A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs

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Natural products represent a rich reservoir of potential small chemical molecules exhibiting antiproliferation and anticancer properties. An example is berberine, a protoberberine alkaloid widely distributed in medical plants used in traditional Chinese prescriptions. Recent advances have shown that berberine exerts anticancer activities both *in vitro* and *in vivo* through different mechanisms. Berberine shows inhibitory effects on the proliferation and reproduction of certain tumorigenic microorganisms and viruses, such as *Heliobacter pylori* and hepatitis B virus. Transcriptional regulation of some oncogene and carcinogenesis-related gene expression and interaction with both DNA and RNA are also well documented. Besides, berberine is a broad spectrum enzyme inhibitor, which affects *N*-acetyltransferase, cyclooxygenase-2, and topoisomerase activities and gene/protein expression. These actions, together with the regulation of reactive oxygen species production, mitochondrial transmembrane potential, and nuclear factor-κB activation might underlie its antiproliferative and proapoptotic effects. More importantly, the suppression of tumor growth and metastasis, the beneficial application in combined medication, and the improvement of multidrug resistance both *in vivo* and *in vitro* clearly show its potential as an alternative medicine for tumor chemotherapy. *Anti-Cancer Drugs* 20:757–769 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

A number of plant-derived agents are currently successfully used in cancer treatment, such as vinca alkaloid, etoposide, taxanes paclitaxel, etc., whereas some are currently under investigation [1]. Berberine (Fig. 1), an isoquinoline alkaloid, belongs to the structural class of protoberberines. It is present in the roots, rhizome, and stem bark of a number of important medicinal plant species including *Berberis vulgaris* (barberry), *Hydrastis canadensis* (goldenseal) (Ranunculaceae), *Coptis chinensis* (Coptis or goldenthread) (Ranunculaceae), *Arcangelisia flava* (Menispermaceae), *B. aquifolium* (Oregon grape), and *B. aristata* (tree turmeric) [2]. *Coptis chinensis* (*Rhizoma coptidis*) and Baical Skullcap Root (*Radix scutellariae*), which contain a large amount of berberine and other protoberberines, have been widely prescribed by traditional Chinese physicians as heat-clearing and detoxicating medicine for thousands of years. Since the last century, berberine has been extensively investigated and was found to possess a wide variety of pharmacological and biological activities, such as antimicrobial, antihelmintic, anti-inflammatory, and anti-oxidative effects [3–5]. Recently, many researchers have been particularly interested in the antineoplastic activities of berberine and have obtained some promising and interesting results both *in vitro* and *in vivo*, which will be discussed in detail in this review.

The anticancer activity of berberine

Inhibition of tumorigenic microorganisms

The strong antimicrobial activities of extracts from *Rhizoma coptidis* and *Radix scutellariae*, two important sources of berberine in nature, have been firmly established by inhibiting the growth of *Klebsiella pneumoniae*, *Proteus vulgaris*, *Mycobacterium smegmatis*, *Candida albicans* [6], *Heliobacter pylori* [7], and the intestinal protozoan parasite *Blastocystis hominis in vitro* [8]. Further studies have shown that this antimicrobial effect was mainly because of one of the active compounds in these herbs, berberine, which displayed significant antibacterial and antifungal activities against *Staphylococcus aureus* and different *Candida* spp., *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, and *Leishmania donovani* [2]. Berberine inhibits the growth of *Helicobacter pylori in vitro* with a minimum inhibitory concentration at 12.5 μg/ml [9]. In hepatitis B virus permanently transfected HepG2 2.2.15 cells, berberine not only markedly reduces viral production, but also induces toxicity in host cells [10], but does not show inhibitory activity against HbsAg and HbeAg [11]. In view of the etiological close relationship between the pathogenic microorganism and tumorigenesis, such as the discovery of the bacterium *Helicobacter pylori* as the main etiologic organism of chronic gastritis, peptic ulcer disease, and gastric cancer [12,13], the antimicrobial activity of berberine might contribute to its anticancer potential.

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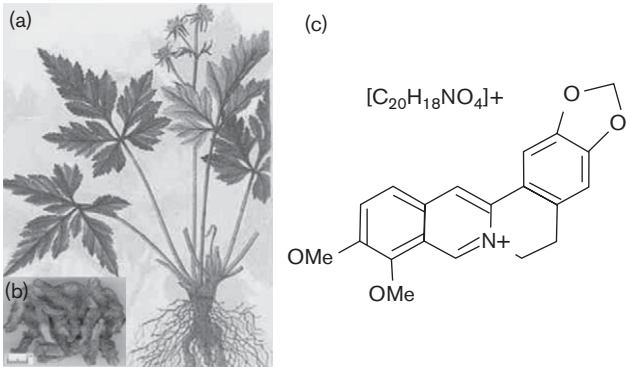
Fig. 1



Plant source and chemical structure of berberine [5,6-dihydro-9, 10-dimethoxybenzo (*g*)-1,3-benzodioxolo (5,6-*a*) quinolizinium], an isoquinoline plant alkaloid, belongs to the structural class of protoberberines. (a) *Rhizoma coptidis* (Huanglian) plant. (b) Chinese medical material of *Rhizoma coptidis*. (c) Chemical structure of berberine.

Regulation of oncogene and carcinogenesis-related gene expression

Some human teratocarcinoma cell lines and tumor tissues exhibit amplification and enhanced expression of *c-Ki-ras2* protooncogene [14]. Berberine treatment induced a pluripotent human teratocarcinoma cell clone NT2/D1, which was derived from the Tera-2 cell line, to differentiate into cells with neuronal cell morphology. This effect was absent in murine teratocarcinoma cell line, F9 [15] and might be because of its downregulation of *c-Ki-ras2* gene mRNA expression [16]. More recently, it was discovered that berberine could increase the activity of AMP-activated protein kinase, which lead to phosphorylation activation of the tumor suppressor gene p53 in vascular smooth muscle cells (VSMCs) [17]. Furthermore, a very new study revealed that p53 showed a cooperative effect on berberine-induced growth inhibition and apoptosis of non-small cell human lung cancer cells *in vitro* and tumor xenograft growth *in vivo* [18].

