Brevilaterin B from *Brevibacillus laterosporus* has selective antitumor activity and induces apoptosis in epidermal cancer

Zhou Chen
Beijing Technology and Business University
Lulu Wang
Beijing Technology and Business University
Yangliu Liu
Beijing Technology and Business University
Panpan Han
Beijing Technology and Business University
Dan Hong
Beijing Technology and Business University
Siting Li
Beijing Technology and Business University
Aijin Ma
Beijing Technology and Business University
Yingmin Jia (jiayingmin2016@163.com)
Beijing Technology and Business University https://orcid.org/0000-0002-7402-262X

Research Article

Keywords: Brevibacillus laterosporus, antimicrobial peptide, anticancer, epidermal cancer cells, apoptosis

Posted Date: November 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-955399/v1

Abstract

Brevibacillus laterosporus, a newly discovered genus, had been shown to be one of the best candidates for producing multiple antimicrobial peptides (AMPs). Herein, we discovered a new bioactivity of Brevilaterin B, an AMP produced by *Br. laterosporus* S62-9, and investigated the details about its Anticancer. Proliferation, membrane permeability and apoptotic rate were checked using CCK-8 Assay, LDH Assay and Annexin V-FITC/PI Kits. ROS levels and mitochondrial membrane potential were detected using the fluorescent probe DCFH-DA and JC-1. Brevilaterin B exhibited broad anticancer activity in a dose-dependent manner. It selectively inhibited the proliferation of epidermal cancer cell A431 but had no effect on normal cells at 2 μg/mL. Typical morphological characteristics of apoptosis and an apoptotic ratio of 71.0% in A431 were observed after treatment with 2-3 μg/mL of Brevilaterin B. In A431 cells, 21.3% ROS generated and 48.8% reduction in mitochondrial membrane potential further occurred, indicating the main site of action was the mitochondrion. Brevilaterin B secreted by *Br. laterosporus* S62-9 has potential application as an anticancer medicament, increasing its commercial value. It's believed to be the first report of the anticancer activity of this type of AMPs.

Highlights

Brevilaterin B was first report to exhibit anticancer activity.

Brevilaterin B induces apoptosis of epidermal cancer cell by action on mitochondrion.

Brevilaterin B has potential application as an anticancer medicament.

Introduction

Despite unprecedented successes in the field of medicine in the last fifty years, human cancer remains a major cause of high morbidity and mortality worldwide (Deslouches et al. 2017). Chemotherapy drugs are still the principal agents used to treat cancer in the advanced or metastatic stages, but the severe side effects to normal cells and tissues, and the easy formation of multi-drug resistance remain problems with the use of chemotherapeutic drugs (Siegel et al. 2014). In the last few decades, tremendous efforts have been devoted to creating new therapies that are more selective and less harmful for patients. There is increasing evidence that antimicrobial peptides (AMPs) have antitumor activity. AMPs are an untapped resource with a low propensity to elicit the development of resistance and low propensity to cause toxicity to healthy cells undergoing rapid proliferation (Deslouches et al. 2013; Steckbeck et al. 2014; Deslouches et al. 2015). AMPs, as cationic and amphipathic peptides may bind to the outer membranes of cancer cells, which have been reported to carry more negatively charged molecules, by electrostatic interactions, and hence cause cytotoxicity toward cancer cells with either a necrotic or apoptotic phenotype (Giuliani et al. 2007; Ting et al. 2014). AMPs are therefore potential therapeutic drugs for the prevention and treatment of cancer. AMPs have efficient tissue penetration and uptake by heterogeneous cancer cells, and treatment with AMPs, either alone or acting synergistically with existing therapeutics, is excepted to

result in improved anticancer therapies with higher selectivity for neoplastic cells and fewer harmful effects on healthy tissues (Oyston et al. 2009).

Several AMPs from bacteria, filamentous fungi, and actinomycetes have been reported to display anticancer properties (Zhao et al. 2018). In particular, AMPs from *Bacillus* sp. have been the focus of many studies because of their interesting antitumor properties. For example, Surfactin secreted by *Bacillus subtilis* has shown good anticancer ability in various cancer cell lines, including the breast cancer cell lines MCF-7 and MDA-MB-231, the human leukemia cell line K562, the cervical cancer cell line HeLa, the rectal cancer cell line LoVo, the histiocytic lymphoma cell line U-937, and the mouse monocyte macrophage leukemia cell line RAW264.7 (Kim et al. 2007; Park et al. 2013). *Br. laterosporus*, previously classified as *Bacillus laterosporus*, is a newly discovered genus and had been shown to be one of the best candidates for producing multiple short sequence AMPs (Krachkovskii et al. 2002). Such peptides contain three structurally unrelated families, a lipopeptide (tauramamide), five linear cationic peptides (bogorols A–E), and four cyclic decapeptides (loloatins A–D) (Barsby et al., 2006; Gerard et al. 1999). Originally, the most valuable feature of these types of AMPs was the broad-spectrum activity against vegetative and nonmultiplying cells of gram-positive and gram-negative bacteria (Singh et al. 2015), drug-resistant bacteria, and pathogenic fungi, such as MRSA, VER, PRSP, and *Candida albicans* (Barsby et al. 2006; Wang et al. 2017). The potential anticancer ability of these AMPs has also been explored. For example, Bogorol B-JX has shown strong inhibition of the proliferation of the human histiocytic lymphoma cell line U-937 and ConA-activated spleen cells (Jiang et al. 2017), and Spergualin has exhibited potential antitumor activity against transplantable leukemias in mice (Nishikawa et al. 1996). However, to the best of our knowledge, these are the only studies of the anticancer activity of AMPs reported to date. Therefore, further systematic research into the selective anticancer activity of AMPs, including the mode of the anticancer action on specific cancer cell lines is desirable.

We selected a recently discovered species *Br. laterosporus* S62-9, which can produce multiple AMPs, and focused on the potential antitumor property of Brevilaterin B, the most valuable component isolated from this species. Thus, the aim of the present work was to evaluate the activity exerted by the AMP Brevilaterin B against various human cancer cell lines and the ability of Brevilaterin B to induce apoptosis in epidermal cancer cell lines in vitro.

Materials And Methods

Strains and AMPs. *Br. laterosporus* S62-9 (CGMCC No. 18629) was used in our research and was stored in the Laboratory of Enzyme Engineering. Brevilaterin B, produced by *Br. laterosporus* S62-9, was obtained according to the method described in our previous report (Ning et al. 2021). The yielding titer of *Br. laterosporus* S62-9 toward *Staphylococcus aureus* (ATCC 25923) was 1888.67 AU/mg.

Cell lines and culture conditions. Human cancer cell lines (n = 27) and several corresponding normal cell lines were used. The cell lines were purchased from Bnbio (Beijing, China), except for the HCT-116, KB, A549, LoVo, and K562 cell lines, which were kindly provided by Prof. Zhenghua Ren from Sun Yat-Sen

University (Guangzhou, China). All cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 and in media containing 10% FBS and 1% penicillin. All standard reagents, including FBS, DEME, RPMI 1640, Ham's F-12, and EGM were obtained from Gibco (Big Cabin, Oklahoma, ME, USA). The cell counting kit 8 (CCK-8), annexin V/propidium iodide (PI) staining kit, and lactase dehydrogenase (LDH) and reactive oxygen species (ROS) assay kits were obtained from Biodee (Beijing, China), BD Biosciences (San Jose, CA, USA), and Jiancheng Bioengineering Institute (Nanjing, China), respectively.

