Berbamine suppresses cell viability and induces apoptosis in colorectal cancer via activating p53-dependent apoptotic signaling pathway

Heng Zhang · Yunping Jiao · Chunyang Shi · Xiao Song · Ying Chang · Yong Ren · Xiaolin Shi

Received: 9 April 2017 / Accepted: 11 September 2017 / Published online: 30 September 2017
© Springer Science+Business Media B.V. 2017

Abstract Berbamine has been shown to exhibit anti-cancer activities in various types of cancers. The effects of berbamine on colorectal colon cancer (CRC) have not been examined, and the present study aimed to investigate the anti-cancer effects of berbamine in CRC and explore its underlying molecular mechanisms. The effect of berbamine on the CRC cells was determined by MTT assay. Flow cytometry was performed to examine the effect of berbamine on cell apoptosis and cell cycle as well as mitochondrial

membrane potential in CRC cell lines. The specific apoptosis-related factors were evaluated by western blot assay. In vivo anti-cancer effect of berbamine was assessed in SW480 xenografts. Berbamine suppressed the cell viability of CRC cells in concentration-dependent and time-dependent manners. Flow cytometry experiments showed that berbamine increased cell apoptotic rate and induced cell cycle arrest at G₀/G₁ phase. Berbamine treatment also decreased the mitochondrial membrane potential in CRC cells.

H. Zhang · X. Song · Y. Chang · X. Shi (✉)
Department of Pharmacy, the Northwest Women and Children's Hospital, Xi'an 710065, Shaanxi Province, China
e-mail: shixiaolin80@outlook.com

H. Zhang
e-mail: hengzhang_dof@outlook.com

X. Song
e-mail: 278403640@qq.com

Y. Chang
e-mail: 286834152@qq.com

Y. Jiao
Department of Pharmacy, Shaanxi No. 2 People's Hospital, Xi'an, Shaanxi Province, China
e-mail: 286977712@qq.com

C. Shi
School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, Shaanxi Province, China
e-mail: shichunyang@sust.edu.cn

Y. Ren
Department of Pharmacy, the First Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi Province, China
e-mail: renyong_alex@outlook.com

Western blot assay showed that berbamine increased the protein levels of p53, caspase-3, caspase-9, Bax and poly ADP ribose polymerase, and decreased the protein levels of Bcl-2 in CRC cells. Berbamine failed to increase the cell apoptotic rate in p53 mutant CRC cell lines. Tumor growth by grafted SW480 cells were significantly suppressed in berbamine group. Expression of p53, caspase-3 and -9 in tumor tissues was significantly up-regulated by berbamine. Berbamine exerts anti-cancer effects in vitro and in vivo via induction of apoptosis, partially associated with the activation of p53-dependent apoptosis signaling pathway.

Keywords Berbamine · Colorectal cancer · Cell viability · Apoptosis · Cell cycle · p53

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and is one of the leading causes of cancer-related death worldwide (Gill et al. 2003), accounting for about 1.4 million new cases and almost 700,000 deaths in 2012, and more than two-thirds of all cases and about 60% of all deaths occurring in developed countries (Arnold et al. 2017). Though surgical treatments and chemotherapy are employed for the treatment of CRC, the recurrence rate is high and the 5-year survival rate is low (Frouws et al. 2017). Therefore, it is urgent for scientists to develop effective chemotherapeutic treatments for CRC.

Recently, natural compounds from microorganisms, plants and marine organisms provide rich resources for the novel anti-cancer compound discovery. Up to date, a lot of anti-cancer drugs were initially identified from natural compounds (Aung et al. 2017; Nobili et al. 2009). For example, the anti-cancer drug, paclitaxel that was originally isolated from *Taxus Brevifolia* was effective against various types of cancers via inhibiting the spindle function of tumor cells (Bernabeu et al. 2017). Vincristine is a clinically used anti-cancer compound and it is an alkaloid compound, which was originally isolated from *Catharanthus roseus* (Greenwell and Rahman 2015). In addition, compound Kushen Injection (which contains alkaloids, flavonoids, saccharides, and organic acids) which is extracted from *Radix Sophorae Flavescentis*

