Anticancer effect of berberine based on experimental animal models of various cancers: a systematic review and meta-analysis

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Abstract

Background: Numerous studies have explored the anti-tumor effect of berberine (BBR), but little clinical evidence guides the use of BBR in cancer patients.

Objectives: Our aim was to investigate the impact of BBR on various cancers in healthy animals to promote the transformation from bench to bed.

Search methods: PubMed, Embase, Springer, and Cochrane databases were searched from January 2000 to October 2018 for relevant articles.

Selection criteria: Only published studies focusing on the relationship between BBR and various cancers in vivo were qualified. Two review authors independently assessed the risk of bias for each study, and any disagreement was resolved by discussion or by involving a third assessor.

Results: A total of 26 studies from 2000 to 2018, focusing on various cancer types, including breast cancer, liver cancer, colorectal cancer, nasopharyngeal carcinoma, lung cancer, gastric cancer, neuroepithelial cancer, endometrial carcinoma, esophageal cancer, tongue cancer, cholangiocarcinoma, and sarcoma were included. Overall, BBR reduced tumor volume (SMD = 3.72, 95% CI: 2.89, 4.56, Z = 8.73, \( p < 0.00001 \)) and tumor weight (SMD = 2.35, 95% CI: 1.51, 3.19, \( Z = 5.50, p < 0.00001 \)) in a linear dose–response relationship (Pearson \( r = -0.6717, p < 0.0001 \) in tumor volume analysis; Pearson \( r = -0.7704, p < 0.0005 \) in tumor weight analysis). BBR inhibited angiogenesis in tumor tissues (SMD = 4.29, 95% CI: 2.14, 6.44, \( Z = 3.92, p < 0.00001 \)), but it had no significant effect on the body weight of experimental animals (SMD = 0.11, 95% CI: \(-0.70, 0.92, Z = 0.27, p = 0.78\)). Publication bias was not detected.

Conclusion: BBR exerted anti-tumor effects in a variety of tumors in vivo, especially breast cancer and lung cancer, and the evidence was still insufficient in colorectal cancer and gastric cancer.

Keywords: Berberine, Cancer, Experimental animals, Meta-analysis

Background

Berberine (BBR) is a natural component purified from the species of the genus Berberis, which has long been used as an anti-diarrheal drug in gastrointestinal disorders in traditional Chinese medicine [1]. At the same time, the anti-tumor effect of BBR has been a hot topic in experimental research in recent years. In the past 3 years, latest studies have shown the anticancer actions of BBR against several high-risk cancers, including lung cancer [2], breast cancer [3], prostate cancer [4], colorectal cancer [5], and gastric cancer [6].

However, little clinical evidence guides the use of BBR in cancer patients. Thus, systematic reviews and meta-analyses of animal studies may help to clarify whether cancer patients could benefit from this approach and promote...
the transformation of animal studies into humans at the same time [7].

Our aim was to investigate the impact of BBR on cancer growth and its adverse effects in randomized controlled trials in healthy animals.

Methods

Identification of studies

From January 2000 to October 2017, relevant literature from PubMed, Embase, Springer, and Cochrane databases was systematically screened. The following Mesh terms and textwords were used: “Neoplasms”[Mesh], “Neoplasia,” “Neoplasias,” “Neoplasm,” “Tumors,” “Tumor,” “Cancer,” “Cancers,” “Malignant Neoplasms,” “Malignant Neoplasm,” “Neoplasm, Malignant,” “Neoplasms, Malignant,” “Malignancy,” “Malignancies,” “Berberine”[Mesh], “Berberine,” “Umbellatine,” and “BBR.” The “AND” or “OR” operator was used to combine these terms in varying combinations. At the same time, references in the articles were also included in the screening. We did not set a language limit during the process. Two authors (Jianhao Xu, Yuming Long) independently reviewed the titles and abstracts identified in the search. In this process, we discussed the articles to incorporate the differences. If problems still could not be resolved, a third assessor (Yusong Zhang) was invited to make a decision. Only published articles were included. No protocol was developed for this review.

Selection criteria

The inclusion criteria were as follows: (1) participants: experimental animals including rodent, mouse, rat, rabbit, guinea pig, dog, horse, sheep, and monkey; (2) intervention: BBR only; (3) outcomes: the effects of BBR in animal models after tumor implantation, including tumor volume, tumor weight, tumor vessel density, and body weight; and (4) study design: experiments should be prospectively controlled. The exclusion criteria were as follows: (a) literature published as letters, editorials, abstracts, reviews, and expert opinions; (b) non-animal-based studies; (c) articles with missing data information; (d) similar and repeated studies; and (e) outdated articles with little significance and credibility. Cohen's kappa statistic was used to assess chance-corrected agreement between reviewers (SPSS Inc. Chicago, IL) [8].

Study characteristics and data extraction

A detailed form was designed for data extraction: first author, publication year, country, cancer type, animals’ baseline characteristics, intervention, duration, and the data of specific outcomes (tumor volume, tumor weight, tumor vessel density, and body weight). Two review authors extracted the data by using the agreed form.

Quality of evidence and risk of bias

For risk of bias of individual studies, the ARRIVE checklist was used to assess pre-clinical animal studies [9]. For risk of bias among studies, such as publication bias and selective reporting, funnel chart analysis, subgroup analysis, and sensitive analysis were all conducted. Two review authors (Jianhao Xu, Yuming Long) independently assessed the risk of bias for each study.

Data synthesis and statistical analysis

We carried out statistical analysis by using the Review Manager software (RevMan 5.3) and STATA statistical software package version 12.0 (Stata Corporation, College Station, TX). The primary outcomes were tumor volume, tumor weight, and tumor vessel density of BBR group compared with the control group. The secondary outcome was the change of body weight. Mean value and standard difference (SD) were used as summary statistics. Standard mean difference (SMD) was measured for continuous data. Linear regression and Pearson’s correlation analysis were used to study the dose–response relationship between BBR and the four outcomes. The heterogeneity among studies was measured by using the $I^2$ test. The latent publication bias was assessed by using a funnel plot. All statistical tests were two-tailed, and $p < 0.05$ was considered statistically significant.

Results

Search results

A total of 969 potential articles were identified from the literature search. After selection, 26 studies [10–35] matched the inclusion criteria and were suitable for our meta-analysis. The flow diagram in Fig. 1 showed the selection process. A review of the study selection and data extraction indicated excellent agreement between reviewers ($k = 0.820$).

Study characteristics and quality assessment

Study characteristics are summarized in Table 1. A total of 26 studies [10–35] from 2000 to 2018, focusing on various cancer types, including breast cancer [10–16], liver cancer [17–19], colorectal cancer [20–22], nasopharyngeal carcinoma [23, 24], lung cancer [25, 26], gastric cancer [27, 28], neuroepithelial tumor [29, 30], endometrial carcinoma [31], esophageal cancer [32], tongue cancer [33], cholangiocarcinoma [34], and sarcoma [35] were included. The studies used rats [14], hamsters [34], and mice [10–13, 15–33, 35] modeled via subcutaneous tumor implantation [10–13, 16–35] or induced tumor formation [14, 15]. BBR was administered in doses ranging between 2.5 mg/kg and 200 mg/kg body weight through intraperitoneal injection [10, 15–17, 19, 21, 23, 24, 30, 33, 35] and gavage [11–14, 18, 20, 22, 25–29, 31, 32].
or from 1000 ppm to 5400 ppm in drinking water [13, 20, 25]. The size of the study sample ranged from 6 to 20, while the follow-up ranged from 1 week to 32.5 weeks. Quality assessment based on the ARRIVE guideline is presented in Table 2. Overall, the studies included in our analysis were of moderate quality.