The cellular signaling cascades mediated by transcription factors, including nuclear factor E2-related factor 2 (Nrf2), nuclear factor-κB (NF-κB), and activator protein-1 (AP-1), have been shown to play pivotal roles in tumor initiation, promotion, and progression processes [19]. A reporter gene assay showed that berberine exhibited an inhibitory effect on AP-1 activity in a dose-dependent and time-dependent manner in human hepatoma cells [20]. However, another study suggested that berberine had no effect on AP-1 activity under the conditions it suppresses NF-κB in Jurkat cells [21]. This inconsistence might be because of the cell difference, but is more likely to result from the incubation time, as the former study showed that berberine inhibited AP-1 activity almost completely at a concentration as low as 10 μmol/l after 48 h treatment, whereas in the latter study the cells were treated with berberine only for 30 min [20,21]. This interpretation was further supported by another study, which showed that berberine inhibited constitutively expressed and TPA-induced binding of AP-1 in human oral epidermal carcinoma cell, KB, and oral squamous cell carcinoma cell, OC2, after 2 h treatment [5]. These results suggested that berberine exhibited pharmacological effects in a slow and smooth manner at lower concentrations ($\leq$ 25 μmol/l), which was also observed in its apoptosis-promoting and antiproliferative effects in human epidermoid carcinoma cells, A431, U937cells, B16 cells, HL-60 cells, etc. [22–24]. In fact, as will be discussed below, berberine showed high affinity to both DNA and RNA. Hence, its integration with DNA in nuclear material might consequently hinder AP-1 binding.

Interaction with DNA and RNA

The interaction between berberine and DNA or RNA to form a berberine–DNA complex or a berberine–RNA complex might be one of its anticancer mechanisms. Earlier studies have shown that a single berberine molecule binds to DNA to form a complex with DNA [25] in a pH-dependent manner [26]. Furthermore, it showed the greatest affinity for polyadenylic acid [poly(A)] and did not seem to associate significantly with polycytidylic acid [poly(C)] or polyuridylic acid [poly(U)] [27]. It is estimated that berberine binds strongly with polyguanylic acid and polyinosinic acid with an affinity in the order 10^{-5} mol/l, whereas its binding to poly(C) and poly(U) is very weak or practically nil [28]. This characteristic was once used to stain DNA and RNA in earlier studies, as berberine is a fluorescent compound with absorbance peaks at 230, 267, 344, 420 nm, and peak emission at 550 nm [29]. Recently, the binding of berberine with DNA and RNA, and its binding affinities have been extensively studied by using several novel analytical techniques, including absorption, fluorescence, nuclear magnetic resonance, and electrospray ionization mass spectrometries. Islam and Suresh Kumar [28] determined the binding affinity, energetics, and conformational aspects of the interaction of berberine to four single stranded polyribonucleotides, polyguanylic acid, polyinosinic acid, poly(C), and poly(U), by absorption, fluorescence, isothermal titration calorimetry, and circular dichroism spectroscopy. Xia *et al.* [30] studied the interaction of berberine with DNA and the competitive interactions of daunorubicin and berberine with DNA by alternating penalty trilinear decomposition algorithm combined with excitation–emission matrix fluorescence data. Bhadra *et al.* [31] examined the equilibrium binding of berberine to various DNAs and the energetics of the interaction, which showed that the binding of berberine to DNA is dependent on base pair heterogeneity in the DNA conformation. Islam *et al.* [32,33] studied the interaction of berberine and palmatine with tRNAphe and compared with the binding of the classical DNA intercalator, ethidium, which showed that the binding of berberine

and palmatine on the tRNA structure appears to be mostly by partial intercalation, whereas ethidium intercalates fully on the tRNA. Tian *et al.* [34] investigated the interaction of berberine with double-strand DNA (dsDNA) and single-strand DNA in solution, dsDNA immobilized on a glassy carbon electrode. The binding of berberine with DNA, when analyzed in terms of the cooperative Hill model, yields the binding constant $K_a = 2.2(\pm 0.2) \times 10^{-4}$ mol/l, corresponding to the dissociation equilibrium constant $K_d = 4.6(\pm 0.3) \times 10^{-5} =$ mol/l. A recent study showed that treatment of osteosarcoma cells and normal osteoblasts with berberine resulted in DNA double-strand breaks, which in turn triggered the activation of p53 and the p53-dependent cellular responses, including cell cycle arrest and apoptosis [35].

Inhibition of carcinogenesis-related enzymes

Inhibition of N-acetyltransferase

It is well established that exposure to environmental and occupational chemicals is an important cause of chemical carcinogenesis. The arylamines, which are metabolized by cytosolic arylamine *N*-acetyltransferase (NAT) using acetyl coenzyme A as an acetyl donor to form reactive carcinogenic metabolites, represent one of the critical documented classes of chemicals known to induce tumors in humans [36,37]. The important role of NAT in drug detoxification and carcinogen activation makes it a potential drug target [38]. Recently, it was discovered that berberine could dose-dependently inhibit NAT activity in several tumor cells, such as human bladder tumor (carcinoma) cells (T24) [39], human colon tumor (adenocarcinoma) cells [40], HL-60 human promyelocytic leukemia cells [41], human malignant astrocytoma (G9T/VGH) and brain glioblastoma multiform (GBM 8401) cells [42], and mouse lymphocytic leukemia cells (L1210) [43]. In addition, the gene and protein expression of NAT was also inhibited by berberine in a dose-dependent and time-dependent manner *in vitro* [42,44].

Inhibition of cyclooxygenase-2

Accumulated evidence suggests that cyclooxygenase-2 (COX-2) plays a key role in colon [43], skin [45], prostate [46], liver [47], and lung [48] tumorigenesis and is supposed to be a new potential target for multiple cancer therapy [47,49,50]. Potentially, compounds inhibiting COX-2 transcriptional activity have, therefore, a chemopreventive property against tumor formation. Berberine inhibits COX-2 transcriptional activity effectively in a dose-dependent and time-dependent manner in colon cancer cells [51], oral cancer cell line OC2 and KB cells [5,52], breast cancer MCF-7 cells, but not in MDA-MB-231 cells [53]. Berberine dose-dependently reduced prostaglandin E_2 production, which was mediated by the direct inhibition of AP-1 binding leading to the transcriptional suppression of COX-2 and reduced COX-2 protein, but not enzyme activity [5]. The effect of berberine on COX-2 expression and activity was supposed to be the basis of its anti-inflammatory effects and involved in the berberine-induced apoptosis [52]. However, in a human Cayman COX inhibitor screening assay, berberine showed no inhibitory effect on either COX isoform activities [54].

Inhibition of telomerase

Telomeres, the ends of linear chromosomes, preserve genome stability and cell viability by preventing aberrant recombination and degradation of DNA. One of the hallmarks of advanced malignancies is continuous cell growth, and this almost universally correlates with the reactivation of telomerase [55]. Therefore, telomerase is an attractive cancer therapeutic target, as it seems to be essentially required in all tumors for immortalization of a subset of cells, including cancer stem cells [56].

In HL-60 cells and human nasopharyngeal carcinoma (NPC) CNE-2 cells, berberine dose and time dependently inhibited telomerase activity [24,57], which was not because of the presence of inhibitors of telomerase activity [24]. Berberine also suppressed *Plasmodium falciparum* telomerase activity in a dose-dependent manner over a range of 30–300 μmol/l indicating that telomerase might be a potential target for future malaria chemotherapy [58]. However, its inhibitory effect on human telomerase in normal cells and other tumor cells need further study to be fully elucidated.

