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Berberine combined with 2-deoxy-D-glucose synergistically enhances cancer cell proliferation inhibition *via* energy depletion and unfolded protein response disruption

Li-xia Fan [a], Chang-mei Liu [a], An-hui Gao [a,b], Yu-bo Zhou [b,*], Jia Li [a,b,*]

[a] School of Life Science, East China Normal University, Shanghai 200237, PR China
[b] National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

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ABSTRACT

Background: Targeting multiple aspects of cellular metabolism, such as both aerobic glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), has the potential to improve cancer therapeutics. Berberine (BBR), a widely used traditional Chinese medicine, exerts its antitumor effects by inhibiting OXPHOS. 2-Deoxy-D-glucose (2-DG) targets aerobic glycolysis and demonstrates potential anticancer effects in the clinic. We hypothesized that BBR in combination with 2-DG would be more efficient than either agent alone against cancer cell growth.
Methods: The effects of BBR and 2-DG on cancer cell growth were evaluated using the Sulforhodamine B (SRB) method. Cell death was detected with the PI uptake assay, and Western blot, Q-PCR and luciferase reporter assays were used for signaling pathway detection. An adenovirus system was used for gene overexpression.
Results: BBR combined with 2-DG synergistically enhanced the growth inhibition of cancer cells *in vitro*. Further mechanistic studies showed that the combination drastically enhanced ATP depletion and strongly disrupted the unfolded protein response (UPR). Overexpressing GRP78 partially prevented the cancer cell inhibition induced by both compounds.
Conclusions: Here, we report for the first time that BBR and 2-DG have a synergistic effect on cancer cell growth inhibition related to ATP energy depletion and disruption of UPR.
General significance: Our results propose the potential use of BBR and 2-DG in combination as an anticancer treatment, reinforcing the hypothesis that targeting both aerobic glycolysis and OXPHOS provides more effective cancer therapy and highlighting the important role of UPR in the process.

1. Introduction

Tumor cell metabolism was first described as cancer's Achilles' heel in 2008 [1]. In fact, all of the seven hallmarks of cancer are linked to tumor metabolism [1]. The view of cancer as primarily a metabolic disease has been widely accepted [2]. Correspondingly, targeting cancer metabolism has become an attractive cancer therapy strategy in both academic settings and industry [3–5]. Since its discovery in the 1920s by Nobel Prize winner Dr. Otto Warburg, the Warburg effect, also known as aerobic glycolysis, has become regarded as one of the most important metabolic features of cancer cells. Mitochondrial oxidative phosphorylation (OXPHOS), the other main biochemical pathway that generates metabolic energy in cells, also contributes to energy production in cancers and may play a major energy-producing role in certain cancers [6,7].

Berberine (BBR) is an isoquinoline derivative alkaloid that can be isolated from many medicinal herbs. It is widely used in traditional Chinese medicine for the treatment of inflammatory diseases and for its antimicrobial activities. BBR can lower blood glucose as effectively as the drug metformin, although it uses a different mechanism from that of metformin and rosiglitazone [8]. Furthermore, BBR has been reported to arrest cell cycle progression and inhibit tumor cell proliferation, thereby having an anti-cancer effect [9–13]. Hampering the mitochondrial respiratory chain complex I and inhibiting OXPHOS play important roles in both its anti-diabetic and anti-cancer effects [14,15].

2-DG has long been recognized as an effective inhibitor of glucose metabolism by targeting hexokinase. Multiple preclinical and clinical studies have demonstrated its anti-cancer effect and safety [16–18]. However, 2-DG not only induces glycolysis inhibition but also reverts the metabolic phenotype toward OXPHOS [19].

Here, we hypothesized that targeting both OXPHOS and aerobic glycolysis with BBR and 2-DG would be more efficient than either agent alone against cancer cell growth. The detailed mechanism was further studied, which showed that the combination could enhance the ATP depletion drastically and disrupt unfolded protein response (UPR) strongly, but independent of AMPK activation.

* Corresponding authors at: 189 Guo Shou Jing Road, Shanghai 201203, China. Tel.: +86 2150801313.
E-mail addresses: ybzhou@mail.shcnc.ac.cn (Y. Zhou), jli@mail.shcnc.ac.cn (J. Li).