Effect of Brevilaterin B on the proliferation of cell lines. The CCK-8 assay was used to determine the proliferation of the cell lines. First, 100 μL of each cell line (5×10^4 cells/mL) in logarithmic growth phase were seeded on 96-well plates and cultured for 24 h. Cells were then mixed and reacted with Brevilaterin B (0, 1, 2, 4, 8, 16, and 32 μg/mL) for 48 h. Finally, CCK-8 solution was used to dye the above mixtures for 4 h, then the cells were examined for absorption at 490 nm using a microplate reader (INFINITE Spark 10M, TECAN, Switzerland). Afterwards, the IC_{50} values were calculated for each cell line.

Effect of Brevilaterin B on the membrane permeability of the cell lines. The membrane permeability of the cell lines was evaluated by the release rate of LDH. First, 500 μL of cells (10^5 cells/mL) in logarithmic growth phase from each cell line were seeded on 48-well plates and cultured for 24 h. The cells and Brevilaterin B (0, 2, and 3 μg/mL) were mixed and reacted for 24 h. Then, the supernatant was harvested after centrifugation (300 × g) for 5 min. Finally, the LDH assay kit was used to calculate the release rate of LDH.

Ultrastructure observation of the cell lines. Transmission electron microscopy (TEM) was used to observe microscopic changes in the cell lines. Cells (3×10^5 cells/mL) in logarithmic growth phase were cultured for 24 h, then mixed and reacted with Brevilaterin B (0, 2, and 3 μg/mL) for another 24 h. After the treatment, the cells were fixed with 5% glutaraldehyde for 12 h, followed by treatment with osmium tetroxide for 90 min. Ethanol was used to dehydrate the fixed cells and the cells were embedded in epon812 epoxy resin. Ultrathin sections prepared using an ultrathin slicer were dyed by 0.5% uranyl acetate and lead citrate. Finally, the microscopic changes in the cell lines were observed using a JEOL TEM-1400 transmission electron microscope (Hitachi, Tokyo, Japan).

Induction of apoptosis in the cell lines. To evaluate the ability of Brevilaterin B to induce apoptosis in the cell lines, the apoptotic rate, level of ROS, and mitochondrial membrane potential were evaluated.

The annexin V-FITC/PI kit was used for calculation of the apoptotic rate. Cells (10^5 cells/mL) in logarithmic growth phase were seeded on six-well plates and incubated for 24 h. These cells were mixed and reacted with Brevilaterin B (0, 2, and 3 μg/mL) for 24 h. Annexin V-FITC (5 μL) was added to the treated cells (100 μL) followed by 5 μL of PI for staining. Then, 400 μL of binding buffer was added and the mixture was quantitatively measured using a BD C6 flow cytometer from BD Bioscience.

DCFH-DA was the fluorescent probe used for examination of the ROS levels. Cell lines (10^5 cells/well) in logarithmic growth phase were seeded on a six-well plate and incubated overnight. Then, these cells were

treated with various concentrations (0, 2, and 3 μg/mL) of Brevilaterin B for 24 h. Next, the treated cells were harvested and washed with PBS (1×) buffer followed by further treatment with DCFH-DA for 20 min. Then, all the cells were washed twice with PBS (1×) buffer, harvested by trypsin digestion, and collected by centrifugation (300 × g, 3 min). Finally, a BD C6 flow cytometer was used to determine the levels of ROS in the cells.

We also evaluated the mitochondrial membrane potential of the cell lines after treatment with Brevilaterin B. Cell lines (10^5 cells/well) in logarithmic growth phase were treated and incubated according to the method described above. The treated cells were resuspended with the fluorescent probe JC-1 and reacted for 15 min. Next, cells were harvested by trypsin digestion and collected by centrifugation (300 × g, 3 min). Finally, the prepared cell lines were resuspended twice in staining solution and evaluated by a BD C6 flow cytometer.

Results

Effect of Brevilaterin B on the proliferation of cells. The effect of Brevilaterin B on the proliferation of 27 human cancer cell lines and 12 corresponding normal cell lines was examined (Table 1). The IC_{50} values determined in our study strongly indicated that Brevilaterin B could effectively inhibit the proliferation of all the tested cancer cells in a dose-dependent manner. However, 8 μg/mL of Brevilaterin B exhibited an obvious cytotoxicity in most of the cancer cell lines. The lowest IC_{50} value observed for Brevilaterin B was 1.40 μg/mL. The IC_{50} values of Brevilaterin B toward the epidermal carcinoma cell line A431 and a gastric cancer cell line were 2.75 and 5.68 μg/mL, respectively and the values toward the corresponding normal cell lines were 12.78 and 10.62 μg/mL, respectively. The results described above indicated that Brevilaterin B could effectively inhibit the proliferation of specific cancer cell lines.

Table 1
IC_{50} values of Brevilaterin B to cancer and normal cell lines

Cell lines	IC_{50}(μg/mL)	Cell lines	IC_{50}(μg/mL)
Skin fibroblasts HSF	12.78±0.33	Normal mammary epithelial cells MCF-10A	1.58±0.33
Epidermal carcinoma cell A431	2.75±0.07	Breast cancer cells MDA-MB-231	4.16±0.27
Esophageal epithelial cells HEEC	2.66±0.34	Breast cancer cells MCF-7	4.94±0.28
Esophageal cancer cell EC109	2.13±0.23	Normal cervical cells HIBEPIC	2.37±0.38
Esophageal cancer cell EC9706	3.23±0.16	Cervical cancer cells Hela	2.60±0.24
Pancreatic duct epithelial cells HPDE6-C7	3.45±0.20	Cervical cancer cells HelaS3	3.16±0.45
Pancreatic cancer cells SW1990	13.40±0.08	Normal ovarian epithelial cells IOSE80	1.12±0.04
Pancreatic cancer cells PANC-1	3.11±0.49	Ovarian cancer cells A2780	7.40±0.36
Normal hepatocytes Lo-2	2.91±0.07	Ovarian cancer cells SKOV3	4.26±0.25
Hepatoma cells SMMC7721	13.81±0.31	Normal prostatic epithelial cells RWPE-1	1.07±0.34
Hepatoma cells HepG2	9.84±0.39	Prostate cancer cells DU145	2.56±0.23
Normal colonic epithelial cells NCM460	3.04±0.10	Prostate cancer cells PC-3M	6.17±0.24
Colon cancer cells HT-29	1.40±0.09	Human normal gastric epithelial cells GES-1	10.62±0.73
Colon cancer cells sw480	17.15±0.97	Human gastric cancer cells BGC-823	5.68±0.25
Colon cancer cells HCT-116	7.67±0.57	Human gastric cancer cells SGC-7901	5.73±0.21
Rectal cancer cells LoVo	4.16±0.53	Squamous cell carcinoma of tongue Tca8113	5.82±0.25
Human embryonic lung fibroblasts CCD-19 Lu	4.76±0.03	Melanoma cells A375	4.16±0.15
Human non-small lung cancer cells A549	5.54±0.55	Human leukemia cells K-562	5.86±0.66
Embryonic kidney cell HEK-293	4.78±0.32	Oral cancer cells KB	3.14±0.07
Renal cell carcinoma A-498	2.33±0.49	-	-

Effect of Brevilaterin B on membrane permeability. On the basis of the above findings, the epidermal carcinoma cell line A431 and normal human skin fibroblasts (HSF) were selected for further study of Brevilaterin B. First, we investigated the effect of Brevilaterin B on the membrane permeability of A431 and HSF as determined from the LDH release rates (Fig. 1). The results showed that 2 μg/mL of Brevilaterin B could induce LDH release from the cancer cell line A431, with a release rate of 107.21%. However, Brevilaterin B did not affect the membrane permeability of the HSF cell line.

