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REVIEW

Transforming Cancer Treatment with Nanotechnology: The Role of Berberine as a Star Natural Compound

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Abstract: Berberine (BBR), recognized as an oncotherapeutic phytochemical, exhibits its anti-cancer properties via multiple molecular pathways. However, its clinical application is hindered by suboptimal tumor accumulation, rapid systemic elimination, and diminished bioactive concentration owing to extensive metabolic degradation. To circumvent these limitations, the strategic employment of nanocarriers and other drugs in combination with BBR is emerging as a focus to potentiate its anti-cancer efficacy. This review introduced the expansive spectrum of BBR's anti-cancer activities, BBR and other drugs co-loaded nanocarriers for anti-cancer treatments, and evaluated the synergistic augmentation of these amalgamated modalities. The aim is to provide an overview of BBR for cancer treatment based on nano-delivery. Berberine (BBR), recognized as an oncotherapeutic phytochemical, exhibits its anti-cancer properties via multiple molecular pathways. However, its clinical application is hindered by suboptimal tumor accumulation, rapid systemic elimination, and diminished bioactive concentration owing to extensive metabolic degradation. To circumvent these limitations, the strategic employment of nanocarriers and other drugs in combination with BBR is emerging as a focus to potentiate its anti-cancer efficacy. Nano-delivery systems increase drug concentration at the tumor site by improving pharmacological activity and tissue distribution, enhancing drug bioavailability. Organic nanocarriers have advantages for berberine delivery including biocompatibility, encapsulation, and controlled release of the drug. While the advantages of inorganic nanocarriers for berberine delivery mainly lie in their efficient loading ability of the drug and their slow release ability of the drug. This review introduced the expansive spectrum of BBR's anti-cancer activities, BBR and other drugs co-loaded nanocarriers for anti-cancer treatments, and evaluated the synergistic augmentation of these amalgamated modalities. The aim is to provide an overview of BBR for cancer treatment based on nano-delivery.

Keywords: berberine, anti-cancer, nano drug delivery system, combination therapy

Introduction

According to the latest epidemiological data from the World Health Organization in 2022, cancer remains a predominant etiological factor in global mortality, contributing to over 20 million fatalities over a decade. Confronting this oncological crisis, the United States Food and Drug Administration has authorized a variety of therapeutic agents, including tyrosine kinase inhibitors, angiogenesis inhibitors, oncolytic virotherapy, and monoclonal antibodies. However, the clinical deployment of these pharmacotherapies is fraught with challenges, such as multidrug resistance and systemic toxicities, which impede their therapeutic efficacy.[1,2] Emerging researches have proven that bioactive compounds of natural origin are gaining recognition as efficacious alternatives, offering substantial therapeutic benefits against various cancers.

Berberine (BBR), an isoquinoline alkaloid extracted from Chinese herbal medicine such as *Phellodendron chinense* Schneid., *Coptidis chinensis* Franch., and *Mahonia bealei* (Fort). Carr.,[3] has been acknowledged for its pharmacological efficacy over thousands of years. Historically exploited for heat-clearing and detoxicating, *Coptis chinensis* has been crucial in

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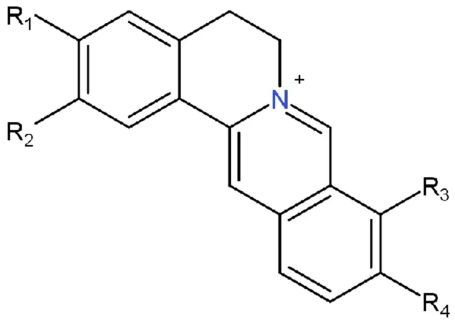
phytotherapy. Modern oncological researches elucidate that BBR exerts a profound inhibitory impact on tumorigenesis through mechanisms including anti-inflammation, anti-angiogenesis, anti-metastasis and anti-invasion, induction of apoptosis and autophagy, and reversal of drug resistance.[2] Although the efficacy of BBR has been acknowledged, its clinical application is hindered by several pharmacokinetic limitations, including low concentration at the site of action, short retention time in vivo, and poor absorption which is related to the extensive metabolism BBR after oral administration, leading to extremely low plasma concentrations.[4–6] To surmount these obstacles, scientific investigators have been relentlessly pursuing novel methodologies. These methodologies include the co-administration of BBR with other pharmaceutical agents to potentiate its therapeutic effect and the employment of advanced nanocarrier to facilitate targeted oncologic interventions, thereby amplifying the cytotoxic impact on malignant cells with heightened specificity and efficacy.

While previous reviews have discussed different types of BBR loaded nanocarriers and the combination of BBR with other drugs, they have not thoroughly explored the efficacy and mechanisms of each nano drug delivery system (NDDS). Our review provided a comprehensive overview of the application of BBR-based NDDSs for cancer treatment, analyzed and compared the efficacy of different nanocarriers in improving the therapeutic outcomes of BBR in cancer therapy, and also elaborated on the pharmacological mechanisms of these delivery systems. By focusing on the combination therapy of BBR and other drugs' co-loaded nano-delivery systems, we aim to provide valuable insights into the potential of these co-delivery systems in improving cancer therapy.

Chemical Properties of BBR

Berberine (BBR), a benzyl tetraisoquinoline alkaloid, is a 5.6-dihydro-dibenzo [a,g] quinolizinium derivative with the chemical formula $C_{20}H_{18}NO_4^+$ (Figure 1). In vivo, BBR undergoes rapid metabolism, resulting in the formation of more than 20 metabolites in the body. These metabolites include berberrubin, thalifendine, demethyleneberberine, dihydroberberine (dhBBR), columbamine, and jatrorrhizine (Figure 1). Interestingly, some of these metabolites have been found to exhibit similar pharmacological effects to BBR:[3] For example, demethyleneberberine, which is a Phase I metabolite of BBR, has been shown to possess mitochondrial-targeted antioxidant and anti-inflammatory effects. It has also demonstrated potential in the treatment of autoimmune hepatitis;[7] Berberrubine has been shown to exhibit cholesterol-lowering and blood lipid-lowering effects by upregulating the expression of low-density lipoprotein receptor (LDLR) in HepG2 cells;[8] Some other metabolites of BBR such as jatrorrhizine and columbamine have also been proved to produce similar pharmacological actions to BBR.[4] However, the extremely poor bioavailability of BBR and its metabolites remains a major obstacle to their efficacy in vivo. Studies have shown that after a single oral administration of 400 mg of BBR, the maximum concentration (Cmax) of BBR in human plasma was determined to be 0.4 ng/mL. Furthermore, studies in rats had oral bioavailability of BBR to be less than 1%, with values ranging from 0.36% to 0.68%.[9,10] The low oral bioavailability of BBR can be attributed to various factors, including its extensive metabolism and interaction with efflux transporters. The first-pass clearance of BBR primarily occurs



	R1	R2	R3	R4
1.Berberine	-OCH2O-		OCH_3	OCH_3
2.Berberrubin	-OCH2O-		OH	OCH_3
3.Thalifendine	-OCH2O-		OCH_3	OH
4.Dihydroberberine	-OCH2O-		OCH3	OCH3
5.Demethyleneberberine	OH	OH	OCH3	OCH3
6.Jatrorrhizine	OH	OCH_3	OCH3	OCH3
7.Columbamine	OCH_3	OH	OCH3	OCH3

Figure 1 Chemical structures of BBR and its main metabolites.

