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Review Article

Anticancer Potential of Natural Isoquinoline Alkaloid Berberine

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Abstract

Despite the availability of several therapeutic strategies and many drugs, the ability to cure most cancers remains a challenge. Natural products have been used for the treatment of numerous diseases, including cancer. The present review delineates various preclinical studies performed *in vitro* and *in vivo* that explore the anticancer potential of berberine, an isoquinoline alkaloid found in numerous plants as a secondary metabolite. Berberine can kill various types of human cancer cells in an optimal concentration- and duration-dependent manner and inhibit the growth of various types of cancers in animal models by elevating oxidative stress. In addition, berberine suppresses cell migration, invasion and epithelial-to-mesenchymal transition in different types of cancer cells. Mechanistically, berberine can induce cancer cell DNA fragmentation/apoptosis through extrinsic and intrinsic pathways, autophagy and necrosis. The cytotoxic effects of berberine in different types of cancer cells are mediated by its ability to induce oxidative stress and cell cycle arrest, and inhibit cell migration, invasion and epithelial-to-mesenchymal transition as well as matrix metalloproteinases through the modulation of Wnt and β-catenin signaling. A single clinical study has shown some promise in gastric cancer patients. Though berberine is a relatively safe compound, it should not be prescribed to pregnant or lactating women to avoid adverse effects on developing fetuses and neonates.

Keywords: Berberine; Apoptosis; Cylins; Reactive oxygen species; beta-catenin; Caspase.

Abbreviations: ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; AIF, apoptosis inducing factor; AMPK, AMP-activated protein kinase; AP, activator protein; Apaf, apoptotic protease activating factor; ATF, activating transcription factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; Bad, Bcl-2 agonist of cell death; Bak, Bcl2-antagonist/killer; Bax, BCL2 associated X apoptosis regulator; Bcl, B-cell lymphoma; BID, BH3 interacting domain death agonist; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; CCR, C-C chemokine receptor type; cdc, cell division cycle; CDK, cyclin-dependent kinase; CDKIs, cyclin-dependent kinase inhibitors; COX, cyclooxygenase; CXCR, C-X-C motif chemokine receptor; DDIG, DNA damage-inducible gene; DSBs, double-strand breaks; EBNA1, Epstein–Barr nuclear antigen 1; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ETIF, eukaryotic translation initiation factor; FADD, FAS-associated death domain; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GLP, glucose-regulated protein; GSH, glutathione; GSK, glycogen synthase kinase; HDAC, histone deacetylase; hERG, human ether-à-go-go-related gene; HIF, hypoxia inducible factor; IHCBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, c-Jun N-terminal kinase; LC3, microtubule associated proteins 1A/1B light chain 3B; LDH, lactate dehydrogenase; MEK/MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MRP, mitochondrial ribosomal protein; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; NAT, N-acetyltransferase; NCAM, neural cell adhesion molecule; Nestin, neuroectodermal stem cell marker; NF-κB, nuclear factor kappa B; Notch, neurogenic locus notch homolog protein; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PTCD, pentatricopeptide repeat domain; PTTG, pituitary tumor transforming gene; RAF, rapidly accelerated fibrosarcoma; Ras, retrovirus-associated DNA sequences; ROS, reactive oxygen species; SCAP, SREBP cleavage-activating protein; Skp, S-phase kinase-associated protein; SQSTM1, sequestosome-1; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TCF, T-cell factor; TGF, transforming growth factor; TIF, translation initiation factor; TRAIL, tumor necrosis factor-(TNF) related apoptosis-inducing ligand; TUFM, Tu translation elongation factor; ULK, Unc-51-like autophagy activating kinase; VASP, vasodilator-stimulated phosphoprotein; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Wif, Wnt inhibitory factor; WTX, Wilms tumor gene on X chromosome; XIAP, X-linked inhibitor of apoptosis protein; Δψm, mitochondrial membrane potential.

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Introduction

The word cancer evokes fear among patients, their families, and loved ones since effective cures remain elusive for many types of cancer. The incidence of cancer has been consistently increasing due to lifestyle changes and increasing environmental pollution. Modern electronic devices have also added to the increasing global cancer burden. In the United States, 1,806,590 new cancer cases were diagnosed, and 606,520 cancer patients died from the disease in 2020 alone.[1] In India, approximately 1,392,179 cases were detected in 2020 and 880,000 cancer patients died, and this figure is expected to increase fivefold by the year 2025.[2,3] The global cancer scenario is much higher, where the number of expected cancer cases was 19.3 million and mortality due to cancer reached

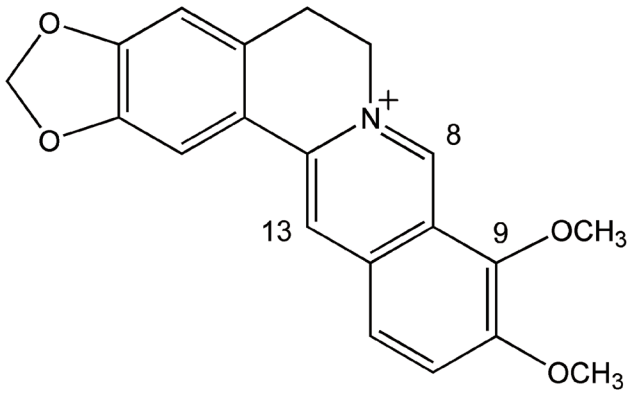

Fig. 1. Chemical structure of berberine. 7,8,13,13a-tetradehydro-9,10-dimethoxy-2,3-(methylenedioxy) berbinium.