and *rhizoam Smilacis Glabrae* has been used to treat different types of cancers in combination with Western anti-cancer agents (Tan et al. 2016). Berbamine (a bisbenzylisoquinoline alkaloid) is the major bioactive compound isolated from the *Berberis amurensis* plant, which has been commonly used as traditional Chinese medicine (Duan et al. 2010). Berbamine has been shown to possess multiple bioactivities such as anti-inflammatory, anti-hypertensive and anti-antiarrhythmic effects (Khan et al. 1969; Wong et al. 1992; Zhang et al. 2012). Recently, various studies have demonstrated the anti-tumor effects of berbamine in myeloma, prostate cancer, lung cancer, pancreatic cancer, liver cancer and gastric cancer (Hou et al. 2014; Jin and Wu 2014; Liang et al. 2009; Zhao et al. 2016; Zhu et al. 2013). However, the molecular mechanisms underlying berbamine-mediated anti-cancer effects are largely unknown. Berbamine was found to exert its anti-tumor activity via triggering the apoptotic pathway as well as modulating the signaling pathways involved in cancer progression. For example, berbamine was found to act as a novel nuclear factor kappa B inhibitor to inhibit growth and induce apoptosis in human myeloma cells (Liang et al. 2009). Yang et al. (2013) showed that berbamine inhibits cell viability and induces apoptosis of human osteosarcoma cells through activating KNK/Ap-1 signaling. However, as far as we know, the anti-cancer effects of berbamine have not been investigated in the CRC.

The current study performed both in vitro and in vivo experiments to elucidate the anti-tumor activities of berbamine in CRC. Two colorectal cancer cell lines were used to examine the in vitro functional effects of berbamine by using MTT assay, flow cytometry and western blot assay. Moreover, the tumor xenograft model in the nude mice was employed to investigate the anti-tumor effect of berbamine in vivo.

Materials and methods

Cells and culture conditions

The CRC cell lines, HCT116 and SW480, and the p53 mutant cell lines, HCT116^{-/-} and SW480^{-/-} were purchased from the American Type Culture Collection (Manassas, VA, USA). All cells were cultured in the Dulbecco's modified Eagle's medium supplemented

with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma, St.Louis, MO, USA). Cells were kept in a humidified incubator with 5% CO₂ at 37 °C.

MTT assay

Cells were seeded in 96-well plates at a density of 5000 cells/well, after 24 h incubation, cell were treated with different concentrations of berbamine (0–64 µg/ml, Sigma). After 24, 48, or 72 h, berbamine-treated cells were induced with MTT for 4 h at room temperature. After removing the medium and MTT reagent, 200 µl DMSO was added to each well, and then thoroughly mixed for 5 min at room temperature. The absorbance at 570 nm of the mixture was determined by a microplate reader (BioTek, Winooski, VT, USA). Cell viability was calculated based on the following formula: cell viability (%) = mean experimental absorbance/mean control absorbance × 100%.

Flow cytometry for determine cell apoptosis and cell cycle

The cell apoptosis and cell cycle of CRC cells were determined by flow cytometry experiments. Briefly, cells were treated with berbamine (20 µg/ml) for 48 h, and cells were collected and washed with phosphate buffer saline (PBS) twice. For the cell apoptosis evaluation, cells were incubated with FITC-Annexin V (BD Biosciences, San Jose, CA, USA) and propidium iodide (PI) (BD Biosciences) for 5 min in the dark at room temperature according to the manufacturer's protocol. Cell apoptosis was analyzed by a FACScan flow cytometer (BD Biosciences). For the cell cycle evaluation, cells were fixed overnight with cold 70% ethanol and followed by incubation with PI and RNase A for 15 min at room temperature. The cell cycle was determined by a FACScan flow cytometer (BD Biosciences) and analyzed using CellQuest software (BD Biosciences).

Determination of mitochondrial membrane potential (MMP)

Cells were treated with berbamine (20 µg/ml) for 48 h, and cells were collected and washed with PBS twice, and then the cells were incubated with

Rhodamine123 (Sigma) for 10 min in the dark. The MMP of the cells was analyzed by flow cytometry.