in the small intestine, and the liver is the main site of its tissue distribution. UDP-glucuronosyltransferase has been identified as the major drug-metabolizing enzyme responsible for the formation of Phase II metabolites of BBR. After oral administration, BBR undergoes oxidative demethylation (M1 generation) followed by glucuronidation as the major mechanisms of intestinal metabolism. Furthermore, the co-ingestion of quinine, which is an inhibitor of P-glycoprotein (Pg-P) and organic cation transporters (OCT), has been found to significantly reduce the biliary excretion of unbound BBR. This suggests that efflux transporters expressed in the liver, such as Pg-P, may play a crucial role in the biliary excretion of BBR. Additionally, efflux transporters involved in the excretion of BBR into the intestinal lumen, bile, and urine may also contribute to its in vivo metabolism, which further hinders the absorption of BBR and leads to low exposure after oral administration. In summary, the intestinal first-pass elimination of BBR, its interaction with Pg-P pump, and its high distribution in the liver are the major obstacles to its oral bioavailability.[10]

Overview of Anti-Cancer Properties and the Involved Mechanisms of BBR

BBR can significantly inhibit the growth rate of various tumors such as gastric cancer, liver cancer and colon cancer. BBR has been reported to possesses various biological antitumor activities, including anti-inflammation, inhibition of angiogenesis, inhibition of metastasis and invasion, induction of apoptosis and autophagy, and reversal of drug resistance.[2] The mechanisms have been summarized below (Figure 2).

Anti-Inflammation

Inflammation plays a crucial role in cancer development and progression. It is now recognized as one of the hallmarks of cancer. Inflammation in cancer involves intricate crosstalk between malignant cells and nonmalignant cells present in the tumor microenvironment. Various mediators, such as cytokines, chemokines, and prostaglandins, participate in this crosstalk. These mediators are secreted by both malignant and nonmalignant cells and act in an autocrine and paracrine manner. They create a pro-inflammatory environment that facilitates tumor growth, survival, angiogenesis, and metastasis. The inflammatory tumor microenvironment is characterized by an influx of immune cells, including macrophages, neutrophils, and lymphocytes. These immune cells release inflammatory mediators that promote tumor cell proliferation, survival, and migration. Additionally, the inflammatory environment can cause genetic alterations, leading to further

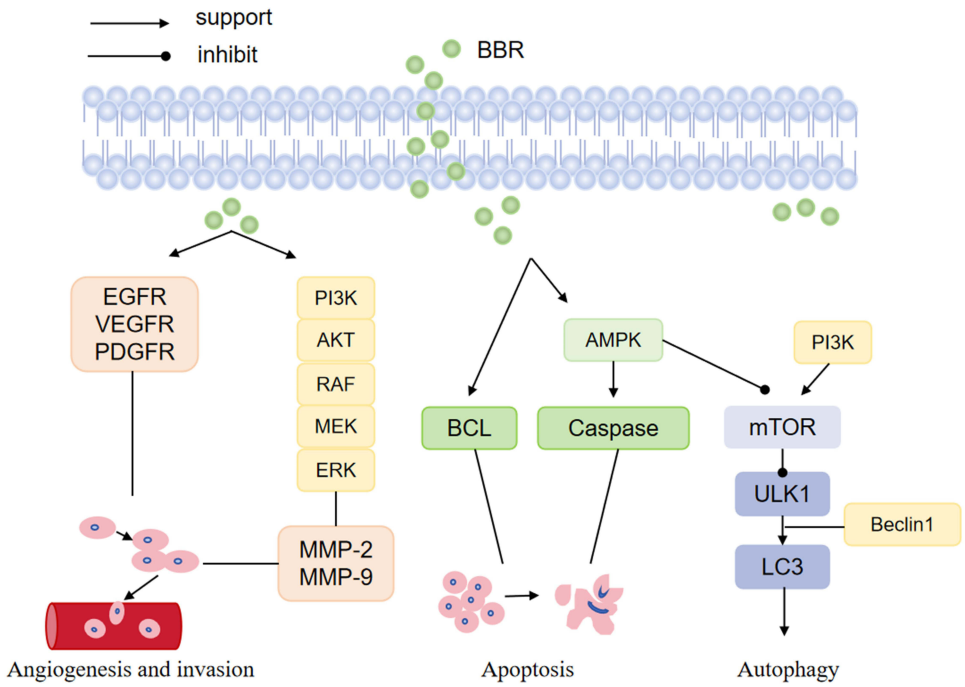


Figure 2 Anti-cancer Mechanisms of BBR.

tumor progression. The activation of various signaling pathways, such as NF-κB, STAT3, and COX-2, is commonly observed in cancer-associated inflammation. These pathways regulate the expression of genes involved in cell proliferation, angiogenesis, and immune evasion.[11] By regulating signaling pathways such as NF-κB, MAPK, and PPAR, BBR can inhibit the transcription of genes encoding inflammatory factors such as IL-1, IL-6, IL-8, IL-17, and TNF-α. These factors are known to drive inflammation and contribute to tumor progression. By reducing the levels of inflammatory proteins, such as IL-1, IL-6, and TNF-α, BBR can create an environment that is less favorable for tumor growth and survival.[12] In 2019, Luo et al[13] conducted a study using BBR to treat Hepatocellular carcinoma (HCC) by modulating inflammatory cytokines involved in the p38MAPK/ERK-COX2 pathway. The results of the study demonstrated that BBR inhibited the high expression of CD68 and F4/80 proteins, effectively reducing the proliferation of HCC cells.

Inhibition of Angiogenesis

Angiogenesis is typically initiated from the capillaries and plays an important part in tumor growth, maintenance and metastasis. Matrix metalloproteases (MMPs) play a crucial role in tumor blood vessel formation, with MMP-2 promoting endothelial cell migration and MMP-9 and other metalloproteinases promoting vascular endothelial growth factor (VEGF) expression.[14,15] In a study by Hamsa, BBR was applied to B16F-10 cells, and the results showed that BBR could inhibit cancer cell metastasis by interfering with angiogenesis mediated by MMP-2 and MMP-9. Additionally, BBR has demonstrated effectiveness in various cancer cell types by suppressing the activity and expression of transcription factors, such as VEGF, thereby reducing cancer cell proliferation.[16–18]

Inhibition of Metastasis and Invasion

Malignant tumors are characterized by the ability of cancer cells to invade and metastasize to other tissues or organs in the body. This process involves the migration and invasion of cancer cells through the blood or lymphatic system, leading to the formation of secondary tumors.[19] The extracellular matrix (ECM) is a well-known cell motility disorder, and when MMPs invade, MMPs causes ECM degradation and drugs can also more readily permeate into the tumor site.[20] In the context of gastric cancer, a study demonstrated that BBR can inhibit the migration and invasion of AGS and SGC7901 cancer cells by downregulating MMP-3, which is an inducer of epithelial-mesenchymal transition (EMT)[21].Another study by Kim et al found that BBR could inhibit the expression of MMP-1 and MMP-9, which are involved in breast cancer metastasis and invasion induced by TPA (a tumor promoter), by inhibiting PKC-α in breast cancer cells.[22]

Apoptosis

In tumor cells, BBR can induce cell death by activating both the intrinsic and extrinsic pathways of apoptosis.[23] The activation of the extrinsic apoptotic pathway is due to the induction of death receptors (such as Fas) and subsequent cleavage of caspase-8, −9, and −3. In addition, BBR can contribute to the generation of reactive oxygen species (ROS), leading to protein activation in response to ER stress. Furthermore, BBR can alter the mitochondrial membrane potential, stimulating the release of cytochrome c from mitochondria and inhibiting the expression of anti-apoptotic proteins such as BCL-2 and BCL-XL. The release of cytochrome c triggers the activation of caspases, ultimately leading to apoptosis.[24] Yip et al proposed that BBR is capable of inducing apoptosis in liver cancer cells by activating procaspase-9 and its effector caspases, procaspase-3 and procaspase-7.[10,25] Similarly, in a study on non-small cell lung cancer (NSCLC), BBR was found to induce apoptosis in NSCLC cells.[26]