**Tumor volume**

Of the 26 screened articles [10–35], 20 [11, 13, 14, 16–19, 21–33] reported the relationship between BBR and tumor volume in animals with breast cancer [11, 13, 14, 16], liver cancer [17–19], colorectal cancer [21, 22], nasopharyngeal carcinoma [23, 24], lung cancer [25, 26], gastric cancer [27, 28], neuroepithelial cancer [29, 30], endometrial carcinoma [31], esophageal cancer [32], and tongue cancer [33]. The SMD and the 95%CI in each study are shown in Fig. 2. The pooled SMD remained statistically significant in breast cancer (SMD = 3.32, 95% CI: 1.29, 5.36; Z = 3.2, p = 0.001), liver cancer (SMD = 7.36, 95% CI: 3.45, 11.27; Z = 3.69, p = 0.0002), colorectal cancer (SMD = 0.70, 95% CI: 0.26, 1.15; Z = 3.10, p = 0.002), nasopharyngeal carcinoma (SMD = 3.85, 95% CI: 1.21, 6.49; Z = 2.86, p = 0.004), lung cancer (SMD = 7.18, 95% CI: 4.26, 10.10; Z = 4.82, p < 0.00001), neuroepithelial tumor (SMD = 1.66, 95% CI: 0.41, 2.92; Z = 2.59, p = 0.010), and endometrial cancer (SMD = 4.65, 95% CI: 1.55, 7.74; Z = 2.94, p = 0.003). The pooled SMD remained statistically insignificant in gastric cancer (SMD = 1.47, 95% CI: −1.01, 7.08; Z = 1.47, p = 0.14). For total studies, the pooled result suggested that the SMD was 3.72 (95% CI: 2.89, 4.56) with statistical significance (Z = 8.73, p < 0.00001).

In view of the obvious heterogeneity (I^2 = 80% for breast cancer; I^2 = 81% for liver cancer; I^2 = 63% for nasopharyngeal carcinoma; I^2 = 89% for lung cancer; I^2 = 61% for endometrial cancer), we conducted a subgroup analysis of different characteristics mainly on the following aspects: gender, animals, BBR dose, administration, duration, and cell lines (Fig. 3). In breast cancer, the BBR dose was a potential influencing factor (I^2 decreased to 0% in one subgroup. Another two I^2 were missing due to the limited study). In liver cancer, the cell line was a potential influencing factor (I^2 decreased to 34% in one subgroup. Another two I^2 were missing due to the limited study). In nasopharyngeal carcinoma, gender, duration, and cell line were potential influencing factors (I^2 decreased to 0% in one subgroup. Another I^2 was missing due to the limited study). In lung cancer, the BBR dose was a potential influencing factor (I^2 decreased to 87, 86, and 0% in three subgroups respectively). In nasopharyngeal carcinoma, gender, duration, and cell line were potential influencing factors (I^2 decreased to 0% in one subgroup. Another I^2 was missing due to the limited study). In endometrial cancer, no potential influencing factor was filtered.

