Anticancer mechanisms of Berberine: a good choice for glioblastoma multiforme therapy

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Abstract

The most typical malignant brain tumor, glioblastoma multiforme (GBM), seems to have a grim outcome, despite the intensive multi-modality interventions. Literature suggests that biologically active phytomolecules may exert anticancer properties by regulating several signaling pathways. Berberine, an isoquinoline alkaloid, has various pharmacological applications to combat severe diseases like cancer. Mechanistically, Berberine inhibits cell proliferation and invasion, suppresses tumor angiogenesis, and induces cell apoptosis. The effect of the antitumoral effect of Berberine in GBM is increasingly recognized. This review sheds new light on the regulatory signaling mechanisms of Berberine in various cancer, proposing its potential role as a therapeutic agent for GBM.

Keywords: Glioblastoma multiforme; Phytomolecules; Berberine; Autophagy; Apoptosis

1. Introduction

Globally, the most frequent type of malignant brain tumor with multiple hereditary and epigenetic defects is glioblastoma multiforme (GBM) [1]. Consequently, patients generally die a few months after their diagnosis, primarily associated with GBM invasion and proliferation [2]. The established protocols for managing GBM (maximal surgical resection and localized radiation) are palliative [3, 4]. Several chemotherapeutic agents, like alkylating agents (such as temozolomide; TMZ) or nitrosoureas (such as carmustine), only result in a slight improvement in the patients' survival [5]. Also, GBM poses a substantial degree of chemotherapy and radiation treatment resistance, which is why it is crucial to identify unique, effective agents for the management of GBM [6].

Natural bioactive plant products are gradually attracting interest as alternative and adjuvant agents for preventing and treating numerous human diseases, like cancer [2]. This strategy has various advanced benefits compared to conventional therapeutic approaches, including reduced toxicity, availability, and cost-effectiveness. Consequently, many studies emphasize that tumor cell development could be suppressed by potential phytomolecules like Zerumbone, Auraptene, Quercetin, Curcumin, and Berberine [5, 7-11]. Recently, Berberine (Fig. 1), an isoquinoline alkaloid from *Coptidis Rhizoma*, has been established as a promising anticancer phytochemical [12-14]. Pharmacologically, Berberine reduces the cell growth, invasion, and metastasis of multiple cancers [15]. Besides, Berberine has gained significant attention, as it is nontoxic and shows substantial clinical benefits without severe adverse reactions [16-19]. Berberine is not cytotoxic on human bronchial epithelial cells and peripheral blood mononuclear cells, suggesting that this compound could be safe for cancer treatment [20-22]. Hence, the main aim of this review was to carry out a detailed evaluation of the therapeutic potential of Berberine in cancer, especially GBM, accompanied by mechanistic findings obtained from preclinical and clinical models.

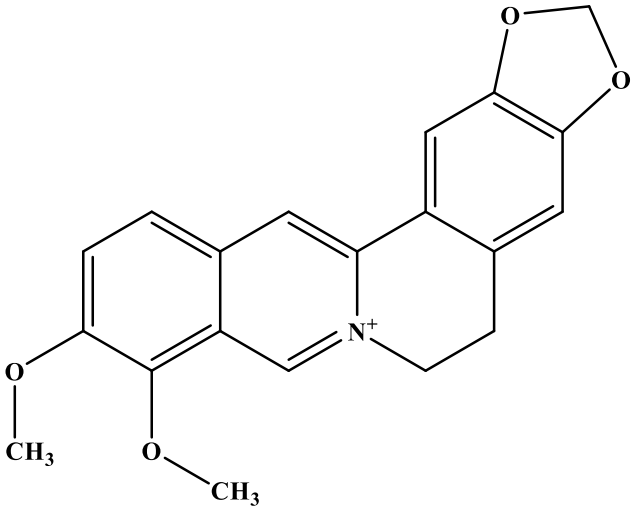

Figure 1. Chemical structure of Berberine (molecular weight 336.3612 g/mol).

2. Anticancer mechanisms of Berberine

It has been reported that Berberine has an anticancer effect via regulating the various type of signaling pathways. Berberine can induce apoptosis via activation of p38 mitogen-activated protein kinase (MAPK), intracellular and mitochondrial reactive oxygen species (ROS) production, caspase activation, suppression of nuclear factor-kappa-B (NF-κB) translocation, decreasing the depolarization of the transmembrane potential, inhibition of the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, consequently, promoting death receptor (DR)-mediated extrinsic pathway and the mitochondria-dependent intrinsic pathway [13, 23, 24]. Furthermore, Berberine can induce apoptosis via cell cycle arrest induction (G1 arrest at lower concentrations and G2/M arrest at higher concentrations) [25]. Berberine was also identified as an autophagy suppressor (via modulating the PTEN/Akt/mTOR, AMP-activated kinase (AMPK) activation, elevating levels of GRP78, etc.) that suppresses tumor development [26]. Moreover, several studies have shown that Berberine can inhibit angiogenesis, invasion, and metastasis by inhibiting different proangiogenic factors, proinflammatory factors, E-cadherin and N-cadherin, urokinase-type plasminogen activator (u-PA), and MMPs, various growth factors, PI3K/Akt, transforming

growth factor (TGF)-β, and Wnt/β–catenin pathway [27, 28]. The detailed anticancer mechanisms of Berberine are reported and discussed below.

2.1. Cell death

2.1.1. Apoptosis-inducing effects

Apoptosis, the process of programmed cell death or cellular suicide [29, 30], is regulated by the DR-mediated extrinsic pathway and the mitochondria-dependent intrinsic pathway [31]. In particular, the apoptotic signaling pathways' regulation could provide significant insight into apoptosis targeting during the invention of novel chemotherapeutic natural agents. The most apparent impact of these substances is to promote apoptosis of tumor cells [32]. Recently, the anticancer activities of Berberine have been thoroughly investigated, and DR-specific ligands (FasL and tumor necrosis factor-related apoptosis-inducing ligand) were identified to activate a sequence of pro-apoptotic factors (caspase-8, caspase-3, and poly (ADP-ribose) polymerase; PARP), leading to cell death. Activated caspase-8 resulted in the loss of mitochondrial transmembrane potential, thereby activating the mitochondria-dependent intrinsic apoptotic signaling pathway. Upregulation of Bad expression and activation of caspase-9, in turn, stimulate downstream pro-apoptotic factors, causing cell death [33-35].

Further, the downregulation of anti-apoptotic factors like Bcl-2 and Bcl-xL accelerates mitochondria-dependent intrinsic apoptotic cell death [36]. For instance, in leukemia, Berberine led to cell apoptosis by upregulation of caspase-8 and caspase-9 and suppressing the expression of Bcl-2 by caspase-3 activation [37, 38]. Furthermore, Berberine-induced FasL upregulation was mediated by the p38 MAPK signaling pathway [33].