Autophagy

Autophagy is a highly conserved mechanism, which can either serve as a form of programmed cell death type II (PCD II) or as a means of cell survival under certain conditions. In this lysosomal degradation system, proteins that are weak or aged, as well as cellular organelles, are enclosed in a double-membrane vesicle known as the autophagosome. Under normal physiological conditions, these components are degraded by lysosomal hydrolysis enzymes to maintain cellular homeostasis. Numerous studies have shown that increased autophagy is associated with improved tumor survival and growth in pathological states, indicating that tumor cells can evade apoptosis through the regulation of autophagy.[27] In the case of BBR, it has been found to inhibit autophagosome formation in MCF-7/ADR cells by modulating the PTEN/Akt/mTOR signaling pathway.[28] Another study demonstrated that BBR can induce autophagic cell death, exerting its

anti-cancer effects by downregulating the PI3K/Akt/mTOR signaling pathway and upregulating ROS-mediated mitochondrial dysfunction in hepatocellular carcinoma Hep3B cells.[29]

Reversal of Drug Resistance

Resistance to chemotherapy drugs is a significant factor contributing to the failure of tumor chemotherapy. Tumors possess various capabilities, such as high fitness, activation of survival pathways, inactivation of death signaling pathways, and evasion of growth suppressors.[30] Research has suggested that BBR has the potential to reverse drug resistance in cancer therapy, implying that BBR may be able to overcome the resistance developed by tumors against certain chemotherapy drugs, thereby enhancing the effectiveness of the treatment. Zhang et al discovered that BBR had the ability to reverse lapatinib resistance in HER2-positive breast cancer cells. This was attributed to the fact that lapatinib activates the c-Myc/ pro-Nrf2 pathway and GSK-3β signaling, which stabilize Nrf2 and maintain low levels of ROS in resistant cells. However, BBR was able to counteract lapatinib resistance by downregulating c-Myc, thereby disrupting the balance of ROS.[31] What's more, BBR could reverse MDR of DOX in breast cancer by inhibiting the efflux function of ATP-binding cassette transporters and downregulating their expression levels.[32] Another study has further confirmed that BBR, functioning as a MET inhibitor, could effectively overcome acquired resistance to osimertinib caused by MET amplification.[33]

Nano-Scale Drug Delivery Systems for BBR

Despite the promising biological effects, BBR is characterized by a low local concentration, short retention time, and poor absorption. Its oral bioavailability is less than 5% in plasma.[5] The rapid clearance of insoluble drugs necessitates large doses, which in turn leads to their wide distribution in non-targeted organs. This distribution is neither beneficial nor economical. Furthermore, increasing the doses of BBR as a single agent would result in unusually high toxicity.

Currently, various types of nano-scale drug delivery systems have been utilized in numerous studies to enhance the efficacy of BBR. These systems include organic systems such as lipid-based NPs, dendrimers, nanoemulsions, nanosuspensions, micelles. In addition, inorganic systems like mesoporous silica NPs, metal NPs, crystalline NPs, heterocyclic aromatic cations, carbon nanotubes have also been employed in BBR delivery, as shown in Figure 3.

Compared to traditional molecular treatment methods of cancer drugs, NDDS offer several advantages. Firstly, these nanodelivery systems can greatly enhance the bioavailability of BBR in anti-cancer treatment. They achieve this by improving pharmacological activity and tissue distribution, as well as preventing physical and chemical degradation both in vivo and in vitro.[34] Secondly, NDDS can increase the concentration of the drug at the tumor site, thereby improving the therapeutic index and reducing adverse reactions. This is possible due to the high permeability and the enhanced permeability and retention (EPR) effect and active targeting, which collectively enhance the treatment efficacy.[35]

Notably, BBR can form nanomedicines with various chemotherapeutic drugs (eg, adriamycin and indomethacin) through intermolecular interactions, which improves the distribution of the drugs in tumors and enhances tumor-killing effects,[36] and there are several studies that take advantage of this feature, with some researchers self-assembling BBR and other drugs to form nano-formulations to exert anticancer effects. In 2020, Cheng et al[37] combined chemotherapeutic and photothermal treatments by combining Camptothecin (CPT) and BBR via a GSH-reactive disulfide bond (CPT-ss-BBR), and then co-assembled with the photosensitizer indocyanine green (ICG) to form nanomedicines (CPT-ss-BBR/ICG NPs).BBR enabled the formulations to have excellent mitochondrial targeting properties and time-dependent uptake properties. The formulation can specifically target the mitochondria of cancer cells and induce rapid photothermal conversion, high levels of ROS, and substantial loss of $\Delta\Psi$ upon NIR irradiation treatment. Thus, the nanomedicine exhibited potent inhibitory effects on A549 cells in the presence of light compared to CPT. And in 2022, the group continued to study berberine self-assembly by inducing glucose oxidase (GOD) self-assembly with ferrocene-berberine coupling (FC-BBR) and indomethacin (IND)), which was then encapsulated by hyaluronic acid (HA) and formed nanopharmaceuticals (FC-BBR/IND@GOD@HA NPs). The prepared nano-assemblies inhibited the proliferation and induced apoptosis in HepG2 cells, which may be the result of targeted chemodynamic therapy and starvation therapy. In addition, FC-BBR/IND@GOD@HA NPs could promote the production of reactive oxygen species and the loss of mitochondrial membrane potential, and block S-phase cells. More importantly, it can inhibit the movement and migration of cancer cells, thus potentially preventing tumor metastasis.[38]

