Research Article

Han-Qing Liu, Ya-Wen An, A-Zhen Hu, Ming-Hua Li, Guang-Hui Cui*

Photodynamic Therapy Enhanced the Antitumor Effects of Berberine on HeLa Cells

<https://doi.org/10.1515/chem-2019-0048>

received November 15, 2018; accepted February 19, 2019.

Abstract: In this study we investigated the antineoplastic effects of Berberine (BBR)-mediated photodynamic therapy (PDT) on HeLa cells and its related mechanisms. The CCK-8 assay and flow cytometry were used to evaluate the proliferation and apoptosis of cells respectively. In addition, changes in protein expression levels were assessed using western blot. BBR at dose of 10 mg/kg was injected intraperitoneally to mice with tumors and PDT treatments were performed 24 hours later. *In vivo* imaging systems were used to evaluate the fluorescence of BBR. *In vitro*, PDT significantly enhanced the effects of BBR on inducing cell apoptosis and inhibiting proliferation. The *in vivo* results showed that the fluorescence intensity in the PDT group was decreased compared with that in the BBR group. Tumor weights and tumor size in the PDT group were less than those in the control group; however, when BBR was applied without PDT, no significant differences were observed between the BBR and control group. The results of western blot showed that PDT enhanced the inhibitory effects of BBR on the mammalian target of rapamycin (mTOR) signaling pathway, that may partly explain the potential underlying mechanisms.

Keywords: Berberine; photodynamic therapy; HeLa cell; mTOR.

1 Introduction

Berberine (BBR) is a quaternary ammonium compound extracted from the Traditional Chinese Medicine *Coptis*. BBR has a wide range of pharmacological effects including antiseptic, antiarrhythmic, anti-hypertensive, and antitumor activities and is used in the treatment of digestive tract diseases, and obstetrical and gynecological diseases. Recently, the antitumor effects of BBR, including inhibition of tumor cell proliferation, invasiveness and metastasis, induction of apoptosis, and its influence on cell growth cycle and signaling pathways, have been widely observed in various tumor models [1-4].

Photodynamic therapy (PDT) is a non-invasive cancer treatment which involves the administration of photosensitizers and illumination by specific wavelengths to produce photochemical and thermal reactions. The sensitization of photosensitizers leads to the conversion of reactive oxygen species from the ground-state, mainly active singlet oxygen (1O_2). 1O_2 can react with many biological molecules, such as nucleic acids, lipids and proteins, and these reactions lead to cell death. In the past decade, PDT has been used in the clinic as a new method of treating malignant tumors, and its definite curative effect and low toxicity are well known [5-8].

BBR is proven as a naturally occurring phototoxic alkaloid, which can be activated by near-ultraviolet light (NUV) and far-UV (FUV), and was found to be a 1O_2 generator in experiments using the chemical trap 2,5-dimethyl furan. Studies on BBR-PDT in the treatment of cancers began in 1986 [9-12].

In the present study, the antitumor effects of BBR-PDT on HeLa cells were evaluated *in vitro* and *in vivo* using a mouse xenograft model. Furthermore, the effects of BBR on the PI3K (Phosphoinositol 3 kinase)- AKT (protein kinase B)- mTOR (mammalian target of rapamycin) signaling pathway was investigated.

*Corresponding author: Guang-Hui Cui, Shenzhen Peking University - The Hong Kong University of Science and Technology Medical Center, Shenzhen City, Guangdong, China, 518036, E-mail: cuiguanghui175@aliyun.com

Han-Qing Liu, A-Zhen Hu, Ming-Hua Li: Shenzhen Peking University - The Hong Kong University of Science and Technology Medical Center, Shenzhen City, Guangdong, China, 518036

Ya-Wen An: The State Key Laboratory of Chemical Oncogenomics, Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen City, Guangdong, China, 518055

2 Materials and Methods

2.1 Animals

BALB/c nude mice were provided by the Medical Laboratory Animal Center of Guangdong (Foshan City, Guangdong, China). The animals were housed in specific pathogen free (SPF) conditions with a 12 hour light/12 hour dark cycle, at 19 – 25°C, a relative humidity of 42 – 59% and free access to food and water. Treatments were carried out after 7 days of quarantine. The experiments were performed in accordance with the international guidelines on experimental animals and approved by the Ethics Committee of Shenzhen Peking University – The Hong Kong University of Science and Technology Medical Center (license number was SYXK 2015-0106).