10 million in the year 2020.[4] The availability of state of art treatment regimens in modern therapy has not significantly reduced the cancer burden in society. The cost of cancer treatment has seen a phenomenal increase in recent years due to the approval of high-cost oncology drugs and other related expenditures.[5] Modern cancer treatment regimens put a heavy economic burden on the families of cancer patients as it drains the majority of their financial resources, and the cost of cancer treatment will still see an upward trend moving forward. Therefore, it is necessary to search for new cost-effective chemotherapeutic agents with fewer toxic implications.

Many modern cancer chemotherapeutic drugs were initially derived from natural resources before the chemical synthesis was undertaken.[6] Natural products may be a great resource to aid in the search for novel cancer treatments as they may be more economic than high-cost exotic chemotherapeutic drugs. Moreover, they may also overcome the drug resistance induced by modern chemotherapeutic agents, which is a major cause of treatment failure. Berberine is a natural isoquinoline alkaloid synthesized by numerous plants including goldenseal (*Hydrastis canadensis*), yellowroot (*Phellodendron amurense*), Chinese goldthread (*Rhizoma coptidis*), Oregon grape (*Berberis aquifolium*), goldthread or savoyane (*Coptis groenlandica*), Indian goldthread (*Coptis teeta*), Indian barberry (*Berberis aristata*), bayberry (*Berberis vulgaris*), barberry (*Berberis napalensis*), Baical skullcap root (*Radix scutellariae*), Amur cork tree (*Coptis chinensis*), tree turmeric (*Berberis aristata*), giloe (*Tinospora cordifolia*), Californian poppy (*Eschscholzia californica),* Lopez root or Forest pepper or wild orange tree (*Toddalia aculeata*), false calumba (*Coscinium fenestratum*) and prickly poppy *(Argemone mexicana).*[7,8]

Berberine (natural yellow 18), also known as 7,8,13,13a-tetradehydro-9,10-dimethoxy-2,3-(methylenedioxy)berbinium or 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-*a*) quinolizinium, is an isoquinoline alkaloid with a molecular weight of 336.367 g/mol (Fig. 1). Berberine chloride is soluble in warm water at a concentration of 3.2 mg/mL, but its solubility is less in cold water (2 mg/mL). The solubility in organic solvents is 75 mg/mL in DMSO, greater than 50 mg/mL in methanol, and greater than 2 mg/mL in ethanol; however, it is sparingly soluble in chloroform. Berberine belongs to the family of protoberberine alkaloids. It is a bright yellow fluorescent powder, which has been used in India and other countries to dye wool, wood, and leather.[9] Berberine possesses yellow fluorescence under ultraviolet light and it is also used as a stain for histological examinations.[10,11]

Berberine-containing plants have been used in traditional Indian Ayurvedic and Chinese systems of medicine to treat various disorders in humans for a long time.[7,12–14] Berberine acts as an antimicrobial, anti-oxidant, antibacterial, anti-inflammatory, antidiarrheal, antidepressant, antidiabetic, antihypertensive, anti-arrhythmic, anti-osteoarthritic, chemo-sensitizing, hepatoprotective, and neuroprotective agent.[15–22] It is active against ischemia-reperfusion injury,[23,24] and clinical trials have shown that berberine can control dyslipidemia, dementia, ocular Behcet's disease, hyperlipidemia, and non-fatty liver disease.[21,25–29] The focus of this review will be to delineate the anticancer activities of berberine alone *in vitro* and *in vivo*.

In vitro studies

The anticancer potential of berberine has been studied *in vitro* using numerous neoplastic cell lines of different tissue origins with specific studies detailed below (Table 1).

Brain cancer

Treatment of human glioblastoma T98G cells with 50, 75, 100, 150, and 200 µg/mL berberine reduced cell proliferation and increased cell death in a concentration-dependent manner with an IC_{50} of 134 µg/mL. Berberine arrested cells in the G_1 phase of the cell cycle, owing to a rise in p27 and decline in cyclin-dependent kinase (CDK) 2/4 and cyclin D/E (Table 1). Berberine triggered apoptosis in T98G cells by elevating the Bcl-2-associated X (Bax)/B cell lymphoma 2 (Bcl-2) protein ratio, in addition to procaspase-9, caspase 9/3, and poly(ADP-ribose) polymerase (PARP), and by disrupting the mitochondrial membrane potential (Δψm). T98G cells treated with berberine had increased reactive oxygen species (ROS), intracellular Ca^{2+} generation, phosphorylation of endoplasmic reticulum (ER) stress-associated ER kinase, eukaryotic translation initiation factor-2α (ETIF-2α), glucose-regulated protein (GRP)78, immunoglobulin heavy chain binding protein (IHCBP), CCAAT/enhancer-binding protein (C/EBP)-homologous protein, growth arrest and DNA damage-inducible gene 153 (GADD153), and activation of caspase 3.[30,31]

Treatment of C6 rat glioma cells with 50, 100, 200, and 500 µM berberine stimulated morphological changes and increased apoptosis (Table 1). Berberine upregulated the expression of Wee1 and suppressed cyclin B, CDK1, and cell division cycle (Cdc)25c, thereby arresting the cells in the G_2/M phase of the cell cycle. Berberine triggered mitochondrial cytochrome c release and elevated caspase 9/3/8, DNA fragmentation, Bax, GADD153 and GRP78, but suppressed Bcl-2 and reduced Δψm.[32]

Treatment of human glioblastoma U87, U251, and U118 cells with 15, 25, 50, 100, and 150 µM berberine reduced cell viability depending on the length of treatment time and drug concentration, and the IC_{50} values were 21.76, 9.79, and 35.54 µM, respectively. Berberine increased senescence in U87 cells, and in U251 cells (lacking PTEN) up to day seven when the S-phase cells were minimal (Table 1). Berberine elevated DNA double-strand breaks (DSBs) indicated by a rise in phosphorylated H2A histone family member X (γ-H2AX) in U251 cells but not in U87 cells. Berberine reduced the expression of epidermal growth factor receptor (EGFR) as well as the phosphorylation of RAF, mitogen-activated protein kinase kinase (MEK), and extracellular signal-regulated kinase (ERK) in U87 and U251 cells.[33] Exposure of U87, U251, and P3 human glioma and astrocyte cells to 50, 100, 150, 200, and 250 µM berberine decreased cell proliferation in a concentration-