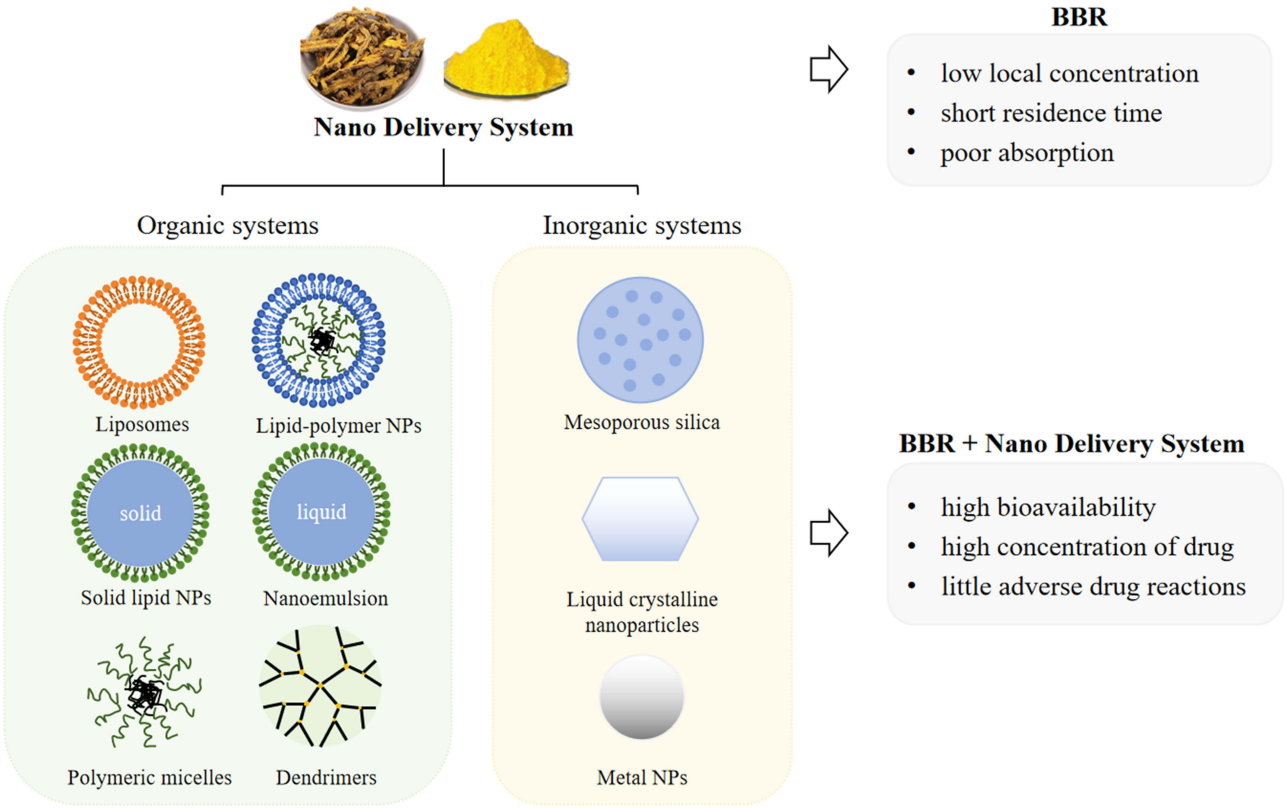


Figure 3 Nano-Scale Drug Delivery Systems for BBR.

Organic Systems

Lipid-Based NPs

Among various types of nanodelivery systems, the lipid-based nanocarriers have been extensively studied. As a nanocarrier, liposomes have good encapsulation rate and stability, which can effectively control drug release and improve drug bioavailability. Compared to other nanosystems, such as inorganic nanoparticles, lipid-based nanopreparations have better cell compatibility and less toxicity to cells. Studies have shown that the hydrophobic core of the lipid-based nanocarrier can significantly enhance the solubility of drugs. Additionally, its small size enables improved permeability for drug delivery.[5] However, liposomes require biological materials such as liposomes phospholipids during production, which is relatively costly. In 2013, Ling et al[39] developed a liposome formulation of BBR with 5 mol% polyethylene glycol (PEG) using the thin-film hydration/extrusion method. The liposome exhibited a high encapsulation efficiency up to 14%. In vitro studies demonstrated that the growth inhibition effect of the BBR liposome on HepG2 cells was 2.5 times higher compared to the BBR solution. The IC_{50} of BBR liposome was only 1.67 μg/mL, indicating a potent inhibitory effect. This effect was attributed to the activation of the caspase/mitochondrial pathway.

Lipid-polymer nanoparticles (LPNPs) are a type of lipid-based nanocarrier that comprises a polymer core and a biocompatible lipid shell. Compared to general liposomes, LPNPs offer improved stability of drug release and a longer half-life. In a study conducted by Song et al, PEG and HA were utilized to double decorate BBR derivatives, resulting in enhanced drug loading, reduced carrier toxicity, and significant anti-tumor efficacy. The LPNPs specifically targeted the mitochondria of tumor cells and were tested on the human pulmonary adenocarcinoma cell line A549.[40]

While polymeric micelles are nanocarriers with a nuclear-shell structure formed through the self-assembly of amphipathic copolymers in an aqueous medium, they offer advantages such as simple preparation, stable processing, and small particle size. However, an independent study has found that the cytotoxicity of lipid-based polymer materials is significantly lower than that of polymeric micelles. This finding is worth considering when using these materials.[41] In a study conducted by Shen et al,[5] they investigated stealth amphiphilic micelles of polymeric phospholipid conjugates as a

promising strategy to improve BBR's delivery to tumors. Specifically, physically and chemically stable mixed micelles formulated from 1.2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (PEG -PE) and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (PEG-succinate ester of vitamin E) in a 3:1 ratio, increased the solubility of BBR by 300% and significantly amplified cell apoptosis and overall cytocidal efficacy in human prostate cancer monolayer (concentration values of 16 to 18 times lower than that of free BBR).

Dendrimers

Dendritic macromolecules have hyperbranched structures that allow them to encapsulate small hydrophobic drug molecules in the polymer voids through non-covalent forces (electrostatic interactions, hydrophobic interactions, and hydrogen bonding), as well as to attach drugs to polymer chains through covalent bonding interactions with the surface of the dendritic macromolecules.[42] Dendrimers have also been shown to enhance transdermal permeation and enable specific drug targeting by attaching targeting ligands and imaging molecules.[43] The toxicity of dendrimers varies depending on their generation and the surface properties conferred by the end-functional groups.[44] Generally, cationic amine dendrimers exhibit stronger cytotoxicity compared to negative ones. In a study conducted by Gupta et al[45] in 2017, BBR was delivered using PAMAM dendrimers to treat breast cancer, resulting in significantly improved anti-cancer activity. The PAMAM-BBR conjugate exhibited a much longer reaction half-time of 14.33 hours compared to 6.7 hours for free BBR.

Inorganic Systems

Mesoporous Silica NPs

Mesoporous silica nanoparticles (MSNs) are a type of orderly mesoporous material prepared using methods such as sol-gel and template methods.[46] This material possesses characteristics such as good biocompatibility, high specific surface area, controllable size, and degradation. MSNs can actively target tumors and facilitate drug delivery by utilizing different internal or external stimuli, such as the local pH conditions, enzymes in the peripheral tissues, or oxidation-reduction potential. External stimuli like a magnetic field or light can also be employed.[47] Lin et al[48] pre-loaded BBR into a folate-targeted Janus gold mesoporous silica nanocarrier (FA-JGMSNs) to overcome the poor bioavailability of BBR. BBR could significantly reduce the organic damage caused by radiation, and using high-Z nanomaterials as a radiosensitizer could effectively inhibit the transmission of ionizing radiation at the tumor site and achieve a higher radiotherapy effect. FA-JGMSNs could not only be used as a radiosensitizer to enhance the radiotherapy effects but also could be used as a photothermal agent to complement the chemoradiotherapy effect through topical photothermal therapy. In vitro and in vivo results demonstrated that the nanoplatform exhibited efficient antitumor effects, good biological safety, and effective protection against normal tissues. Yue et al[49] prepared disulfide bond (S-S) -bridging mesoporous silica nanoparticles (ss-MONs), which showed efficient biodegradability under glutathione conditions and promoted BBR release by breaking the disulfide bond in ss-MONs. CM-ss-MONs-BBR selectively killed cancer cells but not normal cells, and it exhibited higher anti-cancer efficacy than ss-MONs-BBR due to its excellent isotype targeting ability.

Metal NPs

Metal NPs, another popular nanodelivery system, can assist in monitoring and visualizing tumors through light- and magnetic-based methods, while also causing cell death by generating reactive oxygen.[50] Several characteristics of Metal NPs, such as elemental composition, electric charge, and shape, can potentially affect the toxicity of NPs.[51] Among Metal NPs, AuNPs are the most widely used in biological applications due to their ease of synthesis and surface modification. For instance, Chiu et al developed a physically gold nanoparticle-collagen nanocarrier to deliver BBR for the treatment of breast cancer. This nanocarrier exhibited cytotoxicity and endocytosis, inducing apoptosis through targeted delivery of high purity BBR[52] Additionally, AgNPs have been combined with BBR and polyethylene glycol-functionalized folic acid to induce apoptosis and demonstrate molecular-based precise targeting in breast cancer.[53]

Various types of nano-delivery systems have also been utilized to deliver BBR for the treatment of different cancers. These systems have been categorized in Table 1 and include crystalline nanoparticles, nanoemulsions, nanosuspensions, Heterocyclic aromatic cations, and more.[54,55]