Western blot assay

A quantity of 30 µg of protein from cell lysates per sample was separated on a 10% SDS-PAGE by electrophoresis, and then transferred to the PVDF membranes. The PVDF membranes were blocked by using 5% skimmed milk in Tris-buffered saline. After blocking, the membranes were incubated with the primary antibodies at 4 °C overnight, and were immunodetected by incubating with horseradish peroxidase-linked related secondary antibody using an ECL detection kit (Pierce Biotechnology, Waltham, MA, USA). The antibodies are shown below: rabbit polyclonal anti-caspase-3 antibody (1:1500 dilution), rabbit polyclonal anti-caspase-9 antibody (1:2000 dilution), rabbit polyclonal anti-Bax antibody (1:2000 dilution), rabbit polyclonal anti-Bcl-2 antibody (1:1500 dilution), rabbit polyclonal anti-poly ADP ribose polymerase (PARP) antibody (1:1000), rabbit anti-p53 polyclonal antibody (1:1500), and rabbit anti-β-actin polyclonal antibody (1:4000 dilution), all the antibodies were purchased from Abcam (Cambridge, MA, USA).

In vivo study using xenograft model

BABL/C nu/nu mice were obtained from the Laboratory Animal Center of Shanghai Institute for Biology Science. All animal procedures were approved by the Ethics Committee of the Northwest Women and Children's Hospital. For this study, SW480 cells were injected subcutaneously in the midline dorsal region of nude mice. At twenty-four hours post injection, mice were randomly assigned to two groups (treated group and control group, 6 mice per group). Berbamine was administered twice daily at a dosage of 60 mg/kg, s.c. for 4 weeks. The mice in the control group were given equal volumes of isotonic saline. Tumor volumes (length × width² × 0.5) were measured with calipers every 7 days. Mice were euthanized on 35 days and the excised tumors were weighed and harvested from each mouse for further experimentation.

Statistical analysis

The data analysis was performed by using the GraphPad Prism software. Data were presented as mean $\pm$ standard deviation. Significant differences among groups were analyzed by Student's *t* test (comparison for two groups) or One-way ANOVA (comparison for more than two groups). $P < 0.05$ was considered to be statistically significant.

Results

Berberamine suppresses cell proliferation in CRC cell lines (HCT116 and SW480)

The effects of berberamine on the cell proliferation of colorectal cancer cells were examined by MTT assay. The CRC cell lines, HCT116 and SW480, were treated with different concentrations for the duration of 24, 48 or 72 h. As shown in Fig. 1, when treated for 24, 48 or 72 h in the concentration range from 0 to 64 $\mu\text{g/ml}$, berberamine inhibited the cell proliferation of HCT116 cells and SW480 cells. For HCT116 cells, berberamine treatment suppressed the cell proliferation in concentration- and time-dependent manners (Fig. 1a). The effect of berberamine on the cell proliferation in SW480 was similar to that in HCT116 cells (Fig. 1b). The IC_{50} for berberamine in CRC cells after treatment for 24, 48 and 72 h were shown in Table 1.

Fig. 1 Effects of berberamine on the cell viability in CRC cells. **a** The cell viability of HCT116 cells was examined by MTT assay after treatment with different concentrations of berberamine (0–64 $\mu\text{g/ml}$) for 24, 48, or 72 h. **b** The cell viability of SW480

Table 1 The IC_{50} values for berberamine in CRC cell lines (HCT116 and SW480)

Cell lines	24 h (IC_{50})	48 h (IC_{50})	72 h (IC_{50})
HCT116	15.5 ± 1.32	$12.3 \pm 1.02^*$	$9.45 \pm 0.78^{\&.\#}$
SW480	20.5 ± 1.26	$16.4 \pm 0.89^{**}$	$13.7 \pm 0.68^{\&.\#}$

Significant differences between 24 and 48 h groups are shown as * $P < 0.05$, and ** $P < 0.01$; between 24 and 72 h groups as $\&$ $P < 0.05$; and between 48 and 72 h groups as $\#$ $P < 0.05$