2.2 Materials

BBR was provided by Sigma-Aldrich (Burlington, MA, USA) and dissolved in phosphate buffer solution (PBS). The primary antibodies of phosphatidylinositol 3 kinase (PI3K, cat. no. PA5-38904), phospho-PI3K (cat. no. PA5-17387), protein kinase B (AKT, cat. no. AH01112), mammalian target of rapamycin (mTOR, cat. no. PA5-34663), p70 ribosomal s6 kinase (p70s6k, cat. no. MA5-15141), phospho-ribosomal protein s6 (p-rps6, cat. no. MA5-15140) were purchased from Invitrogen (Carlsbad, CA, USA). Primary antibodies of phospho-Akt (cat. no. #4060), phospho-mTOR (cat. no. #5536), phospho-p70s6k (cat. no. #5536), rps6 (cat. no. #2217), B-cell lymphoma-2 Associated X (Bax, cat. no. #2772) and β -actin (cat. no. #4970) were provided Cell Signaling Technology (Danvers, MA, USA).

2.3 Cell proliferation and apoptosis

Absorption of BBR solutions was measured using a Micro-Spectrophotometer (Q5000, Quawell Technology, Inc., Sunnyvale, CA, USA). The concentrations of BBR used were 1.6, 3.1, 6.3, 12.5, 25, 50, 100 and 200 $\mu\text{g/mL}$, and absorbance-concentration curves were obtained by matching the data.

1×10^6 cells/mL of HeLa cells were cultured in 96-well plates, 24 hours later, BBR at concentrations of 0, 1.6, 3.1, 6.3, 12.5, 25 and 50 $\mu\text{g/mL}$ were added to the culture medium. The cells were divided into the BBR and BBR-PDT groups. Six hours later, the cells in the BBR-PDT group were exposed to a 405 nm laser unit at a luminous

flux of 40 mW/cm² for 3 minutes (total energy 7.2 J/cm²) as described previously [13,14]. Cells in the BBR group were not exposed to the laser. Protocols for U-118 MG, U-251 MG and HaCaT cells were the same as HeLa.

The Cell Counting Kit-8 (CCK-8) (DOJINDO, Tokyo, Japan) was used to measure cell proliferation 24 hours after treatment. The concentrations of BBR used were 0, 1.6, 3.1, 6.3, 12.5, 25 and 50 $\mu\text{g/mL}$. According to the instructions of manufacturer, 10 μL CCK-8 reagent was added to 100 μL medium and the cells were cultured at 37°C. One hour later, a microplate reader (Spectra Max i3x, Shanghai, China) was used to detect the absorbance at 450 nm with a reference wavelength of 405 nm.

The Annexin V-PE apoptosis detection kit I (BD Pharmingen™, San Diego, CA, USA) was used to detect apoptotic cells. BBR concentrations were 1.6, 6.3 and 25 $\mu\text{g/mL}$. Following treatment for 6 hours, the cells were washed twice with ice-cold PBS. 1×10^6 cells/mL were resuspended in $1 \times$ binding buffer, and then 5 μL PE Annexin V was added followed by 5 μL 7-AAD. Cells were incubated in the dark for 15 minutes at room temperature (RT), and apoptotic cells were detected using Flow Cytometry (FCW).

2.4 Xenograft models

HeLa cells (1×10^7 cells/mL) suspended in PBS containing 50 % Matrigel (BD Biocoat™, San Diego, CA, USA) were subcutaneously implanted into the flanks of female BALB/c mice (4 weeks old; $n = 10$ in each group) [15,16]. Mice with tumors were randomly divided into the following four groups; the control group, BBR group, laser group and the BBR-PDT group. The mice were treated when the tumor diameter reached 1 cm. BBR 10 mg/kg was injected intraperitoneally into mice in the BBR and BBR-PDT groups. 24 hours later, mice in the laser and BBR-PDT group were exposed to the laser unit with a luminous flux of 1000 mW/cm² for 1 minute (total energy 60.0 J/cm²).

