with other anti-AML drugs would be a promising strategy to AML therapy.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease. With the development of tumor genetics and molecular biology, varied molecular characteristics of oncogenes have been disclosed along with applicable prognostic and therapeutic value¹. However, there are still many patients with undetected molecular abnormalities, indicating the complex and heterogeneity of cancer cases. Thus, identification of new prognostic genes and therapeutic targets would be of help to the individualized clinical treatment of cancer, including AML patients.

Phosphatase of regenerating liver-3 (PRL-3), also known as PTP4A3, belongs to the protein tyrosine phosphatase (PTP) family². In this family, there are 107 members that modulate the phosphorylation and dephosphorylation of many biologically significant molecules with numerous kinases, which all participates in signaling pathways and play roles in development and tumor progression³. We previously showed that PRL-3 is highly expressed in AML patients affect the cell cycle, cell proliferation and apoptosis in AML cell lines, demonstrating PRL-3 as an independent prognostic marker for AML⁴. Thus, we suspect that usage of PRL-3 inhibitors would be a potential therapeutic for AML.

Rhodanine- and 1, 3-thiazolidine-2, 4-dione-based compounds compose of a very important group of heterocyclic compounds in drug development⁵. The rhodamine derivatives have been characterized with ability to inhibit cancer cell migration and the enzymatic activity of PRL-3⁵. But there are few clinical studies on them in cancer therapy study, and no mention its application on AML.

FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) is a common gene mutation in acute myeloid leukemia and patients with this mutation are not sensitive to chemotherapy^{6, 7}. Impressively, FLT3 inhibitor, such as sorafenib can provide an effective treatment to AML patients with this mutation⁸. However, the overall poor efficacy of sorafenib limits its large-scale application on AML patients^{9, 10}. Therefore, overcome of drug resistance and improvement of curative efficacy are urgent needs in clinic.

Evidence shows that the combination of sorafenib and other anti-tumor drugs can play a synergistic anti-tumor effect to improve therapy efficacy^{11–13}. Thus in this study, we try to utilize PRL-3 inhibitors BR-1 and sorafenib to characterize their therapeutical effects and the underlying mechanisms in AML, in order to provide a novel and efficient strategy to AML therapy.

Materials And Methods

Patients

According to the NCCN Clinical Practice Guidelines in Oncology for myeloid neoplasms and acute leukemia in 2019¹⁴, the newly diagnosed AML at the First Affiliated Hospital of Sun Yat-sen University from September 2020 to December 2020 were classified and sampled. Each patient underwent bone marrow puncture at the time of diagnosis with informed consent. Prior to this study, this protocol was submitted and approved by the Ethic Committee of the First Affiliated Hospital of Sun Yat-sen University. Leukemia cells were extracted from the bone marrow of each patient for culture, using lymphocyte separation fluid and erythrocyte lysate. Bone marrows from 10 patients were eventually succeeded to be steadily cultured for the later experiments. The main clinical features of these 10 patients are listed in Table S1.

Cell lines and Reagents

Human leukemia lines (ML-1 and MOLM-13) were obtained from the American Type Culture Collection (ATCC) and grown in the RPMI medium (Hyclone) cultures with 10% fetal bovine serum (FBS), 100U/ml penicillin and 0.1 mg/ml streptomycin. The ML-1-PRL3 cells were established in our lab previously⁴. BR-1 and sorafenib was purchased from GLP BIO. All drugs were dissolved at a 10 mM concentration in dimethyl sulfoxide (DMSO) and stored in small aliquots at – 20°C until further use.

Cell Proliferation, Cell Cycle and Apoptosis Assays

The CCK-8 cell proliferation kit (Dojindo Laboratories, Kumamoto, Japan) was used to examine growth rate of cells. Flow cytometry was carried out for cell-cycle analysis with a flow cytometer (Beckman, Danvers, MA) upon propidium iodide staining. Cell apoptosis was also analyzed by flow cytometry using the VIFC/PI Apoptosis Detection kit (4A Biotech Co., Ltd) as previously described⁴. All experiments were carried out in triplicate each time, and at least 3 independent experiments were repeated.