Berberamine induces apoptosis and causes cell cycle arrest at G_0/G_1 phase in colorectal cancer cell lines

The effects of berberamine on the cell apoptosis and cell cycle of colorectal cancer cells were examined by flow cytometry experiments. The CRC cell lines, HCT116 and SW480, were treated with 20 $\mu\text{g/ml}$ berberamine for the duration of 48 h. As shown in Fig. 2, berberamine treatment for 48 h significantly increased the percentage of apoptotic cells in both HCT116 and SW480 cells (Fig. 2a, b). The cell cycle in HCT116 and SW480 cells were also examined, and berberamine treatment for 48 h significantly increased the cell population at G_0/G_1 phase and decreased the cell population at G_2/M phase, and had no effect on the cell population at S phase in both HCT116 and SW480 cells (Fig. 2c, d).

The effect of berberamine on the MMP in colorectal cancer cell lines

The effect of berberamine on the MMP in HCT116 and SW480 cells was determined after cells were treated

cells was examined by MTT assay after treatment with different concentrations of berberamine (0–64 $\mu\text{g/ml}$) for 24, 48, or 72 h. $N = 3$

Fig. 2 Effects of berbamine on the cell apoptosis, cell cycle and MMP in CRC cells. The cell apoptosis of **a** HCT116 and **b** SW480 cells was assessed by flow cytometry after treatment with berbamine (20 $\mu\text{g/ml}$) for 48 h. The cell cycle of **c** HCT116 and **d** SW480 cells was assessed by flow cytometry after treatment with berbamine (20 $\mu\text{g/ml}$) for 48 h. The MMP of **e** HCT116 and **f** SW480 cells was assessed by flow cytometry after treatment with berbamine (20 $\mu\text{g/ml}$) for 48 h. N = 3. Significant differences between groups were shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

with 20 $\mu\text{g/ml}$ berbamine for the duration of 48 h, and berbamine treatment caused a rapid dissipation of MMP in both HCT116 and SW480 cells (Fig. 2e, f).

Berbamine induces apoptosis via p53 dependent apoptotic signaling pathway

The effect of berbamine on the apoptosis-related proteins in CRC cells (HCT116 and SW480) was determined by the western blot assay. The protein levels of caspase-3, caspase-9, Bcl-2, Bax, PARP, and p53 were determined in HCT116 and SW480 cells that have received 20 $\mu\text{g/ml}$ berbamine for a duration of 48 h. The results showed that berbamine treatment significantly increased the protein levels of caspase-3,

caspase-9, Bax and PARP, and decreased the protein levels of Bcl-2 in both HCT116 cells and SW480 cells (Fig. 3a, b). In addition, the protein level of p53 was also examined, and berbamine treatment caused a significant reduction of p53 protein levels in both HCT116 cells and SW480 cells (Fig. 3a, b). More importantly, berbamine failed to increase the cell apoptotic rate in p53 mutant CRC cell lines (HCT116^{-/-} and SW480^{-/-}) (Fig. 4a, b).

The effect of berbamine treatment on the tumor growth in vivo

To examine the anti-tumor effect of berbamine, nude mice were treated with 60 mg/kg berbamine twice

Fig. 3 Effects of berbamine on the protein levels of cell apoptosis-related factors in CRC cells. The protein levels of caspase-3, caspase-9, Bax, Bcl-2, PARP, and p53 in **a** HCT116 and **b** SW480 cells were determined by western blot assay after treatment with berbamine (20 µg/ml) for 48 h. N = 3

daily for up to 4 weeks. Nude mice in the control group were given equal volumes of isotonic saline. Mice from both groups had a gradually body weight loss (Fig. 5a). The tumor volume in the berbamine-treated mice was significantly smaller at 21, 28 and

35 days after SW480 cells inoculation (Fig. 5b). The mean tumor weight of the excised tumors was also lower in the berbamine-treated animals (0.95 ± 0.14 g) when compared to the control group (2.01 ± 0.68 g). In addition, the protein levels of caspase-3, caspase-9 and p53 were also examined in the tumor tissues. The protein levels of caspase-3, caspase-9 and p53 were increased in the tumor tissues from berbamine-treated groups (Fig. 5c).

