OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.

Research Article
Antitumor Ability of Berberine Accompanied by Modulation of Gut Microbiome in Sarcoma-180 Tumor-bearing Mice

[1]Yanhong Yang, [2]Zili Lei, [2]Li Huang, [2]Fei Yang, [3]Na Zhang, [4]Jian Yuan, [3]Kundong Li, [3]Juan Chen and [3]Jufeng Zhang

[1]The First Affiliated Hospital (School of Clinical Medicine), Guangdong Pharmaceutical University, Nong-Lin-Xia Road #19, Yue-Xiu District, 510080 Guangzhou, People's Republic of China
[2]Guangdong Research Center of Metabolic Diseases of Integrated Western and Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Centre, 510006 Guangzhou, People's Republic of China
[3]School of Life Science and Biopharmacology, Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Centre, 510006 Guangzhou, People's Republic of China
[4]Department of Pathology and Guangdong Key Laboratory for Bioactive Drugs Research, Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Centre, 510006 Guangzhou, People's Republic of China

1st and 2nd author contributed equally to this paper

Abstract

Background and Objective: Berberine (BBR) is an alkaloid with many pharmaceutical activities. The BBR inhibits the proliferation and induces apoptosis in many cancer cell lines. In current study, the objective was to evaluate the effects of BBR on tumor *in vivo* and its potential role in modulation of gut microbiome. **Materials and Methods:** The effects of BBR on the cell cycle and apoptosis of Sarcoma-180 (S-180) cell line were checked by flow cytometry. Hematoxylin-eosin staining and immunohistochemistry staining were used to check the effects of BBR on the S-180 tumor tissues based on S-180 tumor-bearing mice model. The effects of BBR on the gut microbiome of S-180 tumor-bearing mice were studied by 16S rDNA gene analysis. **Results:** The BBR could induce apoptosis and S phase arrest of S-180 cells *in vitro*. Tumor index of the BBR-treated group was significantly decreased and BBR-treated tumors showed more necrosis and decreased Ki-67 expression. The 16S rDNA gene analysis demonstrated that the control group of tumor-bearing mice had higher abundance of *Prevotellaceae* than the BBR treated group ($p<0.05$). Heat map showed the variations of some dominant bacterial family in the BBR-treated group, the tumor control group and the healthy group. The KEGG pathway analysis showed that several metabolism-related pathways were significantly changed in the three groups. **Conclusion:** The BBR can inhibit S-180 tumor cells *in vitro* and *in vivo*, accompanied by modulation of gut microbiome. The BBR may become a novel agent for prevention and treatment of tumors.

Key words: Berberine, antitumor, Sarcoma-180, gut microbiome, 16S rDNA gene analysis

Received: Accepted: Published:

Citation: Yanhong Yang, Zili Lei, Li Huang, Fei Yang, Na Zhang, Jian Yuan, Kundong Li, Juan Chen, Jufeng Zhang, 2018. Antitumor ability of berberine accompanied by modulation of gut microbiome in Sarcoma-180 tumor-bearing mice. Int. J. Pharmacol., CC: CC-CC.

Corresponding Author: Jufeng Zhang, School of Life Science and Biopharmacology, Guangdong Pharmaceutical University, Wai-Huan-Dong Road #280, Guangzhou Higher Education Mega Centre, 510006 Guangzhou, People's Republic of China Tel: +86-18620588938

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chemical compounds derived from plants have been used for centuries to counteract all kinds of diseases. Plants have wide biological and medicinal properties, characterized by high safety, availability, accessibility and low cost, thus representing an invaluable source of chemicals with potential therapeutic effects[1]. Berberine (BBR) is a bioactive alkaloid which can be isolated from several Chinese herbs, such as *Hydrastis canadensis*, *Berberis aristata*, *Coptis chinensis*, *Coptis japonica*, *Phellondendron amurense* and *Phellondendron chinense* Schneid[1-3]. It has been reported that BBR has a wide range of pharmacological and biochemical effects and is effective against a number of diseases including gastroenteritis, diarrhea[1], metabolic syndrome[3], hyperlipidemia[4], diabetes[5] and Alzheimer's disease[6].

Recently, *in vitro* studies using cancer cell lines have shown that BBR inhibits cancer cell proliferation and migration and induces apoptosis in a variety of cancer cell lines[3,7-10]. Most of the studies about the anti-tumor activity of BBR were carried out in cell lines, in current study, the effects of BBR on tumor *in vivo* based on Sarcoma-180 tumor-bearing mice were studied. Moreover, since BBR has been used for treatment of gastroenteritis and diarrhea for a very long time, BBR might exert its pharmaceutical effects by modulation of gut microbiome was proposed. Actually, it has been reported that BBR could improve the lipid and sugar metabolisms by alteration of gut microbiome. Wang *et al*.[11] reported that BBR could lower blood lipid and glucose levels by working through the short chain fatty acids of the gut microbiota. Li *et al*.[12] reported that the lipid lowering effect of BC treatment in hyperlipidemic rats was associated with a global change in the metabolism of lipids, carbohydrates and amino acids, as well as the structure of microbiota.

In present study, the effects of BBR on tumor *in vitro* and *in vivo* based on Sarcoma-180 cell line and Sarcoma-180 tumor-bearing mice was conducted. Furthermore, the difference of gut microbiota between healthy mice and S-180 tumor-bearing mice and the effects of BBR on the gut microbiota of S-180 tumor-bearing mice, were studied, stimulating further development of natural plants derivatives for cancer prevention and treatment.

MATERIALS AND METHODS

This study project was conducted from June, 2016 to October, 2017.

Cell culture: The mouse Sarcoma-180 cell line was purchased from the Cell Resource Center of Shanghai Institute of Life Sciences, Chinese Academy of Sciences. Cells were cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Hyclone, USA) at 37°C in a humidified atmosphere containing 5% CO_2. When needed, cells were treated with BBR (Beyotime Biotechnology Company, China) at different concentrations.

Cell cycle and apoptosis detection by flow cytometry: The S-180 cells were seeded in six-well plates at a density of 1×10^5/well and incubated with BBR at different concentrations for 24 h. Cells were harvested and washed with phosphate-buffered saline (PBS) and fixed with 70% ethnol at 4°C overnight. After staining with propidium iodide (PI) solution for 30 min, cells were collected on a FACScan flow cytometer (Accuri™ C6 Flow cytometer, BD Biosciences, USA) equipped with a 488 nm argon laser and analyzed using Modfit LT. Cells for detection of apoptosis were treated with BBR at different concentrations for 24 h and stained with Annexin-V and PI (BD PharmingenTM, USA) according to the manufacturer's instruction. Flow cytometry analysis was also performed by FACScan flow cytometer and analyzed by FlowJo 7.6.