Effect of Brevilaterin B on cell nuclear morphology and ultrastructure. The effect of Brevilaterin B on the cell nuclear morphology was observed using Hoechst 33258 dye solution (Fig. 2). After treatment with 2–3 μg/mL of Brevilaterin B for 24 h, the number of cancer cells was decreased markedly, and the number of irregular cells and the intensity of the blue fluorescence were increased significantly. Later, the typical morphological characteristics of apoptosis appeared, including the occurrence of pyknosis in the nucleus and the fragmentation of some chromatin, while the HSF cells remained in a normal state.

We further observed the ultrastructure changes in the treated cancer cell line A431 and the HSF cell line using TEM (Fig. 3). The intracellular organelles and microvilli on the cell surface of the HSF cells were complete and abundant, indicating that Brevilaterin B had no effect on these normal cells. In comparison, after treatment with Brevilaterin B, some typical apoptotic characteristics gradually appeared in the A431 cells, including a decrease in the superficial microvilli, the appearance of autophagic vacuoles, "wrinkling" of the nuclear membrane, an increase in nuclear heterochromatin, and the disappearance of the internal structure of the organelles.

Brevilaterin B induced apoptosis in A431 cells. The apoptotic rate, ROS level, and mitochondrial membrane potential of A431 cells were all affected by treatment with Brevilaterin B in a dose-dependent manner. When the concentration of Brevilaterin B was increased from 2 to 3 μg/mL, the apoptotic ratio of the A431 cell line was increased from 17.4–71.0% (Fig. 4). Similarly, treatment with Brevilaterin B caused the intracellular ROS level of A431 cells to increase up to 21.3%, when the Brevilaterin B concentration was increased to 3 μg/mL (Fig. 5). Furthermore, the aggregates of JC-1 were detected to evaluate the ability of Brevilaterin B to induce apoptosis in A431 cells. As shown in Fig. 6, 3 μg/mL of Brevilaterin B could induce 48.8% reduction in the mitochondrial membrane potential in A431 cells, while a reduction of only 24.0% occurred after treatment with 3 μg/mL of Brevilaterin B.

Discussion

Cancers pose a great threat to human health worldwide. Many functional medicaments have been discovered for the treatment of cancer, but these drugs can have severe side effects on normal cells and multi-drug resistance can easily be developed to many drugs currently used to treat cancer (Siegel et al. 2014). AMPs have the potential to be developed as anticancer drugs that are more selective and less harmful toward the human body than many current therapeutics (Deslouches et al., 2013; Steckbeck et al., 2014; Deslouches et al. 2015). The AMPs from *Br. laterosporus* are currently of interest because of their excellent antibacterial and antifungal activities. These AMPs have also been shown to inhibit the

proliferation of the human histiocytic lymphoma cell line U-937 (Jiang et al. 2017). However, to the best of our knowledge, no other studies of the antitumor properties of these AMPs have been reported to date. Herein, we found that our previously reported AMP from *Br. laterosporus* S62-9, Brevilaterin B, could inhibit the proliferation of 27 cancer cell lines in a dose-dependent manner, showing a broad spectrum of anti-cancer activity (Ning et al. 2021). Subsequently, we investigated the primary mechanism of action of Brevilaterin B against the epidermal carcinoma cell line A431, and demonstrated that Brevilaterin B could induce apoptosis in this cell line but was not toxic toward the corresponding normal human cell line.

When cultured cells undergo apoptosis, the cell membrane is the first to be destroyed, and then various enzymes, including LDH, are released and can be rapidly detected in the culture medium (Flores-Guzmán et al. 2019). Accordingly, the rate of released LDH can be used to evaluate the integrity of cell membranes (He et al. 2013). In our study, the levels of LDH detected in the supernatants were obviously increased when epidermal carcinoma A431 cells were treated with Brevilaterin B, which was a similar result to that observed in human promyelocytic leukemia HL-60 cells after treatment with the AMP cecropin A (Cerón et al. 2010). In comparison, normal HSF cells, which were regarded as a control group, were not affected by treatment with Brevilaterin B at the same dose. These results indicated that Brevilaterin B could rupture the cancer cell membrane in a selective way, which was also observed by TEM.

The cell nucleus is an important organelle in active cells. Cell nuclei pyknosis occurs when active cells enter into the apoptotic phase and this can be used as a specific morphological sign to indicate apoptotic cells (Xue et al. 2017). We found that cell nuclei pyknosis occurred in A432 cells, but not in HSF cells, after treatment with Brevilaterin B (2–3 μg/mL), indicating that Brevilaterin B may damage the cell nuclei of A431 cells but not those of HSF cells.

Alterations in the cell morphology, including the changes in the cell membrane and cell nucleus described above, are usually the first changes to occur when cancer cells undergo apoptosis. More changes in the morphological details appear with the arrival of the apoptotic phase, and these changes can be directly observed by TEM.

Apoptosis is a pivotal homeostatic mechanism that causes programmed cell death to prevent the uncontrolled proliferation of cancer cells (Li et al. 2017). At the beginning of cell apoptosis, phosphatidylserine in the cell membrane is exposed, and can be easily stained by annexinV-FITC. Measurement of the amounts of such stained cells can be used to evaluate the apoptotic rate of cancer cell lines, and this rate is usually used for the rapid detection of apoptosis. In our results, the addition of Brevilaterin B obviously induced apoptosis and increased the apoptotic rate, in a similar manner to that reported for the AMP ranatuerin-2PLx against the PC-3M cell line (Chen et al. 2018). Wang et al. have also shown that the AMP temporin-1CEa could efficiency induce apoptosis of the breast cancer cell line Bcap-37. The apoptotic rate of Bcap-37 was increased to 80% after treatment with 40 μM of the AMP, which was similar to the effect of Brevilaterin B (Wang et al. 2013).