Discussion

As far as we know, the anti-tumor effect of berbamine in CRC has not been investigated, though the anti-tumor effects of berbamine have been shown in several other types of cancers including myeloma, prostate cancer, lung cancer, pancreatic cancer, liver cancer and gastric cancer (Hou et al. 2014; Jin and Wu 2014; Liang et al. 2009; Meng et al. 2013; Zhao et al. 2016; Zhu et al. 2013). In the present study, our results showed that berbamine suppressed the cell viability of CRC cell lines in a dose-dependent and time-dependent manner as measured by MTT assay. The flow cytometry experiments showed that berbamine increased the cell apoptotic rate and induced cell cycle arrest at G₀/G₁ phase, and berbamine failed to increase the cell apoptotic rate in p53 mutant CRC cell lines. In addition, berbamine also increased the MMP in the CRC cell lines. The western blot assay further showed that berbamine activated p53-dependent apoptosis signaling pathway. The in vivo experiments showed that berbamine treatment suppressed the

Fig. 4 Effects of berbamine on cell apoptosis in the p53 mutant CRC cells. Cell apoptosis of **a** HCT116^{+/+} and HCT116^{-/-}, and **b** SW480^{+/+} and SW480^{-/-} cells was determined by flow cytometry after treatment with berbamine (20 µg/ml) for 48 h.

+/+ = p53 wild type cells, -/- = p53 mutant cells. N = 3. Significant differences between groups were shown as * $P < 0.05$, ** $P < 0.01$

Fig. 5 Effects of berbamine on tumor growth in vivo. **a** The body weight of the nude mice was measured every 7 days for up to 35 days in both the control and the berbamine group. **b** The tumor volume of the nude mice was measured for 7 days for up to 35 days in both the control and the berbamine group. **c** The protein levels of caspase-3, caspase-9 and p53 in tumor tissues from the control and the berbamine group were examined by western blot assay. N = 6. Significant differences between groups were shown as * $P < 0.05$, ** $P < 0.01$

tumor volume and tumor weight in the nude mice, and berbamine treatment also induced apoptosis in the tumor tissues.

Apoptosis is a process of programmed cell death that happens in multicellular organisms. Disruption of apoptosis signaling pathway is associated with various diseases including cancer (Afshar-Kharghan 2017). Many anti-cancer compounds have been shown to exert their anti-cancer effects via activating apoptotic pathway. In the pancreatic cancer, berbamine treatment induced cell apoptosis and also increased the expression of bax/bcl-2, active caspase-3 and active caspase-9 in the pancreatic cancer cells (Jin and Wu 2014). Liang et al. (2009) showed that berbamine induced cell apoptosis and increased the levels of pro-apoptotic Bax and decreased the anti-apoptotic proteins Bcl-2 and Bcl-xL in myeloma cells. Wang et al. (2009) showed that berbamine induced apoptosis and increased the expression level of Fas, p53, active caspase-3, -8, and -9 in HepG2 cells. Moreover, lidamycin, a member of the enediyne antibiotic family, induced p53-dependent apoptosis of HCT116 cells at low concentrations (Chen et al. 2007). Aciculatin, a naturally isolated compound from *Chrysopogon aciculatus*, induces p53-dependent apoptosis in HCT116 cells in vitro and in vivo (Lai et al. 2012). Consistently, our results showed that berbamine induced cell apoptosis and increased protein levels of p53, caspase-3, caspase-9, Bax and PARP, and decreased the protein levels of Bcl-2, and berbamine failed to increase the cell apoptotic rate in p53 mutant CRC cell lines suggesting that berbamine induced apoptosis via activating the p53-dependent apoptosis signaling pathway in CRC cells.