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Table 1. Anticancer activity of berberine in various cultured cell lines and its mechanism of action

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
Neuroblastoma				
T98G/134 μg/mL	50, 75, 100, 150, or 200 μg/mL	Decreased cell proliferation, increased cell death, ER stress, apoptosis	G_1 arrest, increased p27; reduced CDK2/4, cyclin D,E; increased Bax, procaspase-9, caspase-9, caspase-3, and PARP; ROS, Ca^{2+}, ER kinase, ETIF-2α, GLP78, C/EBP, DDIG153, disrupted Δψm	30,31
C6 (rat)	50, 100, 200, or 500 μM	Increased cell death and apoptosis, DNA fragmentation, ER stress, G_2/M arrest	Increased Wee1, cytochrome c, caspase-9/3/8, Bax, GADD153,GRP 78, decreased cyclin B, CDK1, Cdc25c, Bcl-2, Δψm	32
U87/21.76; U25/19.79; U118/35.54 μmol/L; P3	15, 25, 50, 100, or 150 μmol/L; 50, 100, 150, 200, or 250 μM	Decreased cell viability, cell proliferation, migration, invasion, EMT, increased senescence, cell death, apoptosis, autophagy	Increased DNA DSBs, Bax, cytochrome c release, caspase 3, LC3B-II, AMPK, ULK-1, Beclin-1, oxidative phosphorylation, SQSTM1/P62, decreased IL-18, IL-1β, EGFR, RAF, MEK, ERK1/2, Bcl-2, L-lactate, LDH	33–35
Na2 (mouse); IMR-32	10 or 20 μg/mL	Decreased cell proliferation, EMT increased cell differentiation, cell cycle arrest	Decreased CD133, β-catenin, n-myc, sox2, notch2, nestin, CDK-2, CDK-4, cyclin D1/E, PI3/Akt, Ras-Raf-ERK, increased, p27, p53, NCAM, laminin, Smad, Hsp70, p38- ATP MAPK	36
T98G, LN18; LN229, C6, SHG 44	25, 50, 100, 200, or 400 mg/L	Decreased cell viability, oxygen consumption rate, mitochondrial respiration, increased autophagy, apoptosis, necrosis	Decreased ATP, GSH, NADPH, aerobic oxidation, p-ERK1/2, increased aerobic glycolysis	37
LN229/40 μM; U251/30 μM	5, 10, 20, 40, 80, 160, or 320 μM	Decreased cell proliferation, increased apoptosis	Increased Wif-1, decreased Bcl-2, Wnt/β-catenin signaling, β-catenin/TCF-4 transcription	38
U87MG	10, 25, 100, or 250 μM	Decreased cell viability, cell proliferation, increased apoptosis	Increased ROS, thiobarbituric acid reactive substance, protein carbonylation	39
Head and neck cancer				
KB	1, 10, or 100 μM; 0.01, 0.1 or 1 μg/mL	Decreased cell viability, cell migration, increased apoptosis, DNA fragmentation, cell cycle arrest	Increased caspase 3/7/8/9, PARP, FasL, Bax, Bad, Apaf-1, decreased COX-2, Mcl-1, Akt, Bcl-2, Bcl-xL, MMP-2/9, p38 MAPK	40,41
HSC-3	5, 10, 25, 50 or 75 μM	Decreased cell growth, DNA synthesis, increased apoptosis, G_0/G_1 arrest	Increased ROS, Ca^{2+}, p53, cytochrome c, decreased Bcl2	42
SCC-4	65.2 or 125 μM	Decreased cell viability, cell migration, invasion, increased DNA damage, apoptosis	Decreased MMP-2/9, u-PA, FAK, pJNK, pERK, IKK, NF-κB, Bcl-2, Bcl-xL, Δψm, increased ROS, Ca^{2+}, cytochrome c, Bax, Bad, Bak, caspase 8/9/3, Apaf1, Fas, FADD, AIF, EndoG	43,44
5-8F	2.5, 5, 10, 20, 40, 80, or 100 μM	Decreased cell viability, motility, increased LDH, filopodia formation	Reduced Ezrin phosphorylation (Thr^{567})	45,46
CNE-1	2.5, 5, 10, 20 or 40 μg/mL	Decreased cell viability, cell migration, invasion, EMT	Reduced Twist, increased caspase 3	47

(continued)

Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
HONE1; HK1-EBV	12.5, 25, 75, 150, or 300 μM; 25, 50, 100, or 200 μM	Decreased cell proliferation, cell migration, invasion, stress fiber formation, increased apoptosis G_2/M arrest	Increased cdc2 (p-cdc2; Tyr15), PARP, caspase 3/9, decreased RhoGTPases. p-histone 3, EBNA1, STAT-3	48,49
KYSE-30	1, 2, 4, 8, 16, 32, 64, 128 or 256 μM	Decreased cell viability, cell migration	Decreased CCR7, CXCR4	50
KYSE-70; SKGT4	20, 40, 60, 80, and 100 μmol/L	Decreased cell growth, increased apoptosis, G_2/M arrest	Decreased Akt^{Ser47}, $mTOR^{Ser2448}$, $p70S6K^{Thr389}$, increased $AMPK^{Thr172}$	51
KYSe-450; TE-1; Eca109	NR	Decreased cell migration, invasion, EMT	NR	52
FaDu	12.5 or 25 μM	Increased cytotoxicity, nuclear condensation, apoptosis, decreased cell migration	Increased FasL, TRAIL, caspase 8/7/3, PARP, p53, Bax, Bad, Apaf-1, caspase-9, decreased Bcl-2,and Bcl-xL, VEGF, MMP-2/9, MAPK	53
SSC-15/ 235, SSC-4/242 μM	100, 150, 200, 200, 250, or 300 μM	Decreased cell viability, colony formation, increased autophagy, apoptosis	Conversation LC-3I to LC-3II, decreased SQSTM1 protein p62, miR-21, increased caspase 3, PARP, miR-155	54
Gastrointestinal cancer				
SW620	5, 10, 25, or 50 μM	Decreased cell viability, increased apoptosis	Increased caspase 3/8, PARP, cytochrome c, ROS, JNK, p38 MAPK, phospho-c-Jun, FasL, t-Bid, decreased Bid, c-IAP1, Bcl-2, Bcl-XL	55
HCT-116 SW480	1, 10, or 50 μM	Decreased cell proliferation, increased apoptosis	Increased NAG-1, ATF-3, caspase 3/7	56
SNU5	25, 50, 75, or 100 μM	Decreased cell viability, invasion	Increased ROS, decreased NF-κB, MMP-1/2/9	57
HCT118; SW480	1, 2, 5, 10, 20, 50, or 100 μM	Decreased cell viability and cell migration	Increased ROS, AMPK, decreased, integrin β1, Src, FAK, p130Cas	58
SW480	0.5, 1, 2.5, 5, 10, 25, or 50 μM	Decreased cell proliferation, increased apoptosis, G_0/G_1 arrest	Increased p21, cytochrome c, Bax, caspase 8/9/3, PARP, decreased VEGF, AIF, NF-κB, COX-2	59
HCT116	5, 10, 20, 40, or 80 μM	Decreased cell proliferation, increased apoptosis, G_1 arrest,	Decreased β-catenin	60
HCT-8	0.03, 0.06, 0.12, 0.24, or 0.47 mmol/L	Decreased cell proliferation, S-arrest	Increased, LDH, alkaline phosphatase, acid phosphatase, TNF-α, FasL, p53, prohibitin, Fas, Bax, caspase-3, decreased Bcl-2, procaspase-3, vimentin	61
HCT116; KM12C	6.25, 12.5, 25, or 50 μM	Decreased cell proliferation, colony formation, glucose uptake	Decreased GLUT1, LDH A, hexokinases 2 mRNA, HIF1, mTOR signaling	62
HCT116	1, 10, or 100 μM	Decreased cell viability, increased apoptosis	Increased caspase-3, decreased miR-21, ITGβ4, PDCD4	63
DLD-1; Caco-2	6.25, 12.5, 25, or 50 μM	Decreased cell proliferation, colony formation, S-phase fraction, G_0/G_1 arrest	Decreased lipogenesis, ACC, ACL, FASN, β catenin signaling, SREBP-1, SCAP	64

(continued)

Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
BGC-823; SGC7901	10, 25, 50, 75, or 100 μM	Decreased cell proliferation, increased apoptosis	Increased PARP, caspase-3, decreased Δψm, Akt/mTOR/p70S6/S6	65
BGC-823/24.16 μM	14, 21, 32, 48, 72, or 108 μM	Decreased cell proliferation, increased autophagy	Increased Beclin-1, LC3-II, p-ULK1, decreased mTOR, Akt, ERK, JNK, p38, mTOR/p70S6K	66
SNU-1/30 μM; GES-1/120 μM	6.25, 12.5, 25, 50, 100, and 200 μM	Increased cytotoxicity, apoptosis decreased cell migration, invasion	Increased caspase-3/8/9, decreased NF-κB	67
CACO2/39.87; LOVO/23.27 μM	10, 20, 40, 60, or 80 μM	Decreased cell viability, colony formation	Increased PARP, caspase 3, citrate synthase, decreased TUFM, PTCD3, MRPL 48	68
LOVO/40.8 μM	1.25, 2.5, 5, 10, 20, 40, 80 or 160 μM	Decreased cell growth, colony formation, G_2/M arrest	Decreased cyclin B1, cdc2/25c, DNA, protein synthesis	69
Liver cancer				
HepG2	1-50 μM	Decreased cell growth, S- and G_2/M arrest	Decreased glucocorticoid receptors, α-fetoprotein	70
HepG2	5, 25, 50, 100, or 200 μM	Decreased cell proliferation, increased apoptosis, G_2/M arrest	Decreased SP1, CCND1, Bcl-2	71
HepG2	10, 50, or 100 μM	Decreased cell viability, increased apoptosis	Decreased NF-κB	72
HepG2; SMMC-7721; Bel-7402	3.125, 6.25, 12.5, 25, 50 or 100 μM	Decreased cell viability, increased apoptosis	Increased Bax, pAMPK, pAkt, cytochrome c, caspase 9/3	73
MHCC97L PLC/PRF/ 5HCC	0.5 mg/mL or 50 or 100 μM	Decreased cell viability, G_2/M arrest	Increased miR-23a, p21, GADD45α	74
HepG2; H22 (mouse)	12.5, 25, 50 or 100 μM	Decreased cell proliferation, increased apoptosis	Increased caspase 9/3, decreased cPLA2, COX-2	75
HepG2; HepB3; SNU-182	10, 20, 50, or 100 μM	Decreased cell proliferation	Increased KLF6, ATF3, p21, decreased E2F, PTTG1	76
Huh-7; HepG2; Hep3B	30, 60, or 120 μM; 50 μM	Decreased cell viability, colony formation, G_0/G_1 arrest	Decreased Akt, Skp2, β-catenin, p-p70S6K^{Thr389}, p4EBP1$^{Thr37/46}$, mTORC1, increased FoxO3a, p21^{Cip1}, p27^{Kip1}, pAKT^{Ser473}, pGSK3βSer9, mTORC2	77,78
KKU-213; KKU-214	2, 4, 6, 8, 20 or 20 μM	Decreased cell growth, G_1arrest	Decreased STAT-3, NF-κB, ERK-1/2	79
Breast cancer				
MCF-7; MDA-MB-231	0.001, 0.01, 0.1, 1, 10, 20, 50, 100 or 500 μM	Decreased cell proliferation, invasiveness, migration, increased apoptosis, G_0/G_1 arrest	Binding with VASP, decreased actin polymerization	80–82
MCF-7/36.91 μg/mL	0–100 μg/mL	Decreased cell proliferation	Increased ROS, protein trafficking proteins, decreased proteins involved in proteolysis, protein folding, cell signaling, redox regulation, electron transport, metabolism, increased protein trafficking protein Hsp27	83
SKBR-3; BT-474; T47D; MDA-MB-231	20, 40, 60, 80, and 100 μM	Decreased cell proliferation, increased apoptosis, DNA fragmentation, G_1 arrest	Decreased cyclins D1/E, HER2/PI3K/Akt signaling, increased caspase 9/3, PARP	84