The dose–response relationship of different cancer types on the relationship between BBR and tumor volume of animals is shown in Fig. 4. For single cancer types, a statistically significant linear relationship in colorectal cancer (Pearson r = −0.8785, p = 0.0499) and lung cancer (Pearson r = −0.6718, p = 0.0459) was observed. For total
| Author, year, country | Species, strain, gender, age | Model cell line | Experiment | Control | Outcome |
|-----------------------|-----------------------------|----------------|------------|---------|---------|
|                       |                            |                | Dosage     | Frequency | Administration | Duration | type | mean0 | Sd0 | n0 | mean1 | Sd1 | n1 | p value |
| Breast cancer          |                             |                |            |          |              |          |      |       |     |    |       |     |    |        |
| Elisa Pierpaoli, 2015, Italy [10] | mice, FVB/N, F, 4w | SK-BR-3 | 2.5 mg/kg | biw | ip | 3.25w | DMSO | VD | 16.81 | 7.24 | 10 | 12.09 | 1.98 | 10 | 0.07 |
| Yuwan Zhao, 2017, China [11] | mice, BALB/c, F, 6w | MDA-MB-231 | 100 mg/kg | tiw | po | 3w | DMSO | TV | 2.70 | 0.18 | 7 | 0.68 | 0.08 | 7 | < 0.01 |
| Alaa Refaat, 2013, Japan [12] | mice, BALB/c, F, 6w | 4 T1 | 100 mg/kg | qd | po | 4.3w | CMC | TW | 0.21 | 0.01 | 6 | 0.15 | 0.01 | 6 | < 0.01 |
| Sangmin Kim, 2018, Korea [13] | mice, Balb/c, F, 6-8w | MDA-MB-231 | 0.1% BBR in the drinking water | Daily free intake | po | 6.6w | – | TV | 0.42 | 0.18 | 5 | 0.21 | 0.08 | 5 | 0.05 |
| Kalyani Chowdary Karnam, 2017, India [14] | rats, SD, F, 6.4-8.3w | Induced by DMBA | 50 mg/kg [pretreatment] | tiw | po | 4w | Corn oil | TW | 3.79 | 0.90 | 6 | 0.63 | 0.30 | 6 | < 0.01 |
| Elisa Damiani, 2015, Italy [15] | miceFVB/NF4w | HER2/neu transgenic mice | 2.5 mg/kg | biw | ip | NR | Sterile saline | VD | 16.77 | 5.31 | 7 | 11.07 | 1.75 | 9 | 0.03 |
| Ke Su, 2016, China [16] | mice, Balb/c, F, 6w | MDA-MB-231 | 10 mg/kg | qtd | ip | 3w | DMSO | TV | 0.59 | 0.27 | 6 | 0.27 | 0.12 | 6 | 0.02 |
| Liver cancer           |                             |                |            |          |              |          |      |       |     |    |       |     |    |        |
| Guan-Yu Wang, 2009, China [17] | mice, Balb/c, M, 6w | HEPG2 | 40 mg/kg | qd | ip | 1.4w | Saline | TV | 3.31 | 0.38 | 5 | 2.21 | 0.22 | 6 | < 0.01 |
| Jing Li, 2015, Canada [18] | mice, Balb/c, NR, 6-8w | H22 | 50 mg/kg | qd | po | 2w | Water | TV | 4.24 | 0.56 | 10 | 0.33 | 0.35 | 10 | < 0.01 |
| Chi Man Tsang, 2015, China [19] | mice, NR, NR, NR | MHCC-97 L-luciferase | 10 mg/kg | qod | ip | 5w | Saline | TV | 1.00 | 0.05 | 7 | 0.21 | 0.03 | 7 | < 0.01 |
| Author, year, country          | Species, strain, gender, age | Model cell line | Experiment | Control | Outcome |
|-------------------------------|------------------------------|----------------|------------|---------|---------|
|                               |                              |                | Dosage     | Frequency Administration | Duration | type | mean0 | Sd0  | n0   | mean1 | Sd1  | n1 | p value |
| Norio Iizuka, 2002, Japan [20] | mice, Balb/c, M, 6w          | Colon26/clone 20 | 0.1% BBR in the driNRing water | Daily free intake po | 2w | TW | 0.22 | 0.15 | 9 | 0.25 | 0.12 | 9 | 0.01 | < 0.01 |
|                               |                              |                 | 0.2% BBR in the driNRing water |                      |     | BW | 18.20 | 1.50 | 9 | 18.40 | 1.80 | 9 | 0.01 | < 0.01 |
|                               |                              |                 | 0.4% BBR in the driNRing water |                      |     | TW | 0.22 | 0.15 | 9 | 0.24 | 0.18 | 9 | 0.80 | < 0.01 |
| H Ruan, 2017, China [21]      | mice, Balb/c, NR, 6-7w        | KM12C/shCtrl    | 10 mg/kg qd ip 2w DMSO | TV | 1.26 | 0.97 | 6 | 0.79 | 0.53 | 6 | 0.32 | 0.01 |
|                               |                              | KM12C/shRXRa   | 30 mg/kg |                 |         | BW | 18.20 | 1.50 | 9 | 20.90 | 4.20 | 9 | 0.10 | < 0.01 |
|                               |                              | KM12C/shRXRa   | 50 mg/kg |                 |         | TW | 0.22 | 0.15 | 9 | 0.24 | 0.18 | 9 | 0.80 | < 0.01 |
| Yuchen Cai, 2013, Japan [22]  | mice, Balb/c, NR, 5w         | HT-29           | 10 mg/kg qd po 2w Sterile water | TV | 6.11 | 3.01 | 10 | 4.33 | 2.42 | 10 | 0.16 | 0.01 |
|                               |                              |                 | 30 mg/kg |                 |         | BW | 6.60 | 3.60 | 10 | 4.90 | 3.20 | 10 | 0.28 | < 0.01 |
|                               |                              |                 | 50 mg/kg |                 |         | TV | 6.11 | 3.01 | 10 | 3.90 | 2.70 | 10 | 0.07 | < 0.01 |
|                               |                              |                 | 70 mg/kg |                 |         | BW | 6.60 | 3.60 | 10 | 3.60 | 2.50 | 10 | 0.04 | < 0.01 |
| nasopharyngeal carcinoma      |                              | HONE-1          | 10 mg/kg tw ip 3w DMSO | TV | 0.56 | 0.06 | 5 | 0.10 | 0.03 | 5 | 0.05 | < 0.01 |
| Chao Wang, 2017, China [23]   | mice, NOD/SCID, F, 8w        | C666-1          | 5 mg/kg qd ip 6w DMSO | TV | 0.15 | 0.05 | 5 | 0.04 | 0.03 | 5 | 0.01 | < 0.01 |
| Chi Man Tsang, 2013, China [24]| mice, NR, M, 6-8w            | HONE-1          | 10 mg/kg qd po 6w PBS | TV | 1.40 | 0.07 | 10 | 0.99 | 0.04 | 10 | < 0.01 | < 0.01 |
| Lung cancer                   |                              |                 | A549 1800 ppm Daily free intake po 4w DMSO | TV | 0.06 | 0.02 | 3 | 0.02 | 0.02 | 4 | 0.05 | < 0.01 |
| Michael A. James, 2011, Missouri [25] | mice, Balb/c, M, 4-6w   | A549            | 5400 ppm Daily free intake po 4w DMSO | TV | 0.06 | 0.02 | 3 | 0.01 | 0.01 | 2 | 0.04 | < 0.01 |
| Santosh K. Katiyar, 2009, Alabama [26] | mice, Balb/c, F, 6-7w | A549            | 50 mg/kg qd po 7w PBS | TV | 2.32 | 0.27 | 10 | 2.02 | 0.30 | 10 | 0.03 | < 0.01 |
| Author, year, country                      | Species, strain, gender, age | Model cell line | Dosage | Frequency | Administration | Duration | Control | Experiment  | n0  | Sd0  | n1  | Sd1  | p       |
|-------------------------------------------|-------------------------------|-----------------|--------|-----------|----------------|----------|---------|-------------|-----|-------|-----|-------|---------|
| Junxiong Wang, 2016, China [27]           | mice, Balb/c, F, 5w           | BGC823          | 50 mg/kg | qd        | po             | 4w       | NR      | TV          | 1.40| 0.07  | 0.06| 1.03| < 0.001 |
|                                          |                               |                 |         |           |                |          |         | TW          | 2.32| 0.27  | 1.16| 0.21| < 0.001 |
|                                          |                               |                 |         |           |                |          |         | H1299       | 1.40| 0.07  | 0.30| 0.06| < 0.001 |
|                                          |                               |                 |         |           |                |          |         | TW          | 2.32| 0.27  | 0.62| 0.09| < 0.001 |
| Hongli Li, 2016, China [28]               | mice, Balb/c, M, 4w           | MGC803          | 100 mg/kg | qd       | po             | 3.3w     | NR      | TV          | 1.59| 0.10  | 1.36| 0.05| < 0.001 |
|                                          |                               |                 |         |           |                |          |         | TW          | 2.71| 0.31  | 2.36| 0.29| < 0.002 |
| Neuroepithelial tumor                     |                               |                 |         |           |                |          |         | BW          | 2.51| 0.69  | 0.10| 0.46| < 0.001 |
| Juan Wang, 2015, China [29]               | miceBalb/cNN –                |                 | 100 mg/kg | qd       | po             | 3w       | NR      | TV          | 0.04| 0.02  | 0.02| 0.00| < 0.005 |
|                                          |                               |                 |         |           |                |          |         | TW          | 0.02| 0.02  | 0.02| 0.00| < 0.001 |
| Yuxue Sun, 2018, China [30]               | miceBalb/cN6-8w C6            |                 | 10 mg/kg  | qd       | ip             | 1w       | DMSO    | TV          | 0.77| 0.22  | 0.35| 0.06| < 0.001 |
| Endometrial carcinoma                     |                               |                 |         |           |                |          |         | TV          | 1.01| 0.13  | 0.65| 0.06| < 0.001 |
| Yu Wang, 2018, China [31]                 | mice, Balb/c, NR, 6w          | HEC-1-A         | 50 mg/kg  | qd       | po             | 4w       | DMSO    | TV          | 1.45| 0.07  | 0.60| 0.03| < 0.001 |
| Author, year, country | Species, strain, gender, age | Model cell line | Experiment | Control | Outcome |
|----------------------|-----------------------------|----------------|------------|---------|---------|
|                      |                             |                | Dosage     | Frequency | Administration | Duration | type | mean0 | Sd0 | n0 | mean1 | Sd1 | n1 | p value |
| Esophageal cancer    |                             |                | 100 mg/kg |          |         |          |         |      |      |      |      |      |      |      |      |
| Kewei Ren, 2016, China [32] | mice, Balb/c, M, 6-8w | Eca9706 | 20 mg/kg | qd | po | 7w | DMSO | TV | 6.37 | 0.25 | 5 | 5.05 | 0.60 | 5 | < 0.001 |
|                      |                             |                |           |         |         |          |         |      |      |      |      |      |      |      |      |
|                      |                             |                |           |         |         |          |         |      |      |      |      |      |      |      |      |
| Tongue squamous cell carcinoma |                             |                | 20 mg/kg | qd | po | 7w | DMSO | TW | 2.66 | 0.29 | 5 | 1.82 | 0.21 | 5 | < 0.001 |
| Yung-Tsuan Ho, 2009, China [33] | mice, Balb/c, F, 6w | SCC-4 | 10 mg/kg | q4d | ip | 4w | DMSO | TV | 0.18 | 0.06 | 6 | 0.03 | 0.02 | 6 | < 0.001 |
|                      |                             |                |           |         |         |          |         |      |      |      |      |      |      |      |      |
| Cholangiocarcinoma   |                             |                | 20 mg/kg | qd | po | 3w | sterile water | TW | 0.70 | 0.18 | 5 | 0.67 | 0.11 | 5 | 0.79 |
| Nattapong Puthdee, 2013, Japan [34] | hamster, Syrian, M, 4-5w | Ham-1 | 10 mg/kg | qd | po | 3w | sterile water | TW | 0.70 | 0.18 | 5 | 0.67 | 0.11 | 5 | 0.79 |
| Sarcoma              |                             |                | 20 mg/kg | qd | ip | NR | NR | TW | 2.20 | 0.93 | 10 | 1.26 | 0.54 | 9 | 0.002 |
| Lei Zhang, 2012, China [35] | mice, Kunming, NR, 6w | S180 | 30 mg/kg | qd | ip | NR | NR | BW | 2.71 | 2.20 | 10 | −2.54 | 3.24 | 9 | < 0.001 |