Table 1 Examples of BBR Loaded Nanodelivery Systems

Systems	Type	System	Cellular Model	Effect in vitro	Effect in vivo	Ref.
Organic systems	Lipid-polymer NPs (LPNPs)	BBR/PMA NPS	HeLa	IC_{50} 1. BBR: 24h: 94.70 μg/mL 48h: 25.33 μg/mL 72h: 24.91 μg/mL 2.BBR/PMA NPS: 24h: 118.40 μg/mL 48h: 45.85 μg/mL 72h: 2.85 μg/mL	/	[56]
		BH/FA-CTS NPs	CNE-1	Apoptosis 1. BH: 42.05% 2. BH/CTSNPs: 63.58% 3. BH/FA-CTSNPs: 88.05%	Tumor volumn 1.BH: 716 mm^3 2.BH/CTSNPs:400 mm^3 3.BH/FA-CTSNPs: 245mm^3	[57]
		HA/PEG/BD NDs	A549	IC_{50} 1. Free BD 24 h: 4.23±0.20 μg/mL 48 h: 2.19±0.15 μg/mL 72 h: 1.55±0.43 μg/mL 2. BD NDs 24 h: 2.51±0.18 μg/mL 48 h: 1.59±0.02 μg/mL 72 h: 1.01±0.27 μg/mL 3. PEG/BD NDs 24 h: 3.46±0.12 μg/mL 48 h: 1.81±0.08 μg/mL 72 h: 1.07±0.06 μg/mL 4. HA/PEG/BD NDs 24 h: 1.67±0.11 μg/mL 48 h: 1.47±0.03 μg/mL 72 h: 0.81±0.04 μg/mL	tumor inhibition rate 1. Free BD:9.33% 2. PEG/BD NDs:26% 3. HA/PEG/BD NDs:61.7%	[40]
		BBR-loaded PEG-PE/TPGS-mMics	PC3, LNPaC.	IC_{50} (48h) 1. BBR PC3: 87±11.3 μM LNPaC: 107±16.5 μM 2. Brb-loaded PEG - PE / TPGS - mMic PC3: 4.86±0.28 μM LNPaC: 6.4±0.63 μM	Brb-loaded PEG - PE / TPGS - mMic achieve AUC0–∞=69.308 mg/h/L, ~37-fold higher compared to free drug injection.	[5]
	Polymeric micelles	BBR-NLC	H22	IC_{50} (72h) 1. BBR: 22.1 μg/mL 2.BBR-NLC: 6.3 μg/mL	Tumor inhibition rate 1. BBR: 41.4% 2. BBR-NLC:68.3%	[58]
	Liposomes	Liposomal BBR	HepG2	Apoptosis (72h) 1. BBR:2.2% 2. liposomal BBR: 37.6%	Tumor inhibition rate 1. liposomal BBR: 46.5% 2. DOX: 74.53%	[39]
		BBR-NLC	HepG2, Huh7, EC9706.	IC_{50} (72h) 1. BBR HepG2: 18.3 μg/mL Huh7: 6.5 μg/mL EC9706: 12.4 μg/mL 2. BBR-NLC HepG2: 9.1 μg/mL Huh7: 4.4 μg/mL EC9706: 6.3 μg/mL	/	[59]
	Solid lipid NPs (SLN)	BH-loaded SLNs	MCF-7, HepG 2, A549.	IC_{50} (48h) 1. BH MCF-7: 40 μM HepG 2: 10.3 μM A549: 40 μM 2. BH-loaded SLNs MCF-7: 20.5 μM HepG 2: 4.8 μM A549: 15.2 μM	/	[60]
		BBR-MRs	HepG2, SMMC-7721, H22 cells		tumor inhibition rate 1. BBR(L): 19.12% 2. BBR MRs: 33.28% 3. BBR(H):45.25% 4. BBR MRs: 75.52%	[61]

(*Continued*)

Table 1 (Continued).

Systems	Type	System	Cellular Model	Effect in vitro	Effect in vivo	Ref.
	Nanoemulsion	BBH	Caco-2	The absorption of BBH and showed a 4.4-fold higher relative oral bioavailability in rats.	/	[62]
	Nanosuspension	BBR-NS	HepG2, Huh7.	IC_{50} (72 h) 1. BBR HepG2: 18.3 μg/mL, Huh7: 6.5 μg/mL. 2. BBR-NS HepG2: 8.1 μg/mL, Huh7: 4.7 μg/mL.	Tumor inhibition rate 1. CTS: 79.1% 2. BBR-NS: 63.7%. 3. BBR:41.4%	[63]
	Dendrimers	PAMAM-BBR	MCF-7, MDA-MB-468.	IC_{50} (24h) 1. BBR MCF-7: 6.9 μg/mL MDA-MB-468:4.17 μg/mL 2. BPC MCF-7: 4.03 μg/mL, MDA-MB-468: 2.79 μg/mL	Half-life (t1/2) (h) 1. BBR: 6.70 2. BPC: 14.33 AUC (μg/mL/h) 1. BBR: 1424.42 2. BPC: 2471.17	[45]
Inorganic systems	Mesoporous silica	CM-ss-MONs-BBR	HepG2	CM-ss-MONs-BBR showed the best antitumor effect in all groups, and the NPs have selective killing of cancer cells but not normal cells.	ss-MONs-BBR showed the most obvious antitumor growth efficiency.	[49]
		FA-JGMSNs-BBR	SMMC-7721	FA-JGMSNs-BBR showed the best anti-cancer effect.	Tumor inhibition rate FA-JGMSNs-BBR + RT + NIR > JGMSNs-BBR + RT + NIR > FA-JGMSNs-BBR + RT > FA-JGMSNs + RT > RT > FA-JGMSNs-BBR> BBR > PBS	[48]
	Liquid crystalline nanoparticles (LCNs)	BBR-loaded LCNs	MCF7	IC_{50} (24h) 1. BBR: 1.7 ± 0.02 mM 2. BBR-LCN: 150.9 ± 1.3 μM 3. BBR-LCN-THP: 31.8 ± 1.5 μM 4. BBR-LCN-PEG: 29.1 ± 0.2 μM	/	[64]
	Heterocyclic aromatic cations	Triazolyl berberine derivatives	MCF-7, SW-1990, SMMC-7721, HUVEC.	IC_{50} (48h) 1. BBR MCF-7: 121.91±11.26 μM, SW-1990: 27.64±3.04 μM, SMMC-7721: 68.06±7.76 μM, HUVEC: 18.33±2.31 μM. 2. 9-O-1-(4-tert-butylbenzy1)-4-ethyl-1H-1, 2, 3-triazole berberine chloride (compound 16): MCF-7: 15.80±2.14 μM, SW-1990: 8.54±1.97 μM, SMMC-7721: 11.87±1.83 μM, HUVEC: 25.49±3.24 μM.	/	[65]
	Metal NPs	BBR loaded AgNPs	MCF-7, MDA-MB-231.	IC_{50} (48h) 1. BBR MCF-7: 30 μg/mL, MDA-MB-231: 40 μg/mL. 2. BBR loaded AgNPs MCF-7: 20 μg/mL, MDA-MB-231: 30 μg/mL.	The results show that BBR loaded AgNPs significantly inhibited the proliferation of MCF-7 cells in a dose dependent manner, as represented by the decrease in tumor volume and tumor weight.	[66]
		Au-Col-BB	Her-2	Apoptosis 1. BB: 6.15% 2. Au-Col-BB: 80.6%	Au-Col-BB could suppress the growth of tumors significantly more than Au-Col and control group	[52]
		The triple system DNA-Nanosilver-BBR	CCRF-CEM	IC_{50} 24h: 22 ± 4 μg/mL 48h: 9 ± 1 μg/mL 72h: 5 ± 1 μg/mL	/	[67]
		SeNPs-BBR	ESTs	/	The treatment with SeNPs-BBR significantly reduced ($P < 0.05$) the tumor volume, compared to treatment with BBR alone.	[68]

(*Continued*)

Table 1 (Continued).