(continued)

Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
MDA-MB-231, MCF-7	10, 25, 50, 75, or 100 µM	Increased cytotoxicity, decreased cell migration, invasion	Decreased MMP 2/9, Akt, NF-κB, c-Jun, AP-1	85
MCF-7-memopspheres	10, 20, 30, 40, or 50 µM	Decreased cell survival, targeted berberine was more effective	Decreased ABCC1, ABCC2, ABCC3, ABCG2, Bcl-2, mitochondrial permeability, increased cytochrome c release, caspase 9/3	86
MCF-7/106 µM; MDA-MB-231/85 µM	20, 40, 80, 120, or 160 µM; 10, 20, 40, 80, or 120	Decreased cell viability, colony formation, G_1arrest (MCF-7)	Upregulated 1318 (MCF-7), 1662 (MDA-MB-231) downregulated 2079 (MCF-7), 1044 genes (MDA-MB-231), increased CCNG1, CYP1A1, GADD45A, decreased ANGPTL4, CSF1R (MDA-MB-231), CXCR4, increased CYP1A1, GADD45A	87
MCF-7; MDA-MB-231	10, 25, 50, 75, or 100 µM	Decreased cell viability, increased apoptosis	Increased ROS, cytochrome c, JNK, AIF, Bcl-2, caspase-3, decreased Δψm	88
BT549/16.57 µg/mL; MDA-MB-231/18.52 µg/mL	5, 10, 20, 40, and 80 µM	Decreased colony formation, cell migration, increased apoptosis	Increased caspase-3/9, cytochrome c, Bax, decreased Bcl-2, TGF-β1, MMP-2, DNA DSBs	89,90
Hs578	25 or 50 µM	Decreased cell invasion	Decreased IL-8, EGFR, MEK, ERK	91
MDA-MB-231 MCF-7	20, 40, or 80 µM	Decreased cell proliferation, G_1 arrest	Increased $p2^{cip1}$, $p27^{kip1}$, p53, p-$p53^{Ser15}$, GSK3β, decreased cyclin D1/E, CDK2/4/6, p-Akt^{Thr308}, total Akt, c-Myc	92
MCF-7; MDA-MB-231	6.25, 12.5, 25, 50 or 100 µM	Decreased cell proliferation, increased apoptosis, necrosis	Decreased EGFR, AKT, ERK1/2, p38 kinases, Akt kinase	93
MDA-MB-468; MDA-MB-231	3, 6, or 12 µM; 6.25, 12.5, or 25 µM	Decreased cell proliferation, S+G_2/M, G_0/G_1 arrest (MDA-MB-468/BT-549 cells)	Decreased cyclin A/D, CDK1/4 (MDA-MB-468/BT-549 cells)	94
MCF-7; MCF12A	1, 10 or 100 µM	Decreased cell proliferation, G_0/G_1 arrest, increased MCF-7 death (100 µM)	Increased p53, p21, nucleolar stress, loss of ribosomal protein (RP)L5	95
MCF-7; MDA-MB-231	25 or 50 µM	Decreased colony formation, cell migration, invasion, increased apoptosis, G_2/M arrest	Increased miR-214-3p, Bax, decreased secretin	96
Cervical cancer				
HeLa/4.8 µg/mL L1210/74.6 µg/mL	0.1, 1, 5, 10, 50, 100, or 150 µg/mL	Decreased cell growth, S-phase fraction, G_2/M arrest, increased apoptosis	Increased DNA fragmentation	97
CaSki	50, 100, or 150 µM	Decreased cell viability, increased apoptosis	Increased ROS, Ca^{2+}, p53, Bax, GADD153, caspase-3, decreased Δψm	98
SiHa; HeLa	1-250 µg/mL	Decreased cell viability, growth, increased apoptosis	Increased caspase 3, PARP, p53, Rb, decreased AP-1, HPV oncogenes, hTERT, c-Fos, E6, E7 l	99
HeLa/283 µM	150, 175, 200, 225, 250, 275, or 300 µM	Decreased cell viability, cell migration, wound healing, increased apoptosis, DNA fragmentation	Decreased tubulin network, Δψm, HDAC1/2, HPV-18 E7, CDKs, cyclin, NF-κB, SMAD4, increased p53	100