Berberine was reported to activate intrinsic apoptosis in multiple cancer cells through several pathways. Caspases-3/-8, as well as the cleavage of PARP, are some of these pathways. In line with this, a study showed that Berberine-induced cancer cell apoptosis was mediated by a

mitochondrial-dependent intrinsic apoptotic signaling pathway [39]. In cervical cancer, Berberine reduced cell proliferation through the mitochondria-mediated path and induced apoptosis [40]. Berberine also inhibited the growth of human osteosarcoma cancer cells (MG‑63) through induction of apoptosis (Bax upregulation) and DNA damage [41, 42]. Berberine also has been shown to activate caspases by rising levels of cytochrome C, AMPK activation, MAPK initiation, and ROS generation [23, 43, 44]. Berberine inhibited proliferation and induced apoptosis of non-small cell lung cancer (NSCLC) cells through activation of the p38 MAPK signaling pathway, followed by p53 expression [45].

It has been shown that Berberine increases ROS production in human pancreatic cancer cell lines, facilitating apoptosis [46]. Recently, the 13-ethyl-Berberine, as a more potent Berberine, induced breast cancer cell apoptosis by promoting intracellular and mitochondrial ROS and regulating the apoptosis-related proteins involved in the intrinsic but not extrinsic pathway [47]. In the colon cancer cell line, Berberine could stimulate ROS production, leading to cathepsin B release and PARP activation-dependent apoptosis-inducing factor (AIF) activation, resulting in caspase-independent cell death [48]. In multiple myeloma cells (U266) and human gastric cancer cells (SNU-5), Berberine suppressed NF-κB nuclear translocation, resulting in ROS generation and, consequently, apoptosis [49, 50].

Mitochondrial dysfunction is involved in apoptosis and is essential to the apoptotic pathway process [51]. Indeed, it has been shown that the opening of mitochondrial permeability pore induces depolarization of the transmembrane potential ($\Delta\psi m$) and release of apoptosis factors; hence, loss of $\Delta\psi m$ may be an early event in the apoptotic process [52]. In this regard, Berberine in human gastric carcinoma cells decreased the $\Delta\psi m$ and then led to the release of mitochondrial cytochrome C into the cytoplasm and caused the activation of caspase-3, leading to apoptosis [25]. We have summarized the results reporting the effects of Berberine on apoptosis in cancer cells in Table 1.

Table 1. Summary of results reporting effects of Berberine on apoptosis.

Cell line or Animal	Experimental model	Main effects	Reference
MGC803	Gastric cancer	Inducing apoptosis	[5]
HPV16, SiHa, HPV18, HeLa	Cervical cancer	Reducing the cell viability Inducing apoptosis by activating caspase-3	[40]
MG‑63	Osteosarcoma cancer	Inducing apoptosis and DNA damage	[41]
A549, PC9, H1650 and H1299	Lung cancer	Inhibiting cell proliferation Inducing apoptosis	[45]
IMCE and YAMC	Colon cancer	Stimulating a caspase-independent cell death mediator Releasing AIF from mitochondria	[48]
U266	Multiple myeloma	Inhibiting the proliferation of human myeloma cells Increasing the intracellular ROS level Inducing G2/M phase arrest and consequently apoptosis	[49]
SK-N-SH and NB-1691	Neuroblastoma cancer	Inhibiting the transcription of DAXX Induced cancer cell apoptosis	[53]
ACHN, 786-O	Renal cell carcinoma	Promoting autophagy and apoptosis	[54]
SNU-5	Gastric carcinoma	Activation of caspase-3, inducing apoptosis	[25]
VCAR3 and POCCLs	Ovarian cancer	Inducing apoptosis and necroptosis Activating the RIPK3–MLKL pathway	[55]
A549	Lung cancer	Inhibiting cell proliferation and promoting apoptosis of tumor cells Suppressed the Bcl‑2/Bax	[56]
MCF-7/MDR	Breast cancer	Inducing apoptosis by activating the AMPK-p53 pathway	[57]
HCC70, BT-20, MDA-MB-468, HCC1143, HCC38, BT-549, HCC1937, MDA-MB-231	Triple-negative breast cancer	Inducing apoptosis via G1- and G2-M-arrest	[58]
PANC-1 and MIA-PaCa2	Pancreatic cancer	Inducing G1-phase arrest and apoptosis	[46]
KB cells	Oral Cancer Cells	Apoptosis by inhibition of cyclooxygenase-2 and Mcl-1 expression	[59]
H22, HepG2, and Bel-7404 cells	hepatocellular carcinoma	Inducing the translocation of AIF between the mitochondria and the nucleus	[60]
AN3 CA, HEC-1-A, and KLE cells	Endometrial carcinoma cells	Inducing cell apoptosis through activation of the mitochondrial/caspase pathway and modulating PI3K/Akt signal transduction	[61]

Combination therapy, which incorporates a combination of medicinal agents, is an essential therapeutic strategy in cancer treatment. Compared to the monotherapy approach, the

combination of anticancer agents increases its efficiency, given that it approaches main routes in a characteristically synergistic or additive fashion [62]. In this regard, a synergistic apoptosis-inducing effect by Curcumin and Berberine in the growth inhibition of both MCF-7 and MDA-MB-231 breast cancer cells was reported [63, 64]. Also, it was found that Berberine significantly reversed cisplatin sensitivity and induced caspase-dependent apoptosis in human gastric cancer cells [65]. A similar study found that Berberine improves chemosensitivity to cisplatin by promoting cell apoptosis and inhibiting the PI3K/AKT/mTOR signaling pathway in gastric cancer [66]. Also, Berberine facilitates rapamycin-mediated human hepatoma cell death by inhibiting the mTOR signaling pathway [67]. Berberine, combined with ginsenoside Rg3, also promoted apoptosis by upregulating Bax/Bcl-2 protein [68]. In another study, Kim *et al.* revealed that the combined treatment of As2O3 with Berberine induced apoptotic signaling pathways in human neuroblastoma SH-SY5Y cells via reduction of $\Delta\psi m$, alteration of Bcl-2 family proteins, and ROS generation [69]. The sensitivity of drug-resistant human breast cancer MCF-7/MDR cell to doxorubicin was evaluated in the presence or absence of Berberine. Results revealed that Berberine could induce apoptosis through the AMPK-p53 pathway [57]. Overall, these findings point to the potential of combining two chemotherapeutic agents to improve cytotoxicity in cancer cells, indicating that Berberine-based combination therapy regimens might be beneficial in various cancer cells.