Inhibition of topoisomerase

DNA topoisomerases (Tops) represent a unique class of nuclear enzymes that alter the topological state of DNA by breaking and rejoining the sugar–phosphate backbone bonds of DNA and adjust the topological states of the DNA helix. Topoisomerase I (TopI) is capable of altering the topology of DNA by transiently breaking one DNA strand [59], whereas topoisomerase II (TopII) catalyzes the ATP-dependent relaxation of negative and positive supercoils, knotting, unknotting, catenation, and decatenation of DNA by passing the dsDNA helix through a transient double-stranded break and then resealing the strand break [60]. The anticancer activity exhibited by camptothecin, a quinoline-based alkaloid found in the barks, seeds, and leaves of the Chinese Camptotheca tree (Xi Shu), against a broad spectrum of solid tumors has highlighted the Tops as a pragmatic molecular target for anticancer drugs and stimulated the exploration of Top inhibitors from traditional natural products.

The citations of the effect of berberine on Tops activity in some earlier publications were misleading to some extent, as these researchers did not make a distinction between berberine and one of its metabolites, berberrubine [22,61,62]. The water extract of *Coptis chinensis* was found to have the ability to stabilize the cleavable complex with mammalian DNA TopI, which was because of two

protoberberine alkaloids, epiberberine and groenlandicine, whereas the berberrubine accounted for TopII-mediated DNA cleavage *in vitro* [63]. Coralyne and its analog 5,6-dihydrocoralyne, which have appreciable structural similarity to berberine, showed TopI and TopII inhibitory activities suggesting that berberine might have TopI and/or TopII poisons [59,64]. Berberrubine, a protoberberine, induces DNA cleavage in a site-specific and concentration-dependent manner, which results from its specific poison to TopII *in vitro* by stabilizing TopII-DNA cleavable complexes [60]. Four protoberberine analogs showed potent TopI poisoning activities but exhibited markedly different efficiency with a mechanistic model in which both ligand–DNA and ligand–enzyme interactions are important [65]. These differences in specificity and structure–activity to Tops were because of the structural rigidity associated with the ring system and the substituents [59,65]. In addition, AMC5 cells were resistance to berberrubine, which is associated with decreased level of catalytically active TopIIα, suggesting that TopIIα was the cellular target of berberrubine *in vivo* [66]. In fact, to the best of our knowledge, only recently Kettmann *et al.* [62] proposed a structural model for the ternary berberine–DNA–TopI cleavable complex and Qin *et al.* [67] showed that berberine inhibits TopI by stabilization of the enzyme-mediated DNA cleavable complex, just as camptothecin does.

Suppression of tumor cell proliferation

The antiproliferation/cytotoxicity of berberine has been extensively studied in various cell lines and primarily cultured cells, including multiple tumor cell lines and normal cells (Table 1). Summarizing and analyzing these results, several special characteristics are obvious: (i) berberine exhibits different antiproliferation effects on different cells. For example, the murine melanoma B16 cell line was more sensitive to berberine treatment than the human promonocytic U937 cells (the values were 75–119 times lower) [23]. Similarly, the murine leukemia L1210 cells growing in suspension were more sensitive to berberine (IC_{100} values were 2.3–4.1 times lower) than the human cervical carcinoma HeLa cell line growing as a monolayer [96]. Furthermore, different antiproliferative effect was clear even in the same category of tumor cells. The proliferation of six types of human esophageal cancer ECC cell lines (YES-1 to YES-6) was inhibited by berberine in a dose-dependent manner, but with the IC_{50} varied from 0.11 to 0.90 μg/ml after 72 h treatment [87]. A study showed the cell sensitivity to berberine in increasing order to be B16 < EAC < V79 < U937 < L1210 < NIH-3T3 < HeLa cells [69]. (ii) Berberine seems to be more active in inhibiting tumor cell proliferation, but shows minor cytotoxicity to normal cells. Berberine significantly inhibits human liver cancer cell line HepG2 [83,89,94] proliferation with an IC_{50} of less than 50 μmol/l, whereas has little or no effect on primarily cultured hepatocyte isolated from Sprague–Dawley rats even at a concentration as high as 1 mmol/l [84]. A similar phenomenon was also observed in human GBM cell lines (SF188, SF210, SF126, and U87MG) and the primary cultures of normal human glia cells [79]. However, this might also be because of the short incubation time (half an hour) in berberine-treated primarily cultured hepatocytes [84], as, in primarily cultured VSMCs, berberine also showed an inhibitory effect in a dose-dependent manner [17,75]. (iii) A different IC_{50} value for the same cell type was reported from different labs (Table 1), which might result from different culture conditions and detection methods. (iv) The effect of berberine is relatively slow and gentle, which means it generally exerts significant effects after 24 h treatment. Therefore, most investigators extend the incubation time to 72 h or even longer. However, in S180 cells, berberine exerts a marked but short-lived inhibitory action on cell growth [27]. (v) Berberine also shows potent inhibitory action on some cell proliferation inducer-promoted proliferation. Berberine significantly suppresses growth factor, angiotensin II, and heparin binding epidermal growth factor-induced VSMCs proliferation and migration *in vitro* by delaying or suppressing activation of Akt pathway [75], whereas its inhibition of platelet-derived growth factor-induced VSMCs growth was mediated by activation of AMP-activated protein kinase/p53/p21Cip1 signaling and inactivation of Ras/Rac1/Cyclin D/Cdks pathways [17].

Several potential mechanisms underlying this antiproliferation/cytotoxicity have been proposed. Sethi [97] suggested that the antileukemic activity of the protoberberine alkaloids (including berberine) might be because of its inhibition of reverse transcriptase activity, which interferes with DNA synthesis. Inhibition of DNA, RNA, proteins, and lipids biosyntheses, as well as the oxidation of glucose might also contribute to this [27,98]. In the human myeloma cell line RPMI-8226, the cytotoxic effect may be partial because of its direct blockade of voltage-dependent and Ca^{2+}-dependent K^+ channels [88]. However, the most widely investigated mechanism involved is its role in cell cycle arrest, which has been studied by many groups in tumor and nontumor cells but with controversial results. Berberine induces G_2/M phase arrest in nontumor Balb/c 3T3 cells [96], which is also observed in human gastric carcinoma SNU-5 cell line [70], leukemia cells [99]. Berberine also induces G_1-phase cell cycle arrest in human epidermoid carcinoma A431 cells [22], human HSC-3 oral cancer cells [100], T98G cells [101], murine leukemia L1210 cell lines [68]. However, in U937 and B16 cells, berberine shows no effect on cell cycle profile [23,69]. It is also interesting to note that berberine induces G_1-phase cell cycle arrest in human prostate carcinoma cell lines DU145, but results in a significant accumulation of cells in the G_2–M phase in human prostate carcinoma cell lines, LNCaP and PC-3, and shows no effect on non-neoplastic human prostate