Systems	Type	System	Cellular Model	Effect in vitro	Effect in vivo	Ref.
		BBR-ZnO NPs	A549	Laser irradiation with BBR-ZnO NPs provided improved cytotoxicity rather than BBR-ZnO NPs group (without laser irradiation) at 10 and 25 mg/mL BBR concentrations ($p < 0.05$).	/	[69]
		C60-BBR	CCRF-CEM	IC_{50} 1. BBR 24 h: 58 ± 5 μM 48 h: 23 ± 2 μM 72 h: 19 ± 2 μM 2. C60-BBR (2:1) 24 h: 21 ± 2 μM 48 h: 5.0 ± 0.6 μM 72 h: 3.0 ± 0.2 μM	/	[70]

Nano-Scale Drug Delivery Systems for Combined Therapy

As we all know, single-drug treatment often leads to the activation of alternative pathways, which eventually results in tumor recurrence. Considering the varying efficacy and targeting pathways of different drugs, combination therapy has been proven to alleviate multiple drug resistance (MDR) and reduce the toxic side effects by lowering the dosage of individual drugs.[71] Numerous studies have demonstrated that combination treatments can induce tumor cell apoptosis and inhibit their proliferation, thus achieving a more effective anti-cancer outcome.

BBR itself plays a role in inhibiting the proliferation and metastasis of various types of cancer cells, and it can also act as a photosensitizer to fight against cancers.[72,73] However, BBR has low oral bioavailability, intestinal malabsorption, and other disadvantages. As a result, BBR has been combined with other drugs or treatment methods to achieve synergistic efficacy, as shown in Figure 4. For example, when combined with DOX, BBR has shown a significant effect in treating melanoma (apoptosis after 48h: 17.7%), compared to being treated with BBR or DOX alone (50 μM of BBR: 13.7%, 25 nM of DOX: 10.8%).[74] Wang et al found that the combination of BBR and Evodiamine (EVO) resulted in better apoptosis of hepatocellular carcinoma SMMC-7721, with an inhibition effect of 50.00%, compared to using BBR or EVO separately (20.24% and 16.33%).[75] Additionally, BBR has shown substantial phototoxic effects in photodynamic therapy (PDT) when activated by visible light or LED irradiation.[76,77] Therefore, by combining the synergistic efficacy of multidrug combinations with BBR's characteristics as a photosensitizer and utilizing appropriate nano-delivery methods, a complete drug delivery system can be established (Figure 4). This system can accurately target tumors, control drug release, and assist in combination therapy.

Combination with the Chemotherapeutic Drugs

Chemotherapeutic drugs, which are based on toxic compounds, have played important roles in antitumor therapy since the 1940s.[78] The mechanism of chemotherapeutic agents is complicated, including their effect on the chemical constitution of DNA in tumor cells, inhibition of nucleic acid synthesis, and interference with mitotic tubulin synthesis. However, these drugs also inhibit the growth of normal cells, such as hair follicles, bone marrow, and gastrointestinal cells, which leads to damage to the body during chemotherapy. Additionally, the effectiveness of tumor chemotherapy can be influenced by MDR. Consequently, multidrug combination therapy based on the delivery of nanosystems has become a viable strategy that is recommended for oncotherapy. This strategy has the capability of promoting therapeutic effects and reducing side effects. In this paper, the effects of combining BBR with chemotherapeutic agents based on nanosystems have been summarized in Table 2. The results confirm that this treatment modality is effective in reducing the side effects induced by chemotherapy and, more importantly, reducing tumor cell activity and inducing cell apoptosis.

For example, Mozaffari et al prepared composite nanofibers of the anti-cancer DOX and BBR with Poly-caprolactone (PCL) through electrospinning. Combination therapy with targeted drugs (BBR) not only enhanced the cytotoxic effects of

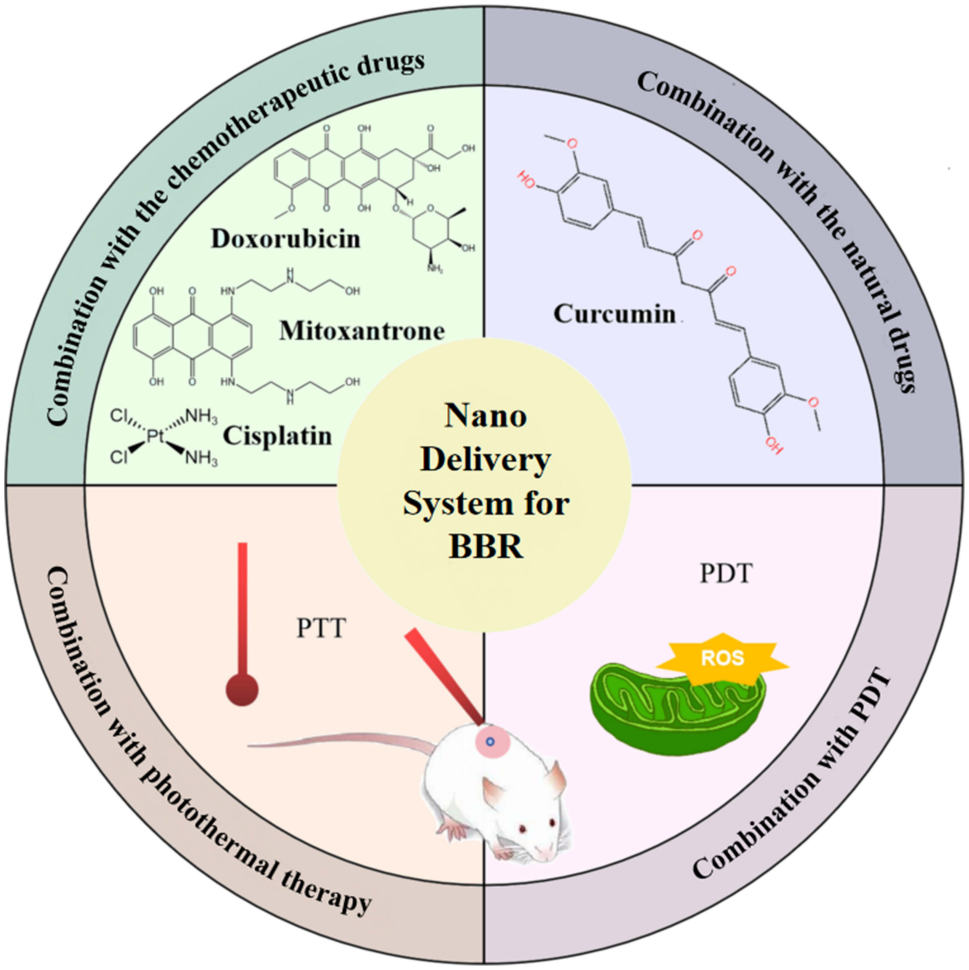


Figure 4 Nano-Scale Drug Delivery Systems of BBR Combined with Other Therapy.

DOX on cancer cells, but also reduced the toxicity of DOX in normal cells. Their study demonstrated controlled and sustained release for up to 72 hours, significant synergistic antitumor effects on the MCF-7 cell line.[79] In 2021, Zimeng et al[84] co-encapsulated mitoxantrone (MIT) and BBR in a liposomal formulation to treat 4T1 breast and L1210 ascitic tumor models. MIT is a synthetic anthraquinone antibiotic drug, and as a cell cycle nonspecific drug, it can kill cancer cells in any phase of the cell cycle, inhibiting both proliferating and non-proliferating cells. However, its clinical application is primarily limited by its cardiotoxicity, bone marrow suppression, hepatotoxicity, and nephrotoxicity. BBR protects the heart and reduces myocardial injury by regulating the expression of Cdk9 and cell cycle protein T1. In addition, BBR can inhibit the proliferation of tumor cells, induce apoptosis and cell cycle arrest of cancer cells. The results suggested that this liposomal formulation could synergistically improve anti-tumor efficiency, reduce cardiotoxicity, and prolong drug circulation time.