An IVIS® Lumina LT imaging system (Series III, Caliper Life Sciences, San Francisco, CA, USA) was used to evaluate the *in vivo* fluorescence of BBR in the BBR and BBR-PDT group after treatment. The Vevo 2100 Imaging System (FUJIFILM Visual Sonics Inc., Toronto, ON, Canada) was used to measure tumor size and evaluate therapeutic effects. Treatments were performed once a week for two weeks, the mice were sacrificed 2 weeks later, and the tumors were collected and measured.

Figure 1: Light absorption characteristics of BBR. (A). Structural formula of BBR. (B). Absorption spectrum of BBR, showing 4 absorption peaks at 228, 263, 344 and 417 nm. The concentration of BBR was 100 µg/mL. (C). Absorption peaks consistent with BBR at different concentrations. (D). Absorbance-concentration curves of BBR. For (C) and (D), the concentrations of BBR were 1.6, 3.1, 6.3, 12.5, 25, 50, 100 and 200 µg/mL.

2.5 Western blot

Protein expression levels were detected using western blot. Briefly, HeLa cells were inoculated into 6-well plates (1 mL/well) for 24 hours. The cells were then divided into the BBR group and BBR-PDT group with BBR concentrations of 0, 1.6 and 25 µg/mL. After 6 hours incubation, treatments were carried out under the conditions described above. 24 hours after treatment, the cells were collected and washed with ice-cold PBS. Lysis buffer (Thermo Fisher Scientific, USA) containing PMSF (phenyl methane sulfonyl fluoride, 1 mM, Thermo Fisher Scientific, USA), Na_3VO_4 (1 mM, Sigma-Aldrich, USA), and NaF (20 mM, Sigma-Aldrich, USA) was added at a volume of 80 µL/well. The mixture was incubated for 30 minutes at 4°C and then centrifuged for 30 minutes at 12,000 r/min. Protein concentrations were measured using a Micro-Spectrophotometer Q5000 and adjusted to 15 µg/mL. Samples of 10 µL were loaded into the spacer gel, the electrophoresis voltages for concentration and separation were 80 V and 120 V, respectively, and the constant current for transfer was 330 mA which lasted 90 minutes. The block time for membranes was 1 hour

at RT, and the membranes were then washed three times with Tris-buffered saline. Primary antibodies were added to the membranes and incubated overnight at 4°C. The incubation time for the secondary antibody was 1 hour at RT. The Chemiluminescence Detector (Tanon 5220S, Guangzhou EVELL Bio-technology Co., Ltd., Guangzhou, Guangdong, China) was used to measure the gray values of the protein bands.

2.6 Statistics

Data are shown as mean ± SD (standard deviation) and SPSS 17.0 software was used to perform *One-way ANOVA* analyses for inter-group differences. The *P* value was used to determine the significance of differences, and $P < 0.05$ was considered statistically significant.

Figure 2: Effects of BBR-mediated PDT on proliferation and apoptosis of cells. (A). Cell proliferation inhibition, the concentrations of BBR were 0, 1.6, 3.1, 6.3, 12.5, 25 and 50 $\mu\text{g/mL}$, and the CCK-8 assay showed that absorbance was detected at 450 nm using a reference wavelength of 405 nm. (B). The PE Annexin V positive cell populations were considered apoptotic cells. (C). Concentrations of BBR in the apoptosis test were 1.6, 6.3 and 25 $\mu\text{g/mL}$. ** $P < 0.01$, vs BBR group.