Modulation of the cell cycle in cancer cells is one of the strategies to develop new anti-cancer therapies (Corona et al. 2017; Liu et al. 2017). Various studies have suggested that cell cycle arrest may result in apoptosis owing to the existence of cell cycle check point and feedback control (Niknejad et al. 2016). On the other hand, the anti-cancer compounds can also induce apoptosis via a signaling pathway independent of cell cycle arrest (Karimian et al. 2016; Kocab and Duckett 2016). Wang et al. (2007) showed that berbamine induced apoptosis and caused cell arrest at G₀/G₁ phase in human hepatoma cell line. In chronic myeloid leukemia, berbamine was found to induced G₀/G₁ cell cycle arrest and apoptosis in KU812 cells (Liang et al. 2011). Our results as evaluated by flow cytometry showed that berbamine treatment induced cell cycle arrest at G₀/G₁ phase, suggesting that cell

apoptosis induced by berbamine may be related to cell cycle arrest at G₀/G₁ phase in CRC cells.

The in vivo anti-tumor effect of berbamine was also demonstrated in several types of cancers including prostate cancer, lung cancer and liver cancer (Hou et al. 2014; Wang et al. 2009; Zhao et al. 2016). Zhao et al. showed that berbamine significantly suppressed the pancreatic tumor volume in the nude mice, and ratios of apoptotic cells as well as the protein levels of active caspase-3 and -9 in pancreatic tumor from the berbamine-treated group was significantly increased (Jin and Wu 2014). In agreement with previous studies, the present study showed that berbamine treatment significantly reduced the colorectal tumor volume and also increased the protein levels of p53, active caspase-3 and caspase-9, suggesting the involvement of apoptosis in vivo anti-tumor effects of berbamine in CRC.

In summary, our results demonstrated that exhibited a strong anti-cancer effect in CRC. Our data suggest that berbamine exerts anti-cancer effects in vitro and in vivo via induction of apoptosis, associated with the activation of p53-dependent apoptosis signaling pathway.

Acknowledgements This work was supported by the Research and Development Project for Science and Technology of Shaanxi Province (Project No. 2013SF2-14).