Trypan blue Assays

AML cells were seeded into the 96-well plates with a density of 5×10^3 cells per well. After 24 hours drug treatment, cells were fully mixed with 0.4% trypan blue with a ratio of 10:1 (V/V) for another 3 minutes, followed by observation and photographing under microscope immediately.

Soft Agar Assay for Colony Formation

For soft agar plate preparation, 2 ml agar mixture (1% Agar, 2X RPMI with 20% FBS and 2X antibiotics) was plated onto each culture petridish with 35 mm diameter and set aside for 5 min to be solidified. For cell seeding on the soft agar plate, 0.35% agarose (W/V) was prepared with RPMI with 10% FBS and 1X antibiotics. Until the temperature of this solution lowers than 40°C but higher than 37°C, every 5000 ML-1 cell were mixed with this agar solution and plated on top of each prepared soft agar plate with final 10 μ M BR-1 or with the equal volume of DMSO as control. Plates were incubated at 37°C in a humidified incubator for 7 to 21 days. During this culture process, cells were feed for 1–2 times with 500 μ l RPMI per week. After colonies could be observed on plates, the colonies were stained with 0.5 ml 0.5% crystal violet (W/V) for about 20 minutes and the colonies were counted and photographed under a microscope.

Western Blots

PRL-3 antibody and its western blot analysis were described previously⁴. The blots were detected by ECL (Clarity Western ECL Substrate, Cat. #170–5060; BioRad) and visualized with ChemiDoc (MP, Serial #731BR00765; BioRad).

Tumor Models in Immunodeficient Mice

Four-week old nude mice were purchased from the Experimental Animal Center of Sun Yat-sen University and randomly divided into two groups, of which, one group was used for subcutaneous injection and the other for intravenous injection. For subcutaneous injection, each mouse was injected subcutaneously with 1×10^6 ML-1 cells. When tumor volume reached to 0.8 cm³, half of the mice were randomly selected and injected with BR-1 at 15mg/kg dose intraperitoneally once every three days, for 4 times in total, while the other half was injected with the same volume of corn oil as control group. Mice were sacrificed on the 13th day after the first injection, and the tumor size and weight were measured and compared between the two groups. For intravenous injection, all mice were injected with 1×10^6 ML-1 cells into caudal vein. After 14 days, mice were also randomly assigned into two groups (n = 5 per group) and then treated with corn oil and BR-1(15 mg/kg) respectively once every three days, 3 time in total. For another 10 days from the first drug administration, mice were sacrificed and the dissected spleen and liver were analyzed as above. This animal experiment was strictly performed in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Sun Yat-Sen University.

Statistical Analysis of Clinical Samples

The SPSS 25.0 software (SPSS, Chicago, Ill) was adopted to perform the statistical analysis. Comparisons between two groups were analyzed using Student's t-test, while Bonferroni test was used for comparisons between three or more groups. Differences were considered to be statistically significant at $P < 0.05$.

Results

BR-1 Enhances AML Cell Apoptosis

To investigate whether BR-1 could induce apoptosis of AML cells, we treated ML-1 and MOLM-13 cells with BR-1 at a concentration gradient (0 μ M, 5 μ M, 10 μ M, 20 μ M). Immunoblotting results revealed that with BR-1 concentration increase, AML cells were accompanied with the increasing activation of apoptosis-related signaling, including the cleaved amounts of caspase-7 and PARP as well as the activated p-H2A.X (Fig. 1A, B). Flow cytometry analysis also confirmed the indicated apoptosis induced by BR-1 (Fig. 1C,D, $p \leq 0.0001$, respectively). Given that JAK/STAT5 and PI3K/AKT pathways are associated with anti-apoptosis effect in tumor¹⁵⁻¹⁷, we sought to clarify this PRL-3 inhibitor BR-1 could suppress these two pathways to induct the apoptosis of tumor. Our results showed that BR-1 can effectively suppress the phosphorylation of STAT5 and AKT. As expected, the expression of PI3K was decreased along with BR-1 treatment (Fig. 1E).