The mitochondrion is the main organelle in cells that plays an important role in cell growth, proliferation, and differentiation. Perturbation of mitochondrial function is a key event in the apoptotic cascade, and

the development of a mitochondrial permeability transition state is necessary for cell death to proceed (Gaspar et al. 2013). Additionally, the mitochondrion is the “factory” for ROS release, which is the first important feature for apoptosis evaluation. On stimulation from an external factor, intracellular ROS levels can be increased substantially, which can cause oxidative damage to the cell membrane, proteins, and nucleic acids, until apoptosis occurs (Shahrestanaki et al. 2019). In the present study, a 10-fold increase in the amount of ROS released was observed when the concentration of Brevilaterin B was increased from 2 to 3 μg/mL. Li et al. have studied the effect of the AMP CM4 on the breast cancer cell lines MX-1, MCF-7, and MDA-MB-231. The results demonstrated that CM4 could cause the release of high levels of ROS, which then induced apoptosis (Li et al. 2018). The mitochondrion is also the “factory” for cell energy generation. Its pathway could be activated when drugs act on cancer cell lines, resulting in changes in the mitochondrial membrane potential, which is the second most important feature for apoptosis evaluation. Liu et al. have found that the AMP B11 could infiltrate into cervical cancer HeLa cells, and cause mitochondrial disorder, even cell apoptosis, by effectively decreasing the mitochondrial membrane potential (Xia et al. 2016). In the present study, Brevilaterin B caused a decrease in the mitochondrial membrane potential of A431 cancer cells, resulting in mitochondrial depolarization, and finally inducing apoptosis of the cancer cells.

In conclusion, we investigated the selective anti-cancer activity of the AMP Brevilaterin B from *Br. laterosporus* S62-9. Brevilaterin B exhibited selective anti-cancer activity toward the epidermal carcinoma cell line A431 but had no effect on normal HSF cells. Our study revealed that 2 μg/mL of Brevilaterin B could inhibit the proliferation of A431 cells and cause damage to the cell morphology, including the cell membrane and cell nucleus. In addition, we have shown that Brevilaterin B could induce the apoptosis of A431 cancer cells, and the mitochondrion was the main site of action. Overall, these findings indicate that Brevilaterin B may have potential application as an anti-cancer medicament. To the best of our knowledge, the present study is the first report of anti-cancer activity for this type of AMP.

Declarations

Acknowledgements

Funds: This study was supported by Beijing Natural Science Foundation [No. KZ201810011016]; the National Natural Science Foundation of China [No. 31771951, No. 31801510 & No. 32072199].

Statements and Declarations

There are no conflicts of interest.

References

Barsby T, Warabi K, Sørensen D, Zimmerman WT, Kelly MT, Andersen RJ (2006) The Bogorol family of antibiotics: template-based structure elucidation and a new approach to positioning enantiomeric pairs of amino acids. J Org Chem 71(16): 6031-6037. PMID: 16872185. DOI: 10.1021/jo060667p

Cerón JM, Contreras-Moreno J, Puertollano E, Cienfuegos A, Puertollano MA, Pablo MA (2010) The antimicrobial peptide cecropin A induces caspase-independent cell death in human promyelocytic leukemia cells. Peptides 31(8): 1494-1503. DOI: 10.1016/j.peptides.2010.05.008

Chen XL, Zhang LY, Ma CB, Zhang YQ, Xi XP, Wang L, Zhou M, Burrows JF, Chen TB (2018) A novel antimicrobial peptide, Ranatuerin-2PLx, showing therapeutic potential in inhibiting proliferation of cancer cells. Bioscience Reports 38(6). DOI: 10.1042/BSR20180710

Deslouches B, Di YP (2017) Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications. Oncotarget 8(28): 46635-46651. DOI:10.18632/oncotarget.16743

Deslouches B, Steckbeck JD, Craigo JK, Doi Y, Burns JL, Montelaro RC (2015) Engineered cationic antimicrobial peptides to overcome multidrug resistance by ESKAPE pathogens. Antimicrob Agents Chemother 59:1329-33. DOI:10.1128/AAC.03937-14

Deslouches B, Steckbeck JD, Craigo JK, Doi Y, Mietzner TA, Montelaro RC (2013) Rational design of engineered cationic antimicrobial peptides consisting exclusively of arginine and tryptophan, and their activity against multidrugresistant pathogens. Antimicrob Agents Chemother 57(6): 2511-2521. DOI:10.1128/AAC.02218-12

Flores-Guzmán F, Alvarado-Sansininea JJ, López-Muñoz H, Escobar ML (2019) Antiproliferative, cytotoxic and apoptotic activity of the bentonite transformation of sesquiterpene lactone glaucolide B to 5β-hydroxy-hirsutinolide on tumor cell lines. European Journal of Pharmacology 856: 172406. DOI:10.1016/j.ejphar.2019.172406

Gaspar D, Veiga AS, Castanho MARB (2013) From antimicrobial to anticancer peptides. A review. Front Microbiol 4: 294. PMID: 24101917. Doi: 10.3389/fmicb.2013.00294

Gerard JM, Haden P, Kelly MT, Andersen RJ (1999) Loloatins A-D, Cyclic decapeptide antibiotics produced in culture by a tropical marine bacterium. J Nat Prod 62(1): 80-85. DOI: 10.1021/np980219f

Giuliani A, Pirri G, Nicoletto SF (2007) Antimicrobial peptides: an overview of a promising class of therapeutics. Central European Journal of Biology 2(1):1-33.

He NW, Shi XL, Zhao Y, Tian LM Wang DY, Yang XB (2013) Inhibitory effects and molecular mechanisms of selenium-containing tea polysaccharides on human breast cancer MCF-7 cells. Journal of Agricultural and Food Chemistry 61(3): 579-588. DOI:10.1021/jf3036929

Jiang HX, Ji C, Sui JK, Sa RB, Wang XH, Liu XL, Guo TL (2017) Antibacterial and antitumor activity of Bogorol B-JX isolated from *Brevibacillus laterosporus* JX-5. World J Microbial Biotechnol 33: 177. DOI: 10.1007/s11274-017-2337-z

Kim S, Kim JY, Kim S, Bae HJ, Yi H, Yoon SH, Koo BS, Kwon M, Cho JY, Lee C, Hong S (2007) Surfactin from *Bacillus subtilis* displays anti-proliferative effect via apoptosis induction, cell cycle arrest and

survival signaling suppression. FEBS Letters 581(5): 865-871. DOI: 10.1016/j.febslet.2007.01.059

Krachkovskii SA, Sobol AG, Ovchinnikova TV, Tagaev AA, Yakimenko ZA, Azizbekyan RR, Kuznetsova NI, Shamshina TN, Arseniev AS (2002) Isolation, biological properties, and spatial structure of antibiotic loloatin A. Russ J Bioorg Chem 28(4): 269-273. DOI: 10.1023/A:1019531505769

Li X, Fan XX, Jiang ZB, Loo WTY, Yao XJ, Leung ELH, Chow WC, Liu L (2017) Shikonin inhibits gefitinib-resistant non-small cell lung cancer by inhibiting TrxR and activating the EGFR proteasomal degradation pathway. Pharmacol Res 115:45-55. DOI: 10.1016/j.phrs.2016.11.011