Mean0: mean value in control group (cm³ for tumor volume, g for tumor weight and body weight, mm/mm² for vessel density); Sd0: standard difference in control group; N0: sample size in control group; Mean1: mean in berberine group; Sd1: standard difference in berberine group; N1: sample size in berberine group; M: male; F: female; NR: non reported; TV: tumor volume; TW: tumor weight; VD: vessel density; BW: body weight.
Table 2: Quality assessment of eligible studies with ARRIVE checklist

|                                | Breast cancer | Liver cancer | Colon cancer | nasopharyngeal carcinoma | Lung cancer | Gastrointestinal cancer | Neuroepithelial tumor | Endometrial carcinoma | Esophageal cancer | Tongue carcinoma | Cholangiocarcinoma | Sarcoma | Total |
|--------------------------------|---------------|--------------|--------------|---------------------------|------------|-------------------------|----------------------|----------------------|------------------|------------------|-------------------|---------|-------|
|                                | n%            | n%           | n%           | n%                        | n%         | n%                      | n%                   | n%                   | n%               | n%               | n%                | n%      | n%    |
| Number of publication          | 7             | 3            | 3            | 2                         | 2          | 2                       | 2                    | 1                    | 1                | 1                | 1                  | 1       | 2     |
| Title                          | 10            | 3            | 10           | 3                         | 10         | 2                       | 10                   | 0                    | 2                | 0                | 1                  | 10      | 2     |
| Abstract                       | 10            | 3            | 10           | 3                         | 10         | 2                       | 10                   | 0                    | 2                | 0                | 1                  | 10      | 2     |
| Background                     | 43            | 2            | 67           | 3                         | 10         | 1                       | 50                   | 2                    | 0                | 0                | 1                  | 100     | 0     |
| Objectives                     | 10            | 3            | 10           | 3                         | 10         | 2                       | 10                   | 0                    | 2                | 0                | 1                  | 100     | 0     |
| Ethical statement              | 51            | 1            | 50           | 1                         | 50         | 1                       | 50                   | 1                    | 50               | 0                | 0                  | 1       | 100   |
| Study design                   | 71            | 1            | 33           | 2                         | 67         | 2                       | 100                  | 1                    | 50               | 0                | 0                  | 1       | 100   |
| Experimental procedures        | 86            | 10           | 3            | 10                        | 1          | 100                     | 1                    | 100                  | 1                | 100              | 1                  | 100     | 1     |
| Experimental animals           | 10            | 2            | 67           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |
| Housing and husbandry          | 86            | 1            | 33           | 2                         | 67         | 0                       | 0                    | 1                    | 50               | 1                | 50                | 0                  | 1       | 100   |
| Method                         | 4             | 1            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 50               | 1                | 50                | 0                  | 1       | 100   |
| Sample size                    | 57            | 3            | 10           | 1                         | 33         | 0                       | 0                    | 1                    | 50               | 1                | 50                | 0                  | 1       | 100   |
| Allocating animals to experimental groups | 57 | 2 | 67 | 3 | 10 | 0 | 0 | 1 | 50 | 1 | 50 | 0 | 1 | 10 | 0 | 5 |
| Experimental outcomes         | 86            | 10           | 3            | 10                        | 1          | 100                     | 1                    | 100                  | 1                | 100              | 1                  | 100     | 1     |
| Statistical methods            | 10            | 3            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |
| Baseline data                  | 10            | 3            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |
| Numbers analysed               | 86            | 3            | 1            | 10                        | 1          | 100                     | 1                    | 100                  | 1                | 100              | 1                  | 100     | 1     |
| Outcomes and estimation        | 10            | 3            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |
| Adverse events                 | 57            | 1            | 33           | 2                         | 67         | 0                       | 0                    | 1                    | 50               | 1                | 50                | 0                  | 0       | 0     |
| Discussion                     | 10            | 3            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |
| Interpretation/ scientific implications | 10 | 3 | 10 | 3 | 10 | 2 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 | 100 | 1 |
| Generalisability/y translation | 4             | 2            | 67           | 2                         | 67         | 1                       | 50                   | 2                    | 0                | 0                | 0                  | 1       | 100   |
| Funding                        | 71            | 3            | 10           | 3                         | 10         | 2                       | 100                  | 1                    | 100              | 1                | 100              | 1                  | 100     | 1     |

The colours indicate where the proportion of studies meeting that criteria are less than 25% (red), 25%–50% (pink), 50%–75% (light green) and more than 75% (green).
studies, the SMD values of all studies showed a statistically significant decreasing trend with increasing concentration of BBR (Pearson r = −0.6717, p < 0.0001).