Table 1 The in-vitro antiproliferation effect of berberine

Cells (line)	Inhibitory effect (IC_{50})	Method	TCR	TIR (h)	Reference
HeLa	6.1 ± 0.5 μg/ml (24 h), 7.2 ± 0.3 μg/ml (48 h), 4.8 ± 0.5 μg/ml (72 h)	TBS	0.1–150 μg/ml	0–72	[68]
L1210	2.7 ± 0.1 μg/ml (24 h), 3.5 ± 0.2 μg/ml (48 h), 1.0 ± 0.5 μg/ml (72 h)	TBS	0.1–150 μg/ml	0–72	[68]
U937	Dose dependent inhibition	TBS	0–75 μg/ml	24	[69]
SNU-5	Dose dependent inhibition	TBS	0–200 μmol/l	0–72	[70]
A431	45% 75 μmol/l (24 h), 58% 75 μmol/l (48 h), 78% 75 μmol/l (72 h)	MTT	0–75 μmol/l	0–72	[22]
DU145 PC-3 LNCaP	40% 100 μmol/l (24 h) DU145, 75% 100 μmol/l (48 h) DU145, 80% 100 μmol/l (72 h) DU145	MTT	0–100 μmol/l	0–72	[61]
U937	15.19 ± 3.06 μg/ml (24 h), 4.48 ± 0.22 μg/ml (48 h)	TBS	0–100 μg/ml	0–72	[23]
B16	0.0032 ± 0.0003 μg/ml (24 h), 0.0059 ± 0.0006 μg/ml (48 h), 0.0149 ± 0.0007 μg/ml (72 h)	TBS	0–100 μg/ml	0–72	[23]
SMMC-7721	Dose dependent inhibition	MTT	0–89 μmol/l	48	[71]
MCF-7	56% 20 μmol/l (72 h)	TBS	0–20 μmol/l	72	[72]
SC-MI, CL1-5	7.5 μmol/l (72 h)	MTT	0–100 μmol/l	72	[73]
Colo205	50% 80 μmol/l (72 h)	TBS	0–1600 μmol/l	0–72	[40]
C6; U-87	88% 10 μmol/l (24 h) C6; ~60% 20 μmol/l (24 h) U87	MTT	0–20 μmol/l	24	[74]
VSMC	Dose dependent inhibition	TBS	0–10 μmol/l	0–72	[17]
VSMC	~50% 200 μmol/l	MTT	0–300 μmol/l	30 min	[75]
NHK	No effect	MTT	0–100 μmol/l	48	[76]
B16	64.9% 1 μg/ml (24 h), 86.1% 1 μg/ml (48 h)	TBS	0–25 μg/ml	0–72	[77]
HL-60	No effect <40 μmol/l for 24 h	CNC	0–100 μmol/l	24, 48	[78]
Normal human glial cells	No effect 100 μg/ml for 24 h	TBS	100 μg/ml	24	[79]
SF210, SF188, SF126, U87	SF210>SF188>SF126>U87>60% 100 μg/ml for 24 h	TBS	100 μg/ml	24	[79]
MDA-MB231	25 μmol/l (48 h)	Crystal violet staining	0–100 μmol/l	48	[80]
S180	Marked but short-lived inhibitory action	CNC	0–5 μg/ml	0–96	[27]
NPC/HK1	40% 200 μmol/l (5 h)	CNC	0–200 μmol/l	0–5	[81]
K1735-M2	Dose dependent inhibition	SBA	0–25 μmol/l	0–96	[30]
EAC	0.358 ± 0.0201 μg/ml (12 h), 0.813 ± 0.0569 μg/ml (24 h), 0.870 ± 0.0466 μg/ml (36 h), 0.272 ± 0.0135 μg/ml (48 h)	TBS	0–100 μg/ml	0–48	[82]
HepG2	Dose dependently inhibition	FC	0–50 μmol/l	72	[83]
Primary cultured rat hepatocytes	No effect for 4 h incubation	MTT	0–1 mmol/l	4	[84]
A549; MRC-5	No effect <40 μmol/l	MTT	0–100 μmol/l	24, 48	[85]
LLC	No effect <1 μmol/l	CNC	0–10 μmol/l	24	[86]
YES-1 to YES-6	0.11–0.90 μg/ml (72 h)	MTT	0–10 μg/ml	72	[87]
RPMI-8226	5 μmol/l (48 h)	MTT	0–100 μmol/l	0–48	[88]
HepG2	13.0 ± 0.73 μg/ml (48 h)	MTT	0–40 μg/ml	0–48	[89]
K1735-M2	5 μmol/l (72 h)	SBA	0–100 μmol/l	0–96	[90]
NIH-3T3	11.43 ± 0.32 μmol/l (24 h), 30.10 ± 1.70 μmol/l (48 h)	CNC	0–134.5 μmol/l	0–72	[91]
EAC	2.69 ± 0.19 μmol/l (24 h), 2.29 ± 0.10 μmol/l (48 h)	CNC	0–134.5 μmol/l	0–48	[91]
SVKO3, Fadu HepG2, HeLa Fibroblast	$LC_{50} \leq 0.03$ μmol/l	MTT	0–1 μg/ml	24	[92]
A7r5	22.9 ± 0.4 μmol/l (24 h)	[^{3}H] Assay	0–10 μmol/l	0–72	[93]
HepG2, Hep3B	3.1 ± 0.3 μg/ml (48 h), 15.2 ± 0.4 μg/ml (48 h)	XTT	0–10 μmol/l	0–72	[94]
SK-Hepl, PLC/PRF/5	3.3 ± 0.3 μg/ml (48 h), 13.9 ± 0.8 μg/ml (48 h)				
K562, U937 P3H1, Raji	14.1 ± 1.0 μg/ml (48 h), 9.0 ± 2.4 μg/ml (48 h), 7.9 ± 1.9 μg/ml (48 h), 0.6 ± 0.3 μg/ml (48 h)	XTT	0–10 μmol/l	0–72	[94]
L929	40 μg/ml (72 h)	MTT	0–100 μg/ml	72	[95]

CNC, cell number counting; FC, flow cytometric analysis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SBA, sulforhodamine B assays; TBS, trypan blue staining; TCR, tested concentration range; TIR, tested incubation time range; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide.

epithelial cell line PWR-1E [61]. In addition, berberine exhibits protective effects against G_0/G_1 phase arrest induced by SIN-1 in porcine kidney cell line LLC-PK1 [102]. The different concentrations used in different studies may account for the conflicting information in the literature, as berberine at low doses (12.5–50 μmol/l) is concentrated in mitochondria and promotes G_1 arrest, whereas higher doses (over 50 μmol/l) result in cytoplasmic and nuclear accumulation and G_2 arrest [29]. In fact, as early as 1996, berberine was found mainly in cytoplasm during berberine-induced (100 μg/ml) cell cycle G_2/M arrest, whereas it was highly concentrated in nuclei in the induction of apoptosis under high dose (200 μg/ml) [96]. In addition, different cell lines exhibit significantly different sensitivities to this alkaloid, as discussed above.