Li CY, Liu HY, Yang YQ, Xu XX, Lv TT, Zhang HD, Liu KH, Zhang SQ, Chen YQ (2018) N-myristoylation of antimicrobial peptide CM4 enhances its anticancer activity by interacting with cell membrane and targeting mitochondria in breast cancer cells. Frontiers in pharmacology 9: 1297. PMID: 30483133. DOI: 10.3389/fphar.2018.01297

Ning YW, Han PP, Ma JM, Liu YL, Fu YN, Wang ZX, Jia YM (2021) Characterization of brevilaterins, multiple antimicrobial peptides simultaneously produced by *Brevibacillus laterosporus* S62-9, and their application in real food system. Food Bioscience 42: 101091. Doi: 10.1016/j.fbio.2021.101091

Nishikawa K, Shibasaki C, Takahashi K, Nakamura T, Takeuchi T, Umezawa H (1996) Antitumor activity of Spergualin, a novel antitumor antibiotic. J Antibiot 39(10): 1461-1466. PMID: 3781914. DOI: 10.7164/antibiotics.39.1461

Oyston PC, Fox MA, Richards SJ, Clark GC (2009) Novel peptide therapeutics for treatment of infections. J Med Microbiol 58(Pt8): 977-987. DOI:10.1099/jmm.0.011122-0

Park SY, Kim J, Lee YJ, Lee SJ (2013) Surfactin suppresses TPA-induced breast cancer cell invasion through the inhibition of MMP-9 expression. International Journal of Oncology 42(1): 287-296. DOI:10.3892/ijo.2012.1695

Shahrestanaki MK, Bagheri M, Ghanadian M, Aghaei M, Jafari SM (2019) *Centaurea cyanus* extracted 13-O-acetylsolstitialin A decrease Bax/Bcl-2 ratio and expression of cyclin D1/Cdk-4 to induce apoptosis and cell cycle arrest in MCF-7 and MDA-MB-231 breast cancer cell lines. Journal of Cellular Biochemistry 120(10): 18309-18319. DOI: 10.1002/jcb.29141

Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics 2014. CA Cancer J Clin 64:9-29. doi: 10.3322/caac.21208 PMID: 24399786

Singh PK, Solanki V, Sharma S, Thakur KG, Krishnan B (2015) The intramolecular disulfide-stapled structure of laterosporulin, a class IId bacteriocin, conceals a human defensin-like structural module. FEBS J 282(2): 203-214. DOI: 10.1111/febs.13129

Steckbeck JD, Deslouches B, Montelaro RC (2014) Antimicrobial peptides: new drugs for bad bugs? Expert Opin Biol Ther 14:11-14.

Ting CH, Huang HN, Huang TC, Wu CJ, Chen JY (2014) The mechanisms by which pardaxin, a natural cationic antimicrobial peptide, targets the endoplasmic reticulum and induces c-FOS. Biomaterials 35(11):3627-3640. doi: 10.1016/j.biomaterials.2014.01.032 PMID: 24477193.

Xue ML, Ji XQ, Xue CX, Liang H, Ge YL, He XJ, Zhang L, Bian K, Zhang LH (2017) Caspase-dependent and caspase-independent induction of apoptosis in breast cancer by fucoidan via the PI3K/AKT/GSK3β pathway in vivo and in vitro. Biomedicine & Pharmacotherapy 94: 898-908. DOI:10.1016/j.biopha.2017.08.013

Wang S, Wang QW, Zeng XF, Ye QH, Huang S, Yu HT, Yang TR, Qiao SY (2017) Use of the antimicrobial peptide sublancin with combined antibacterial and immunomodulatory activities to protect against methicillin-resisitant *Staphylococcus aureus* infection in mice. J Agric Food Chem 65(39): 8595-8605. PMID: 28906115. DOI: 10.1021/acs.jafc.7b02592

Wang C, Zhou Y, Li S, Li HB, Tian LL, Wang H, Shang DJ (2013) Anticancer mechanisms of temporin-1CEa, an amphipathic α-helical antimicrobial peptide, in Bcap-37 human breast cancer cells. Life Sciences 92(20-21): 1004-1014. DOI:10.1016/j.lfs.2013.03.016

Xia LJ, Wu YL, Ma J, Yang JH, Zhang FC (2016) The antibacterial peptide from Bombyx mori cecropinXJ induced growth arrest and apoptosis in human hepatocellular carcinoma cells. Oncology letters 12(1): 57-62. DOI:10.3892/ol.2016.4601

Zhao HB, Yan L, Xu XG, Jiang CM, Shi JL, Zhang YW, Lei SZ, Shao DY, Huang QS (2018) Potential of *Bacillus subtilis* lipopeptides in anti-cancer I: induction of apoptosis and paraptosis and inhibition of autophagy in K562 cells. Amb Express 8(1):1-16. PMID: 29777449. DOI: 10.1186/s13568-018-0606-3