**Tumor weight**
Of the 26 screened articles [10–35], 12 [12, 14, 16, 20, 23, 26–28, 32–35] reported the relationship between BBR and tumor weight in animals with breast cancer [12, 14, 16], colorectal cancer [20], nasopharyngeal carcinoma [23], lung cancer [26], gastric cancer [27, 28], esophageal cancer [32], and tongue cancer [33]. The SMD and the 95%CI in each study are shown in Fig. 5. The pooled SMD remained statistically significant in breast cancer(SMD = 3.71, 95% CI: 2.18, 5.25; Z = 4.74, p < 0.00001), lung cancer(SMD = 3.65, 95% CI: 1.86, 5.44; Z = 4.00, p < 0.0001), and gastric cancer(SMD = 1.90, 95% CI: 0.61, 3.20; Z = 2.88, p = 0.004). The pooled SMD remained statistically insignificant in colorectal cancer(SMD = −0.17, 95% CI: −0.71, 0.36; Z = 0.63, p = 0.53). For total studies, the pooled result suggested that the SMD was 2.35(95% CI: 1.51, 3.19) with statistical significance (Z = 5.50, p < 0.00001).

In view of the obvious heterogeneity($I^2 = 89\%$ for lung cancer), we conducted a subgroup analysis of different characteristics mainly on the following aspects: dose of BBR and cell lines(Fig. 6). In lung cancer, the dose of BBR was a potential influencing factor ($I^2$ decreased to 0, 57, and 0% in three subgroups respectively).

The dose–response relationship of different cancer types on the relationship between BBR and tumor weight of

| Study     | Std. Mean Difference | Random, 95%CI | Favours control group | Favours berberine group |
|-----------|----------------------|---------------|-----------------------|------------------------|
| 1. Breast cancer |                       |               |                       |                        |
| Ke Xu 2016 | 1.40                 | 0.52-2.27     |                       |                        |
| Sangmei Kim 2018 | 1.35            | −0.13-3.81 |                       |                        |
| Kalyani Chowdry Kamal 2017 | 4.35         | 1.94-6.75 |                       |                        |
| Kalyani Chowdry Kamal 2017 | 2.99        | 1.14-4.84 |                       |                        |
| Yuxiao Zhao 2017 | 3.98            | 2.78-5.23 |                       |                        |
| Subtotal (95% CI) | 3.32           | 1.28-5.36 |                       |                        |
| Heterogeneity: P = 80% |                     |               |                       |                        |
| 2. Liver cancer |                       |               |                       |                        |
| Guo-Yu Wang 2009a | 5.32          | 2.29-8.34 |                       |                        |
| Guo-Yu Wang 2009b | 5.92            | 2.96-8.87 |                       |                        |
| Jing Li 2015 | 5.02               | 3.11-6.92 |                       |                        |
| Chi-Min Tang 2015 | 18.61          | 9.28-23.94  |                       |                        |
| Subtotal (95% CI) | 7.36            | 3.45-11.27 |                       |                        |
| Heterogeneity: P = 81% |                     |               |                       |                        |
| 3. Colorectal cancer |                   |               |                       |                        |
| Hu Ruan 2017a | 0.56               | −0.61-1.72 |                       |                        |
| Hu Ruan 2017b | 0.17               | −0.96-1.31 |                       |                        |
| Yuchun Cai 2013a | 0.62            | −0.28-1.53 |                       |                        |
| Yuchun Cai 2013b | 0.76            | −0.13-1.70 |                       |                        |
| Yuchun Cai 2013c | 1.17             | 0.23-2.11 |                       |                        |
| Subtotal (95% CI) | 0.70             | 0.26-1.15 |                       |                        |
| Heterogeneity: P = 6% |                    |               |                       |                        |
| 4. Nasopharyngeal carcinoma |               |               |                       |                        |
| Chi-Min Tang 2013a | 2.53            | 0.64-4.42 |                       |                        |
| Chi-Min Tang 2013b | 2.96            | 0.72-5.24 |                       |                        |
| Chao Wang 2017 | 5.64               | 3.34-8.94 |                       |                        |
| Subtotal (95% CI) | 3.85            | 1.14-6.54 |                       |                        |
| Heterogeneity: P = 63% |                    |               |                       |                        |
| 5. Lung cancer |                   |               |                       |                        |
| Michael A. James 2011a | 1.06         | −0.33-3.45 |                       |                        |
| Michael A. James 2011b | 2.43            | −3.09-8.14 |                       |                        |
| Santosh K. Kalyari 2009a | 6.67           | 4.26-9.05 |                       |                        |
| Santosh K. Kalyari 2009b | 15.64          | 8.84-18.44 |                       |                        |
| Santosh K. Kalyari 2009c | 16.32          | 10.51-22.14 |                       |                        |
| Santosh K. Kalyari 2009d | 2.69            | 1.41-3.97 |                       |                        |
| Santosh K. Kalyari 2009e | 6.37            | 4.00-8.75 |                       |                        |
| Santosh K. Kalyari 2009f | 12.87           | 8.33-17.40 |                       |                        |
| Subtotal (95% CI) | 7.18            | 4.28-10.10 |                       |                        |
| Heterogeneity: P = 69% |                    |               |                       |                        |
| 6. Gastric cancer |                    |               |                       |                        |
| Hong-Li Li 2015 | 1.80               | 0.36-3.23 |                       |                        |
| Jun-Hung Wang 2016 | 6.47         | 4.02-8.93 |                       |                        |
| Subtotal (95% CI) | 3.03            | −1.01-7.08 |                       |                        |
| Heterogeneity: P = 48% |                    |               |                       |                        |
| 7. Neuroepithelial tumor |               |               |                       |                        |
| Juan Wang 2015 | 1.12               | 0.04-2.19 |                       |                        |
| Yuexue Sun 2018 | 2.42               | 0.53-3.90 |                       |                        |
| Subtotal (95% CI) | 1.66            | 0.41-2.92 |                       |                        |
| Heterogeneity: P = 48% |                    |               |                       |                        |
| 8. Endometrial cancer |                  |               |                       |                        |
| Yu Wang 2010a | 3.34               | 1.36-5.33 |                       |                        |
| Yu Wang 2010b | 6.56               | 3.16-9.96 |                       |                        |
| Subtotal (95% CI) | 4.66            | 1.50-7.84 |                       |                        |
| Heterogeneity: P = 81% |                    |               |                       |                        |
| 9. Esophageal cancer |                  |               |                       |                        |
| Kewei Ren 2016 | 2.59               | 0.88-4.51 |                       |                        |
| Total (95% CI) | 3.72            | 2.89-4.56 |                       |                        |
| Heterogeneity: P = 65% Test for overall effect: P = 0.00001 | | | | |

Fig. 2 Forest plot of the tumor volume
Fig. 3 Subgroup analyses of the tumor volume

Fig. 4 The dose–response relationship of BBR and tumor volume
animals is shown in Fig. 7. For single cancer types, a statistically significant linear relationship in lung cancer (Pearson $r = -0.9623$, $p = 0.0021$) was observed. For total studies, the SMD values of all studies showed a statistically significant decreasing trend with increasing concentration of BBR (Pearson $r = -0.7704$, $p < 0.0005$).