Berberine-induced G_1 cell cycle arrest is mediated through the increased expression of Cdki proteins (Cip1/p21 and Kip1/p27), a simultaneous decrease in Cdk2, Cdk4, Cdk6, and cyclins D1, D2, and E, and enhanced binding of Cdki–Cdk [22,61,101]. The G_2/M cell cycle arrest induced by berberine might be mediated by suppression of the cyclin B1 expression, inhibition of Cdc2 kinase activity, and together with increased Wee1 expression through upregulation of p53 gene [70,99,103]. Berberine exhibits cyclin E inhibitory effect in human glioblastoma T98G cells, human prostate carcinoma DU145 cells, and human epidermoid carcinoma A431 cells [22,61,101], whereas Huanglian extract (50% is berberine) does not suppress the protein expression of cyclins A or E in human gastric cancer cell line MKN-74

[103]. Besides the cell differences, the concentration of berberine might account a lot for this inconsistence, as the highest concentration in latter studies is only 10 μg/ml of Huanglian extract (approximately 15 μmol/l berberine), which is much lower than that of the former study. The effects of berberine on the cell cycle are shown in Fig. 2.

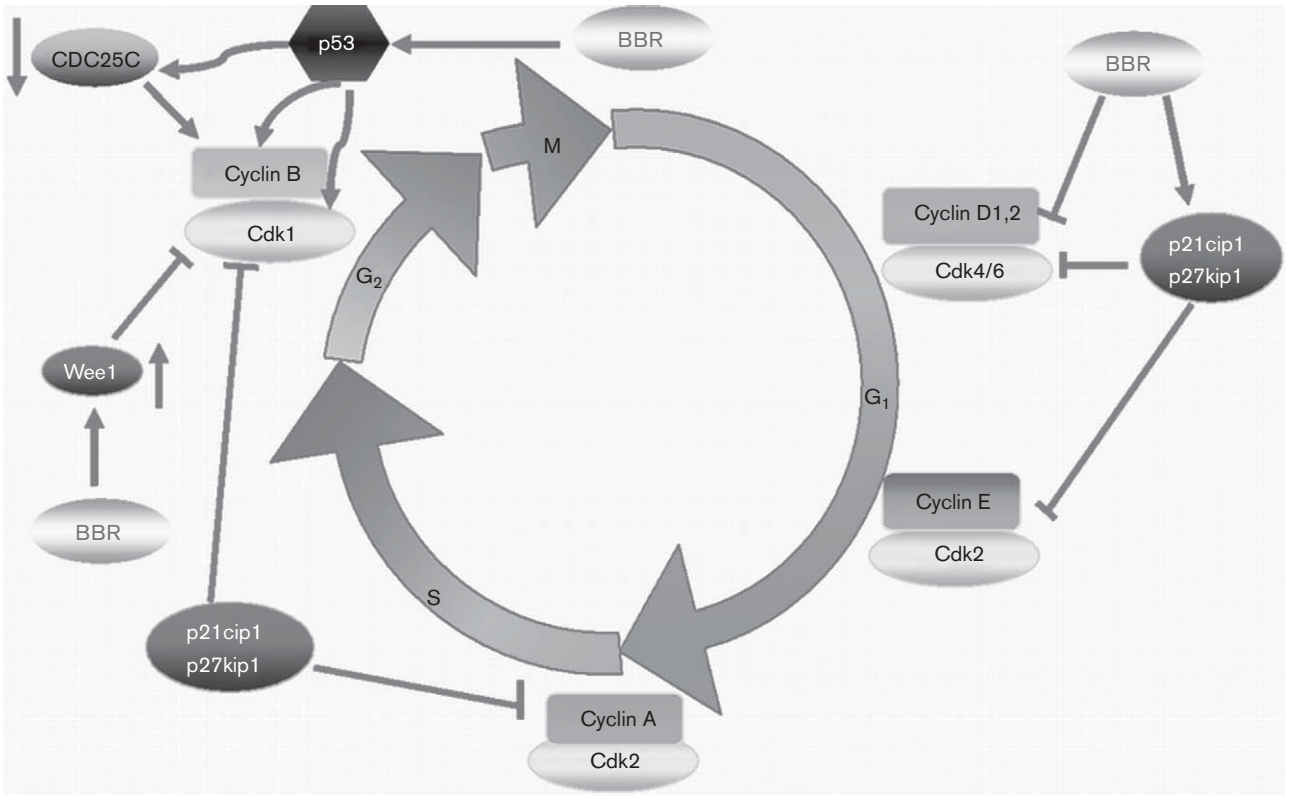
Induction of apoptosis

Many potential cancer-protective agents can be broadly categorized as blocking agents, which impede the initiation stage, or suppressing agents, which arrest the promotion and progression of tumor, presumably by affecting or disturbing crucial factors that control cell proliferation, differentiation, or apoptosis [104]. Recent detailed knowledge on molecular carcinogenesis provided the potential for therapeutic intervention in cancer by specifically targeting and sensitizing cancer cells to apoptosis [105]. Berberine shows proapoptotic effects in many cancer cell lines and nontumor cells, including HL-60 cells [24,106,107], Balb/c 3T3 cells [96], HeLa and L1210 cells [68], SNU-5 cells [70], U937 cells [23,69], B16 cells [23], Ehrlich ascites carcinoma (EAC) cells [82], WEHI-3 cells [107], A431 cells [22], prostate cancer cells [61], human oral epithelioid carcinoma cell lines (KB) [52], SW620 cells [104], and SMMC-7721 cells [71]. Its roles in apoptosis are described below.