S-180 solid tumor model: Sixty female C57BL/6 mice (12 weeks old) (The Laboratory Animal Center of Guangdong Pharmaceutical University) were housed in a temperature-controlled room (25). The mice had free access to standard diet and water. The mice were divided randomly into 3 groups and each group had 20 mice. The S-180 cells were washed with physiological saline and the density of the cells within the fluid was adjusted to between 2×10^7 and 3×10^7 cells mL^{-1} before 0.2 mL was injected into the right armpit of the mice of the BBR-treated group and the tumor control group. When tumors became a diameter of about 4 mm, 1 μg $\mu L^{-1}\times$ g (weight) BBR was fed per mouse everyday of the BBR-treated group and the tumor control group was fed with water. Another group was healthy mice without tumors. The tumor volume, body weight and survival rate were recorded throughout the experiment period. The mice were sacrificed after 6 weeks and the tumor, spleen and thymus of each mouse was removed and weighed. The ratio of tumor suppression was calculated: The weight of the tumors obtained from the mice treated with BBR/ the weight of the tumors removed from the mice administered with water. The tumor index was calculated by dividing the

respective tumor weight by body weight multiply by 100. The spleen and thymus indices were calculated by dividing the respective organ weight by body weight multiply by 100. The animal experiments were approved by the Laboratory Animal Center of Guangdong Pharmaceutical University (Guangzhou, China).

Hematoxylin-eosin (H and E) staining and immunohistochemistry (IHC) staining: The H and E staining was performed on 4 μm sections, stained with hematoxylin and eosin. IHC staining of Ki-67 was performed by the Department of Pathology of the First Affiliated Hospital of the Guangdong Pharmaceutical University.

16S rDNA gene analysis: Fecal bacterial DNA extraction, 16S rDNA gene PCR amplification and sequencing and 16S rDNA gene analysis were carried out by Gene Denovo Biotechnology Company (Guangzhou, China). The fecal bacterial DNA of each sample was extracted and the total DNA samples were characterized by 1% agarose gel electrophoresis for integrity and size. The DNA extracts were stored at -80°C before being used as templates for 16S rDNA analysis. The primers 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT) were used to amplify the V3 and V4 domain of bacterial 16S rDNA. The PCR reactions contained 5-100 ng DNA template, 1×GoTaq Green Master Mix (Promega, Madison, WI), 1 mM $MgCl_2$ and 2 pmol of each primer. Reaction conditions consisted of an initial 94°C for 3 min followed by 35 cycles of 94°C for 45 sec, 50°C for 60 sec and 72°C for 90 sec and a final extension of 72°C for 10 min. Amplicons were purified and then sequenced on the Hiseq2500 PE250 platform. Sequence data were analyzed using Quantitative Insights into Microbial Ecology (QIIME). The 16S rDNA gene sequences were assigned to operational taxonomic units (OTUs) using UCLUST with a threshold of 97% pair-wise identity and classified taxonomically using the Ribosomal Database Project (RDP) classifier 2.0.1. Alpha diversity estimates were calculated with the Shannon value. Principal coordinates analysis (PCA) and heat map were performed to present differences between the gut microbial communities of different groups.

Statistical analysis: Statistical differences were determined using Statistical Product and Service Solutions (SPSS) Software (IBM SPSS Statistics 23.0 for Windows, IBM Corp, Armonk, NY, USA). The p-value less than 0.05 was considered to be significant.

RESULTS

Effect of BBR on the cell cycle and apoptosis of S-180 cells detected by flow cytometry: The effect of BBR on the cell cycle of S-180 cells was detected by flow cytometry and it was shown that the cells were accumulated in S phase and less cells entered G1 phase (Fig. 1). The BBR could also induce the apoptosis of S-180 and the apoptosis was obvious at different concentrations (Fig. 2).

Effect of BBR on tumor inhibition and immune system: The tumor index of BBR-treated mice was significantly lower than that of the control group (Fig. 3) and the ratio of tumor suppression was 23.58%. There was no difference of the spleen index and thymus index between the two groups (Fig. 3). The results of H and E staining showed that necrosis was obvious in BBR-treated tumors compared with the control group (Fig. 4). The IHC staining demonstrated that the expression level of Ki-67 was significantly decreased in the BBR-treated tumors (Fig. 4).

Variations of fecal microbial communities in the BBR group, the control group and the normal group: The relative abundances of bacterial phylum in different groups were presented in Fig. 5. Most samples had high abundance of *Bacteroidetes* and tumor samples (the control group) had higher abundance of *Tenericutes* compare with the normal mice ($p = 0.057$). The bacterial family composition presented an obvious alteration between the BBR group and the tumor control group (Fig. 6). The control group had higher abundance of *Prevotellaceae* than the BBR treated group ($p<0.05$). No significant variations in the abundances of *Bacteroidales*, *Lachnospiraceae*, *Ruminococcaceae*, *Rikenellaceae*, *Porphyromonadaceae*, *Helicobacteraceae*, *Alcaligenaceae* and *Lactobacillaceae* were observed.

The variation of some dominant bacterial family was presented with a heat map to figure out their contribution to the variation of the bacterial community (Fig. 7). According to the results of the family heat map, *Bacteroidaceae*, *Acidaminococcaceae*, *Lactobacillaceae*, *Rikenellaceae*, *Anaeroplsmataceae*, *Streptococcaceae*, *Rhodospirillaceae*, *Coriobacteriaceae*, *Erysipelotrichaceae*, *Peptococcaceae* and *Desulfovibrionaceae* were enriched in the BBR treated samples and contributed most to the separation of the communities. *Porphyromonadaceae*, *Spirochaetaceae* and *Alcaligenaceae* were enriched in the tumor samples of the control group. *Verrucomicrobiaceae*, *Clostridiaceae*-1 and *Deferribacteraceae* were enriched in the normal mice samples.