BR-1 Arrests AML Cell Cycle and Inhibits AML Cell Proliferation

We then explored the role of BR-1 in AML cell proliferation. CCK-8 assay indicated that BR-1 significantly repressed ML-1 and MOLM-13 cell proliferation respectively (Fig. 2A,B, $p \leq 0.0001$). Soft agar colony formation assay showed that BR-1 treatment led to clearly less and smaller colonies of ML-1 cells, compared to the DMSO control group (Fig. 2C-E, $p = 0.0024$), which confirmed the inhibition of BR-1 to AML cell proliferation. Next, flow cytometry analysis further verified the inhibitory effect of BR-1 on AML cell cycle progression, while demonstrated that BR-1 could arrest cell cycle progression by inducing cell cycle arrest at the S phase in ML-1 and MOLM-13 cells a dose-dependent manner (0 μ M, 5 μ M, 10 μ M, 20 μ M, Fig. 2F,G). Moreover, we also determined the expression of cell cycle-related proteins of S phase and showed that BR-1 clearly attenuated the expressions of CDK2 and CDC25A (Fig. 2H,I), further validating the inhibitory effect of BR-1 on AML cell cycle progression and proliferation.

BR-1 renders more cytotoxicity efficacy to PRL-3 highly expressing cells

Considering BR-1 was characterized as an inhibitor of PRL-3⁵, and PRL-3 is also elevated in AML patients⁴, we treated the parental ML-1 cells with the PRL-3-overexpressing ML-1 cells with BR-1 (25 μ M) respectively. More apoptosis in PRL-3-overexpressing cells were observed than that of the wild-type parental cells upon BR-1 treatment (Fig. 3A). Accordingly, BR-1 also exerted obvious cell proliferation inhibition to PRL-3 forced expression cells (Fig. 3B). Statistically analysis further proved that BR-1 presented more inhibitory effect on PRL-3 highly expressing cells than PRL-3 low cells (Fig. 3C, $p \leq 0.0001$), indicating the promising application on AML therapy.

Combination of BR-1 with Sorafenib renders synergetic cytotoxicity on AML cells

To tentatively promote the cytotoxicity efficacy of BR-1, we used and compared BR-1 with a FLT3 inhibitor, sorafenib as well as their combination to examine their therapeutical potential in both ML-1 and MOLM-13 cells. CCK-8 assay and flow cytometry both demonstrated that combination of BR-1 with sorafenib could significantly inhibit AML cell proliferation, compared to both the individual BR-1 or Sorafenib, respectively (Fig. 4A,B, $p \leq 0.0001$). As sorafenib generally blocks cells in the G1 phase, combination of Sorafenib with BR-1 majorly enhanced G1 phase arrest of AML cells, compared to the singly treated cells

(Fig. 4C, D). Likewise, the supplemental addition of sorafenib efficiently prompted the apoptotic cell ratio, compared to each individual agent (Fig. 4E-H, $p \leq 0.0001$). As MOLM-13 cell is a FLT3-ITD-positive cell, sorafenib showed more cytotoxicity to this cell than ML-1 cell which is a FLT3-negative cell. However, the combination of BR-1 was capable to enhance the apoptotic effect of sorafenib (Fig. 4E-H). We also checked the expression status of FLT3 and found that BR-1 could down-regulated FLT3 (Fig. 4I), suggesting the mutual regulation between PRL-3 and FLT3 expressions as reported¹⁸. Overall, our results revealed the evident synergetic effect of BR-1 and Sorafenib.

BR-1 represses AML Progression in vitro and in vivo

Eventually, to confirm the therapy potent of BR-1 on AML, we performed xenograft experiments in nude mice. The experimental design was shown in Fig. 5A. Results showed that, in the subcutaneous inoculation of AML cell groups, BR-1 efficiently inhibited tumor formation of AML cells, as the tumor size were smaller, compared with the corn oil control group, although there was no statistical significance, which might be due to the short tumor formation time after cell inoculation (Fig. 5B, $p = 0.5594$). However, the tumor weight of the mice after treating with BR-1 was lighter than that of the corn oil control group (Fig. 5C, $p = 0.0144$). In the intravenous injection groups, the treatment was not harmful to mice, as there was no significant weight loss observed, compared to the control group (Fig. 5D). Results show that the parental ML-1 cells could invade into other mouse tissues and caused the typically enlarged spleens (Fig. 5E, $p = 0.0067$) and livers (Fig. 5F, $p = 0.0117$), respectively. In contrast, BR-1 significantly abrogated the cell invasiveness and maintained relatively normal spleen and liver sizes, compared to those of control. Interestingly, we also noted that mice with leukemia could develop hind limb paralysis, but BR-1 can effectively delay this.