2.1.2. Cell cycle regulation

Besides inducing apoptosis, various cancer drugs are now used to induce permanent cellular cycle arrest [70]. Cell proliferation is regulated by multiple cyclin-dependent kinases (CDKs) [71]. CDK's activities are tightened and regulated by mitogenic signals and can be inhibited when cell cycle control points are activated in response to DNA damage [72]. Several studies have shown that Berberine induces cell cycle arrests, which initiates apoptosis of bladder cancer, colon cancer, thyroid carcinoma, lung cancer, endometrial cancer, and prostate

carcinoma [73-80]. A study has revealed that Berberine has been shown to induce a p53-dependent G1-cell cycle arrest and apoptosis of human osteosarcoma cells by inflicting DNA damage [81]. Samad *et al.* have shown that Berberine treatment caused G0/G1 cell cycle arrest due to high cyclin D1 (CCND1) and low CDK4 protein and mRNA levels in colorectal cancer cells [82]. In a similar study on colorectal cancer, Berberine promoted cell cycle arrest at the G1 phase through CyclinD1 and CDK4 downregulation [83]. Besides, studies of the cell cycle have shown that in a cell subpopulation (S phase), Berberine causes rapid apoptosis [84]. Lin *et al.* assessed the cytotoxic activity and apoptotic pathway of Berberine and demonstrated that Berberine induced a significant G2/M cell cycle arrest [25]. Also, a concentration-dependent decrease of S phase cells and an increase in the G2/M phase were detected with Berberine treatment [85]. Notably, Berberine can inhibit the proliferation by inducing G1-phase arrest and apoptosis by a mechanism involving ROS production rather than caspases-3/-7 activation in pancreatic cell lines (46). Numerous findings proved that Berberine induces G1 arrest at low concentrations (12.5–50 μM) and, in higher doses, results in G2 arrest (83). In this regard, Ren et al. demonstrated that Berberine at low concentrations (20 and 40 μM) caused S and G2/M cell cycle arrest, while treatment with high concentrations of Berberine (60 and 80 μM) arrested the G2/M phase of the cell cycle in melanoma cells (81). Besides, Berberine was shown to initiate G1 arrest in parallel with the activation of the p53-p21 cascade (at low concentrations, 5-20 μM). Prostate carcinoma cells were arrested at G2/M at higher doses of Berberine [23]. Based on these data, it could be hypothesized that different concentrations of Berberine cause cell cycle effects in cancer cells.

Recently, novel Berberine derivatives were also evaluated for antiproliferative activities against prostate, breast, and colon cancer cell lines. Their outcomes showed that these derivatives could arrest the cell cycle at the G1 phase [86]. Further, Berberine's encapsulation into liquid crystalline nanoparticles improved its anticancer effects in MCF7 human breast

cancer cells [87]. Pierpaoli *et al.* have shown that the synthetic derivative of Berberine, 13-(4-chlorophenyl ethyl) Berberine iodide, NAX014, exerts potential antiproliferative activity against HER2-overexpressing breast cancer cells through inducing apoptosis and modulating the expression of cell cycle checkpoint molecules involved in cell senescence [88]. Interestingly, because chemotherapy medications have various effects on cancer cells at different stages of the cell cycle, employing a combination of treatments raises the likelihood that all of the cancer cells will be removed. For instance, induction of apoptosis and cell cycle arrest in the G0/G1 phase in bladder cancer T24 cells demonstrated that Berberine might improve the cytotoxicity of epirubicin in these cells [89]. As shown in Table 2, we have demonstrated combination therapy regimens based on Berberine in various cancer cells. By promoting cell cycle arrest at the G1 or G2/M phases, Berberine makes them more sensitive to radiation and chemotherapy.

Table 2. A summary of cell cycle regulation by Berberine in various cancer cells.

Combination regimen	Berberine concentration (s)	Cancer cell line	Main mechanism	Reference
X-rays + Berberine	100 μM	Human hepatocarcinoma cells (HepG2)	G2/M arrest	[44]
Cisplatin + Berberine	100 μM	Ovarian cancer cell lines (VCAR3 and POCCLs cells)	G0/G1 cell cycle arrest	[90]
Cisplatin + Berberine	5 μM	Osteosarcoma cells (MG-63)	G0/G1 cell cycle arrest	[42]
Emodin + Berberine	5 and 10 μM	Breast cancer cells (MCF-7 and MDA-MB-231)	G0/G1 phase cell cycle arrest	[91]
Evodiamine + Berberine	25 μM	Breast cancer cells (MCF-7)	G0/G1 phase cell cycle arrest	[92]
Theophylline + Berberine	50 and 100 μM	Breast cancer cells (MDA-MB-231)	G2/M arrest	[93]
Doxorubicin + Berberine	50 μM	Murine melanoma cells (B16F10)	G2/M arrest	[94]
Galangin + Berberine	90 μM	Esophageal carcinoma cells (Eca9706, TE-1, and EC109)	G2/M arrest	[95]
TPD7[1] + Berberine	40 μM	leukemia cells (Jurkat and H9)	G1 phase cell cycle arrest	[96]
Tamoxifen + Berberine	20 μM	Tamoxifen-sensitive (MCF-7) and tamoxifen-resistant (MCF-7/TAM) breast cancer cells	G1 phase cell cycle arrest	[97]

[1]**TPD7,** (N-(40-Acetyl-30,5,6-trimethoxybiphenyl-3-yl-(N0-[4-(3-morpholin-4-ylpropoxy) phenyl] urea)

2.1.3. Autophagy regulation

Autophagy is an evolutionary mechanism of eukaryotes' life that causes lysosomal degradation or recycling of misfolded proteins and degraded or useless cell components through the development of autophagosomes [15]. Autophagy facilitates the extended longevity of tumor cells with apoptosis defects; therefore, this process is a two-edged sword in tumoral cells because of its potential to promote tumor development and suppress it [98]. Berberine, recently identified as an autophagy suppressor, inhibits autophagosome formation in MCF-7/ADR cells. Wang *et al.* have shown that Berberine reverses doxorubicin resistance in breast cancer cells by inhibiting autophagy through modulating the PTEN/Akt/mTOR signaling pathway [99]. Besides, Berberine induced both apoptotic and autophagic death of HepG2 cells associated with AMPK activation [100]. Further, it was shown that a low dose of Berberine could induce autophagy by promoting the phosphorylation of AMPK [101]. Berberine may also cause autophagic cell death in liver cancer cells [102]. In acute lymphoblastic leukemia, Berberine promoted autophagic cell death by inactivating AKT/mTORC1 signaling [103]. Zhang *e al.* have shown that Berberine represses human gastric BGC-823 cancer cell growth by inducing cytostatic autophagy [104]. Recently, it has been demonstrated that glucose-regulated protein 78 is associated with stress-induced autophagy. High levels of GRP78, a key upstream activator of the unfolded protein response (UPR), result in stress-induced autophagy, and functional blockade of the proteasome induces GRP78 to promote autophagosome formation. Berberine could cause autophagic cancer cell death by elevating levels of GRP78 [105]. In addition, Berberine-induced autophagy in p53-null leukemic cells was inhibited by 3-methyladenine, an autophagy inhibitor [106].