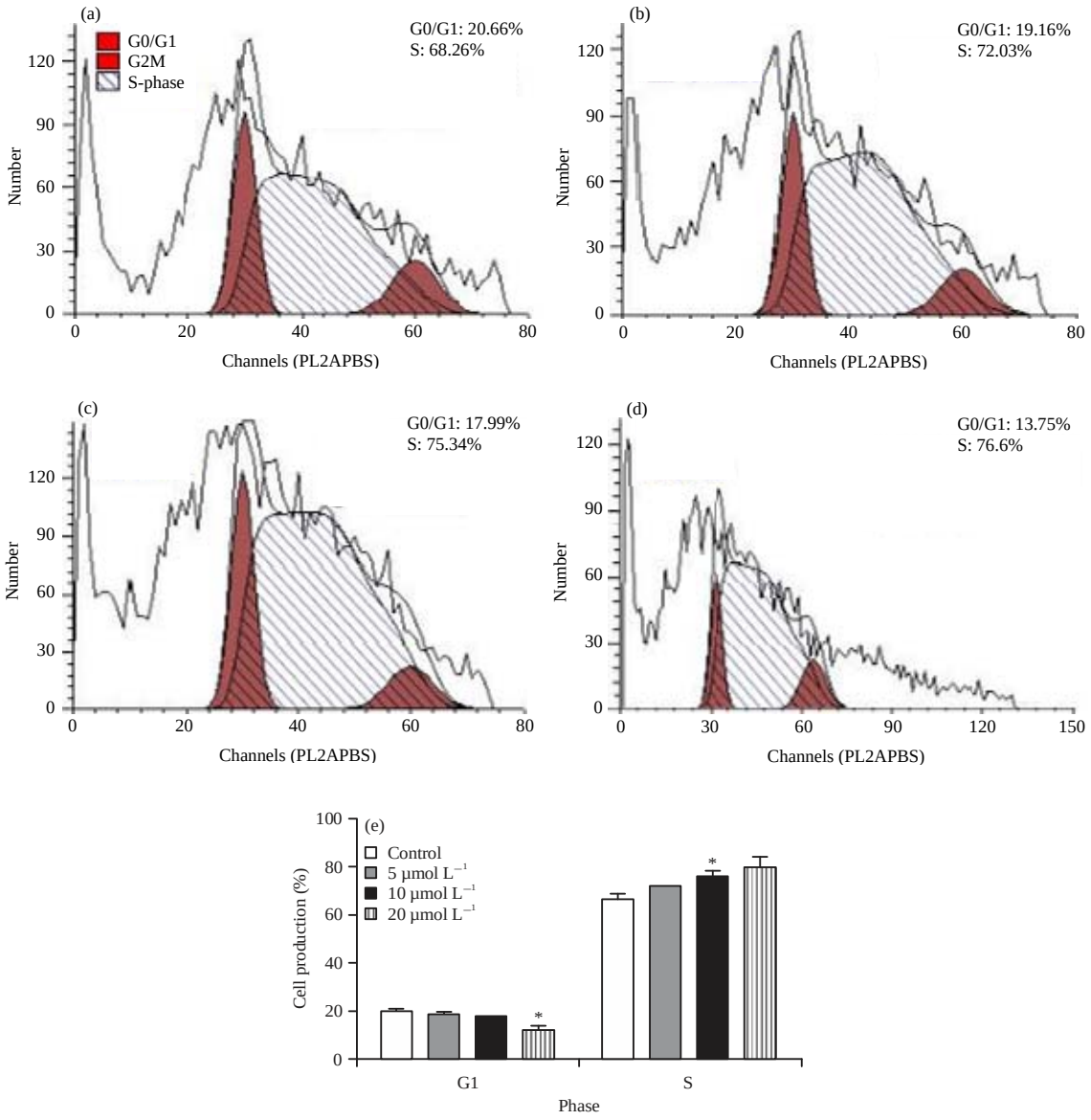

Fig. 1(a-e): Effect of BBR at different concentrations on the cell cycle of S-180 cells detected by flow cytometry, (a) Control, (b) 5 μmol L^{-1}, (c) 10 μmol L^{-1}, (d) 20 μmol L^{-1} and (e) The histogram showed that the S-180 cells treated with BBR were accumulated in S phase and less cells entered G1 phase, *p<0.05

Principal co-ordinates analysis (PCoA) was performed to determine the influence of BBR on the tumors (Fig. 8). The points of the BBR treated group, the control tumor group and the normal group could be distinguished, indicating the difference between the three gut microbial communities, suggesting that the bacterial community changed in the tumor state compared with healthy mice and BBR could alter the bacterial community of the tumor-bearing mice.

The results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that several pathways were significantly changed in the three groups, including aminoacyl-tRNA biosynthesis, amino sugar and nucleotide sugar metabolism, pyrimidine metabolism, porphyrin and chlorophyll metabolism (Fig. 9).

DISCUSSION

Cancer is one of the leading causes of death worldwide. Most of the chemotherapy drugs for cancer are cytotoxic, which have serious adverse reactions and directly affect the curative effect and prognosis of cancer. It is necessary to find some new compounds with little side effects as