To further validate the cytotoxicity of BR-1 on patient-derived primary AML cells, we extracted and isolated AML cells from patients in our hospital. Upon treatment of these cells with BR-1, cells showed evident proliferation inhibition by CCK-8 analysis (Fig. 5G), while presented obvious apoptosis-promoting effect from BR-1 (Fig. 5H). Among these patients, there were two patients with FLT3-ITD mutation, and the combination of BR-1 with sorafenib showed remarkably cytotoxic effect on induction of cell death visualized with trypan blue staining (Fig. 5I). Taken together, our in vitro AML primary cells and in vivo xenograft results evidently manifested that BR-1 is a potential agent for AML therapy.

Discussion

It is reported that the malfunction of PTP family members leads to various diseases including cancer, neurological disorder and diabetes¹⁹. Among them, PRL-3 is known to play roles in cancer progression by involving in invasion and metastasis of cancer cells and tumor angiogenesis². Increasing evidences further suggest that PRL-3 is an important driver of cancer metastasis²⁰⁻²². For instance, PRL-3 reinforces PI3K/Akt activation and consequently promotes epithelial-mesenchymal transition of cancer cell²³. Knockdown of PRL-3 conversely reduces tumor size and inhibits metastasis as well as the invasion and growth of cancer cells^{24, 25}. Our previous study also showed that PRL-3 is an independent

poor prognostic factor for AML⁴, thus the PRL3 inhibitor would be a promising therapy agent for leukemia. Rhodanine- and 1, 3-thiazolidine-2, 4-dione-based compounds have been shown to possess antibacterial, antifungal, antiviral, antimalarial, insecticidal, herbicidal, antitumor, anti-inflammatory and cardiogenic activities²⁶. Garam Min et al identified that one rhodanine-based compound, BR-1, can be used as a PRL-3 inhibitor which strongly inhibits the migration and invasion of PRL-3 overexpressing colon cancer cells through the dephosphorylation of known PRL-3 substrates, including ezrin and cytokeratin 8⁵. However, there is no any report on this BR-1 on leukemia therapy.

To fulfill our suspicion, we tentatively examined the cytotoxicity of BR-1 on AML cells and our experiments clearly demonstrate that BR-1 is an effective anti-tumor agent against AML cells. Mechanistically, it is known that PRL-3 activates both JAK/STAT5 and PI3K/AKT pathways to confer anti-apoptotic effect^{15–17}. Our results here verify the real effect of BR-1 on AML by promoting apoptosis, while effectively represses both JAK/STAT5 and PI3K/AKT pathways, validating the specific inhibition of PRL-3 and its downstream oncogenic signaling by BR-1.

In addition, we disclose that BR-1 can specifically arrest AML cell cycle in the S phase, while promotes the cell apoptosis. Studies have confirmed that cdc25A was one of the intra-S phase checkpoints. The degradation of cdc25A could inhibit the activity of cyclinA/E-CDK2 and blocks the recruitment of CDC45 at the DNA replication origins, so as to inhibit the duplication of DNA^{27, 28}. Our results show that BR-1 can effectively downregulate this checkpoint molecule and indicating BR-1 would be an useful reagent for cell cycle study, though the further exploration should be performed.