Notably, it has been shown that Berberine promotes doxorubicin-induced autophagy and cell death in leukemic cells [106]. Further studies confirmed the synergistic anti-breast cancer

activities of co-treatment of Berberine and Curcumin by induction of autophagic cell death [63]. In combination with radiation, Berberine (5 and 10 μM) demonstrated substantial improvement in the radiation-induced clonogenic inhibition of A549 lunar carcinoma cells compared with radiation alone. Autophagosome involvement and a more significant proportion of the acridine orange stain-positive cells were shown by the cell ultrastructure, demonstrating the increased radiosensitivity of Berberine via autophagy [107]. More interestingly, radiation therapy combined with Berberine was associated with elevated LC3II and p62, consequently blocking autophagy, suggesting its radiosensitizing effects in hepatic malignancies [44].

2.2. Angiogenesis, invasion, and metastasis

2.2.1. Angiogenesis

Angiogenesis develops new blood vessels from pre-existing vessels and is an essential process for the growth, proliferation, and metastasis of tumor cells [108]. The overexpression of proangiogenic factors leads to abnormal angiogenesis in cancer development. This induces a local disequilibrium between proangiogenic and antiangiogenic, creating a new vascular supply for cancer metastasis [109, 110]. Therefore, it is a potentially effective strategy to combat cancer growth by suppressing angiogenesis and human cancer cells' metastasis. A study showed that Berberine could reduce the angiogenic activity of hepatocellular carcinoma (HCC) cells by v) ascular endothelial growth factorVEGF) down-regulation *in vitro*, indicating that Berberine could be a potential antiangiogenic agent for HCC [111]. The extra-tumor invasion of the primary HCC implant into the surrounding normal liver tissue has effectively been suppressed by Berberine, showing its antimetastatic activity *in vivo*. Further, the antitumor activity of Berberine was accompanied by invasiveness inhibition, probably through hypoxia-inducible factor (HIF)-1α/VEGF downregulation [112]. Berberine increases the radiosensitivity of esophageal squamous cancer by inhibiting VEGF and HIF-1 expression, implying that the radiosensitive effect is possibly mediated through its anti-hypoxia effects

[113, 114]. In this regard, Zhang *et al.* have shown that Berberine radiosensitizes nasopharyngeal carcinoma by suppressing HIF-1α expression [115]. Besides, it was found that Berberine could inhibit invasion and angiogenesis by triggering HIF-1α/VEGF in NSCLC cells [116]. Further, Berberine significantly suppressed the VEGF-induced upregulation of matrix metalloproteinase (MMP)-2 at both mRNA and protein levels via VEGF-triggered extracellular regulated protein kinase (ERK)1/2 pathways [117] (Table 3).

Table 3. Summary of the *in vitro* results reporting effects of Berberine on angiogenesis.

Cell line or Animal	Experimental model	Effect	Reference
Hep G2	Hepatocellular carcinoma	Reduced angiogenesis through inhibition of secretion of VEGF from HCC and down-regulation of VEGF mRNA expression	[111]
SiHa, CaSki, and HeLa	Cervical cancer	Inhibited angiogenesis by VEGF downregulation	[113]
HUVEC	Human umbilical vein endothelial cells	Reducing VEGF	[118]
MCF-7	Human breast cancer cells	Reducing VEGF	[119]

Hamsa *et al.,* in their study, have shown that Berberine (*in vivo*, 10 mg/kg) exerted significant inhibition in proangiogenic factors like VEGF and proinflammatory mediators, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, which are involved in tumor angiogenesis. The mRNA expression levels of proangiogenic factors, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and HIF, were also downregulated in tumor cells after treatment with Berberine [120]. Recently, Pierpaoli *et al.* have shown that the antitumor effects of NAX014 (a Berberine derivative) are mainly related to its impact on tumor vascular network against a transgenic murine model of HER2/neu-positive mammary carcinoma [121]. The authors also have indicated that NAX014 reduced HER-2 overexpressing breast cancer cell migration and the frequency of lung metastasis in HER-2/neu transgenic mice, probably through VEGF downregulation [122]. Another type of study showed that

Berberine prevents non-alcoholic steatohepatitis-derived hepatocellular carcinoma by regulating inflammation and angiogenesis genes involving p38MAPK/ERK-COX2 pathways [123](Table 4).

Table 4. Summary of the *in vivo* results reporting effects of Berberine on angiogenesis.

Cell line or Animal	Experimental model	Effect	Reference
Mice	NASH-HCC	Inhibited inflammation and angiogenesis genes through p38MAPK/ERK-COX2 pathways	[123]
B16F-10 and mice	Melanoma	Reduced angiogenesis through the inhibition of HIF, VEGF, COX-2, NO, NF-κB	[120]
transgenic murine model	Breast and lung cancer	Effect on tumor vascular network, tumor cell senescence, VEGF downregulation	[121, 122]

2.2.2. Invasion and Metastasis

Invasion and metastasis are the most prevalent cause of cancer-related mortality in patients [124]. Consequently, it is crucial to examine whether Berberine may influence tumor cell migration and invasion. As modulators during the growth of an organism, the modified expression of cadherins plays an essential role in tumorigenesis, tumor development, tumor metastasis, invasion, and angiogenesis [125]. Cadherins are now considered as possible targets for cancer treatments based on current investigations of the role of cadherin signaling in malignant tumors. Classical cadherins like E-cadherin (an epithelial marker) and N-cadherin (a mesenchymal marker) proteins are well known to be closely linked to cell migration and invasion. E-cadherin is vital for maintaining tissue integrity and supplying power to keep the epithelial cell layers polarized [126]. The influence of Berberine on E- and N-cadherin is one aspect of antimetastatic activity. Berberine affects the expression of the proteins E-cadherin and N-cadherin, and its action depends on time and concentration. Mishra *et al.* showed a significant reduction in vimentin expression, N-cadherin, fibronectin, and increased E-cadherin expression, suggesting its role in inhibiting epithelial-mesenchymal transition (EMT) in