**Tumor vessel density**

Of the 26 screened articles [10–35], 3 [10, 15, 19] reported the relationship between BBR and tumor vessel density in animals with breast cancer [10, 15] and liver cancer [19]. The SMD and the 95% CI in each study are shown in Fig. 8. The pooled SMD remained statistically significant in breast cancer (SMD = 1.09, 95% CI: 0.37, 1.81; $Z = 2.96$, $p = 0.003$). For total studies, the pooled result suggested that the SMD was 4.29 (95% CI: 2.14, 6.44) with statistical significance ($Z = 3.92$, $p < 0.00001$). No statistical heterogeneity was observed ($I^2 = 0\%$ for breast cancer).

The dose–response relationship of different cancer types on the relationship between BBR and tumor weight of animals is shown in Fig. 9. For single cancer types, no linear relationship was concluded because of
the limited studies. For total studies, the SMD values of all studies showed no statistically significant trend (Pearson $r = -0.9866$, $p = 0.1044$).

**Body weight**

Of the 26 screened articles [10–35], 7 [11, 16, 17, 20, 22, 27, 35] reported the relationship between BBR and body weight in animals with breast cancer [11, 16], liver cancer [17], colorectal cancer [20, 22], gastric cancer [27], and sarcoma [35]. The SMD and the 95% CI in each study are shown in Fig. 10. The pooled SMD remained statistically significant in liver cancer (SMD = −2.18, 95% CI: −4.00, −0.36; $Z = 2.35$, $p = 0.02$). The pooled SMD remained statistically insignificant in breast cancer (SMD = 1.41, 95% CI: −0.38, 3.20; $Z = 1.54$, $p = 0.12$) and colorectal cancer (SMD = −0.14, 95% CI: −1.03, 0.75; $Z = 0.30$, $p = 0.76$). For total studies, the pooled result suggested that the SMD was 0.11 (95% CI: −0.70, 0.92) with statistical significance ($Z = 0.27$, $p = 0.78$).

In view of the obvious heterogeneity ($I^2 = 73\%$ for breast cancer; $I^2 = 80\%$ for colorectal cancer; $I^2 = 52\%$ for liver cancer), we conducted a subgroup analysis of different characteristics mainly on the following aspects: dose of BBR, administration, and cell lines (Fig. 11). $I^2$ were missing in breast cancer group and liver cancer group due to limited studies. No potential influencing factor was found in colorectal cancer group.

The dose–response relationship of different cancer types on the relationship between BBR and body weight of animals is shown in Fig. 12. For single cancer types, no statistically significant linear relationship was found. For total studies, the SMD values of all studies showed no statistically significant trend (Pearson $r = -0.1440$, $p = 0.7116$).

**Publication bias and sensitivity analysis**

The publication bias evaluation for the meta-analysis of tumor volume, tumor weight, tumor vessel density, and body weight is shown in Fig. 13. These funnel plots showed that most of the studies are in the upper part of the inverted funnel and approximately symmetrical, suggesting that the publication bias was unobvious.

A sensitivity analysis was performed to assess the stability of our results in terms of tumor volume, tumor weight, tumor vessel density, and body weight. The trim method was used, and the results did not show considerable changes between the previous and new SMDs (Fig. 14). Next, we deleted one individual study at a time, and the results of the rest of the studies were checked for any reversal. The statistical outcomes showed that the pooled SMDs were all still significant although one study was excluded (Fig. 15).

| Study          | Std. Mean Difference | Random, 95% CI | Favours control group | Favours berberine group |
|---------------|---------------------|----------------|-----------------------|------------------------|
| 1. Breast cancer | 0.65                | -0.07-1.77     |                       |                        |
| Elisa Pierpaoli 2015 | 1.45                | 0.30-2.59      |                       |                        |
| Elisa Damiani 2015  | 1.09                | 0.37-1.81      |                       |                        |
| Subtotal (95% CI) |                    |                |                       |                        |
| Heterogeneity: $I^2 = 0\%$ |              |                |                       |                        |
| 2. Liver cancer     | 4.29                | 2.14-6.44      |                       |                        |
| Chi Man Tsang 2015   | 1.90                | 0.36-3.44      |                       |                        |
| Total (95% CI)       |                    |                |                       |                        |
| Heterogeneity: $I^2 = 76\%$ Test for overall effect: $P = 0.02$ | | | | |
Among these included studies, a wide range of molecular targets, which are essential for the anti-cancer effect of BBR, was revealed. Except for three articles [15, 33, 35] that did not involve the discussion of molecular mechanisms, the remaining 23 articles [10–14, 16–32, 34] analyzed the anti-tumor mechanism of BBR. The pharmacological effects of BBR was summarized into five aspects: proliferation (including apoptosis, autophagy, cell cycle arrest, and others), intracellular oxidative stress, inflammation, angiogenesis, and migration. Table 3 shows how BBR works in different scenarios of multiple types of cancers. In addition, in order to understand the anticancer mechanism more clearly and deeply, Table 4 shows the clustering analysis of the common molecular pathways and target proteins between studies.

**Table 3**

| Study          | Std. Mean Difference | Random, 95% CI   | Favours berberine group | Favours control group |
|----------------|----------------------|------------------|-------------------------|-----------------------|
| 1. Breast cancer |                      |                  |                         |                       |
| Ke Su 2016     | 0.55                 | -0.61-1.72       |                         |                       |
| Yuwan Zhao 2017| 2.39                 | 0.91-3.87        |                         |                       |
| Subtotal (95% CI)| 1.41                | -3.39-3.20       |                         |                       |
| Heterogeneity: I² = 73% |                  |                  |                         |                       |
| 2. Liver cancer |                      |                  |                         |                       |
| Guan-Yu Wang 2009a | -3.27              | -5.35-1.19       |                         |                       |
| Guan-Yu Wang 2009b | -1.39              | -2.85-0.08       |                         |                       |
| Subtotal (95% CI)| -2.18               | -4.90-0.36       |                         |                       |
| Heterogeneity: I² = 52% |                  |                  |                         |                       |
| 3. Colorectal cancer |                 |                  |                         |                       |
| Norio 2002a    | -0.11                | -1.04-0.81       |                         |                       |
| Norio 2002b    | -2.54                | -3.86-1.22       |                         |                       |
| Norio 2002c    | -0.82                | -1.79-0.16       |                         |                       |
| Yuchen Cai 2013a | 0.48                | -0.41-1.37       |                         |                       |
| Yuchen Cai 2013b | 0.81                | -0.11-1.73       |                         |                       |
| Yuchen Cai 2013c | 0.94                | 0.03-1.85        |                         |                       |
| Subtotal (95% CI)| -0.14               | -1.03-0.75       |                         |                       |
| Heterogeneity: I² = 80% |                  |                  |                         |                       |
| 4. Gastric cancer |                   |                  |                         |                       |
| Junxiong Wang 2016 | 3.30                | -0.27-6.88       |                         |                       |
| 5. Sarcoma     |                      |                  |                         |                       |
| Lei Zhang 2012 | 1.83                 | 0.72-2.94        |                         |                       |
| Total (95% CI) | 0.11                 | -0.70-0.92       |                         |                       |
| Heterogeneity: I² = 82% Test for overall effect: P = 0.78 | | | | |

**Fig. 9** The dose–response relationship of BBR and tumor vessel density

**Fig. 10** Forest plot of the body weight
The most frequently studied pathways were on cell proliferation and 19 articles focused on this mechanism. Seven of these studies involved tumor cell apoptosis pathways (breast cancer [9, 12], liver cancer [17, 18], lung cancer [26], gastric cancer [27], esophageal cancer [32]), one involved autophagy pathways (neuroepithelial cancer [30]), and five involved cell cycle arrest pathways (colon cancer [22], lung cancer [25], gastric cancer [27], esophageal cancer [32], cholangiocarcinoma [34]). The second most common frequently studied pathways were on cell migration. Four articles in three cancers studied the relationship between BBR and tumor cell migration (breast cancer [13, 16], liver cancer [19], endometrial carcinoma [31]). There was only one study reported the relationship between BBR and intracellular oxidative stress (breast cancer [14]), inflammation (breast cancer [14]), and angiogenesis (liver cancer [19]) respectively.