Zhou et al showed that PRL-3 is involved in FLT3-ITD signaling and can be as an therapy target for AML¹⁸. PRL-3 is also a downstream of FLT3 signaling²⁹ and ectopic expression of PRL-3 conferred therapeutic resistance to FLT3 inhibitor in AML¹⁸. Therefore, here we validate the synergetic effect of BR-1 and sorafenib, a FLT3 inhibitor, and manifest that combination of BR-1 with Sorafenib would be an promising therapy strategy to AML patients, as our in vitro therapy in AML cell lines and the patient-derived primary AML cells results obviously demonstrate the impressive therapy effect, which is better than either one used alone. Mechanistically, we suspect that BR-1 inhibits PRL-3 activity, thus further sensitize AML cells to sorafenib, as blocake of PRL-3 by BR-1 can effectively reduce FLT3 phosphorylation.

Taken together, our results show that BR-1 would be a novel and efficient anti-AML agent, which should be carried out on pre-clinical investigation. Moreover, combination of BR-1 with sorefenib would be an promising strategy to AML therapy.

Declarations

Ethics Approval and Consent to Participate

All patients (or their parents or guardians) have given their written informed consent and that the study protocol was approved by the institute's committee on human research. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. All experiments using nude mice were strictly performed in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Sun Yat-Sen University.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by Guangdong Basic and Applied Basic Research Foundation (No. 2019A1515010294) to TX, and National Science Foundation of China (No. 81472730) to WH.

Acknowledgements

Thank you for the patient and families participated in this project.

Author's contribution

Conceptualization: Xiuzhen Tong; experiment: Xiaomin Chen, Dade Rong and Wanhua Cai; original draft preparation: Xiaomin Chen; review and editing: Haihe Wang; project administration: Xiuzhen Tong and Haihe Wang.

References

1. Prada-Arismendy J, Arroyave JC, Rothlisberger S. Molecular biomarkers in acute myeloid leukemia. *Blood Rev.* 2017;31(1):63-76.
2. Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T. Protein tyrosine phosphatases in the human genome. *Cell.* 2004;117(6):699-711.
3. Zhang ZY. Protein-tyrosine phosphatases: biological function, structural characteristics, and mechanism of catalysis. *Crit Rev Biochem Mol Biol.* 1998;33(1):1-52.

4. Qu S, Liu B, Guo X, Shi H, Zhou M, Li L, Yang S, Tong X, Wang H. Independent oncogenic and therapeutic significance of phosphatase PRL-3 in FLT3-ITD-negative acute myeloid leukemia. *Cancer*. 2014;120(14):2130-2141.
5. Min G, Lee SK, Kim HN, Han YM, Lee RH, Jeong DG, Han DC, Kwon BM. Rhodanine-based PRL-3 inhibitors blocked the migration and invasion of metastatic cancer cells. *Bioorg Med Chem Lett*. 2013;23(13):3769-3774.
6. Zappone E, Defina M, Aprile L, Bartalucci G, Gozzetti A, Bocchia M. FLT3 Inhibitors in the Management of Acute Myeloid Leukemia. *Anticancer Agents Med Chem*. 2017;17(8):1028-1032.
7. Wu M, Li L, Hamaker M, Small D, Duffield AS. FLT3-ITD cooperates with Rac1 to modulate the sensitivity of leukemic cells to chemotherapeutic agents via regulation of DNA repair pathways. *Haematologica*. 2019;104(12):2418-2428.
8. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M, Carter C. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Res*. 2006;66(24):11851-11858.
9. Serve H, Krug U, Wagner R, Sauerland MC, Heinecke A, Brunnberg U, Schaich M, Ottmann O, Duyster J, Wandt H, Fischer T, Giagounidis A, Neubauer A, Reichle A, Aulitzky W, Noppeney R, Blau I, Kunzmann V, Stuhlmann R, Krämer A, Kreuzer K-A, Brandts C, Steffen B, Thiede C, Müller-Tidow C, Ehninger G, Berdel WE. Sorafenib in Combination With Intensive Chemotherapy in Elderly Patients With Acute Myeloid Leukemia: Results From a Randomized, Placebo-Controlled Trial. *Journal of Clinical Oncology*. 2013;31(25):3110-3118.
10. Engen CB, Wergeland L, Skavland J, Gjertsen BT. Targeted Therapy of FLT3 in Treatment of AML- Current Status and Future Directions. *J Clin Med*. 2014;3(4):1466-1489.
11. Zhang S, Doudican NA, Quay E, Orlow SJ. Fluvastatin Enhances Sorafenib Cytotoxicity in Melanoma Cells. *Anticancer Res*. 2011;31:3259-3266.
12. Jafarnejad M, Sove RJ, Danilova L, Mirando AC, Zhang Y, Yarchoan M, Tran PT, Pandey NB, Fertig EJ, Popel AS. Mechanistically detailed systems biology modeling of the HGF/Met pathway in hepatocellular carcinoma. *NPJ Syst Biol Appl*. 2019;5:29.
13. Li ZJ, Dai HQ, Huang XW, Feng J, Deng JH, Wang ZX, Yang XM, Liu YJ, Wu Y, Chen PH, Shi H, Wang JG, Zhou J, Lu GD. Artesunate synergizes with sorafenib to induce ferroptosis in hepatocellular carcinoma. *Acta Pharmacol Sin*. 2020.
14. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellstrom-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-951.
15. Shariati M, Meric-Bernstam F. Targeting AKT for cancer therapy. *Expert Opin Investig Drugs*. 2019;28(11):977-988.
16. Bolli R, Dawn B, Xuan YT. Role of the JAK STAT Pathway in Protection Against Myocardial Ischemia Reperfusion Injury. *Trends Cardiovasc Med*. 2003;13(2):72-79.