Alteration of proapoptotic and antiapoptotic gene expression

As is well known, Fas (APO-1/CD95) is a prototypic death receptor expressed on the surface of a number of cell types, which triggers apoptosis by binding its ligand FasL. Berberine treatment could not only dose-dependently and time-dependently increase Fas protein expression, but also induce FasL expression in tumor cell lines [104,108]. Furthermore, berberine upregulates the pro-apoptotic gene p53 protein expression and activates its phosphorylation *in vitro* [17,70], which might also account for the induction of activating transcription factor 3 in human colorectal cancer cells [109] and the inhibition of platelet-derived growth factor-induced VSMCs growth [17]. Besides, p53 also cooperated in berberine-induced growth inhibition and apoptosis of non-small cell human lung cancer cells *in vitro* and tumor xenograft growth *in vivo* [18]. More recently, several studies have shown that berberine induces cell cycle arrest and apoptosis of human osteosarcoma cells [35], human neuroblastoma cells [110], and prostate cancer cells [111] in a p53-dependent manner. Berberine increases Bax, another

Fig. 2



Effects of berberine on cell cycle. In tumor cells, berberine at low doses promotes G_1 arrest, whereas at higher doses, results in cytoplasmic and nuclear berberine accumulation, and G_2 arrest. BBR, berberine.

proapoptotic gene protein expression in cancer cells [22,61,70,101,107]. Meanwhile, the antiapoptotic Bcl-2 family genes, including Bcl-2, Bid, Bcl-xL, and BID, were significantly decreased [22,61,70,101,104,108] and the ratio of Bax/Bcl-2 protein expression was elevated [101,112]. The alteration of berberine on proapoptotic and antiapoptotic gene expression might be partly mediated by the generation of reactive oxygen species (ROS) and the activation of multiple signaling pathways, such as the JNK/p38 MAPK signaling pathway [104], protein kinase C (PKC), ERK (extracellular signal-regulated kinase), and glycogen synthase kinase-3β [109], etc.

Role of reactive oxygen species

ROS are involved in various biological effects, such as cell activation, proliferation, survival, and apoptosis, mediated by many signaling pathways, such as MAPK, ERK1/2, JNK, NF-κB, Akt, caspases, and calcium [113]. Berberine showed inhibitory effects on lipoxygenase [114] and xanthine oxidase [60], two important ROS-derived sources, suggesting its antioxidative potentials. Recent studies have shown that berberine could prevent Cu^{2+}-induced LDL oxidation and protect oxidized LDL-induced cellular dysfunction [115]. In rat mesangial cells, berberine also significantly increased superoxide dismutase activity and decreased superoxide anion and malondialdehyde (MDA) formation [116]. In cultured rabbit corpus cavernosum smooth muscle cells, berberine inhibits the damaging effects of H_2O_2, with increased cell viability, NO production, superoxide dismutase activity, and decreased lactic acid dehydrogenase release and MDA content [117]. These in-vitro antioxidative results were further verified by some in-vivo studies [84,118,119]. However, in cell-free system, berberine (1 mmol/l) shows only moderate $OH^{\bullet}$-scavenging activity (23%), which is much less than that of berberrubine (85%) and coptisine (79%) [120]. However, some investigations have shown that berberine induces ROS formation, which might play an important role in berberine-induced apoptosis [69,70,104,121]. (i) Berberine treatment increases intracellular ROS production in multiple tumor cell lines [29,69,70,90,100,104,112,121], which might be mediated by enhancement of xanthine oxidase activity and inhibition of respiratory chain complex I in mitochondria [90,121]. Berberine treatment increases ROS generation in prostate cancer cells but not in normal prostate epithelial cells [121], which might partly contribute to explaining the antioxidative effect of berberine in normal cells, as discussed above. Another possibility is that, at lower concentrations, prooxidant action through inhibition of complex I in mitochondria outweighs the antioxidative activity, whereas at higher concentrations, the antioxidative activity prevails over the prooxidant effect [90]. However, the autofluorescence might be neglected by some researchers, as berberine shows intensive fluorescence and its excitation and emission wavelengths are very close to the commonly used ROS probe, such as dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) (excitation/emission 488/525 nm). (ii) Pretreatment of *N*-acetyl-L-cysteine (NAC), a well-known antioxidant, significantly decreases berberine-induced ROS production and prevents berberine-induced apoptosis [104,121]. (iii) NAC administration prevents berberine-induced release of cytochrome *c* and Smac/DIABLO into the cytosol and reversed berberine-induced apoptosis effects through the inhibition of JNK, p38 and c-jun activation, and FasL and t-BID expression [104,121].

Effect on mitochondrial transmembrane potential ($\Delta\psi m$), cytochrome c release, and caspase activation

It is widely accepted that mitochondria are central regulators of intrinsic apoptosis pathways, and alterations in mitochondrial structure and function play an important role in apoptosis. Cytochrome *c* release from mitochondria is a key step in the apoptosis induced by many death stimuli, whereas the caspase family members are central initiators and executioners of apoptosis.

Changes in the mitochondrial membrane potential ($\Delta\psi m$) have been linked to the initiation and activation of the apoptotic cascade. The fall or loss of $\Delta\psi m$ was observed after berberine treatment in tumor cell lines, such as HepG2, SNU-5, T98G, A431, human prostate carcinoma cells, and U937 by JC-1, DiOC6, or rhodamine 123 staining [22,69,70,101,108,121], and in isolated rat liver mitochondria [90]. This was followed by increased cytochrome *c* release from mitochondria [22,61,108,121]. It is certain that caspases 9, 3, and poly(ADP-ribose) polymerase are involved in berberine-induced apoptosis [22,61,69,101], whereas the activation of caspase 8 is still controversial [69,104,108]. In addition, berberine also remarkably downregulates the caspase inhibitor c-IAP1 protein expression in tumor cells [104].