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Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
HeLa	1, 2, 4, 6, or 8 µg/mL	Decreased colony formation	Increased LDH, DNA strand breaks, decreased glutathione transferase	101,102
SiHa; HeLa; CaSki	5, 10, 15, or 20 µM	Decreased cell viability, motility, invasion	Decreased u-PA, MMP-2, NF-κB, TGF-β1, p38, FAK, paxillin, Src, Snail-1, C23, β-catenin, increased TIMP-2	103
SiHa; CaSki	150, 200, or 250 µM	Decreased cell viability, cell migration, invasion, EMT, increased apoptosis	Increased Bax, caspase 3, E-cadherin, KRT17, decreased Bcl-2, MMP-9, N-cadherin, vimentin	104
Leukemia				
HL-60	5, 10, 25, or 50 µg/mL; 10, 50, or 100 µM	Decreased cell viability, increased apoptosis, DNA fragmentation, G_2/M arrest	Complexed with DNA, decreased nucleophosmin/B23 mRNA, telomerase, NAT, 2-aminofluorene (AF)-DNA adduct, N-cadherin, increased E-cadherin	105-108
HL-60; WEHI-3 (mouse)	5, 15, 30, or 60 µM	Increased cytotoxicity, apoptosis, G_0/G_1, G_2/M arrest	Increased Ca^{+2}, caspase-3, Bax, cytochrome c, Wee1, 14-3-3σ, decreased Δψm, Bcl-2, Cdc25c, CDK1, cyclin B1, Src	109,110
HL-60	2.5, 5, 10, 20, 40, 80, or 100 µM; 20, 40, 60, 80, or 100 µM	Decreased cell viability, migration, increased apoptosis, chromatin condensation, DNA fragmentation	No change in CXCR-4, increased PARP, caspase 3/8, ERK, p38	111,112
EU4	1, 10, 50, or 100 µM	Increased cytotoxicity, apoptosis,	Increased caspase 3/9, PARP, Bax, miR-24-3p, PIM-2, decreased XIAP, MDM2	113,114
EU6	12.5, 25, 50, or 100 µM	Decreased cell viability, increased autophagy	Decreased AKT/mTORC1, p-S6, pAKT	115
MM.1S/15-25 µM; RPMI-8266	25, 50, 75, and 100 µM	Decreased cell viability, colony formation	Increased $p16^{INK4A}$, p73, UHRF1 degradation	116
Prostate cancer				
DU145; PC-3; LNCaP	10-100 µM; 25, 50, 75, or 100 µM (PC-3)	Decreased cell proliferation, increased cell death, DNA fragmentation, apoptosis, G_0/G_1 arrest	Decreased cyclins D1/2E, CDK 2/4/6, Δψm, increased $p21^{/Cip}1$, $p27^{/Kip1}$ Bax, caspase 9/3, PARP, ROS, cytochrome c, Smac/DIABLO	117–118
PC-3; LNCaP	5, 10, 20, 50, or 100 µM	Decreased cell growth, increased apoptosis, G_0/G_1 arrest	Increased Bax, caspase 3	119
LnCaP; PC-3	20, 100, or 200 µM	Decreased cell growth, increased apoptosis, G_1arrest	Decreased prostate-specific antigen, EGFR	120
LNCaP; LAPC-4; 22Rv1; C4-2B, PC-3	1.56, 3.125, 6.25, 12.5, 25, 50, or 100 µM	Decreased cell proliferation, increased apoptosis	Decreased androgen receptor	121
RM-1 (mouse); PC-3	5, 10, 20, or 50 µM	Decreased cell proliferation, increased apoptosis, G_0/G_1, G_2/M arrest	Increased DNA DSBs, p53, p21, ATM/Chk1	122,123

(continued)

Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
LNCaP; PC-82	1, 5, 25, 50, or 100 µM	Decreased cell viability, increased apoptosis, necrosis	Increased cyclophilin-D, p53 translocation to mitochondria	124
PC-3; DU145; LNCaP	10, 25, 50, 75 µM	Decreased cell proliferation, motility, migration, EMT	Decreased vimentin, PDGFRβ, COL1A2, BMP7, TGF-β, NODAL, WNT1, Snail	125
22Rv1	12.5, 25, or 50 µM	Decreased cell proliferation, increased apoptosis	Decreased C3 enzyme	126,127
Ovarian cancer				
OVCAR-3/10 µM; SKOV-3/100 µM	1, 10, or 100 µM	Decreased cell proliferation, G_2/M, S-arrest	Increased p27	128
FTE187; A2780; HEY; HO8910	5, 10, or 20 µM	Decreased proliferation, colony formation, increased apoptosis	Increased ROS, PARP, ATM, p53, DNA DSBs, decreased RAD51, homologous recombination DNA repair	129
SKOV3/9.2 µM	5, 10, 30, 50 or 100 µM	Decreased cell proliferation, cell migration, invasion	Decreased hERG1	130
SKOV-3/50 µM; TOV-21G/25 µM; MDAH-2774/32 µM	12.5, 25, 50 and 100 µM	Decreased cell viability, colony formation, cell migration, invasion, increased cytotoxicity,	Decreased EGFR, ErbB2, cyclin D1, MMP 2/9, VEGF, PI3K, Akt	131
SKOV3/78.52 µM; 3AO/125.8 µM	2.5, 5, 10, 20, 40, 80, 160, or 320 µM	Decreased cell proliferation, cell migration, invasion	Increased miR-145, TET3, HK2, decreased MMP16, Warburg effect,	132,133
Osteosarcoma				
U2OS; Saos-2; HOS	1, 5, 10, 20, or 50 µg/mL	Decreased cell proliferation, increased apoptosis, G_1 arrest	Increased p53, p21, p27, Bax, PUMA, FAS, DNA DSBs, decreased cyclin E/D1	134
U2OS	12.5, 25, or 50 µg/mL	Decreased cell proliferation, colony formation, increased apoptosis	Decreased PI3k, AKT, Bcl2, procaspase-3, increased PARP, Bax	135
Saos-2; MG-63	10, 20, 40, 60, 80, or 100 µg/mL	Decreased cell proliferation, increased apoptosis	Decreased caspase 1, IL-1β	136
MG-63	20, 40, 60, or 80 µM; 5, 10, 20, 40, or 80 µM	Increased cytotoxicity, apoptosis, DNA fragmentation, decreased colony formation, EMT	Increased DNA DSBs, decreased MMP-2, N-cadherin, vimentin, fibronectin, β-catenin, snail, EZH2	137,138
Lung cancer				
A549	2.5, 5.0, 10, 20, 40, 80, or 100 µM	Decreased cell proliferation, cell migration, invasion, G_1arrest	Increased TIMP-2, Akt, CREB, MAPK, decreased MMP-2, uPA, NF-κB, cFos, cJun, cyclin B1	139,140
A549; H1299	25, 50, 75, or 100 µM	Decreased cell proliferation, increased DNA fragmentation, apoptosis	Increased Bax, Bak, caspase 3, decreased Δψm, Bcl-2, Bcl-xL	141
H460/5 µM	0.1, 1, 5, or 10 µM	Decreased cell growth, G_0/G_1 arrest	NR	142