3 Results

3.1 PDT enhanced cell proliferation inhibition and apoptosis induction caused by BBR *in vitro*

As shown in Figure 1B, there were 4 absorption peaks for BBR (100 $\mu\text{g/mL}$) in PBS at 228, 263, 344 and 417 nm, and the maximum absorption peak was observed at 344 nm. The shapes of the BBR absorption peaks at different concentrations were similar (Figure 1C) and a good linear relationship between absorbance and concentration ranging from 1.6 to 200 $\mu\text{g/mL}$ was observed (Figure 1D). The 405 nm laser unit was used to perform PDT.

To evaluate the inhibition of cell proliferation and cell apoptosis induction by BBR-PDT, we performed the CCK-8 assay and FCW test. When the concentration of BBR

was less than 1.6 $\mu\text{g/mL}$, the absorbance-concentration curves did not show a good linear relationship. When the concentration of BBR was greater than 50 $\mu\text{g/mL}$, the color of the BBR solution disturbed the CCK-8 assay. Therefore, the concentrations of BBR used in the CCK-8 assay were 0, 1.6, 3.1, 6.3, 12.5, 25 and 50 $\mu\text{g/mL}$. The results showed that PDT increased cell proliferation inhibition by BBR (Figure 2A), the IC_{50} and 95% confidence interval of BBR and BBR-PDT for HeLa cells were 17.49 (7.11 – 72.29) $\mu\text{g/mL}$ and 2.50 (1.88 – 3.45) $\mu\text{g/mL}$, respectively. Similar effects of PDT enhancing cell proliferation inhibition caused by BBR were observed in groups of U-118 MG, U-251 MG and HaCaT cells. In the apoptosis tests, the PE Annexin V positive cell populations were considered apoptotic cells (Figure 2B). The percentage of cell apoptosis induced by BBR-PDT was greater than that induced by BBR (Figure 2C).

Figure 3: In vivo antitumor effects of BBR-mediated PDT in the xenograft model. N = 10, means $\pm$ SD. (A). *In vivo* fluorescence measurement. (B). Anatomical tumors in the different treatment groups. (C). Comparison of tumor size both before and after treatment (cm²). (D). Comparison of tumor weight (g). The dose of BBR was 10 mg/kg. PDT was performed using a 405 nm laser unit with a luminous flux of 1000 mW/cm² for 1 min. * $P < 0.05$, vs control group.

3.2 BBR-mediated PDT inhibited tumor development in xenograft models

Following the *in vitro* study, further *in vivo* validation was carried out using a xenograft model. *In vivo* fluorescence measurements showed that the fluorescence intensity of BBR was reduced in the BBR-PDT group compared with the BBR group, which indicated that BBR was activated by the 405 nm laser and photobleaching occurred after PDT (Figure 3A). These findings showed that BBR-PDT could produce biological effects in tumors. Tumor size and weight in the BBR-PDT group were significantly reduced compared with the control group ($P < 0.05$); however, BBR without PDT showed no significant differences compared with the control groups (Figure 3B,C and D).

3.3 Effects of BBR-mediated PDT on the mTOR signaling pathway

The *in vitro* and *in vivo* results confirmed that PDT enhanced the anti-tumor effects of BBR. Previous studies have shown that PDT, mediated by several photosensitizers, can affect the mTOR signaling pathway [17-20]; however, the influence of BBR and BBR-PDT on the mTOR signaling pathway is still unclear. Therefore, we evaluated the changes in protein expression and phosphorylation levels after BBR and BBR-PDT treatment. As shown in Figure 4, mTOR is one of the main kinases downstream of PI₃K/AKT. Phosphorylation of mTOR compound 1 (mTORC1) decreased when the PI₃K/AKT signaling pathway was inhibited. BBR dose-dependently decreased the pho-PI₃K, pho-AKT and pho-mTORC1 levels. PDT enhanced the inhibitory effects of BBR on the PI₃K-AKT-mTOR pathway.

Figure 4: Inhibition of the PI₃K-AKT-mTOR signaling pathway by BBR-mediated PDT. (A). Diagram of the PI₃K-AKT-mTOR signaling pathway. (B). Representative Western blot analysis. (C). Densitometric quantification of the corresponding Western blot analysis. Concentrations of BBR were 0, 1.6 and 25 μg/mL, and PDT was performed after 6 hours co-incubation. Means ± SD, n = 3, * *P* < 0.05, ** *P* < 0.01, vs BBR group. ^*P* < 0.05, ^^*P* < 0.01, vs concentration 0.