Combination with the Natural Medicines

Natural medicines are considered safer with fewer side effects compared to chemotherapeutic drugs. Moreover, they possess effective therapeutic advantages due to their multi-target sites and multi-channel characteristics.[86] Several natural products, including paclitaxel, curcumin, resveratrol, and genistein, have demonstrated their ability to inhibit cancer cell growth and induce apoptosis by modulating one or more miRNAs and regulating the expression of key apoptotic proteins.[87,88] In contrast to specific inhibitors that typically target only one protein in a signaling pathway, natural medicines, especially when used in combination, have a more significant impact on cancer therapy. Furthermore, the use of nano delivery systems enables precise tumor targeting and control of drug release, ultimately enhancing the effectiveness of combination treatments. For instance, the co-delivery of curcumin and BBR via liposomes has shown

Table 2 Examples of Combination BBR with the Chemotherapeutic Drugs Based on Nano Preparation

Combination	Cellular Model	Carrier	Dosage of Administration in vivo	Effect of Combination in vitro	Effect of Combination in vivo	Ref.
Doxorubicin (DOX)	MCF-7	PCL	/	Cell viability (%) 1. DOX (15 μg): 71.5% DOX (30 μg): 55.9% 1. BBR (30 mg/mL): 33.13% 2. DOX+BBR (15μg+30 mg/mL): 35.95% DOX+BBR (30μg+30 mg/mL): 19.29%	/	[79]
Doxorubicin (DOX)	4T1	Self-assembled nanodrug	BBR, Dox, Dox + BBR, DBNP and DBNP@CM at a final Dox dose of 1 mg/kg and a final BBR dose of 0.25 mg/kg, respectively.	IC_{50} (48h) (5 μg/mL) 1. DOX: 1.147 μg/mL 2. DOX+BBR: 1.186 μg/mL 3. DBNP: 1.105 μg/mL 4. DBNP@CM: 1.110 μg/mL	The inhibition rate 1. BBR: 1.1% 2. Dox: 49.2% 3. Dox + BBR: 51.1% 4. DBNP: 58.3% 5. DBNP@CMz: 69.8%	[80]
Olaparib	Panc-1, Miapaca-2, Aspc-1, Capan-2. (Four types of Pancreatic cancer cells)	/	/	Apoptosis (48 h): no data Two drugs in combination is far greater than BBR or olaparib treatment group.	/	[81]
Cisplatin (DDP)	BGC-823, SGC-7901.	/	In SGC-7901/DDP cells: DDP (3 mg/mg/day) or BBR (10 mg/kg/day) evaluated in a xenograft mice mode for 30 days.	Apoptosis 1. DDP (30 μM) 2. BBR (30 μM) 3. DDP (30 μM) +BBR (30 μM) DDP and BBR co-treatment dramatically increased the BGC-823/DDP and SGC-7901/DDP cell apoptotic rates when compared to the other three groups.	Co-treatment with DDP and BBR suppressed the in vivo tumor growth of SGC-7901/DDP cells by around 50%	[82]

Irinotecan (IRI) Onivyde	BXPC-3	Nanoliposomes	Free IRI and BBR combination, IRI co-loaded BBR liposomes (lipBI), IRI liposomes, and BBR liposome mixture (lipB plus lipI) were injected into 220–250 g SD rats via tail vein and the doses were 4 mg/kg for IRI and 2 mg/kg for BBR.	IC_{50} 1. LipBI BXPC-3: 29.49 μg/mL L02: 92.14 μg/mL 2. Onivyde BXPC-3:39.23 μg/mL L02: 176.5 μg/mL 3. lipB plus lipI BXPC-3: 33.39 μg/mL L02: 78.52 μg/mL	It is worth noting that the anti-tumor effect of lipBI was the best, with significantly reduced tumor volume.	[83]
Mitoxantrone (MIT)	4T1, L1210.	Nanoliposomes	(a) saline (control group), (b) MF+BF, (c) ML, (d) BL, and (e) MBL. Treatments were administrated intravenously on days 11, 14, 17 and 20 after tumor inoculation with a MIT dose of 2 mg/kg and a BBR dose of 4 mg/kg.	IC_{50} (48 h) 1. MIT 4T1: 6.5 ng/mL L1210:. 25.8 ng/mL 1. BBR 4T1: 150.7 ng/mL L1210: 3817.3 ng/mL 1. MIT+BBR 4T1: MIT:BBR (1:1): 3.0/3.0 ng/mL L1210: MIT:BBR (2:1): 25.4/12.7 ng/mL	Mice treated with MBL exhibited the most potent antitumor activity, which might be attributed to the synergistic therapeutics effect of MIT and BBR.	[84]
Icotinib	NSCLC	/	The mice were randomized into 4 groups (n=5 per group) to receive either vehicle control, icotinib (125 mg/kg, one daily) alone, BBR (80 mg/kg, one daily) alone or icotinib plus BBR, by oral gavage.	/	It was significantly suppressed by combination treatment of the two drugs compared to either drug alone.	[85]

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promising results in hepatocellular carcinoma therapy by inhibiting tumor-aHSCs crosstalk and inducing apoptosis in tumor cells.[89] Although this therapy is currently uncommon, its potential and application value are evident.

Combination with Photothermal Therapy

Photothermal Therapy (PTT) applies a laser to irradiate the tumor site in order to heat and kill tumor cells, achieving anti-tumor treatment. The selection of an appropriate light source is crucial in the optical treatment process. Near-infrared (NIR) lasers have advantages over ultraviolet and visible light due to their minimal tissue damage and strong tissue penetration. They are widely used as the primary light source in optical therapy. By applying local NIR irradiation, photothermal agents generate high temperatures (> 42°C), leading to apoptosis in cancer cells through the disruption of cell membranes, interference with the cytoskeleton, and inhibition of DNA synthesis.[90] Although PTT can rapidly reduce tumor volume, it often faces challenges in completely eradicating the tumor. There are several reasons for this. First, the penetration depth of NIR light is limited, making it insufficient for solid tumors.[91] Second, long-term tumor suppression is inadequate, leading to a high risk of tumor recurrence and metastasis.[92] Additionally, PTT may trigger a pro-inflammatory response in dendritic cells (DC) and macrophages, leading to the release of pro-inflammatory cytokines and inducing inflammation.[93] Moreover, PTT may cause tissue damage due to inaccurate laser exposure during treatment and systemic distribution in vivo.[94] Therefore, combining PTT with drug therapy and nanodelivery is expected to overcome the aforementioned challenges.