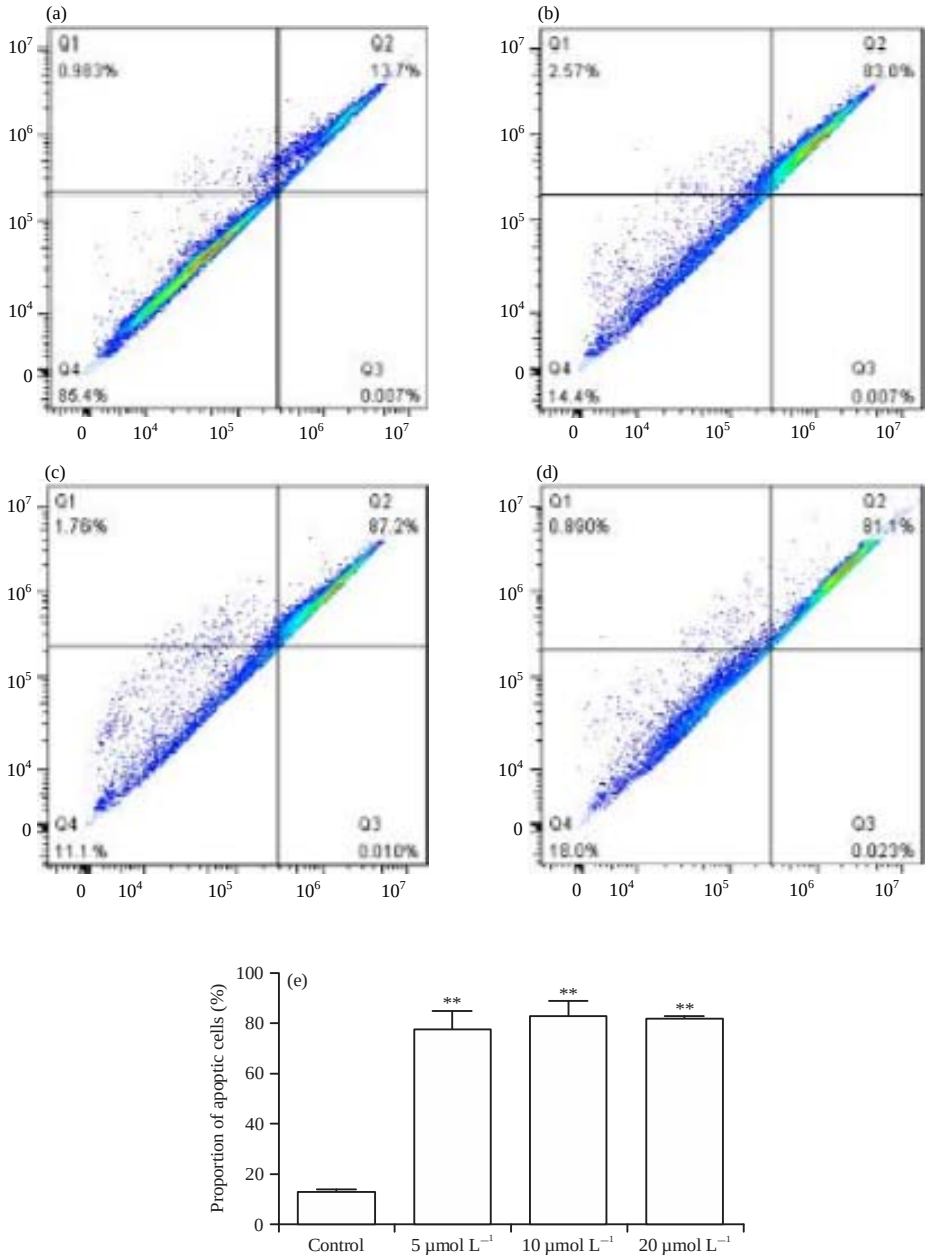

Fig. 2(a-e): Effect of BBR at different concentrations on apoptosis of S-180 cells detected by flow cytometry, (a) Control, (b) 5 μmol L^{-1}, (c) 10 μmol L^{-1}, (d) 20 μmol L^{-1} and (e) The histogram showed that BBR could significantly induce apoptosis of S-180 cells, **p<0.01

complementary or alternative medicine for treatment of cancer. Many plants have wide biological and pharmaceutical activities, including hormonal mimicry, antioxidant, antibacterial, anti-inflammatory effects and anticancer activity[1]. The BBR is an "old" medicine used for treatment of gastroenteritis and diarrhea. Recently it has been reported that BBR could inhibit the proliferation of many cancers, but the underlying mechanisms are still not clear yet.

Present results showed that BBR could induce S phase arrest and apoptosis of S-180 cells *in vitro*. Du *et al.*[13] reported that combination of BBR and evodiamine acted synergistically to suppress the proliferation of MCF-7 cells by inducing G_0/G_1 phase cell cycle arrest and apoptosis. Li *et al.*[14] reported that BBR could modulate PI3K-AKT and MAPK signaling pathways in thyroid carcinoma cells, which leads to mitochondrial apoptosis, G0/G1 cell cycle arrest and suppressive migration.

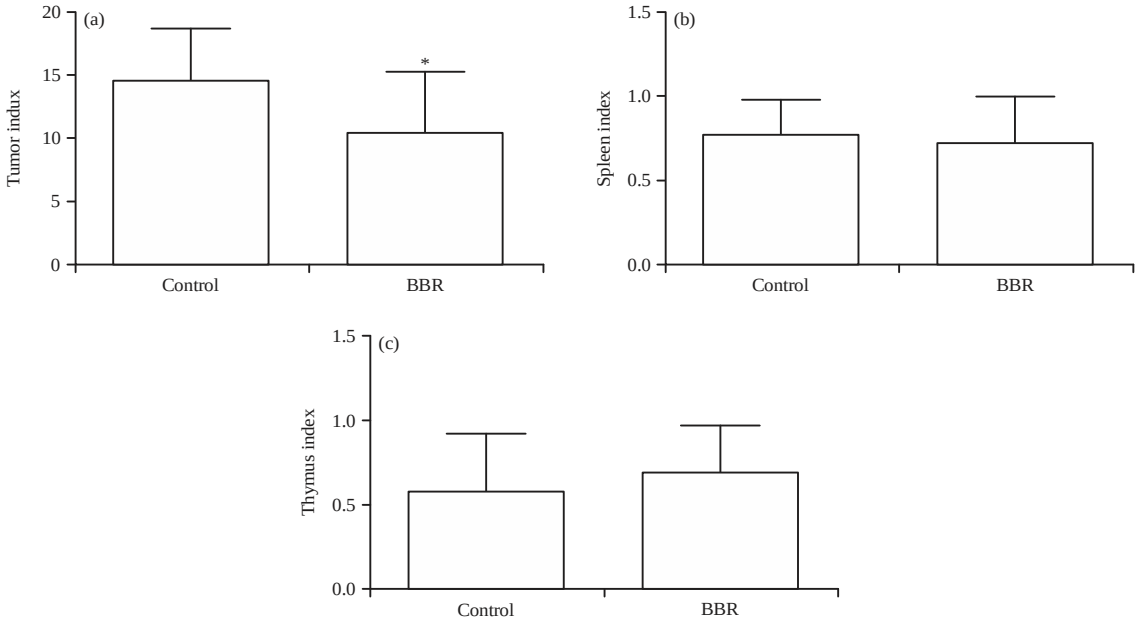

Fig. 3(a-c): Effect of BBR on tumor inhibition and immune system, (a) Tumor index, (b) Spleen index and (c) Thymus index, *p<0.05