Effect on NF-κB activation

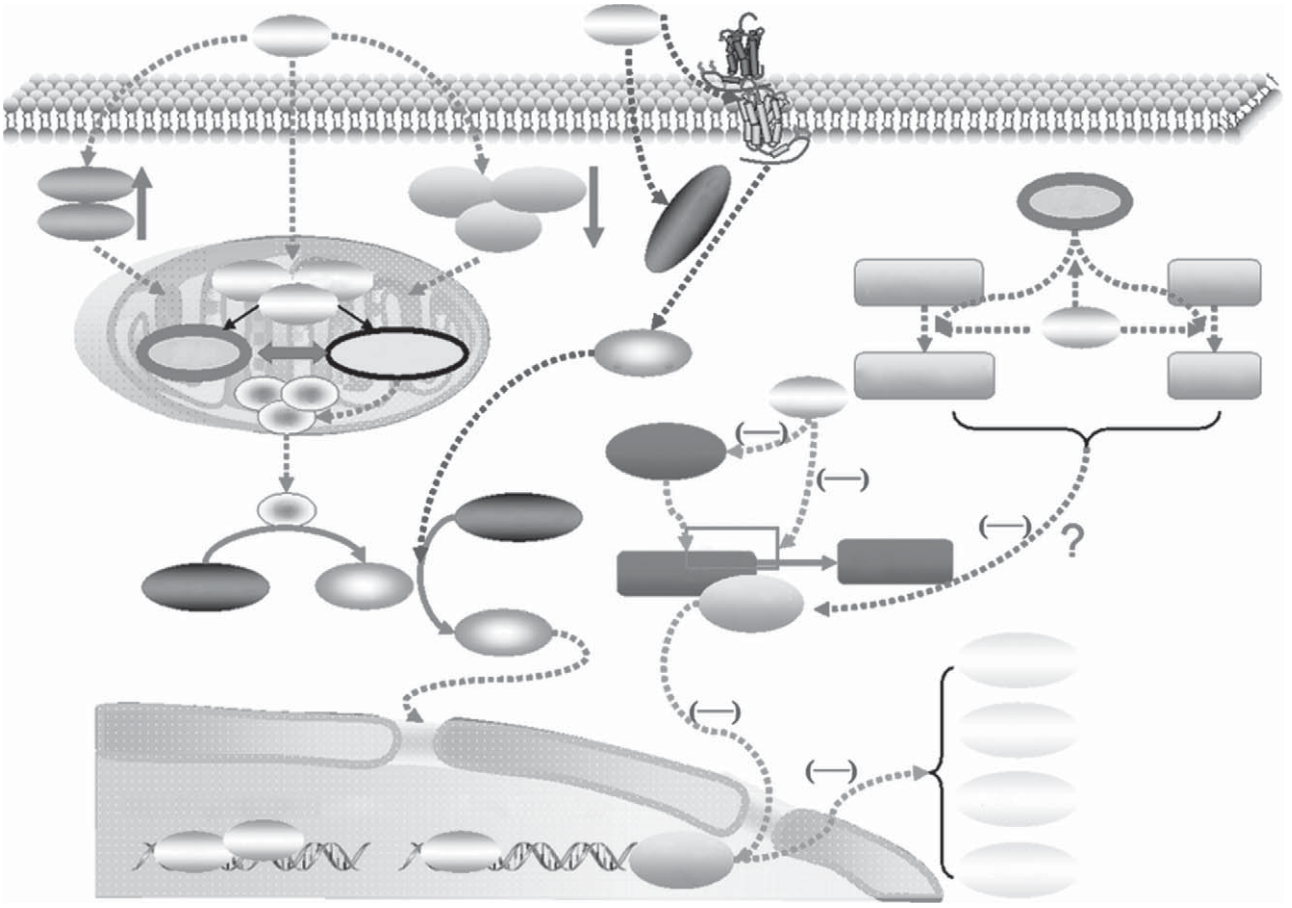
Berberine inhibits NF-κB activation induced by various inflammatory agents and carcinogens, such as TNF-α, PMA, okadaic acid (OA), and cigarette smoke condensate, which is mediated through the inhibition of phosphorylation and degradation of IKBα by the inhibition of IKB kinase activation, leading to the suppression of phosphorylation and nuclear translocation of p65 and finally to inhibition of NF-κB reporter activity. This resulted in the deceased expression of NF-κB-regulated gene products involved in antiapoptosis (Bcl-xL, survivin, IAP1, IAP2, and cFLIP), proliferation (cyclin D1), inflammation (COX-2), and invasion (MMP-9) [21]. Its effect on NF-κB is nonspecific for tumor cells, which is also observed in SW620 colonic carcinoma cells [104], keratinocytes [122], lung epithelial cells (A-549), and fibroblasts (HFL1) [123]. However, the potential mechanisms underlying this inhibition might be different between tumor cells and nontumor cells. Berberine-inhibited NF-κB activation in tumor cells is mediated by its time-dependent phosphorylation of JNK and p38

MAPK and inactivation of ERK [104], whereas in nontumor cells this might be through suppression of Akt activation but not p38 MAPK [124]. The effect of berberine on tumor cell apoptosis is as shown in Fig. 3.

Effect on tumor metastasis

In confrontation cultures consisting of embryoid bodies and multicellular DU-145 prostate tumor spheroids, berberine significantly inhibits MMPs, including MMP-1, MMP-2, MMP-9 protein expression and angiogenesis [125], which is also observed in SNU-5, HL-60, and WEHI-3 cells [76,127]. Furthermore, berberine also decreased basal and UV-induced MMP-1 and TPA-induced MMP-9 expression and activity in human dermal fibroblasts and normal human keratinocytes, respectively [76,127]. This MMP inhibition is partly mediated by decreased intracellular ROS levels, as free radical scavengers, such as vitamin E, also shows similar results [107,126]. In human lung cancer A549 cells, berberine inhibits MMP-2 expression by regulating the tissue inhibitor of metalloproteinase-2 [85]. In rat C6 glioma cells and U-87 human malignant glioma cells, berberine significantly decreases the activation of PKCα and PKCε, and leads to actin cytoskeleton rearrangements. The levels of two downstream transcription factors, *myc* and *jun*, and MMP-1 and MMP-2 are also significantly reduced [74]. The suppression of MMPs partly contributes to the inhibitory effect on the motility and invasion ability of the tumor cells [86]. However, berberine shows no inhibitory effect on the phosphorylation of Akt and enzymatic activity of MMP-2 [128]. A recent study has shown that berberine inhibits migration and invasion of human SCC-4 tongue squamous carcinoma cells, which is mediated by the p-JNK, p-ERK, p-p38, IκK, and NF-κB signaling pathways resulting in inhibition of u-PA, MMP-2 and MMP-9 [129].

Fig. 3



Effect of berberine on apoptosis. Three signaling pathways might be involved in berberine-induced apoptosis: (i) Berberine could accumulate in mitochondria, increase mitochondria ROS formation, and affect mitochondrial transmembrane potential ($\Delta\psi m$), which result in the cytochrome *c* release and activation of caspases 9 and 3. (ii) Berberine activates Fas/FasL death receptor pathway to induce apoptosis. (iii) Berberine induced JNK and p38 MAPK phosphorylation mediated by ROS, which might lead to the suppression of nuclear factor-κB (NF-κB) activation. Furthermore, berberine increases proapoptotic gene, such as Bax, and decreases antiapoptotic gene, such as Bcl-2, Bcl-xL, Bid, Survivin, IAP1, IAP2, and cFLIP. BBR, berberine; ROS, reactive oxygen species.

NM23-H1 and SDF-1 are candidate genes involved in the mobility and migration of tumor cells. Berberine could substantially increase the expression of NM23-H1 and reduce SDF-1 protein level, which results in decreased 5-8F cell motility and leukemic stem cells migration [78,130].

Berberine directly inhibits human umbilical vein endothelial cells tube formation *in vitro*. Migration and modified confrontation culture experiments showed that berberine inhibits the capacity of hypoxic SC-M1 cells to stimulate human umbilical vein endothelial cells migration, which is because of its regulation on vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α, two key factors in mediating tumor angiogenesis. Berberine does not downregulate HIF-1α mRNA but destabilized HIF-1α protein through proteolysis, which abrogates the accumulation of HIF-1 and the *trans*-activation of VEGF gene and subsequently abolishes hypoxia-induced VEGF expression [73].