(continued)

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Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
A549	20, 40, 80, or 160 µM; 6.25, 12.5, 25, 50, or 100 µM	Decreased cell viability, cell migration, invasion, EMT, increased apoptosis, G_0/G_1 arrest	Increased E-cadherin, p21, ROS, $p21^{WAF1}$, IL6, IL1β, TNF-α, p-NF-κB, mTOR, decreased vimentin, Snail-1, Slug, cyclin A/1/2/B1, NF-κB	143-145
A549	20, 40, 80, 100, 120, 140, 160, 180, or 200 µmol/mL	Decreased cell viability, increased nuclear fragmentation, apoptosis, G_0/G_1 arrest	Increased ROS, p21, p53, Bax, cytochrome c, caspase 8/9/3, decreased Δψm, Bcl-xL, Bcl-2, TNF-α, COX-2, MMP-2, & MMP-9, HDAC 1/2/4	147
A549	30, 60, 90, 150, or 200 µM	Decreased cell proliferation, increased apoptosis	Increased Bax, decreased MMP-2, Janus kinase-2, VEGF, NF-κB, AP-1	148
A549; PC9	20, 40, 60, 80, 100, 120, 140, or160 µM	Decreased cell proliferation, colony formation, increased apoptosis	Increased Bax, TF, JNK, p38MAPK, decreased Bcl2, miR-19a	149
NCI-H460/30.3 µM; A549/44.5 µM; NCI-H1299/43.8 µM	10, 20, 40 or 80 µM	Decreased cell proliferation, colony formation, increased apoptosis	Increased DNA DSBs, decreased TOP2B, Sin3A	150
Pancreatic cancer				
BxPC-3/62.8 µM; HPDEE6E7c7	10, 50, 100, 150, or 200 µM	Decreased cell survival, increased apoptosis, DNA damage	Increased caspase 3/7, AIF, 234 genes, decreased 33 genes related to BRCA1-mediated DNA damage response, G_1/S, G_2/M cell cycle checkpoint regulation, p53 signaling	151
PANC-1/15 µM; MiaPaCa-2/10 µM	NR; 1, 5, 7, 10, or 15 µM	Decreased side population of cells, cell proliferation, increased apoptosis, G_1 arrest	Decreased SOX2, POU5F1, NANOG, increased ROS	152,153
PANC-1, MiaPaCa-2	0.15, 0.3, 1.5, 3, or 6 µM	Decreased cell proliferation, G_1 arrest	Decreased DNA synthesis, Δψm, ATP, mTORC1, ERK, increased AMPK, acetyl-CoA carboxylase	154
PANC-1	1, 5, 7.5, 10, 15, or 30 µM	Decreased cell proliferation, migration, increased apoptosis	Decreased TNF-α, K-ras, 3726 genes, increased 3726 genes, CDKN2A, glycolysis	155
Renal cancer				
ACHN	10, or 20 µmol/L	Decreased cell proliferation	Decreased c-Fos	156
G401	5, 10, 20, or 50 µM	Decreased cell proliferation	Increased p21, p27, AMPK, T-ACC, mTOR, S6 kinase, WTX, decreased cyclin E	157
Bladder cancer				
T24	0.8, 8, 80, 800, or 1,600 µM	NR	Decreased NAT	158
BIU-87; T24	1, 5, 10, 25, 50, 75, or 100 µM	Decreased cell viability, increased apoptosis, G_0/G_1 arrest	Increased caspase-3/9, H-Ras, c-fos	159
T24	10, 25, or 50 µg/mL	Decreased cell migration, invasion	Decreased heparanase	160
Thyroid cancer				
8505C/10 µM; TPC1/10 µM	1, 10, or 100 µM	Decreased cell growth, G_0/G_1 arrest	Increased p-27	161

(continued)

Table 1. *(continued)*

Cell line/IC_{50}	Berberine concentration	Outcome	Mechanism	References
FTC-133; 8305C	10, 25, 50, or 100 µM	Decreased cell viability, increased apoptosis, DNA fragmentation	Increased caspase 3, p53, p27	162
TT/1 µg/mL	0.4, 0.8, 1.6, 3.2 and 6.4 µg/mL	Decreased cell viability, S-/G_2 phase fraction, increased apoptosis, G_1 arrest	Increased caspase 3, decreased RET, Akt, Bcl2, Rb, E2F1, cyclin E	163
C643; OCUT1; TPC1	10, 20, 40, 80, or 160 µM	Decreased cell proliferation, increased apoptosis, arrested G_0/G_1 phase	Decreased Δψm, cyclin E1, CDK2, vimentin, p-AKT1, p-AKT1, p-ERK, p-JNK, PI3K, p-Akt, Akt, Nrf2, increased caspase 3, Bax, p21, p-ERK, p-P38, p-JNK	164
K1	10, 40, or 80 µmol/L	Decreased cell proliferation	Decreased PI3K, p-Akt/Akt, Nrf2	165