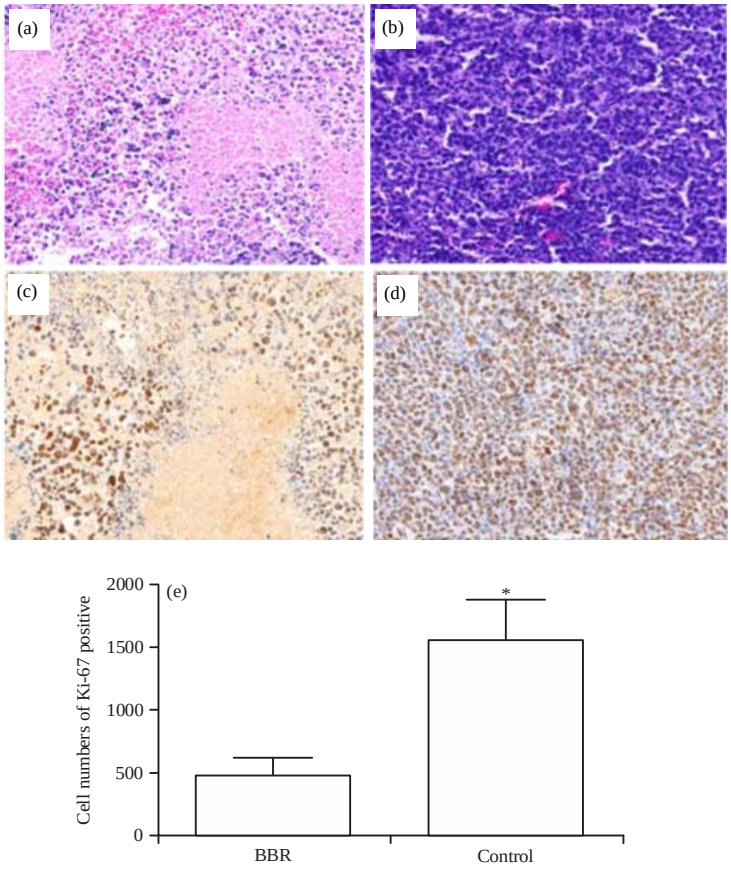

Fig. 4(a-e): H and E staining and IHC of Ki-67, (a) H and E staining of BBR-treated tumor, (b) H and E staining of the control group of tumor, (c) Ki-67 expression of BBR-treated tumor, (d) Ki-67 expression the control group of tumor (200×) and (e) The histogram showed that the cell numbers of Ki-67 positive was significantly decreased in the BBR-treated tumors compared with the control, *p<0.05

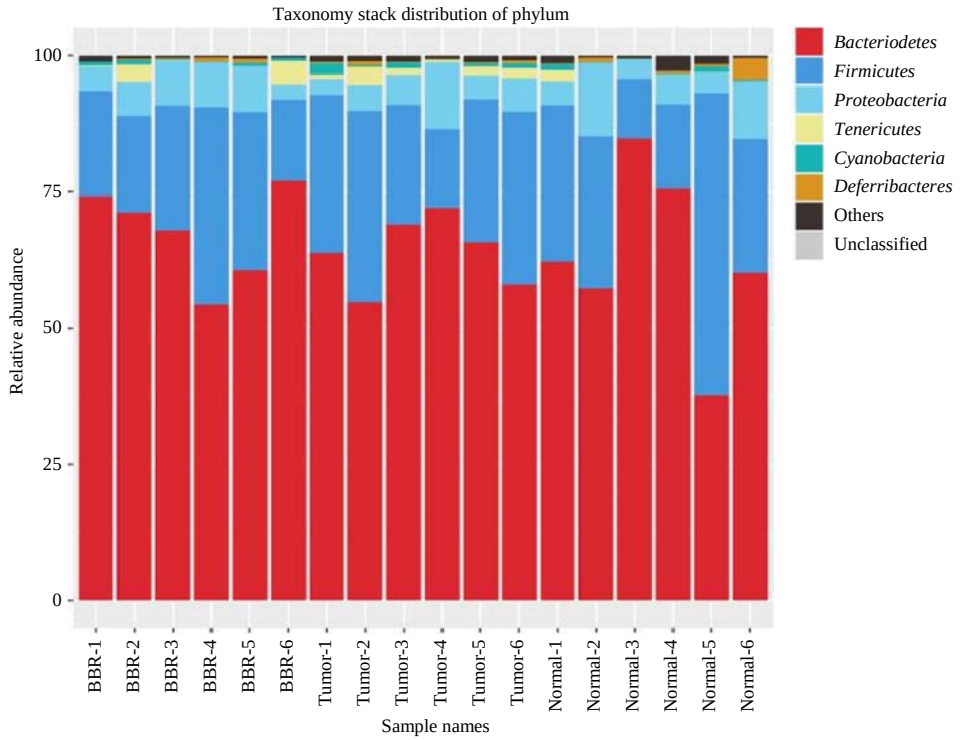

Fig. 5: Relative abundances of the gut microbiota at the bacterial phylum level. BBR 1-6: The group of BBR treated mice, Tumor 1-6: The control group (tumor-bearing mice treated with water), Normal 1-6: The normal healthy mice