Figures

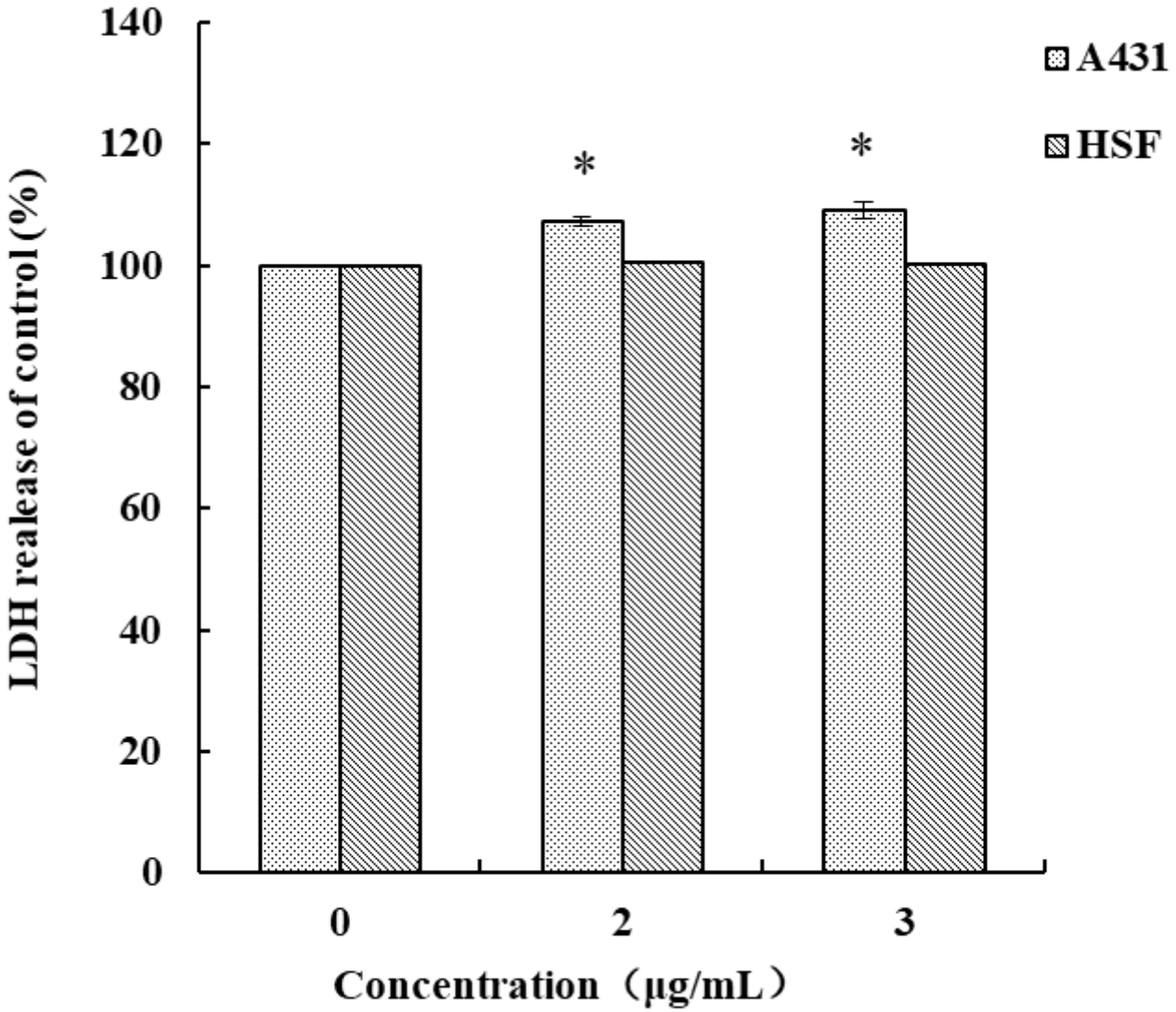

Figure 1

Changes of LDH release rate in A431 cells treated by Brevilaterin B (*p<0.05; **p <0.01).

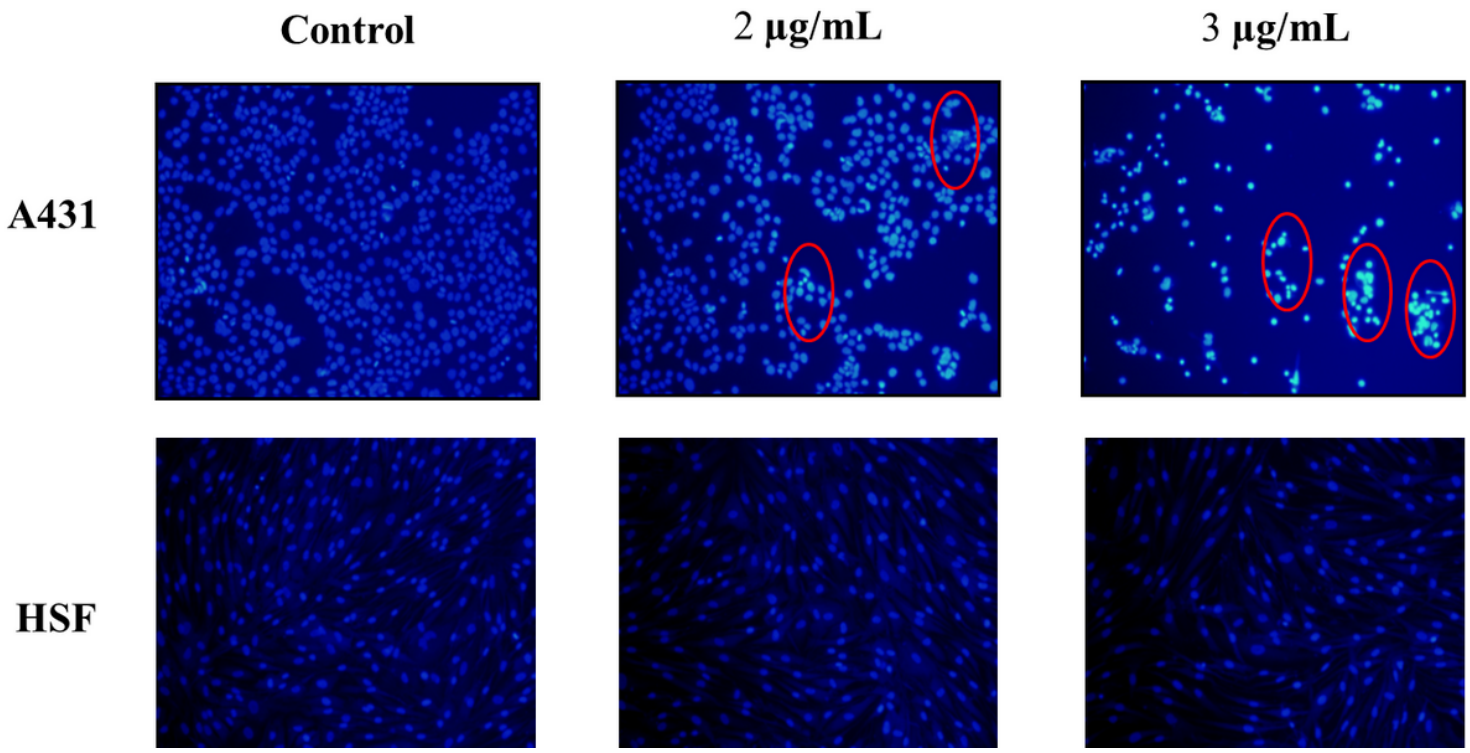

Figure 2

Effects of Brevilaterin B on nucleus in A431 cells and HSF cells (enlargement factor: 100×).