Figure 5: Effects of BBR-mediated PDT on downstream of mTORC1. (A). Diagram of the mTORC1 pathway. (B). Representative Western blot analysis. (C). Densitometric quantification of the corresponding Western blot analysis. Concentrations of BBR were 0, 1.6 and 25 μg/mL, PDT was performed after 6 hours co-incubation. Means ± SD, n = 3, * *P* < 0.05, ** *P* < 0.01, vs BBR group. ^*P* < 0.05, ^^*P* < 0.01, vs concentration 0.

As shown in Figure 5, phosphorylation of p70 ribosomal S6 kinase (p70s6K) was regulated by mTORC1, and it also influenced ribosome protein S6 (rps6). Synergistic-inhibition of p70s6K and rps 6 phosphorylation by BBR and PDT was observed.

4 Discussion

BBR has been known as a naturally phototoxic alkaloid for more than 30 years. Luiza [21] found that delivery of BBR to tumor cells was closely related to low density lipoproteins (LDL), and the physicochemical parameters

of the association between BBR and LDL are important for the antitumor effects of PDT mediated by BBR on glioma cells. Shen [22] revealed the underlying mechanisms of ROS-photogeneration by BBR in a solvent model using the time-dependent density functional theory. The author demonstrated the generation of $^1\text{O}_2$ and $^3\text{O}_2$, which are indicators of PDT, after activation of BBR induced by a light source. Hirakawa [23] proved that BBR was bound to DNA by electrostatic force, and the generation of $^1\text{O}_2$ following BBR activation was switched by the DNA microenvironment. These studies indicated that BBR-PDT is a promising strategy for treating cancers. However, there are few studies on the antitumor effects of BBR-PDT in xenograft models *in vivo*, and the effects of BBR-PDT on HeLa cells are unclear. In this study, we showed the antineoplastic effects of BBR-PDT on HeLa cells not only *in vitro* but also *in vivo*. The *in vitro* results showed that BBR-PDT significantly inhibited proliferation of HeLa, U-118 MG, U-251 MG and HaCaT cells and induced cell apoptosis. The inhibitory effects of BBR-PDT on proliferation in different cell lines may be nonspecific. The nude mouse xenograft model showed that the fluorescence intensity of BBR was reduced in the BBR-PDT group compared with the BBR group, and tumor size in BBR-PDT group was less than that in the control group ($P < 0.05$). However, BBR without PDT showed no significant differences compared with the control group. These findings indicated that PDT enhanced the anti-tumor effects of BBR in HeLa cells.

In previous studies, the antitumor mechanisms of BBR-PDT were mainly attributed to the cell-damaging effects caused by ROS [24-27], and to mitochondria damage. Studies on the influence of BBR-PDT on signaling pathways are lacking. PI₃K-AKT-mTOR is a major pathway that has been confirmed be vital in cancer cells and inhibited by ROS. The Ser/Thr protein kinase mTOR lies downstream in the PI₃K/AKT signaling pathway. All three kinases are important in the regulation of cancer cell growth, survival and angiogenesis. It generally promotes survival by activating anti-apoptotic factors and inhibiting proapoptotic factors. The PI₃K-AKT-mTOR pathway also has a critical role in tumor stem cell self-renewal capacity and resistance to radiotherapy and chemotherapy [28, 29]. Recent studies showed that PDT inhibited the PI₃K-AKT-mTOR signaling pathway. Han [30] found that Upconversion Fluorescent Nanoparticles Encapsulating Chlorin e6 could mediate PDT and induce suppression of *p*-AKT and *p*-mTOR via ROS generation in foam cells.