Discussion

We performed a systematic review and meta-analysis to systematically evaluate the efficacy and adverse effect of BBR on various cancers. The results showed that BBR could inhibit the growth of a variety of cancers in vivo, especially in breast cancer (SMD = 3.32, 95% CI: 1.29, 5.36 in tumor volume; SMD = 3.71, 95% CI: 2.18, 5.25 in tumor weight; SMD = 1.09, 95% CI: 0.37, 1.81 in tumor vessel density) and lung cancer (SMD = 7.36, 95% CI: 3.45, 11.27 in tumor volume; SMD = 3.65, 95% CI: 1.86, 5.44 in tumor weight). Evidence for the benefit of BBR was not sufficient for gastric cancer (SMD = 1.47, 95% CI: −1.01, 7.08 in tumor volume) and colorectal cancer (SMD = −0.17, 95% CI: −0.71, 0.36 in tumor weight). BBR showed a dose–response relationship in tumor volume and weight (Pearson r = −0.6717 and −0.7704, with p < 0.0001 and p < 0.0005, respectively). At the same time, dose was an important influencing factor for heterogeneity from the subgroup analysis. The change in body weight of experimental animals was used as an indicator of the adverse effects of BBR. The above results indicated that no statistically significant difference was observed in terms of body weight under the effect of BBR (SMD = 0.11, 95% CI: −0.70, 0.92).

In the past 3 years, numerous studies have attempted to elucidate the relationship between BBR and breast/lung cancer. By using molecular modeling and in vitro studies, BBR significantly reduced EGFR and AKT phosphorylation and may be a useful alternative to lapatinib, an EGFR inhibitor which can cause acquired drug resistance in breast cancer patients [36]. BBR lowers blood sugar, increases insulin sensitivity, and corrects lipid metabolism disorders; it may reduce the incidence of breast cancer [37]. Single-drug BBR has an obvious inhibitory effect on lung cancer cells; BBR can inhibit doxorubicin (DOX)-mediated STAT3 activation and sensitize lung cancer cells to the cytotoxic effects of DOX treatment. Given the widespread clinical application of BBR and its low toxicity, our findings are important for the development of a new combination of BBR and DOX for the treatment of lung cancer [38]. In addition to medical treatment, BBR has protective effects on radiation-induced lung injury via intercellular adhesion molecular-1 and transforming growth factor-beta-1 in patients with lung cancer [39].
Fig. 13 Funnel plot of tumor volume studies, tumor weight studies, tumor vessel density studies, and body weight studies.

Fig. 14 Metatrim plot of tumor volume studies, tumor weight studies, tumor vessel density studies, and body weight studies.
Although, in the present study, the therapeutic effect of BBR in colorectal and gastric cancer required more evidence, numerous studies have confirmed the gain effect of BBR combined with chemotherapy in recent years. Latest research shows that the combination of the second generation Hsp90 inhibitor NVP-AUY922 and BBR therapy could inhibit a variety of oncogenic signaling pathways of colorectal cancer [40]. Another study showed that BBR as an adjunctive therapeutic agent could attenuate chemical resistance during gastric cancer treatment. The combination of 5-FU and BBR showed synergistic inhibition of survivin and STAT3 levels, thereby enhancing the death of gastric cancer cells [41]. In addition to the 5-FU-based chemotherapy regimen, BBR treatment reduced cisplatin resistance in gastric cancer cells by modulating the miR-203/Bcl-w apoptotic axis [42].

In the present study, body weight index was used to evaluate the growth of experimental animals to indirectly evaluate the adverse effects of BBR. However, studies have shown that BBR could induce weight loss in rodents [43, 44] and humans [45, 46]. In recent years, research has reported that BBR affected body weight by upregulating AMPK and UCP3 expression to control energy expenditure [47]. Therefore, the toxic side effects of BBR cannot be objectively and accurately evaluated by the change of body weight alone.

**Limitations**

There were some limitations to our analysis that deserve discussion. First, we observed considerable heterogeneity between the studies when analyzing tumor volume, tumor weight, and body weight. Although subgroup analysis (Figs. 3, 5, and 11) was performed, some $I^2$ were missing because of the limited studies. Secondly, generally speaking, obviously significant publication bias was not found based on the funnel plot (Fig. 13). However, poor symmetry of the funnel plot on tumor volume suggested more high-quality researches should be included. Thirdly, although PubMed, Embase, Springer, and Cochrane databases had been carefully and comprehensively searched, articles selected for each cancer type were still small which could lead to bias. Fourthly, the anticancer effects of berberine in humans were not identified clearly and further studies in humans were needed to develop it as an anticancer agent.
### Table 3 Molecular pathways and proteins in different cancers