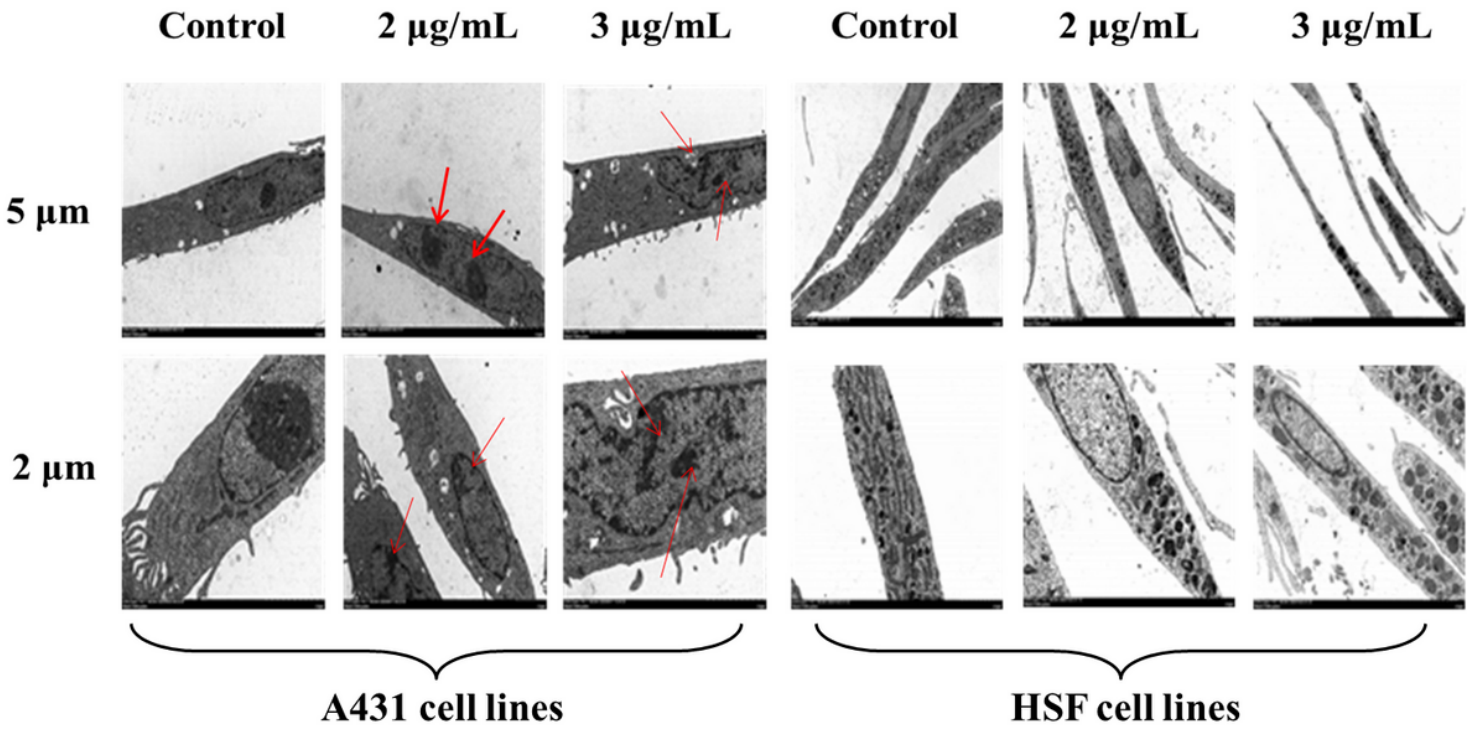

Figure 3

Effects of Brevilaterin B on ultrastructure in A431 and HSF cells.

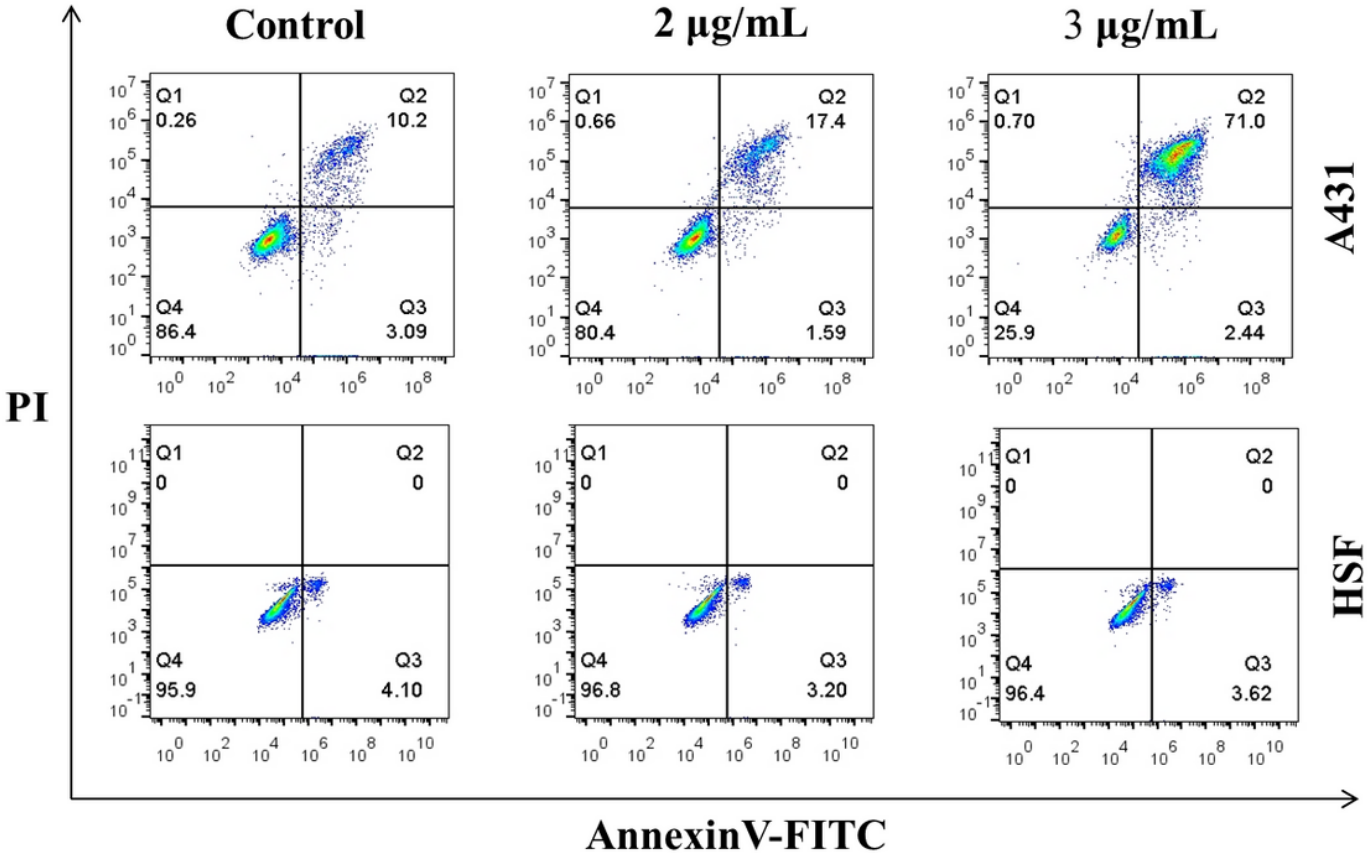

Figure 4

Effect of Brevilaterin B on apoptotic rate of A431 cells (A, FITC/PI double-staining flow chart; B, statistical chart of apoptotic rate; **p <0.01).