References

- Afshar-Kharghan V (2017) The role of the complement system in cancer. *J Clin Invest* 127:780–789
- Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F (2017) Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 66:683–691
- Aung TN, Qu Z, Kortschak RD, Adelson DL (2017) Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. *Int J Mol Sci* 18:656
- Bernabeu E, Cagel M, Lagomarsino E, Moretton M, Chiappetta DA (2017) Paclitaxel: what has been done and the challenges remain ahead. *Int J Pharm* 526:474–495
- Chen L, Jiang J, Cheng C, Yang A, He Q, Li D et al (2007) P53 dependent and independent apoptosis induced by lidamycin in human colorectal cancer cells. *Cancer Biol Ther* 6:965–973
- Corona SP, Ravelli A, Cretella D, Cappelletti MR, Zanotti L, Dester M et al (2017) CDK4/6 inhibitors in HER2-positive breast cancer. *Crit Rev Oncol Hematol* 112:208–214
- Duan H, Luan J, Liu Q, Yagasaki K, Zhang G (2010) Suppression of human lung cancer cell growth and migration by berbamine. *Cytotechnology* 62:341–348
- Frouws MA, van Herk-Sukel MP, Maas HA, Van de Velde CJ, Portielje JE, Liefers GJ et al (2017) The mortality reducing effect of aspirin in colorectal cancer patients: interpreting the evidence. *Cancer Treat Rev* 55:120–127
- Gill S, Thomas RR, Goldberg RM (2003) Review article: colorectal cancer chemotherapy. *Aliment Pharmacol Ther* 18:683–692
- Greenwell M, Rahman PK (2015) Medicinal plants: their use in anticancer treatment. *Int J Pharm Sci Res* 6:4103–4112
- Hou ZB, Lu KJ, Wu XL, Chen C, Huang XE, Yin HT (2014) In vitro and in vivo antitumor evaluation of berbamine for lung cancer treatment. *Asian Pac J Cancer Prev* 15:1767–1769
- Jin X, Wu Y (2014) Berbamine enhances the antineoplastic activity of gemcitabine in pancreatic cancer cells by activating transforming growth factor-beta/Smad signaling. *Anat Rec (Hoboken)* 297:802–809
- Karimian A, Ahmadi Y, Yousefi B (2016) Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. *DNA Repair (Amst)* 42:63–71
- Khan I, Qayum A, Qureshi Z (1969) Study of the hypotensive action of berbamine, an alkaloid isolated from berberis lycium. *Life Sci* 8:993–1001
- Kocab AJ, Duckett CS (2016) Inhibitor of apoptosis proteins as intracellular signaling intermediates. *FEBS J* 283:221–231
- Lai CY, Tsai AC, Chen MC, Chang LH, Sun HL, Chang YL et al (2012) Aciculin induces p53-dependent apoptosis via MDM2 depletion in human cancer cells in vitro and in vivo. *PLoS ONE* 7:e42192
- Liang Y, Xu RZ, Zhang L, Zhao XY (2009) Berbamine, a novel nuclear factor kappaB inhibitor, inhibits growth and induces apoptosis in human myeloma cells. *Acta Pharmacol Sin* 30:1659–1665
- Liang Y, Qiu X, Xu RZ, Zhao XY (2011) Berbamine inhibits proliferation and induces apoptosis of KU812 cells by increasing Smad3 activity. *J Zhejiang Univ Sci B* 12:568–574
- Liu W, Li J, Song YS, Li Y, Jia YH, Zhao HD (2017) Cdk5 links with DNA damage response and cancer. *Mol Cancer* 16:60
- Meng Z, Li T, Ma X, Wang X, Van Ness C, Gan Y et al (2013) Berbamine inhibits the growth of liver cancer cells and cancer-initiating cells by targeting Ca(2+)-calmodulin-dependent protein kinase II. *Mol Cancer Ther* 12:2067–2077
- Niknejad H, Yazdanpanah G, Ahmadiani A (2016) Induction of apoptosis, stimulation of cell-cycle arrest and inhibition of angiogenesis make human amnion-derived cells promising sources for cell therapy of cancer. *Cell Tissue Res* 363:599–608
- Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E et al (2009) Natural compounds for cancer treatment and prevention. *Pharmacol Res* 59:365–378
- Tan CJ, Zhao Y, Goto M, Hsieh KY, Yang XM, Morris-Natschke SL et al (2016) Alkaloids from *Oxytropis ochrocephala* and antiproliferative activity of sophoridine derivatives against cancer cell lines. *Bioorg Med Chem Lett* 26:1495–1497
- Wang GY, Zhang JW, Lu QH, Xu RZ, Dong QH (2007) Berbamine induces apoptosis in human hepatoma cell line SMMC7721 by loss in mitochondrial transmembrane

- potential and caspase activation. *J Zhejiang Univ Sci B* 8:248–255
- Wang GY, Lv QH, Dong Q, Xu RZ, Dong QH (2009) Berbamine induces Fas-mediated apoptosis in human hepatocellular carcinoma HepG2 cells and inhibits its tumor growth in nude mice. *J Asian Nat Prod Res* 11:219–228
- Wong CW, Seow WK, O’Callaghan JW, Thong YH (1992) Comparative effects of tetrandrine and berbamine on subcutaneous air pouch inflammation induced by interleukin-1, tumour necrosis factor and platelet-activating factor. *Agents Actions* 36:112–118
- Yang F, Nam S, Zhao R, Tian Y, Liu L, Horne DA et al (2013) A novel synthetic derivative of the natural product berbamine inhibits cell viability and induces apoptosis of human osteosarcoma cells, associated with activation of JNK/AP-1 signaling. *Cancer Biol Ther* 14:1024–1031
- Zhang CM, Gao L, Zheng YJ, Yang HT (2012) Berbamine protects the heart from ischemia/reperfusion injury by maintaining cytosolic Ca(2+) homeostasis and preventing calpain activation. *Circ J* 76:1993–2002
- Zhao Y, Lv JJ, Chen J, Jin XB, Wang MW, Su ZH et al (2016) Berbamine inhibited the growth of prostate cancer cells in vivo and in vitro via triggering intrinsic pathway of apoptosis. *Prostate Cancer Prostatic Dis* 19:358–366
- Zhu L, Zhang B, Lu X, Shu Y, Liu B (2013) Delivery of paclitaxel and berbamine by polymeric carriers to cure gastric cancer. *Oncol Res* 20:265–274