17. Xin P, Xu X, Deng C, Liu S, Wang Y, Zhou X, Ma H, Wei D, Sun S. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol*. 2020;80:106210.
18. Zhou J, Bi C, Chng WJ, Cheong LL, Liu SC, Mahara S, Tay KG, Zeng Q, Li J, Guo K, Tan CP, Yu H, Albert DH, Chen CS. PRL-3, a metastasis associated tyrosine phosphatase, is involved in FLT3-ITD signaling and implicated in anti-AML therapy. *PLoS One*. 2011;6(5):e19798.
19. Ruddraraju KV, Zhang Z-Y. Covalent inhibition of protein tyrosine phosphatases. *Molecular BioSystems*. 2017;13(7):1257-1279.
20. Zhang Y, Li Z, Fan X, Xiong J, Zhang G, Luo X, Li K, Jie Z, Cao Y, Huang Z, Wu F, Xiao L, Duan G, Chen H. PRL-3 promotes gastric cancer peritoneal metastasis via the PI3K/AKT signaling pathway in vitro and in vivo. *Oncol Lett*. 2018;15(6):9069-9074.
21. Al-Aidaroos AQO, Zeng Q. PRL-3 phosphatase and cancer metastasis. *Journal of Cellular Biochemistry*. 2010;111(5):1087-1098.
22. Duciel L, Monraz Gomez LC, Kondratova M, Kuperstein I, Saule S. The Phosphatase PRL-3 Is Involved in Key Steps of Cancer Metastasis. *J Mol Biol*. 2019;431(17):3056-3067.
23. Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 Down-regulates PTEN Expression and Signals through PI3K to Promote Epithelial-Mesenchymal Transition. *Cancer Research*. 2007;67(7):2922-2926.
24. Gari HH, DeGala GD, Ray R, Lucia MS, Lambert JR. PRL-3 engages the focal adhesion pathway in triple-negative breast cancer cells to alter actin structure and substrate adhesion properties critical for cell migration and invasion. *Cancer Lett*. 2016;380(2):505-512.
25. Matsukawa Y, Semba S, Kato H, Koma Y-i, Yanagihara K, Yokozaki H. Constitutive Suppression of PRL-3 Inhibits Invasion and Proliferation of Gastric Cancer Cell in vitro and in vivo. *Pathobiology*. 2010;77(3):155-162.
26. Tomai T, Mai LP. Rhodanine as a Privileged Scaffold in Drug Discovery. *Curr Med Chem*. 2009;16(13):1596-1629.
27. Christina K-R, Jonathan M. Cdc25 mechanisms of checkpoint inhibition and recovery. *Trends Cell Biol*. 2006;16(6):285-292.
28. Ciardo D, Goldar A, Marheineke K. On the Interplay of the DNA Replication Program and the Intra-S Phase Checkpoint Pathway. *Genes*. 2019;10(2).
29. Park JE, Yuen HF, Zhou JB, Al-aidaroos AQO, Guo K, Valk PJ, Zhang SD, Chng WJ, Hong CW, Mills K, Zeng Q. Oncogenic roles of PRL-3 in FLT3-ITD induced acute myeloid leukaemia. *EMBO Molecular Medicine*. 2013;5(9):1351-1366.