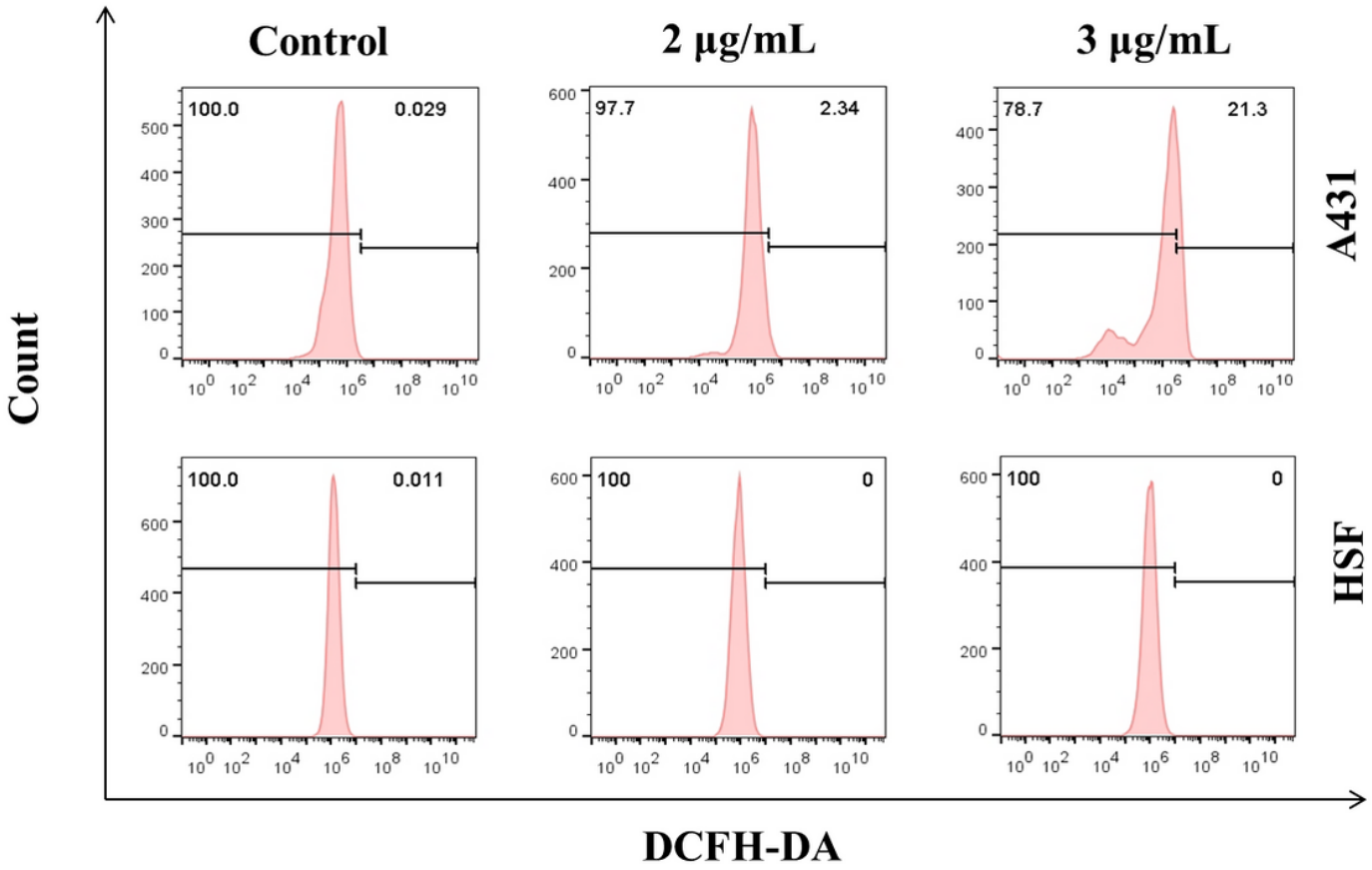

Figure 5

Effect of Brevilaterin B on ROS levels of A431 cells (A, DCFH-DA monochrome flow chart; B, DCFH positive proportion statistical chart; **p <0.01).

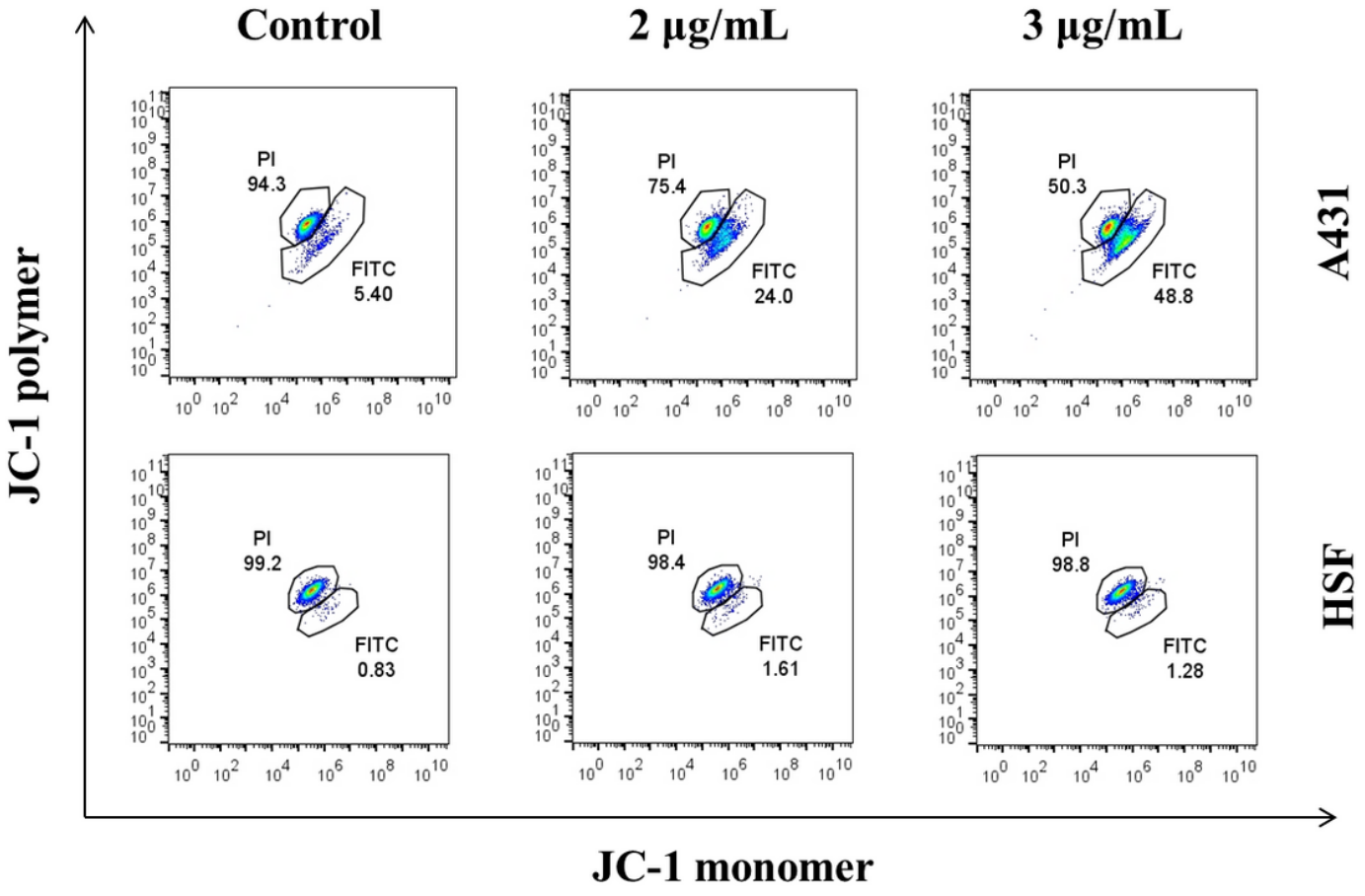

Figure 6

Effect of Brevilaterin B on mitochondrial membrane potential of A431 cells (A, JC-1 monochrome flow chart; B, JC-1 positive aggregate statistical chart; **p <0.01).

Supplementary Files

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

- GraphicalAbstracts.docx
- 11274Authorchecklist.docx