In vitro, berberine exerts a dose-dependent and time-dependent inhibitory effect on the motility and invasion ability of highly metastatic A549 cells under noncytotoxic concentrations. This results from its inhibition of u-PA and MMP-2 expression through regulating the urokinase–plasminogen activator inhibitor and the tissue inhibitor of metalloproteinase-2, respectively, which is meditated by upstream mediators of the effect involved *c-jun*, *c-fos*, and NF-κB [85]. *In vivo*, oral administration of berberine for 14 days significantly inhibits the spontaneous mediastinal lymph node metastasis produced by orthotopic implantation of Lewis lung carcinoma into the lung parenchyma in a dose-dependent manner, but does not affect the tumor growth at the implantation site of the lung. These antimetastatic properties are mediated through repression of AP-1 activity by suppressing the expression of u-PA [86], whose overexpression is correlated with lymphatic metastasis of lung cancer [131,132].

Application in combined medication

In GBM cells, treatment with a nontoxic dose of berberine renders GBM cells more sensitive than vehicle-treated control cells to X-rays (radiosensitization), which is not observed in primary human glial cultures, suggesting that berberine could be integrated with postoperative radiotherapy to selectively promote residual GBM tumor cell death [79]. As_2O_3-induced inhibition of glioma cell growth, reduction in motility and invasion are significantly enhanced by berberine cotreatment [74]. More recently, a study indicated that berberine and *Coptis* extracts enhance the anticancer effect of estrogen receptor antagonists on human breast cancer MCF-7 cells through downregulating the expression of EGFR, HER2, Bcl-2, and COX-2, and upregulating IFN-β and p21 [53]. These studies suggest the potential of berberine in the combined medication in tumor chemotherapy.

Improvement of multidrug resistance

The ATP-binding cassette (ABC)-superfamily multidrug efflux pumps are known to be responsible for chemoresistance: P-glycoprotein (ABCB1), MRP1 (ABCC1), and ABCG2 (breast cancer resistance protein). These transporters play an important role in normal physiology by protecting tissues from toxic xenobiotics and endogenous metabolites [133]. ABCG2 has been identified as a multidrug transporter that confers resistance on tumor cells [134]. The ABC proteins of the MDR-type meditate berberine uptake in cultured *Coptis japonica* cells [135]. Furthermore, the ABCG2 expression and the side population are decreased by berberine in MCF-7 breast cancer cells [72].

The overexpression of human multidrug resistance (MDR1) gene coding for multidrug resistance transporter (pgp-170) has been reported in numerous solid tumors, including colon carcinoma, renal carcinoma, hepatoma, and pancreatic carcinoma. Berberine treatment could upregulate the pgp-170 expression in oral (KB,OC2), gastric (SC-M1,NUGC-3), colon (COLO205,CT26), and HepG2, Hep3B, HA22T/VGH cancer cells [136,137]. Furthermore, paclitaxel induced dose-dependent cytotoxicity, apoptosis and/or G_2/M arrest in OC2, SCM1, and COLO205 cells is blocked by berberine pretreatment, which might be mediated by its modulation of pgp-170 expression and function in these cells [136].

In-vivo anticancer activities

One of the earlier studies suggested that cytostatic activity of berberine against Ehrlich ascites and a lymphomatous ascites tumor is manifested only in culture and not when the tumors are growing in mice [138]. Berberine also exerts an inhibitory action on the growth of S180 cells in culture but when given to tumor-bearing mice by daily injections, there is no prolongation of survival. Rather, the life span decreases with increasing doses [27].

However, later studies have shown that berberine exhibits significant anticancer effects *in vivo*. In Dalton's lymphoma ascites tumor cells-bearing mice, berberine hydrochloride treatment remarkably increases life span and intraperitoneal administration is more effective than oral administration [95]. Berberine and tetrahydroberberine derivatives show no anticancer activity, but berberine, berberrubine, and the ester derivatives of berberrubine exhibit a strong activity against sarcoma-180 ascites [139]. Berberine sulfate inhibits the effects of the tumor promoters 12-*O*-tetradecanoylphorbol-13-acetate, teleocidin, and markedly suppresses the promoting effect of teleocidin on skin tumor formation in mice initiated

with 7,12-dimethylbenz[a]anthracene [140]. 20-Methylcholanthrene or *N*-nitrosodiethyl-amine-induced carcinogenesis is also significantly suppressed by berberine hydrochloride in a dose-dependent manner in small animals, indicating that berberine offers protection against chemical carcinogenesis [141]. In animal studies, berberine also potentiates the anticancer activity of carmustine, cyclophosphamide, or *Alstonia scholaris* extract when used in combination [95,142,143]. Furthermore, this synergistic effect is also observed when berberine and irradiation are applied in combination to treat both in-vivo and in-vitro models of lung cancer [144]. Berberine inhibits azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation and putative preneoplastic lesions of the colon in male F344 rats, which is because of its inhibition of COX-2 activity [145]. Furthermore, berberine administration also improves AOM-induced lipid peroxidation, protein-bound carbohydrates, and antioxidative status in rats [146]. In addition, *Coptidis rhizoma* supplement significantly attenuates weight loss of nude mice carrying a human esophageal cancer cell line YES-2 without a change in food or water intake, and its major component berberine dose-dependently inhibits secretion of IL-6 by YES-2 cells *in vitro* [147]. The same group reported similar results in syngeneic mice bearing colon 26/clone 20 carcinoma cells [148]. In a CC-4 tumor cell-implanted murine xenograft model, berberine treatment results in a reduction in both tumor incidence and tumor size [149].

Furthermore, in Friend murine leukemia virus (FMuLv)-induced erythroleukemia in BALB/c mice, berberine elevates the life span of leukemia-harboring animals by more than 60 days, which might be partly because of the decreased expression of Bcl-2, Raf-1, Erk-1 IFN-γ receptor and erythropoietin and increased expression of p53 [150].

However, there is observation that low dose of berberine (1 mg/kg) might stimulate tumor mass, although higher doses (5 and 10 mg/kg) significantly reduce the tumor volume and tumor weight [77]. It is also interesting to note that oral administration of berberine significantly inhibits the spontaneous mediastinal lymph node metastasis produced by orthotopic implantation of Lewis lung carcinoma into the lung parenchyma in a dose-dependent manner, but does not affect the tumor growth at the implantation site of the lung [86].

Conclusion

Decades of basic research provide evidence that berberine has anticancer potential, which happens at different layers and different stages of tumorigenesis. However, the inconsistence of its anticancer activities *in vivo* needs further systemic study to evaluate. Berberine has been widely used to treat gastroenteritis and diarrhea patients in the Chinese population for a long time and side effects can result from high dosages and may include gastrointestinal discomfort, dyspnea, lowered blood pressure, flu-like symptoms, and cardiac damage [3]. These accumulated data may be helpful for its future clinical trials for cancer chemotherapy.

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