osteosarcoma cells. Also, Berberine inhibited invasion and migration of osteosarcoma cells through the MMP-2 downregulation, proving its inhibitory action on the MMPs required for cancer cell invasion. Furthermore, in mesenchymal cells and neural tissue, N-cadherin is strongly expressed and stimulates increased cellular proliferation and migration by interactions with the epidermal growth factor receptor (EGFR) [127]. In this regard, Cao *et al.* and Kim *et al.* have shown that downregulation of EGFR signaling pathways by Berberine may be involved in its anti-tumorigenic and antimetastatic effects in cancer cells [128, 129]. These findings offer a new perspective that EMT may be involved in malignant tumor development as a driving factor in tumorigenesis. EMTs in epithelial cells generally have strong cell adhesion and invasive properties. The downregulation of E-cadherin and simultaneous upregulation of other cadherins, such as N-cadherin, which plays a crucial role in invasion, are the main elements of the EMT [130]. Various growth factors (EGF, HGF, and TGF-β1) were reported to activate or modulate EMT [131]. It has also been shown that the reorganization of cell adhesion molecules (E-cadherin complexes) causes EMT modifications in various cellular networks. While these components are considered EMT targets, several extracellular matrix (ECM) components like MMPs can act as initiators of EMT modifications in some cell systems. MMPs are a family of essential proteins that degrade the ECM membrane [132].

Experimental evidence suggests that Berberine can impede tumor cell migration and invasion. There is growing evidence of a significant part of the cancer metastasis and angiogenesis played by u-PA and the MMPs. The u-PA and MMP inhibition could suppress cancer cell migration and invasion. Ho *et al.* have shown that Berberine inhibited migration and invasion of human SCC-4 tongue squamous carcinoma cells via inhibition of MMP-2 and MMP-9 and u-PA [133]. Besides, in cervical cancer, Berberine inhibited invasion of highly metastatic SiHa cells through MMP-2 and u-PA downregulation, reversing TGF-β1-induced-EMT, upregulating E-cadherin, and inhibiting N-cadherin and snail-1. In A549 human lung cancer

cells, Berberine increased the concentration and time-dependent expression of the E-cadherin protein and significantly reduced N-cadherin expression; such modifications prevented invasion and metastatic behavior [134]. In human cervical cancer, Berberine suppressed metastasis by inhibiting cell viability, migration, invasion, the expressions of MMP-9, N-cadherin, and Vimentin, and enhancement of E-cadherin expression [135]. In another study, Berberine has been documented as reducing MMP-9 expression, inhibiting cell invasion of breast cancer cells [136]. Further, Berberine inhibited the expression of MMP-9, MMP-1, MMP-13, E-cadherin, N-cadherin, and u-PA in A375.S2 and A375.S2/PLX skin cancer cells, suggesting the potential of Berberine as an antimetastatic agent in melanoma [137]. Berberine also exhibited the ability to inhibit metastasis in prostate cancer cell lines (PC-3 and LNCaP) by suppressing the migration and invasion by inhibiting EMT-related genes [138]. Studies have shown that EMT is a mediator on the PI3K/Akt signaling pathway and has drawn considerable interest as a novel objective for metastatic tumor prevention and treatment [139]. Tyrosine kinase-activated PI3K influences varieties of cellular processes such as cell proliferation, differentiation, apoptosis, angiogenesis, invasion, and migration [140]. Furthermore, the PI3K/Akt pathway has also been shown in several studies to induce EMT directly or indirectly associated with other signaling pathways, e.g., Ras, NF-κB, TGF-β, and Wnt/β–catenin [141]. Furthermore, previous research found that All-trans retinoic acid (ATRA) increased Akt activation regulation through the retinoic acid receptor (RAR)-Akt interaction. The active form of Akt significantly reduces expression levels of the tumor suppressor RAR2. Consequently, the PI3K/AKT signaling pathway may be linked to the retinoid signaling pathway. In this regard, Kou *et al.* have also shown that Berberine suppressed EMT through cross-talk regulation of PI3K/AKT and RARα/RARβ in melanoma cells [142].

Various studies have shown that Berberine inhibits the MMP-2 and MMP-9 expression and reduces the migration and invasion of highly metastatic murine melanoma cells [143]. In the

chondrosarcoma cell line, Berberine could minimize cell migration, wound-healing migration, cell invasion, and cell motility by inhibiting the expression of the $\alpha v\beta 3$ Integrin and activity of the PKCδ and c-Src signaling pathways [144]. Besides that, Berberine suppressed the expression of MMP-2/-9 by downregulating the phosphorylation of VEGFR2 and downstream signaling members (AKT and ERK1/2), highlighting the inhibitory effects of Berberine on cell proliferation and migration of human breast carcinoma [145]. Berberine exerted an anti-invasive influence on HepG2 cells through suppression of MMP-9 expression and the activity of PI3K/AKT and ERK pathways. These data suggest that Berberine may be a potential alternative against invasive hepatoma cells through PI3K/AKT and ERK pathways-dependent downregulation of MMP-9 expression [146]. In conclusion, the results show clearly that Berberine plays a significant role in regulating the pathways mediated by cadherin and MMP, leading to inhibitory impacts on the migration and invasion of GBM (Table 5).

Table 5. Summary of results reporting effects of Berberine on metastasis.

Cell line (s)	Berberine concentration (s)	Experimental model	Effect (s)	Reference (s)
SiHa and Ca Ski cells	150 μM, 200 μM, and 250 μM	Cervical cancer	Inhibiting cervical cancer cell viability and metastasis	[135]
SW620 and LoVo cells	40 μM	Colorectal cancer	Inhibiting invasion and metastasis	[147]
PC-3 and LNCaP cells	0-75 μM	Prostate cancer	Reducing metastasis by inhibiting EMT-related genes	[138]
AN3 CA and HEC-1-A cells	25 and 50 μM	Endometrial cancer	Reducing metastasis via miR-101/COX-2/PGE2 signaling pathways	[79]
B16F-10 cells	0.5, 1, and 2 mg/mL	Melanoma tumor-bearing mice	Inhibiting lung metastasis by ERK and NF-κB signaling pathways	[143]
JJ012 cells	3, 10, and 30 μM	Chondrosarcoma	Reducing metastasis via modulating the $\alpha v\beta 3$ integrin and the PKCδ, c-Src, and AP-1 signaling pathways	[144]
5-8F and 6-10B cells	2.5, 5, 10, or 20 μM[1] 15 and 30 mg/kg[2]	Nasopharyngeal carcinoma	Reducing metastasis by targeting rho kinase-mediated Ezrin phosphorylation at Threonine 567	[148]
A549 cells	5, 10, and 20 μM	Human lung cancer	Reducing ECM proteinase MMP-2 and u-PA Regulating TIMP-2 and PAI	[149]
A549 cells	90 μM	Human lung cancer	Inhibition of metastasis via downregulating VEGF and MMP-2 protein expression Suppressing metastasis through inhibition of TGF-β1-induced EMT	[56, 150]