Many studies have shown that BBR attenuates cell proliferation and apoptosis by influencing the PI₃K-AKT-mTOR pathway and autophagy. For example, Shukla [31] observed that BBR induced down-regulation of the PI3K-

AKT-mTOR pathway, significantly upregulated the mRNA expression of FoxO proteins and evoked mitochondrial apoptosis in HepG2 cells. Chitra [32] found that BBR blocked the activation of PI3K-AKT against bleomycin-induced dysregulation, inhibited *p*-mTOR and stimulated autophagy in pulmonary fibrosis rats. Yi [33] revealed the relationship between BBR induced apoptosis and AKT signaling in human gastric cancer cells, and inhibition of the AKT-mTOR-p70s6K-rps6 pathway in BBR-treated BGC-823 cells was observed. Ai [34] proved that BBR regulated proliferation via the mTOR-p70S6K signaling pathway. These studies indicate that BBR influences the PI3K-AKT-mTOR-p70s6K-rps6 signaling pathway. In the present study, we confirmed that BBR-PDT inhibited activation of the PI₃K-AKT-mTOR pathway, and the phosphorylation of p70s6K and rps6 was inhibited when *p*-mTORC1 was suppressed. In addition, we found that inhibition of this signaling pathway by BBR-PDT was stronger than that by BBR alone. This may explain why the BBR-PDT group showed greater inhibition of tumor proliferation and stronger induction of cell apoptosis. In a recent report, Kou [35] showed that BBR-mediated sonodynamic therapy (SDT) induced autophagy by reducing the phosphorylation of AKT and mTOR in macrophage. It is well known that the mechanisms of SDT are similar to those of PDT [36]; thus, this study partially supported our findings.

In conclusion, we demonstrated that PDT enhanced the inhibitory effects of BBR on HeLa cell proliferation not only *in vitro* but also *in vivo*; the synergistic inhibition of the mTOR signaling pathway may partly explain the potential underlying mechanisms.

Acknowledgements: We thank the Health and Family Planning Commission of Shenzhen (201605018) and Shenzhen Science and Technology Planning Project (JCYJ20160427185055877, JCYJ20160429090753103) financial support.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- [1] Ortiz L.M., Lombardi P., Tillhon M., Scovassi A.I., Berberine, an epiphany against cancer, *Molecules*, 2014, 19, 12349-12367.
- [2] Zou K., Li Z., Zhang Y., Zhang H.Y., Li B., Zhu W.L., et al., Advances in the study of berberine and its derivatives: a focus