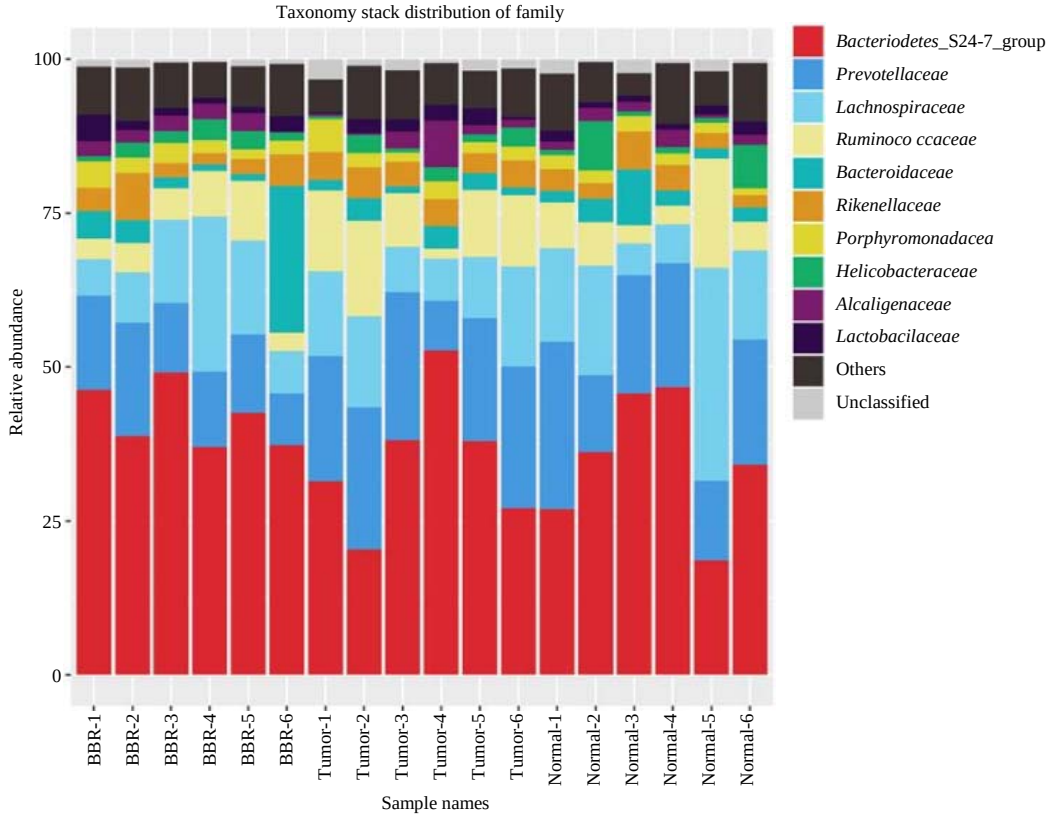

Fig. 6: Relative abundances of the gut microbiota at the bacterial family level. BBR 1-6: The group of BBR treated mice, Tumor 1-6: The control group (tumor-bearing mice treated with water), Normal 1-6: The normal healthy mice

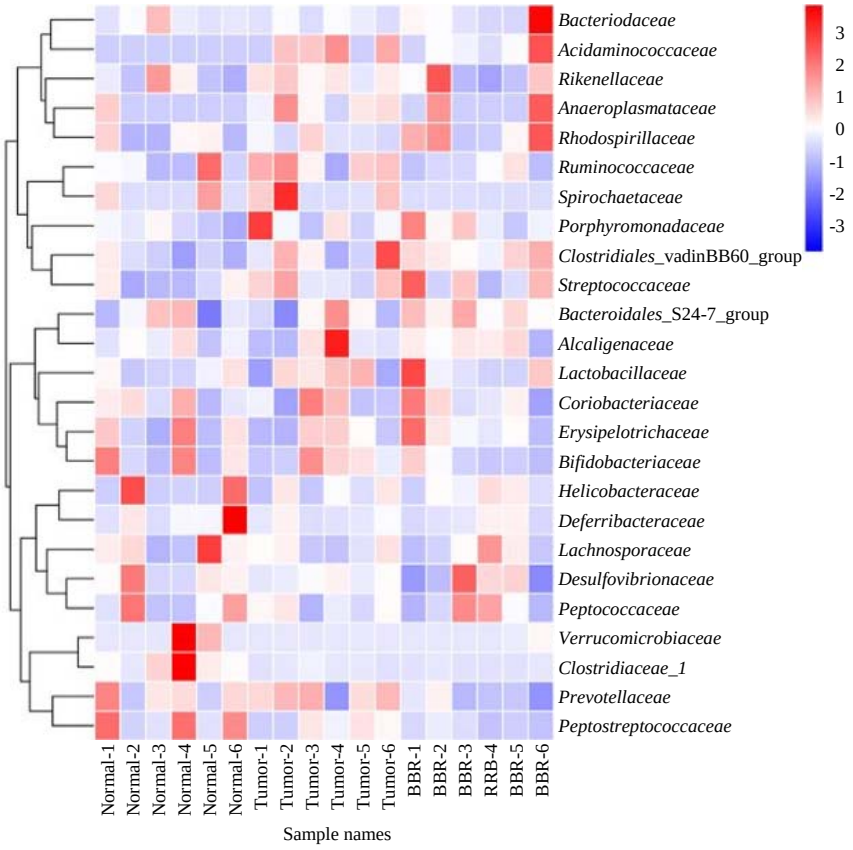

Fig. 7: Heat map of the dominant bacterial family. Columns present the abundances of the selected bacterial family of each mouse. The abundances were clustered using unsupervised hierarchical clustering (Blue: Low abundance, Red: High abundance). BBR 1-6: The group of BBR treated mice, Tumor 1-6: The control group (tumor-bearing mice treated with water), Normal 1-6: The normal healthy mice

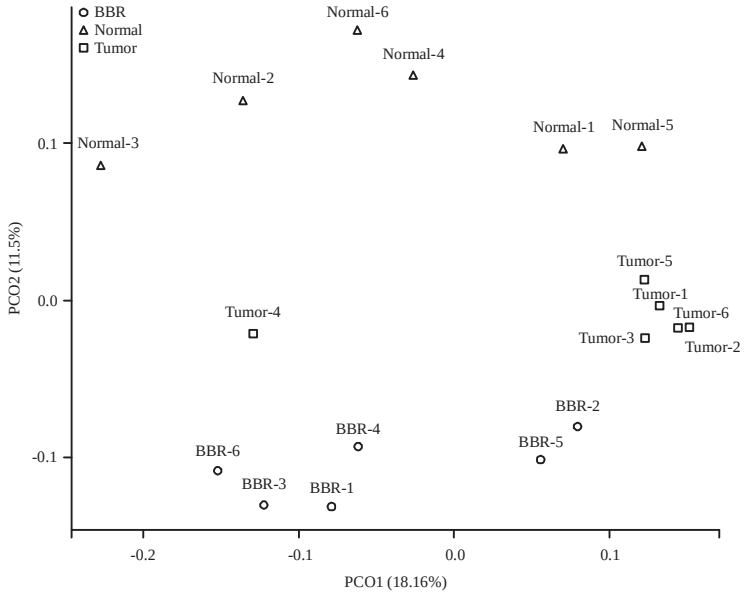

Fig. 8: Principal Co-ordinates Analysis (PCA) of the gut microbiota. BBR 1-6: The group of BBR treated mice, Tumor 1-6: The control group (tumor-bearing mice treated with water), Normal 1-6: The normal healthy mice