MDA-MB-231 cells	60 and 90 μM	Breast cancer	Reducing invasion, IL-8 dependently	[151]
MDA-MB-231, MCF-7, and T-47D cells	50 and 100 μM	Breast cancer	Inhibiting the metastatic behavior via Akt pathway modulation and MMP-2/-9 downregulation	[152]
T24 cells	10, 25, and 50 μM	Bladder cancer	Inhibiting the metastasis and invasion of tumor cells via blocking the heparanase expression	[153]
AN3 CA and HEC-1-A cells	25 and 50 μM	Endometrial cancer	Inhibiting tumor growth and metastasis via miR-101/COX-2/PGE2 signaling pathways	[79]
KB cells	1 μg/mL[2]	Human oral cancer	Downregulation of MMP-2 and MMP-7 activation of the p38 MAPK signaling pathway	[33]
SNU-5 cells	75 μM	Human gastric cancer	Downregulation of MMP-1/-2/-9	[50]

[1] *in vitro*

[2] *in vivo*

3. Antitumor effects of Berberine in GBM

As discussed, GBM, as one of the most lethal brain tumors, is susceptible to natural substances through cell cycle disruption or apoptosis induction; hence, the identification of agents that can improve the survival and effectiveness of chemotherapeutic agents and postoperative radiotherapy for GBM patients remains an obstacle in neuro-oncology [154, 155]. Induction of apoptotic cell death accompanied by caspase-3 and -9 activation is mainly due to the cancer-specific cytotoxic activity of Berberine [13, 156, 157]. However, the pathways influencing apoptosis activation by Berberine are poorly defined. Agnarelli *et al.* have found that Berberine exerted G2-cell cycle arrest in U343 MG cells [158]. In contrast, it was shown that Berberine (IC50 value 134 μg/mL) is associated with a significant rise in the G1 phase through the increased expression of p27 and the decreased expression of CDK2, CDK4, cyclin D, and cyclin E proteins, which impaired cell proliferation in T98G GBM cells. Berberine also markedly enhanced apoptosis dose-dependently in T98G cells by inducing a higher ratio of the Bax/Bcl-2 proteins, the disruption of $\Delta\psi m$, and the activation of procaspase-9, caspase-9, caspase-3, and PARP [159]. These results were in line with Dajiang *et al.* (inducing cell apoptosis via Bcl-2 downregulation) [160]. Furthermore, Berberine therapy (50 and 100 μM)

was proven to contribute to reduced motility and cell death through caspase-1-mediated IL-1β inactivation in U251 and U-87 MG cells [160].

Berberine could reduce cellular viability and cause oncosis-like death, characterized by cytoplasmic vacuoles, cell swelling, and plasma membrane blebbing, in GBM cells (T98G, LN18, C6, SHG44, and LN229), and that these impacts were linked to intracellular adenosine triphosphate (ATP) depletion [161]. Molecularly, AMPK is the "fuel gauge" in the cell and is activated by increasing AMP + ADP over ATP concentration, a condition that often signals cell stress. Recently, Berberine has been reported as a potential activator of AMPK [162]. One target of AMPK is p53, which can influence cell growth and tumor progression via modulating the activity of cell survival signaling such as mTOR and Akt [163, 164]. However, the specific role of AMPK on the metastatic potential of cancer cells remains largely unknown. Kim *et al.* have shown that Berberine-induced AMPK activation inhibits melanoma cells' metastatic potential via reduced ERK activity and COX-2 protein expression [165]. Also, Park *et al.* have shown that Berberine reduced human colon cancer cell lines' migration via AMPK activation [165]. In an analysis, Berberine demonstrated a tendency to induce p53 phosphorylation, but in AMPK inhibition conditions, p53 phosphorylation did not change. These pieces of evidence show that p53 activation does not depend on AMPK in U-87 MG cells [166]. Liu *et al.* have shown that Berberine promoted the phosphorylation of wtp53 (a tumor suppressor protein), increased the expression of p21 protein, reduced cyclin D1 content, and caused G1 phase arrest in U-87 MG cells. Also, it was found that Berberine reduced mutp53 (an oncoprotein) content and caused G2 phase arrest in U251 cells with a concurrent decrease in p21, cyclin D1, and cyclin B1 content. Berberine significantly inhibited GBM growth in the mouse tumor model [167]. Palma *et al.* have demonstrated an increase of the cells in the G1-phase, together with the reduction in the G2/M-phase of U-87 MG cell cycle after treatment with Berberine, suggesting that the accumulation of cells in the G1-phase is associated with Berberine-induced

apoptosis [168]. These findings indicate that Berberine could suppress the progression of GBM cells by interfering with cell cycle markers, showing Berberine as a possible drug for the treatment of GBM.

As discussed, mitochondrial ROS production has been documented to play a role in cell death, inducing apoptosis via mitochondrial permeability transition [166]. Palma *et al.* have shown that Berberine in U-87 MG cells (25 μM) displayed elevated ROS levels, increasing oxidative stress. Also, Berberine was found to reduce the cell viability of U-87 MG cells and induce early apoptosis (10, 25, 100, and 250 μM) without any change in total caspase-3 and p-p53 levels [168]. Ting-Ching *et al.* have found that Berberine has potential cytotoxic effects (>50 μM) against C6 rat GBM cells through G2/M cell cycle arrest, apoptosis induction (increased Bax/Bcl-2 ratio, caspases-3, -8 and -9 activation), and consequently ROS generation [169]. Berberine also was shown to increase ROS production and the level of intracellular Ca^{2+} and cause endoplasmic reticulum (ER) stress as evidenced by the detection of ER stress-associated molecules, which was associated with the activation of caspase-3 and consequently apoptosis [170].

In cancer biology, it is commonly recognized that EMT, a tumor invasion marker, is implicated in invasive cell generation and cancer stem cell acquisition [130]. In various cancers, Berberine can reverse the EMT process [140, 142, 171], which results in migration, invasion, and metastasis inhibition. Consistently, Berberine has been shown to reduce the migration and invasion of TMZ-resistant GBM cells [172], thus indicating a Berberine-dependent disruption of the migratory potential in these cells. Treatment of Berberine has also greatly improved α/β-catenin protein expression, two epithelial markers, and reduced vimentin and α-smooth muscle actin (α-SMA; a tumor invasive marker) protein expression, two mesenchymal markers for both U-87 MG and U251, which suggest the EMT process may have been reversed by Berberine [160].

Fu *et al.* have recently synthesized lipophilic Berberine derivatives and evaluated their anti-GBM effects on C6 and U-87 MG cells. In this promising study, Berberine analogs inhibited cell proliferation, invasion, and migration against rat C6 and human U-87 MG cells (IC50; 1.12–6.12 μM). Furthermore, it has been found that these analogs are preferential for mitochondria, which contributes to an increase in ROS production [173]. These compounds are ideal candidates to fight against GBM by interacting with mitochondria.