- on anti-inflammatory and anti-tumor effects in the digestive system, *Acta Pharmacol Sin*, 2017, 38, 157-167.
- [3] Mohammadi S., Seyedhoseini F.S., Asadi J., Yazdani Y., Effects of berberine on the secretion of cytokines and expression of genes involved in cell cycleregulation in THP-1 monocytic cell line, *Iran J Basic Med Sci*, 2017, 20, 530-537.
 - [4] Gu M., Xu J., Han C., Kang Y., Liu T., He Y., et al., Effects of Berberine on Cell Cycle, DNA, Reactive Oxygen Species, and Apoptosis in L929 MurineFibroblast Cells, *Evid Based Complement Alternat Med*, 2015, 2015, 796306.
 - [5] Chilakamarthi U., Giribabu L., Photodynamic Therapy: Past, Present and Future, *Chem Rec*, 2017, 17, 775-802.
 - [6] Liu H.Q., Wang Y.M., Li W.F., Li C., Jiang Z.H., Bao J., et al., Photobleaching characteristics of α -(8-quinolinoxy) zinc phthalocyanine, a new type of amphipathic complex, *Open Chem*, 2017, 15, 400-411.
 - [7] Liu H.Q., Wang Y.M., Li W.F., Li C., Jiang Z.H., Bao J., et al., Anti-Psoriasis Effects and Mechanisms of A-(8-Quinolinoxy) Zinc Phthalocyanine-Mediated Photodynamic Therapy, *Cell Physiol Biochem*, 2017, 44, 200-214.
 - [8] Cheng J., Xu J., Duanmu J., Zhou H., Booth C.J., Hu Z., Effective treatment of human lung cancer by targeting tissue factor with a factor VII-targeted photodynamic therapy, *Curr Cancer Drug Targets*, 2011, 11, 1069-1081.
 - [9] Philogène B.J., Arnason J.T., Towers G.H., Abramowski Z., Campos F., Champagne D., et al., Berberine: A naturally occurring phototoxic alkaloid, *J Chem Ecol*, 1984, 10, 115-123.
 - [10] Tuveson R.W., Berenbaum M.R., Heininger E.E., Inactivation and mutagenesis by phototoxins using *Escherichia coli* strains differing in sensitivity to near- and far-ultraviolet light, *J Chem Ecol*, 1986, 12, 933-948.
 - [11] Wang M.X., Huo L.M., Yang H.C., Gao Y.J., E Z., An experimental study on the photodynamic activity of berberine in vitro on cancer cells, *J Tradit Chin Med*, 1986, 6, 125-127.
 - [12] Cheng L.L., Wang Y.J., Huang D.H., Yao S.D., Ding G.J., Wang S.L., et al., Radiolysis and photolysis studies on active transient species of berberine, *Spectrochim Acta A Mol Biomol Spectrosc*, 2014, 124, 670-676.
 - [13] Shi R., Li C., Jiang Z., Li W., Wang A., Wei J., Preclinical Study of Antineoplastic Sinoporphyrin Sodium-PDT via In Vitro and In Vivo Models, *Molecules*, 2017, 22, E112.
 - [14] Hu J., Wang X., Liu Q., Zhang K., Xiong W., Xu C., et al., Antitumor effect of sinoporphyrin sodium-mediated photodynamic therapy on human esophageal cancer Eca-109 cells, *Photochem Photobiol*, 2014, 90, 1404-1412.
 - [15] Wang F.F., Shi C., Yang Y., Fang Y., Sheng L., Li N., Medicinal mushroom *Phellinus igniarius* induced cell apoptosis in gastric cancer SGC-7901 through a mitochondria-dependent pathway, *Biomed Pharmacother*, 2018, 102, 18-25.
 - [16] Lu X., Wu Y.M., Yang J.M., Ma F.E., Li L.P., Chen S., et al., Preparation of Rhodium (III) complexes with 2(1H)-quinolinone derivatives and evaluation of their in vitro and in vivo antitumor activity, *Eur J Med Chem*, 2018, 151, 226-236.
 - [17] Kraus D., Palasuberniam P., Chen B., Targeting Phosphatidylinositol 3-Kinase Signaling Pathway for Therapeutic Enhancement of Vascular-Targeted Photodynamic Therapy, *Mol Cancer Ther*, 2017, 16, 2422-2431.
 - [18] Liu W., Wang Q., Tuo J., Chang Y., Ying J., Jiang M., et al., ALA-PDT suppressed the cell growth by Akt-/Erk-mTOR-p70 s6k pathway in human SZ95 sebocytes in vitro, *Photodiagnosis Photodyn Ther*, 2018, 24, 1-6.
 - [19] Tuo J., Wang Q., Zouboulis C.C., Liu Y., Ma Y., Ma L., et al., ALA-PDT suppressing the cell growth and reducing the lipogenesis in human SZ95 sebocytes by mTOR signaling pathway in vitro, *Photodiagnosis Photodyn Ther*, 2017, 18, 295-301.