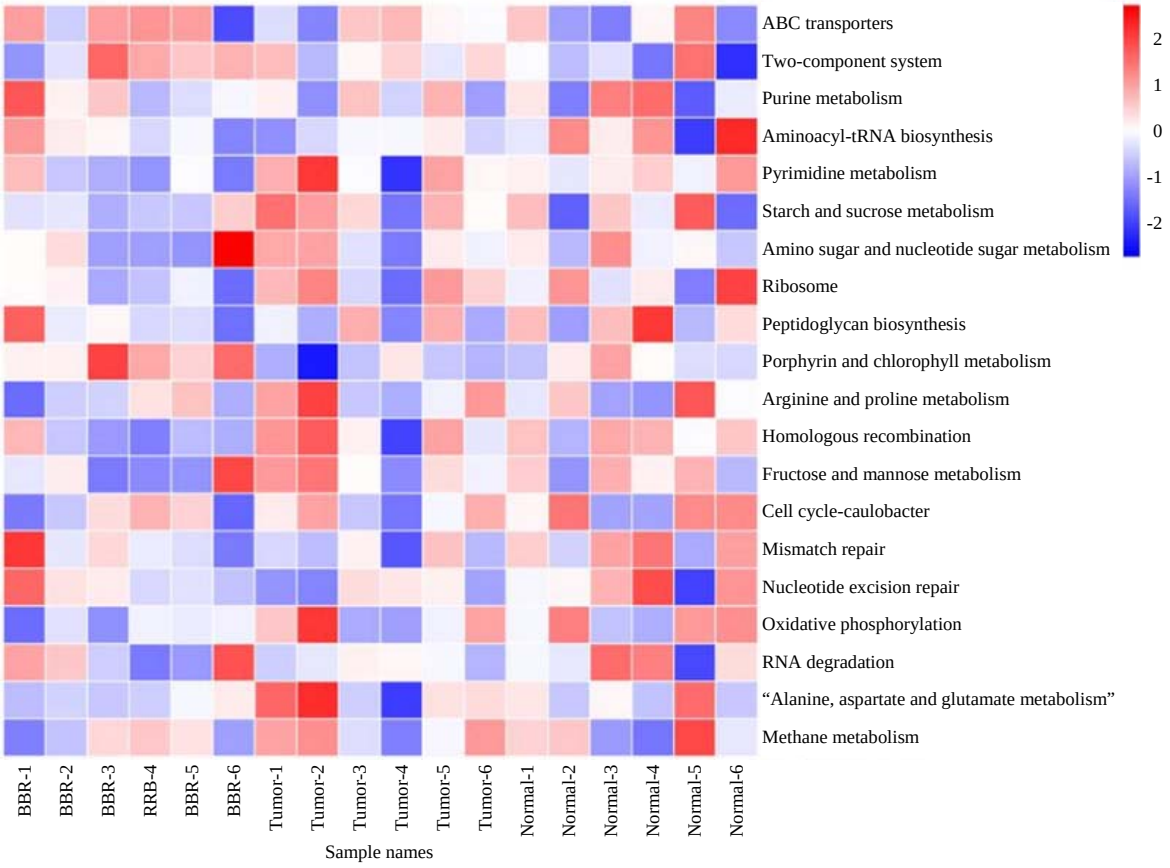

Fig. 9: Presentation of KEGG assignments of the altered pathways in the BBR group, the tumor group of control and the normal group

Jiang *et al.*[15] reported that BBR could induce G2/M arrest and apoptosis in human esophageal cancer cells. It seems that BBR could affect any phase of the cell cycle. The effects of BBR on tumor *in vivo* were further studied and these results demonstrated that the tumor index of the BBR treated group was significantly decreased than the control group, which confirmed the results *in vitro*. There was no difference of the spleen and thymus index between the BBR group and the control group.

People pay more and more attention to gut microbiome nowadays. The gut microbiota is made up mainly of four phyla, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*[16]. Recently, there has been a great deal of research into the relationship between the microbiome and diseases, such as obesity[16], diabetes mellitus[17] and Alzheimer's disease[18]. Flemer *et al.*[19] reported that the heterogeneity of colorectal cancer may relate to microbiota types that either predispose or provide resistance to the disease and profiling the oral microbiome may offer an alternative screen for detecting colorectal cancer. Daniel *et al.*[20] uncovered several metabolic pathways in the microbiome that, when perturbed by host genetics and *H. hepaticus* inoculation, contribute to colon cancer.

Since BBR is usually used for treatment of gastroenteritis and diarrhea by taking orally, the effects of BBR on gut microbiome were further checked. About the relationship between BBR and gut microbiome, most of the study has studied the effects of BBR on gut microbiome of metabolic diseases. Wang *et al.*[11] reported that promotion of butyrate production in gut microbiota might be one of the important mechanisms of BBR in regulating energy metabolism. It has also been reported that the lipid lowering effect of BBR treatment in hyperlipidemic rats is associated with a global change in the metabolism of lipids, carbohydrates and amino acids, as well as the structure of microbiota[12]. Few reports are about the effects of BBR on the gut microbiome of tumors. So, this experiment was designed to study the alteration of the gut microbiome of the healthy mice, S-180 tumor-bearing mice and the BBR treated tumor-bearing mice by 16S rDNA gene analysis. current results showed that the control group

of tumor-bearing mice had higher abundance of *Prevotellaceae* than the BBR treated group at the family level ($p<0.05$) and tumor samples (the control group) had higher abundance of *Tenericutes* compare with the normal mice at the phylum level ($p = 0.057$). It has been reported that the numbers of *Bifidobacterium* is reduced, whereas those of *Prevotellaceae* are increased in human colon cancers than matched adjacent normal tissue[21]. Zackular *et al.*[22] established a murine model of inflammation-associated colorectal cancer that mirrors what is seen in humans and characterized the gut microbiome. The result of the operational taxonomic units (OTUs) analysis showed that members of the *Bacteroides*, *Odoribacter* and *Akkermansia* genera were rich in tumor-bearing mice, whereas, members of the *Prevotellaceae* and *Porphyromonadaceae* families were decreased[23]. It seems that *Prevotellaceae* are increased in cancers and cirrent result showed that BBR could decrease the number of *Prevotellaceae*. *Tenericutes* has also been reported to have higher abundance in colorectal cancer (CRC) than in normal[23], which is consist with result.

CONCLUSION

This study checked the effects of BBR on tumor and gut microbiome using S-180 tumor-bearing mice model. The results demonstrated that BBR could inhibit S-180 cells *in vitro* and *in vivo*. The tumor-bearing mice had altered gut microbiome compared with healthy mice and BBR could modulate the gut microbiome of tumor-bearing mice. Several metabolism-related pathways were also significantly changed in the three groups based on the result of KEGG analysis. The BBR as an "old" medicine for gastroenteritis and diarrhea, may become a novel agent for the prevention and treatment of tumor.