Several experiments have shown that autophagy plays a role in both the survival and apoptotic death of GBM cells, implying that the specific therapy purpose defines the therapeutic importance of this complex physiological mechanism [174]. Nonetheless, previous studies have found a correlation between reduced autophagy and chemotherapy resistance in GBM cells [175]. Multiple reports have proposed that autophagy precedes the death of GBM cells upon application of TMZ, with findings suggesting that the TMZ-sensitivity of GBM cells can also be increased by compounds like rapamycin (which can activate autophagy) [176]; however, it is not confirmed throughout all findings. Therefore, it can be a better approach for minimizing chemoresistance in GBM patients to find new strategies that are sufficient to modify autophagic responses in conjunction with therapeutic chemical compounds such as TMZ. Berberine treatment can downregulate the activation of EGFR/MEK/ERK1/2 signaling, suggesting a potential role for ERK1/2 signaling in the autophagy-dependent system in GBM. In this regard, Qu *et al.* have demonstrated that Berberine is an essential solution for increasing GBM cells' sensitization to TMZ therapy by focusing on the initiation of autophagy-mediated by ERK1/2, rendering Berberine a theoretically helpful GBM therapeutic agent [172]. Besides, the latest proofs indicate that autophagy has a beneficial function in reducing tumor necrosis/inflammation and mitigating tumor cell genome damage as a response to metabolic stress. Wang *et al.* showed that the metabolic state of GBM cells was highly influenced by Berberine, leading to high autophagy flux by targeting the AMPK/mTOR/Unc-51 like

autophagy activating kinase (ULK1) pathway, reducing the invasive potential and inducing apoptotic cell death [177]. This result shows the possible therapeutic benefits of plant alkaloids that modulate autophagy in GBM therapy. Sun *et al.* have also demonstrated that Berberine induced autophagy as a defensive influence and reduced the oxygen consumption rate (OCR) by inhibiting phosphorylated-ERK1/2, preventing mitochondrial aerobic respiration. According to this report, Berberine reduces the metabolic activity of GBM by reducing ERK1/2 activity and causing autophagy, which finally contributes to apoptosis [161].

Substantial molecular studies have highly improved our understanding of the genetic modifications that underlie GBM pathogenesis. Activation of receptor tyrosine kinases (RTKs) controls many essential functions, including cell development and longevity; RTK dysregulation has been discovered in some cancers and has proven to be relevant to the growth and development of multiple cancers [177]. The activation of EGFR, as the upstream RTK regulator, is likely to play a vital role in promoting GBM cell proliferation and survival through the AKT and MAPK pathways upregulation; therefore, EGFR inhibition may prove helpful for GBM therapy [178]. Liu *et al.* have found that in GBM cells treated with Berberine, the level of EGFR and its downstream pathway RAF/MEK/ERK signaling pathway were considerably reduced (IC50 of 21.76 μM). Interestingly, Berberine was found to suppress tumor growth (*in vivo*) through EGFR downregulation and senescence induction, suggesting that successful EGFR inhibition by Berberine indicates a possible application in treating GBM patients [179]. Combination therapy regimens-based Berberine in GBM cells has been shown in Table 6.

Table 6. Combination therapy regimens-based Berberine in GBM cells.

Combination regimen	Type of study	Dose	Main findings	Reference
Berberine + Arsenic trioxide	C6 rat and U-87 MG	0-20 μM	A decrease in the activation of PKCα and ε and led to actin cytoskeleton rearrangements Reducing Myc and Jun, and MT1-MMP and MMP-2 Reduction in motility and invasion	[180]

Berberine + solid lipid curcumin particles (SLCPs)	U-87 MG and U-251MG	Berberine (100 μM) and SLCP (20 μM)	Induction of cell death DNA fragmentation A decrease in ATP levels Reduction in Δψm Inhibiting the PI3K/Akt/mTOR pathway	[181]
Berberine + TMZ	U-87 MG and U-251MG	Berberine (10 μM) and TMZ (100 μM)	Reducing invasion and migration Inducing autophagy Enhancing GBM TMZ-sensitivity	[172]

Angiogenesis is a different era for cancer treatment to suppress cancer development, including sequential stages, including tumor growth and differentiation, cell migration, and endothelial cell tube formation [182]. VEGFR2, the principal mediator for biological effects of VEGF, plays a crucial role in tumor angiogenesis and can thus prevent angiogenesis and tumor development by targeting VEGF/VEGFR2 and downstream signal molecules [183]. In this regard, Jin *et al.* have shown that Berberine could dose-dependently inhibit cell viability of U-87 MG and U251 cells (IC50 of 42 and 32 μM, respectively. More specifically, Berberine's antiangiogenic activity on GBM xenografts was revealed by targeting the VEGFR2/ERK pathway, which shines a spotlight on GBM as a promising alternative therapy (see Table 3 and Table 4) [184].

4. BBB

The blood-brain barrier (BBB) inhibits bacteria entering and large hydrophilic molecules and facilitates the transport of essential substances by the CNS. Penetration across the BBB is a crucial property for compounds targeting CNS diseases. Following intravenous administration of *C. Rhizoma* extract, Berberine's pharmacokinetics in rat hippocampus and the kinetic characteristics of Berberine are dissimilar in the hippocampus and plasma [185]. Berberine effluxes to the brain via P-glycoprotein and is characterized by reduced intestinal absorption and low solubility, related to insufficient bioavailability in the brain. Sobolova *et al.* investigated the BBB penetration of Berberine and its derivate using parallel artificial membrane permeation assay (PAMPA). They found that Berberine cannot be transported into

the brain by passive diffusion [186]. Berberine cannot pass the BBB and can reduce the permeability of BBB [187, 188]; however, some kinds of strategies can be applied to increase the transport of Berberine to the brain.