 - [20] Xu C., Wang Q., Feng X., Bo Y., Effect of evodiagenine mediates photocytotoxicity on human breast cancer cells MDA-MB-231 through inhibition of PI3K/AKT/mTOR and activation of p38 pathways, *Fitoterapia*, 2014, 99, 292-299.
 - [21] Luiza Andreazza N., Vevet-Bizet C., Bourg-Heckly G., Sureau F., José Salvador M., Bonneau S., Berberine as a photosensitizing agent for antitumoral photodynamic therapy: Insights into its association to low density lipoproteins, *Int J Pharm*, 2016, 510, 240-249.
 - [22] Shen L., Ji H.F., The mechanisms of ROS-photogeneration by berberine, a natural isoquinoline alkaloid, *J Photochem Photobiol B*, 2010, 99, 154-156.
 - [23] Hirakawa K., Hirano T., The microenvironment of DNA switches the activity of singlet oxygen generation photosensitized by berberine and palmatine, *Photochem Photobiol*, 2008, 84, 202-208.
 - [24] Tian H., Kang Y.M., Gao H.L., Shi X.L., Fu L.Y., Li Y., et al., Chronic infusion of berberine into the hypothalamic paraventricular nucleus attenuates hypertension and sympathoexcitation via the ROS/Erk1/2/iNOS pathway, *Phytomedicine*, 2019, 52, 216-224.
 - [25] De Sarkar S., Sarkar D., Sarkar A., Dighal A., Staniek K., Gille L., et al., Berberine chloride mediates its antileishmanial activity by inhibiting Leishmania mitochondria, *Parasitol Res*, 2019, 118, 335-345.
 - [26] Song J., Lin C., Yang X., Xie Y., Hu P., Li H., et al., Mitochondrial targeting nanodrugs self-assembled from 9-O-octadecyl substituted berberine derivative for cancer treatment by inducing mitochondrial apoptosis pathways, *J Control Release*, 2019, 294, 27-42.
 - [27] Paul M., Hemshekhar M., Kemparaju K., Girish K.S., Berberine mitigates high glucose-potentiated platelet aggregation and apoptosis by modulating aldose reductase and NADPH oxidase activity, *Free Radic Biol Med*, 2019, 130, 196-205.
 - [28] Zheng R., Jiang H., Li J., Liu X., Xu H., Polyphyllin II Restores Sensitization of the Resistance of PC-9/ZD Cells to Gefitinib by a Negative Regulation of the PI3K/Akt/mTOR Signaling Pathway, *Curr Cancer Drug Targets*, 2017, 17, 376-385.
 - [29] Chen Y.J., Kay N., Yang J.M., Lin C.T., Chang H.L., Wu Y.C., et al., Total synthetic protoapigenone WYC02 inhibits cervical cancer cell proliferation and tumour growth through PIK3 signalling pathway, *Basic Clin Pharmacol Toxicol*, 2013, 113, 8-18.
 - [30] Han X.B., Li H.X., Jiang Y.Q., Wang H., Li X.S., Kou J.Y., et al., Upconversion nanoparticle-mediated photodynamic therapy induces autophagy and cholesterol efflux of macrophage-derived foam cells via ROS generation, *Cell Death Dis*, 2017, 8, e2864.
 - [31] Shukla S., Rizvi F., Raisuddin S., Kakkar P., FoxO proteins' nuclear retention and BH3-only protein Bim induction evoke mitochondrial dysfunction-mediated apoptosis in berberine-treated HepG2 cells, *Free Radic Biol Med*, 2014, 76, 185-199.
 - [32] Chitra P., Saiprasad G., Manikandan R., Sudhandiran G., Berberine inhibits Smad and non-Smad signaling cascades and

- enhances autophagy against pulmonary fibrosis, *J Mol Med (Berl)*, 2015, 93, 1015-1031.
- [33] Yi T., Zhuang L., Song G., Zhang B., Li G., Hu T., Akt signaling is associated with the berberine-induced apoptosis of human gastric cancer cells, *Nutr Cancer*, 2015, 67, 523-531.
- [34] Ai F., Chen M., Yu B., Yang Y., Xu G., Gui F., et al., Berberine regulates proliferation, collagen synthesis and cytokine secretion of cardiac fibroblasts via AMPK-mTOR-p70S6K signaling pathway, *Int J Clin Exp Pathol*, 2015, 8, 12509-12516.
- [35] Kou J.Y., Li Y., Zhong Z.Y., Jiang Y.Q., Li X.S., Han X.B., et al., Berberine-sonodynamic therapy induces autophagy and lipid unloading in macrophage, *Cell Death Dis*, 2017, 8, e2558.
- [36] Pan X., Wang H., Wang S., Sun X., Wang L., Wang W., et al., Sonodynamic therapy (SDT): a novel strategy for cancer nanotheranostics, *Sci China Life Sci*, 2018, 61, 415-426.

Supplemental Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/chem-2019-0048>).