SIGNIFICANCE STATEMENT

This study discovers the effects of BBR on tumor *in vivo* based on S-180 tumor-bearing mice that can be beneficial for the prevention and treatment of tumors. This study will help the researchers to uncover the critical area of the alteration of the gut microbiome between healthy mice and tumor-bearing mice and the effects of BBR on the gut microbiome of tumor-bearing mice that many researchers were not able to explore. Thus, a new theory on the effects of BBR on tumors accompanied by modulation of gut microbiome, may be arrived at.

ACKNOWLEDGMENTS

This study was supported by the Guangdong Science and Technology Program (No. 2014A020212303), the National Natural Science Foundation of China (No. 31671520), the Research Project of the First Affiliated Hospital of Guangdong Pharmaceutical University (No. GYFY201601) and the Guangdong Natural Science Foundation (No. 2016A030313742).

REFERENCES

1. Ortiz, L.M.G., P. Lombardi, M. Tillhon and A.I. Scovassi, 2014. Berberine, an epiphany against cancer. Molecules, 19: 12349-12367.
2. Chen, X.W., Y.M. Di, J. Zhang, Z.W. Zhou, C.G. Li and S.F. Zhou, 2012. Interaction of herbal compounds with biological targets: A case study with berberine. Scient. World J., Vol. 2012. 10.1100/2012/708292.
3. Tillhon, M., L.M.G. Ortiz, P. Lombardi and A.I. Scovassi, 2012. Berberine: New perspectives for old remedies. Biochem. Pharmacol., 84: 1260-1267.
4. Kong, W., J. Wei, P. Abidi, M. Lin and S. Inaba *et al*., 2004. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat. Med., 10: 1344-1351.
5. Tang, L.Q., W. Wei, L.M. Chen and S. Liu 2006. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J. Ethnopharmacol., 108: 109-115.
6. Yao, J., W.J. Kong and J.D. Jiang, 2015. Learning from berberine: Treating chronic diseases through multiple targets. Sci. China Life Sci., 58: 854-859.
7. Refaat, A., S. Abdelhamed, H. Yagita, H. Inoue, S. Yokoyama, Y. Hayakawa and I. Saiki, 2013. Berberine enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast cancer. Oncol. Lett., 6: 840-844.
8. Zou, K., Z. Li, Y. Zhang, H.Y. Zhang and B. Li *et al*., 2017. 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., 38: 157-167.
9. Liu, Q., H. Jiang, Z. Liu, Y. Wang and M. Zhao *et al*., 2011. Berberine radiosensitizes human esophageal cancer cells by downregulating homologous recombination repair protein RAD51. PloS One, Vol. 6, No. 8. 10.1371/journal.pone.0023427.
10. Li, J., L. Gu, H. Zhang, T. Liu, D. Tian, M. Zhou and S. Zhou, 2013. Berberine represses DAXX gene transcription and induces cancer cell apoptosis. Lab. Investig., 93: 354-364.

11. Wang, Y., J.W. Shou, X.Y. Li, Z.X. Zhao and J. Fu *et al*., 2017. Berberine-induced bioactive metabolites of the gut microbiota improve energy metabolism. Metabolism, 70: 72-84.
12. Li, M., X. Shu, H. Xu, C. Zhang, L. Yang, L. Zhang and G. Ji, 2016. Integrative analysis of metabolome and gut microbiota in diet-induced hyperlipidemic rats treated with berberine compounds. J. Trans. Med., Vol. 14, No. 1. 10.1186/s12967-016-0987-5.
13. Du, J., Y. Sun, Y.Y. Lu, E. Lau, M., Zhao, Q.M. Zhou and S.B. Su, 2017. Berberine and evodiamine act synergistically against human breast cancer MCF-7 cells by inducing cell cycle arrest and apoptosis. Anticancer Res., 37: 6141-6151.
14. Li, L., X. Wang, R. Sharvan, J. Gao and S. Qu, 2017. Berberine could inhibit thyroid carcinoma cells by inducing mitochondrial apoptosis, G0/G1 cell cycle arrest and suppressing migration via PI3K-AKT and MAPK signaling pathways. Biomed. Pharmacother., 95: 1225-1231.
15. Jiang, S.X., B. Qi, W.J. Yao, C.W. Gu and X.F. Wei *et al*., 2017. Berberine displays antitumor activity in esophageal cancer cells *in vitro*. World J. Gastroenterol., 23: 2511-2518.
16. Omer, E. and H. Atassi, 2017. The microbiome that shapes us: Can it cause obesity? Curr. Gastroenterol. Rep., Vol. 19. 10.1007/s11894-017-0600-y.
17. Zhang, Q., X. Xiao, M. Li, M. Yu and F. Ping *et al*., 2017. Vildagliptin increases butyrate-producing bacteria in the gut of diabetic rats. PloS One, Vol. 12, No. 10. 10.1371/journal.pone.0184735.
18. Vogt, N.M., R.L. Kerby, K.A. Dill-McFarland, S.J. Harding and A.P. Merluzzi *et al*., 2017. Gut microbiome alterations in Alzheimer's disease. Scientif. Rep., Vol. 7, No. 1. 10.1038/s41598-017-13601-y.
19. Flemer, B., R.D. Warren, M.P. Barrett, K. Cisek and A. Das *et al*., 2017. The oral microbiota in colorectal cancer is distinctive and predictive. Gut. 10.1136/gutjnl-2017-314814.
20. Daniel, S.G., C.L. Ball, D.G. Besselsen, T. Doetschman and B.L. Hurwitz, 2017. Functional changes in the gut microbiome contribute to transforming growth factor β-deficient colon cancer. MSystems, Vol. 2, No. 5. 10.1128/mSystems.00065-17.
21. Sivaprakasam, S., A. Gurav, A.V. Paschall, G.L. Coe and K. Chaudhary *et al*., 2016. An essential role of Ffar2 (Gpr43) in dietary fibre-mediated promotion of healthy composition of gut microbiota and suppression of intestinal carcinogenesis. Oncogenesis, Vol. 5, No. 6. 10.1038/oncsis.2016.38.
22. Zackular, J.P., N.T. Baxter, K.D. Iverson, W.D. Sadler, J.F. Petrosino, G.Y. Chen and P.D. Schloss, 2013. The gut microbiome modulates colon tumorigenesis. MBio, Vol. 4, No. 6. 10.1128/mBio.00692-13
23. Lu, Y., J. Chen, J. Zheng, G. Hu and J. Wang *et al*., 2016. Mucosal adherent bacterial dysbiosis in patients with colorectal adenomas. Scient. Rep., Vol. 6. 10.1038/srep26337.