Intranasal administration of Berberine can prevent hepatic metabolism and bypass the BBB via the olfactory pathway [189]. A study by Wang *et al.* showed that intranasal delivery of Berberine via in situ thermo-responsive hydrogels could be used as a safer and more effective strategy to deliver more Berberine to the brain [190]. Furthermore, Verapamil enhances the CNS uptake of a few drugs; when concomitantly administered with Berberine, it improves brain bioavailability of Berberine in the brain [191]. Another way to increase Berberine influx through BBB is suggested by Gao *et al.* that propose using M2 macrophage-derived exosomes as a drug carrier for Berberine to deliver drugs [192]. Hopefully, the high glucose transporter level in the surface of the tumors, associated with cancer stem cells and cancer metastases, will provide 10-100-fold higher intake through the different models. Also, glucose coating is a promising technique to cross the BBB, a possible application for the treatment of GBM. Moreover, Berberine's effectiveness was explored in many CNS disease models found in the hippocampus and cerebrospinal fluid. Nano drugs have drawn considerable interest for their potential applications, which show a new chapter in medicine development, including target delivery, controllable release, less systematic toxicity, and better drug bioavailability. Interestingly, Berberine-Glu nanoparticles in GBM cells are readily absorbed, and the Berberine-Glu IC50 was much lower in U-87 MG and U251 than Berberine-Water [193]. Berberine-Glu has enhanced cytotoxicity, reduced cell viability by targeting mitochondrion with higher G2/M phase-arrest. Berberine-Glu exhibited improved brain and GBM imaging in primary U-87 MG GBM-bearing mice, which shows more Berberine passing through the BBB. Further, in a more interesting study, Fu *et al.* have shown that introducing the dodecyl to the C-9-O-position or C-13-position of the Berberine scaffold (lipophilic substituents)

significantly improved the BBB penetration of synthetic derivatives, boosting their anti-GBM impacts [173].

5. Concluding remarks and future directions

Because of its pathological features, GBM is much harder to manage than other malignant cancers. Consequently, our study focuses on clarifying the role of Berberine for GBM management by addressing its known biological functions, including apoptosis, autophagy, cell cycle, angiogenesis, migration, invasion, and metastasis (Fig. 2). The present review showed that Berberine has potent anticancer effects and primarily exerts anti-GBM properties that have been tested in *in vitro* and *in vivo* laboratory models. Notably, Berberine has demonstrated that some natural and synthetic anticancer agents have improved its cytotoxic effects. However, the implications and mechanistic pathways of Berberine in GBM should be clarified in more preclinical and clinical trials.

As mentioned, Berberine has low toxicity towards healthy cells, which is very effective in cancer therapy across several clinical stages; however, dissolution, absorption, biochemical distribution, and BBB penetration of Berberine are limited. Therefore, the emergence of new Berberine formulations and derivatives appears to be a significant tendency to transcend their limits in clinical practice. Many Berberine derivatives have been designed and synthesized to enhance passing through the BBB [173]. For instance, Yu *et al.* stated that PEG-lipid-PLGA hybrid Berberine-loaded nanoparticles promote the efficiency of oral delivery, indicating that nano-formulation is a promising technique for the improvement of Berberine pharmacokinetics [194]. Collectively, Berberine appears to be a good naturally occurring compound that has encouraged us to expand our cancer therapy knowledge through several strategies like targeted therapy, combination therapy, novel formulae to enhance its pharmacokinetic profile, and

structural change for improved effectiveness. However, additional preclinical models and clinical studies are required to evaluate the therapeutic efficacy of Berberine for GBM therapy.

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CONFLICT OF INTEREST

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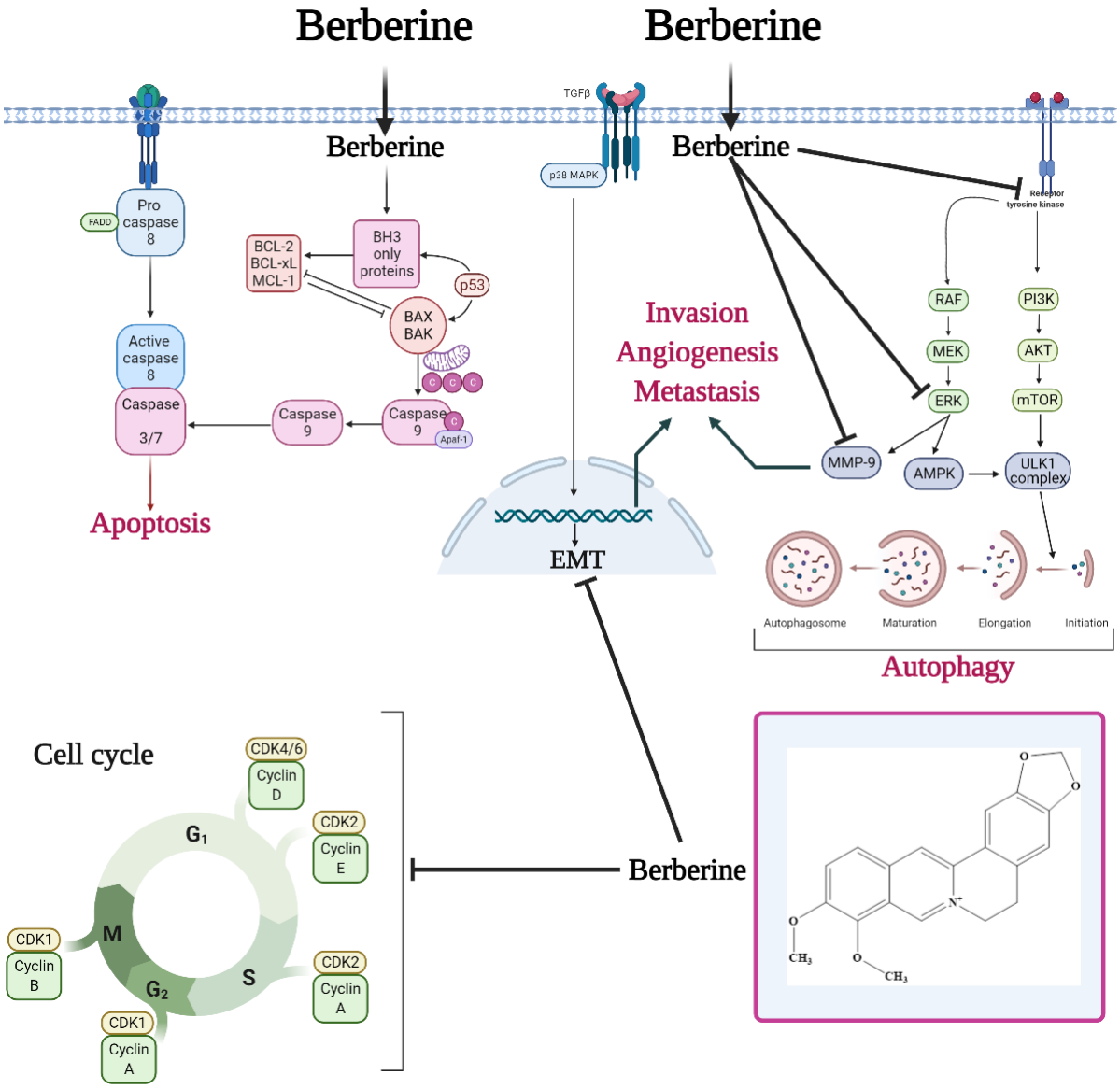

Figure 2. The anticancer mechanisms of Berberine.

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