In 2019, Li et al[48] developed Janus gold-mesoporous silica nanocarriers (FA-JGMSN) for loading BBR to enable radioactive photothermal therapy for liver cancer. Their study demonstrated that this approach effectively prolonged BBR retention within cells and alleviated damage caused by radiation-induced intestinal effects. Cheng et al used electrostatic attraction to co-assemble camptothecin (CPT), BBR, and the photosensitizer indocyanine green (ICG) in order to enhance the combination therapy of chemotherapy and photothermal effects on A549 cells. The co-assembled nano-drugs, due to their lipocationic properties, specifically targeted the mitochondria in the cancer cells, induced rapid photothermal transformation after NIR irradiation, and inhibited the growth of A549 cells.[37]

Combination with PDT

Photodynamic therapy (PDT) utilizes photosensitizers to generate abundant reactive oxygen free radicals (ROS) for the targeted destruction of tumor cells upon specific light exposure. Therefore, photosensitizers play a crucial role in PDT.[95] Interestingly, several natural compounds such as hypocrellin, curcumin, and BBR have been proven to possess photo-dynamic properties, highlighting their potential for extensive application in PDT.[96] BBR, as a photosensitizer, is easily produced and exhibits diverse pharmacological effects. However, it also suffers from drawbacks such as toxicity and low solubility. Moreover, when photosensitizers accumulate poorly in tumor tissues, they tend to have a brief presence in the bloodstream and are swiftly eliminated from the body. Hence, the delivery of photosensitizers using nanocarriers may overcome the limitations associated with photodynamic therapy in cancer treatment.[97] In 2021, Floriano et al[95] incorporated BBR into a nanoemulsion and evaluated its therapeutic efficacy against cervical cancer cells, revealing that the BBR-containing nanoemulsion significantly enhanced cytotoxicity potential and ROS generation. Several examples support the strategies of PTT and PDT (Table 3).

Conclusions and Outlook

Despite these challenges, the potential for nano-targeted delivery of BBR remains vast and untapped. For instance, embracing the traditional Chinese medicine philosophy of "unification of drug and adjuvant" promises a novel approach. This philosophy emphasizes the synergistic effects of combining multiple components, which could be mirrored in advanced nano-formulation strategies. By integrating BBR with carefully selected adjuvants within a nanocarrier system, it is possible to enhance the bioavailability and therapeutic efficacy of BBR while mitigating its stability issues. Leveraging insights from modern precision therapies could lead to the development of targeted, controllable, and pulsatile release mechanisms that enhance drug bioavailability. These advanced delivery systems could be designed to respond to specific stimuli in the tumor microenvironment, such as pH changes, enzymatic activity, or hypoxia, ensuring that BBR is released precisely where and when it is needed. This would not only improve the therapeutic outcomes but

Table 3 Examples of Combination BBR with the PTT/PDT Therapy Based on Nano Preparation

Therapy	Laser	Combined (Drug)	Cellular Model	Carrier	Dosage of Administration in vivo	Effect of Combination	Ref.
PTT	808 nm for 5 min (1 W/cm^2)	/	SMMC-7721, HL-7702.	Gold mesoporous silica	Inject PBS, FA-JGMSNs (20 mg/kg), JGMSNs-BBR (25 mg/kg), FA-JGMSNs-BBR (25 mg/kg), free BBR (5 mg/kg) every 3 days.	FA-JGMSNs-BBR combining with RT displayed higher tumor inhibition rates than BBR, FA-JGMSNsBBR or FA-JGMSNs with RT. (no data)	[48]
	447 nm for 4 min (80 J/cm^2)	/	Caski, HaCaT.	Nanoemulsions	/	Cell viability (24 h) 1. BBR Nps (BN) (1.25 μM): Caski: 90% HaCaT: 83% 1. BN combined with PDT: Caski: 27.59% HaCaT: 46.12%	[98]
	808 nm for 3 min (2W/cm^2)	/	A549	Nanoparticles	5 mg/kg dose of BBR; Collect 2 mL blood from the femoral artery at 24 h postinjection.	With the intrinsic anti-cancer potentials of BBR and ZnO, photothermal effects of ZnO NPs induced by laser irradiation can elevate therapeutic efficacies for A549 cells. (no data)	[69]
	785 nm for 5 min (2 W/cm^2)	/	HT-29	Rod-like keratin nanoparticles (KNPs)	/	IC_{50} (Enough concentration to kill 50% of the cells) (48 h) 1. KNPs: >2000 μg/mL 2. BERB: 62 ± 18 μg/mL 3. KNPs/BERB: 1200 ± 45 μg/mL	[99]

(*Continued*)

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Table 3 (Continued).

Therapy	Laser	Combined (Drug)	Cellular Model	Carrier	Dosage of Administration in vivo	Effect of Combination	Ref.
	808 nm laser for 5 min (1 W/cm^2)	Camptothecin (CPT)	A549	Supramolecular nanodrugs	/	IC_{50} (at the same dosage) 1. CPT 24 h: 4.49 ± 0.34 μM 48 h: 0.90 ± 0.10 μM 72 h: 0.43 ± 0.10 μM 1. BBR-OH 24 h: 33.71 ± 1.79 μM 48 h: 21.12 ± 4.99 μM 72 h: 14.18 ± 3.72 μM 1. ICG 24 h: 134.30 ± 17.86 μM 48 h: 89.91 ± 12.46 μM 72 h: 72.72 ± 7.83 μM 1. ICG (with laser irradiation) 24 h: 13.65 ± 0.49 μM 48 h: 10.56 ± 3.25 μM 72 h: 6.86 ± 0.48 μM 1. CPT-ss-BBR 24 h: 5.93 ± 0.35 μM 48 h: 1.16 ± 0.30 μM 72 h: 0.63 ± 0.10 μM 1. CPT-ss-BBR/ICG NPs 24 h: 5.48 ± 0.38 μM 48 h: 0.93 ± 0.12 μM 72 h: 0.48 ± 0.06 μM 1. CPT-ss-BBR/ICG NPs (with laser irradiation) 24 h: 3.06 ± 0.11 μM 48 h: 0.36 ± 0.11 μM 72 h: 0.21 ± 0.08 μM	[100]
PDT	447 nm LED for 4 min (1.2 mW/cm^2)	/	T98G	PLGA	/	Apoptotic percentages 1. BBR: 5.07% 2. BBR-S: 51.20% 3. BBR-S LED: 69.80%	[101]
	808 nm laser for 10 min (0.75 W/cm^2)	/	A549	Gold nanoclusters	There is a noticeable reduction in cell viability percentage for cells subjected to AuNC@BBR@Ghost-associated PDT, compared to those treated with bare AuNCs, BBR-associated PDT, and the AuNC@BBR complex-associated PDT.	/	[102]

also reduce the potential side effects associated with systemic drug distribution. Furthermore, the integration of cutting-edge technologies such as CRISPR-Cas9 for gene editing and RNA interference (RNAi) could offer additional layers of precision to BBR delivery systems. By targeting specific genetic markers or pathways implicated in cancer progression, these technologies could work in tandem with BBR to achieve a more comprehensive anti-cancer effect. Another promising direction is the use of biomimetic nanocarriers, which are designed to mimic natural biological structures, such as cell membranes, to enhance the delivery and uptake of BBR. These biomimetic carriers can improve the targeting of BBR to cancer cells and facilitate its entry into the cytoplasm or nucleus, where its therapeutic targets reside. Additionally, exploring the use of multi-functional nanocarriers that can co-deliver BBR with other therapeutic agents, such as immunomodulators or anti-angiogenic drugs, could provide a multi-faceted approach to cancer therapy. This strategy could enhance the overall anti-cancer effect by attacking the tumor from multiple angles and overcoming resistance mechanisms. Finally, advances in nanotechnology could also address the scalability issues associated with the production of BBR nano-formulations. Developing robust and reproducible manufacturing processes that maintain the integrity and efficacy of the nanocarriers can pave the way for the transition from laboratory research to clinical applications. In conclusion, by integrating traditional Chinese medicine principles with modern nanotechnology and precision medicine approaches, there is a significant opportunity to unlock the full potential of BBR in cancer therapy. Continued research and innovation in this field could lead to the development of highly effective, targeted, and safe cancer treatments that leverage the unique properties of BBR and the advantages of nano-delivery systems.

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Disclosure

The authors report no conflicts of interest in this work.

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