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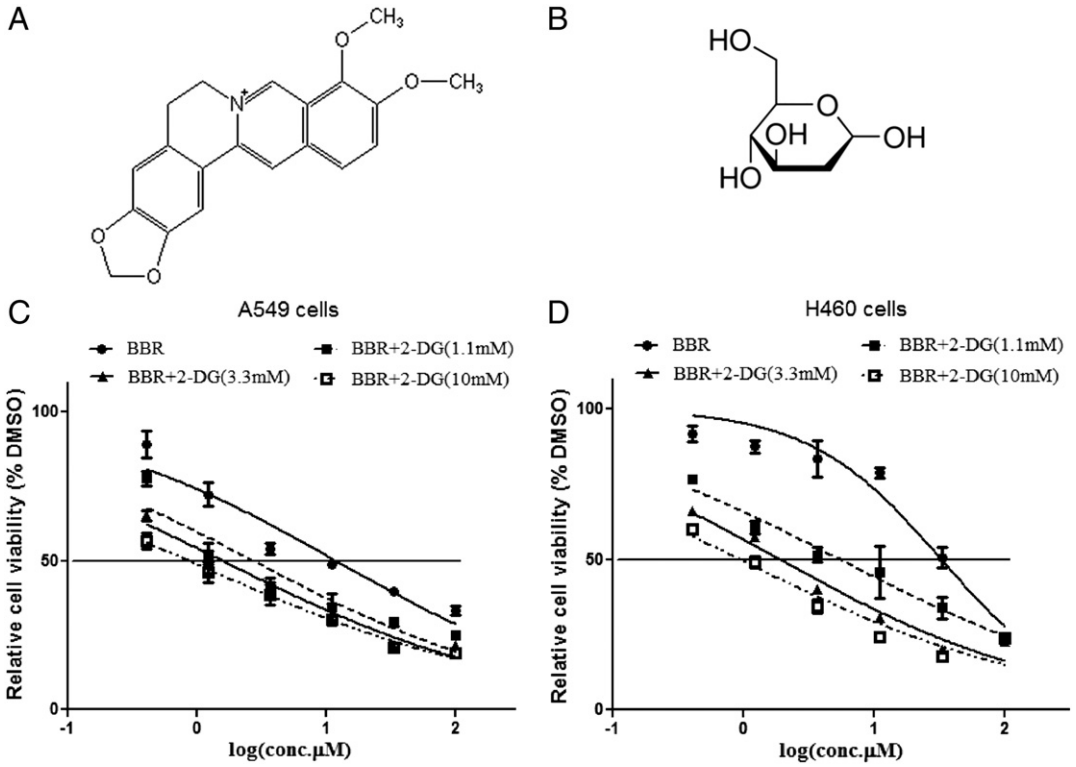

Fig. 1. BBR combined with 2-DG enhance cancer cell growth inhibition. Chemical structure of BBR (A) and 2-DG (B). SRB cell viability assay was used to evaluate the combination effect of BBR and 2-DG on human lung cancer cells A549 (C) and H460 (D). Cells were treated with BBR and 2-DG in different doses for 72 h and evaluated with SRB method. Data were normalized to the untreated control, which represented 100%. The data are presented as the mean ± SD (n = 3).

2. Materials and methods

2.1. Chemicals

2-Deoxy-D-glucose (2-DG) was purchased from Sigma-Aldrich (St. Louis, MO, USA)) and dissolved in sterile distilled water at stock concentrations of 1 M. BBR (a gift from Dr. Lihong Hu, Shanghai Institute of Materia Medica, Shanghai, China) and tunicamycin (Tm), purchased from Millipore (Billerica, MA, USA) were dissolved in DMSO at stock concentrations of 20 mM and 1 mg/ml, respectively. All stock solutions were stored at −20 °C. The final DMSO concentration in the cell cultures was maintained below 1% of the total medium volume throughout the experiments.

2.2. Adenovirus generation

GRP78 recombinant adenovirus vector (Ad-GRP78) was a gift from Dr. Allen Volchuk (University of Toronto, Canada). Dominant-negative AMPK α1 recombinant adenovirus was generated using the AdEasy adenovirus system according to the manufacturer's instructions.

2.3. Cell lines and culture conditions

The A549, H460, H1299, HCT116 and HEK293 cell lines were obtained from the Cell Bank (Chinese Academy of Sciences, Shanghai, China). WI-38 cells were gifts from Dr. Meiyu Geng (Shanghai Institute of Materia Medica, Shanghai, China). HEK293-*CHOP*-promoter-luc and HEK293-*GRP78*-promoter-luc cells were HEK293 cells that were stably transfected with *CHOP*-promoter-luc and *GRP78*-promoter-luc plasmids, which were generous gifts from Dr. Pierre Fafournoux (The National Institute for Agricultural Research (INRA), France) and Dr. Christopher C. Glembotski (San Diego State University, San Diego, CA), respectively. HEK293-*XBP1*-splicing-luc cells were HEK293 cells that were stably transfected with the XBP1-splicing-luc vector, which was subcloned from the pCAX-F-XBP1delDBD-venus plasmid, a gift from Dr. Masayuki Miura (The University of Tokyo) [20]. A549 cells were maintained in F12 medium, H460 and H1299 cells were maintained in RPMI-1640 medium, WI-38 cells were maintained in MEM medium, HCT116 cells were maintained in 5A medium, and HEK293 cells were cultured in HG-DMEM. All culture media were supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone), 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% carbon dioxide (CO_2) at 37 °C.

2.4. Total RNA isolation and reverse transcription PCR

Total RNA was extracted using TRIzol reagent (Invitrogen), according to manufacturer's instructions. The resulting RNA was treated with RNase-free DNase I (Promega, Madison, WI, USA) according to the manufacturer's protocol. RNA concentration and integrity were checked with a UV/VIS spectrophotometer NanoDrop 1000 (Thermo

Table 1
Combination indices of BBR and 2-DG for A549 cells.

	2-DG (mM)		
BBR (μM)	1.1	3.3	10
0.41	0.53	0.33	0.53
1.23	0.17	0.23	0.41
3.70	0.16	0.24	0.36
11.1	0.24	0.24	0.33
33.3	0.41	0.19	0.26
100	0.77	0.52	0.42