Figures

Figure1

Figure 1

BR-1 Enhances AML Cell Apoptosis A-B. Expression of apoptosis-related proteins with varied concentrations of BR-1 treatment in ML-1(A) and MOLM-13(B) cells. C-D. Flow cytometry analysis and cell ratio of apoptosis by VIFC/PI staining in ML-1(C) and MOLM-13 (D) cells with varied concentrations of BR-1 treatment. E-F. Expression of STAT5, PI3K and AKT with varied concentrations of BR-1 treatment in ML-1 (E) and MOLM-13 (F) cells.

Figure2

Figure 2

BR-1 Arrests AML Cell Cycle and Inhibits AML Cell Proliferation A-B. Cell viability analyses of ML-1 (A) and MOLM-13 (B) cells with varied concentrations of BR-1 treatment. C. The soft agar colony formation assay watching using a microscope $\times 40\times$ after crystal violet staining D. The soft agar colony formation assay watching to the naked eye after crystal violet staining. E. Quantification of colony numbers of the control group and the BR-1 group. F-G. Effect of BR-1 on cell cycle progression of ML-1(F) and MOLM-13

(G) cells with varied concentrations of BR-1 treatment. H-I. Expression of the cell cycle regulatory molecules in S phase in ML-1(H) and MOLM-13 (I) with varied concentrations of BR-1 treatment.

Figure3

Figure 3

BR-1 renders more cytotoxicity efficacy to PRL-3 highly expressing cells A. Comparison of the expression of apoptosis-related proteins between ML-1-GFP and ML-1-PRL-3 cells upon BR-1 treatment. B. Cell

viability analyses of ML-1-GFP and ML-1-PRL-3 cells upon BR-1 treatment. C. Comparison of the variation between ML-1-GFP and ML-1-PRL-3 cells upon BR-1 treatment.

Figure4

Figure 4

Combination of BR-1 with Sorafenib renders synergetic cytotoxicity on AML cells A-B. Cell viability analyses of ML-1 (A) and MOLM-13 (B) cells with treatments of BR-1(10μM), Sorafenib(1μM) and their combination C-D. Effect on cell cycle progression of ML-1(C) and MOLM-13 (D) cells with treatments of

BR-1(10μM), Sorafenib(1μM) and their combination E-F. Flow cytometry analysis and cell ratio of apoptosis by VIFC/PI staining in ML-1(E) and MOLM-13 (F) cells with treatments of BR-1(10μM), Sorafenib(1μM) and their combination G-H. Expression of apoptosis-related proteins and AKT in ML-1(G) and MOLM-13(H) cells with treatments of BR-1(10μM), Sorafenib(1μM) and their combination. I. Expression of FLT3 after treating with BR-1(10μM) in ML-1 and MOLM-13 cells.

Figure5

Figure 5

BR-1 represses AML Progression in vitro and in vivo A. Schematic outline of the xenograft experiments. B. Subcutaneous tumor formation with treatment of corn oil and BR-1(15mg/kg). C. Comparison of the tumor weight between treatments of corn oil and BR-1 (15mg/kg). D. Comparison of the mice weight between treatments of corn oil and BR-1(15mg/kg). E. Comparison of the spleen's weight between treatments of corn oil and BR-1 (15mg/kg). F. Comparison of the liver's weight between treatments of corn oil and BR-1 (15mg/kg). G. Cell viability analyses of primary AML cells upon BR-1 treatment (10 μ M). H. Cell death ratio of primary AML cells upon BR-1 treatment (10 μ M) by trypan blue staining assay. I. Cell death ratio of primary AML cells with treatments of BR-1(10 μ M), Sorafenib(1 μ M) and their combination by trypan blue staining assay.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TABLES1.docx](#)