| Molecular Pathway                        | Proteins                                                                 | Functional clustering                               |
|----------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------|
| Breast cancer                          |                                                                           |                                                     |
| ↑ caspase-9/cytochrome c-mediated apoptosis [11]; TRAIL(TNF-related apoptosis-inducing ligand)-mediated apoptosis [12] | ↑ caspase-3 [11, 12]; caspase-9, CIC-C, Bax, Ligase-4 [11]; PARP, PS3 [12] | Proliferation(including apoptosis)                  |
| ↓ cell proliferation [14]              | ↓ Bcl-2 [11]; P65, Mcl-1 [12]; PCNA [14]                                   |                                                     |
| ↓ intracellular reactive oxygen species (ROS) levels [14] | ↑ MDA [14]                                                               | Intra cellular oxidative stress                      |
| ↓ inflammation [14]                    | ↓ SOD, CAT, GSH, Vit-C [14]                                                |                                                     |
| ↓ TGF-β1-induced cell migration [13]; vasodilator-stimulated phosphoprotein (VASP)-induced cell migration [16] | ↓ IL-1β, IL-6, TNF-α, NF-κB [14]                                         | Migration                                            |
|                                        |                                                                           |                                                     |
| Liver cancer                           |                                                                           |                                                     |
| ↑ Fas-mediated apoptosis [17]          | ↑ Fas, PS3, caspase-3, caspase-8, caspase-9 [17]                           | Proliferation(including apoptosis)                  |
| ↓ arachidonic acid metabolic pathway [18]; Id-1-induced cell proliferation [19] | ↓ PGE2, cPLA2, COX-2 [18]; Id-1 [19]                                       |                                                     |
| ↓ Id-1-induced angiogenesis [19]       | ↓ Id-1, VEGF, HIF-1α [19]                                                  | Angiogenesis                                         |
| ↓ Id-1-induced migration [19]          | ↓ Id-1 [19]                                                              |                                                     |
| Colon cancer                           |                                                                           |                                                     |
| ↓ β-catenin - induced proliferation by binding RXR [21]; cell proliferation by inducing the G2/M phase arrest and down-regulated the expression of the related cyclins [22] | ↑ c-Cbl, p21^WAF1/CIP1[^21]                                                 | Proliferation(including cell cycle arrest)          |
|                                        | ↓ Cdc2 [21, 22]; PCNA, β-catenin, Ki-67, c-myc, RXRα [21]; cyclin B1, cdc25C [22] |                                                     |
| Nasopharyngeal carcinoma               |                                                                           |                                                     |
| ↓ cell proliferation via an Epstein-Barr virus nuclear antigen 1(EBNA1)-dependent mechanism [23]; cell proliferation by inhibiting STAT3 activation [24] | ↑ Cleaved PARP [24] | Proliferation                                      |
|                                        | ↓ Mcl-1, p-STAT3 [23, 24]; EBNA1 [23]                                       |                                                     |
| Lung cancer                            |                                                                           |                                                     |
| ↑ G1 cell cycle arrest [25]; PS3-Induced growth inhibition and apoptosis [26] | ↑ PS3 [25, 26]; Bax, Bak, caspase-3 [26]                                  | Proliferation(including apoptosis and cell cycle arrest) |
| ↓ cell proliferation via MAPK pathways [25] | ↓ p-Akt, p-CREB, p-MAPK, cyclin B1 [25]; Bcl-2, Bcl-xL [26]             |                                                     |
| Gastric cancer                         |                                                                           |                                                     |
| ↑ apoptosis and cell cycle arrest via inhibiting EGFR signaling [27] | ↓ pERK [27, 28]; pAKT, pSTAT3, pNF-kB, NF-kB, Bcl-xL, cyclin D1 [27]; p-F38 MAPK, p-JNK, IL-8 [28] | Proliferation(including apoptosis and cell cycle arrest) |
| ↓ cell proliferation via MAPK pathways [28] |                                                                           |                                                     |
| Neuroepithelial cancer                 |                                                                           |                                                     |
| ↑ ERK1/2-mediated impairment of mitochondrial aerobic respiration and autophagy [30] | ↑ C-parp-1, LC3II [30]                                                     | Proliferation(including autophagy)                   |
| ↓ cancer growth by suppressing Hedgehog signaling pathway [29] | ↓ Gli1, PTH1 [29]; Ki-67, p-ERK1/2 [30]                                     |                                                     |
| Endometrial carcinoma                  |                                                                           |                                                     |
| ↓ cell growth via miR-101/COX-2 [31]   | ↓ COX-2, PGE2 [31]                                                       | Proliferation                                       |
| ↓ cell metastasis via miR-101/COX-2 [31] | ↓ COX-2, PGE2 [31]                                                       | Migration                                           |
| Esophageal cancer                      |                                                                           |                                                     |
| ↑ cell growth inhibition, apoptosis and cell cycle arrest at G2/M phase [32] | ↑ P21, P27, PS3, cleaved-PARP, caspase-3, Bax [32]                  | Proliferation(including apoptosis and cell cycle arrest) |
|                                        | ↓ PI3K, Rac, p-JAK2, p-STAT3, Wnt3a, β-catenin, Bcl-2, Mcl-1, XIAP, Ki-67, cyclin B, cyclin D, cyclin E, CDK1, CDK2, CDK4, CDK6 [32] |                                                     |
| Cholangiocarcinoma                     |                                                                           |                                                     |
| ↑ G1 cell cycle arrest [34]            | ↓ PCNA, cyclin D1, cyclin E [34]                                          | Proliferation(including cell cycle arrest)          |
| ↓ cell proliferation [34]              |                                                                           |                                                     |
Conclusion

BBR exerted anti-tumor effects in a variety of tumors in vivo, especially for breast cancer and lung cancer. However, evidence was still insufficient in colorectal cancer and gastric cancer. One of its anti-tumor mechanisms was anti-angiogenesis. There was a dose-response relationship in the anti-tumor effects.

Abbreviations

BBR: Berberine; BW: Body Weight; CI: Confidence Interval; DMSO: Dimethyl Sulfoxide; EBNA1: Epstein-Barr virus nuclear antigen 1; ROS: Reactive Oxygen Species; SD: Standard Difference; Standard Mean Difference; TRAIL: TNF-related apoptosis-inducing ligand; TV: Tumor Volume; TW: Tumor Weight; VASP: Vasodilator-Stimulated Phosphoprotein; VD: Vessel Density

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Authors’ contributions

XJH and ZYS designed this study. XJH, LYM, NLW and YXY collected and analyzed the data. XJH, WRH and YN drafted the manuscript. XJH, TJL and ZYS interpreted the data and revised the manuscript. All authors have read and approved the manuscript.

Table 4 Cluster analysis of molecular pathways and proteins in different cancers

| Functional clustering | Molecular Pathway | Proteins |
|----------------------|------------------|----------|
| Proliferation(apoptosis) | Breast cancer: ↑ caspase-9/cytochrome c-mediated apoptosis [11]; TRAIL(TNF-related apoptosis-inducing ligand)-mediated apoptosis [12] | ↓ ERK1/2-mediated impairment of mitochondrial aerobic respiration and autophagy [30] |
| Proliferation | Colon cancer: ↓ cell proliferation by inducing the G2/M phase arrest and down-regulated the expression of the related cyclins [22] | ↓ cell proliferation via an ERK1/2-mediated impairment of mitochondrial aerobic respiration and autophagy [30] |
| Proliferation(others) | Breast cancer: ↓ cell proliferation [14] | ↑ C-parp-1, LC3II [30] |
| Intracellular oxidative stress | Breast cancer: ↑ intracellular reactive oxygen species (ROS) levels [14] | ↑ MDA [14] |
| Inflammation | Breast cancer: ↓ inflammation [14] | ↓ IL-1β, IL-6, TNF-α, NF-κB [14] |
| Angiogenesis | Liver cancer: ↓ Id-1-induced angiogenesis [19] | ↓ Id-1, VEGF, HIF-1α [19] |
| Migration | Breast cancer: ↓ TGF-β1-induced cell migration [13]; vasodilator-stimulated phosphoprotein (VASP)-induced cell migration [16] | ↓ TGF-β1, MMP-2, MMP-9 [13]; Id-1 [19]; COX-2, PGE2 [31] |
| No effect: | Endometrial carcinoma: ↓ cell metastasis via miR-101/COX-2 [31] | No effect: VASP [16] |

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All data generated or analysed during this study are included in this published article.

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Competing interests
The authors declare that they have no competing interests.

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