NR, not reported; ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; AIF, apoptosis-inducing factor; AMPK, AMP-activated protein kinase; AP, activator protein; Apaf, apoptotic protease activating factor; ATF, activating transcription factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; Bad, Bcl-2 agonist of cell death; Bak, Bcl2-antagonist/killer; Bax, Bcl2 associated X apoptosis regulator; Bcl, B-cell lymphoma; BID, BH3 interacting domain death agonist; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; CCR, C-C chemokine receptor type; Cdc, cell division cycle; CDK, cyclin-dependent kinase; CDKIs, cyclin-dependent kinase inhibitors; COX, cyclooxygenase; CXCR, C-X-C motif chemokine receptor; DDIG, DNA damage-inducible gene; DSBs, double-strand breaks; EBNA1, Epstein–Barr nuclear antigen 1; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ETIF, eukaryotic translation initiation factor; FADD, FAS-associated death domain; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GLP, glucose-regulated protein; GSH, glutathione; HDAC, histone deacetylase; GSK, glycogen synthase kinase; hERG, human ether-à-go-go-related gene; HIF, hypoxia-inducible factor; IHCBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, c-Jun N-terminal kinase; LC3, microtubule associated proteins 1A/1B light chain 3B; LDH, lactate dehydrogenase; MEK/MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MRP, mitochondrial ribosomal protein; mTOR, mechanistic target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; NAT, N-acetyltransferase; NCAM, neural cell adhesion molecule; Nestin, neuroectodermal stem cell marker; NF-κB, nuclear factor kappa B; Notch, neurogenic locus notch homolog protein; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PTCD, pentatricopeptide repeat domain; PTTG, pituitary tumor transforming gene; RAF, rapidly accelerated fibrosarcoma; Ras, retrovirus-associated DNA sequences; ROS, reactive oxygen species; SCAP, SREBP cleavage-activating protein; Skp, S-phase kinase-associated protein; SQSTM1, sequestosome-1; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TCF, T-cell factor; TGF, transforming growth factor; TIF, translation initiation factor; TRAIL, tumor necrosis factor-(TNF) related apoptosis-inducing ligand; TUFM, Tu translation elongation factor; ULK, Unc-51 like autophagy activating kinase; VASP, vasodilator-stimulated phosphoprotein; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Wif, Wnt inhibitory factor; Wnt, wingless-type MMTV integration; WTX, Wilms tumor gene on X chromosome; XIAP, X-linked inhibitor of apoptosis protein; Δψm, mitochondrial membrane potential.

dependent manner, but had a lesser effect on human astrocytes. Berberine treatment reduced the migration and invasion of U87 and U251 cells and induced apoptosis by upregulating Bax, cytochrome c release, caspase 3 activation, and reducing Bcl-2 protein expression. Berberine increased oxidative phosphorylation and reduced markers of glycolysis: adenosine triphosphate (ATP), L-lactate, and lactate dehydrogenase (LDH; Table 1). Berberine increased concentration-dependent autophagy by upregulating microtubule-associated protein 1A/1B-light chain 3 (LC3)-II and downregulating sequestosome-1 (SQSTM1)/p62 proteins. Berberine elevated AMP-activated protein kinase (AMPK), Beclin-1, and phosphorylated Unc-51-like autophagy activating kinase 1 (ULK-1), a downstream target for mechanistic target of rapamycin (mTOR) depending on the concentration in U87 and U251 cells.[34] Berberine (50 or 100 μM) induced the death of U251 and U87 cells and inhibited cell migration, production of interleukin (IL)18 and IL1β, and epithelial-to-mesenchymal transition (EMT) by reducing the activation of ERK1/2 depending on its concentration (Table 1).[35]

Exposure of Na2 (mouse neuroblastoma) and IMR-32 (human neuroblastoma) cells to 10 and 20 μg/mL berberine reduced cell proliferation, increased cell differentiation, and alleviated changes in stemness-related markers of cancer including CD133, β-catenin, n-myc, sex determining region Y-box 2 (Sox2), Notch2, and neuroepithelial stem cell protein (Nestin). Berberine arrested cells in the G_0/G_1 phase of the cell cycle, inhibited CDK-2/4 and cyclin D1/E, enhanced the Bax/Bcl-2 ratio, and increased p27 and p53 expression (Table 1). Berberine exhibited antimigratory potential by inducing neural cell adhesion molecule (NCAM), attenuating its polysialylation, and downregulating matrix metalloproteinase (MMP)-2/9. Berberine treatment enhanced the epithelial marker laminin, Smad, and heat shock protein (Hsp)70 levels and inhibited EMT by attenuating phosphoinositide 3-kinase (PI3K)/Akt and Ras-Raf-ERK signaling and upregulating p38-mitogen-activated protein kinase (MAPK; Table 1).[36]

Human glioma cells (T98G, LN18, LN229, C6, and SHG 44) exposed to 25, 50, 100, 200, and 400 mg/L berberine for 24, 48, and 72 h had reduced cell viability in a time- and concentration-dependent manner. An oncosis-like death was triggered, characterized by cell swelling, vacuolization of cytoplasm, plasma membrane blebbing, and intracellular ATP decline. Berberine stimulated autophagy and decreased the rate of oxygen consumption and mitochondrial respiration, and phosphorylated ERK1/2 (p-ERK1/2) in glioma cells. Berberine induced necrosis and apoptosis as indicated by annexin-V fluorescein isothiocyanate (FITC)/ propidium iodide (PI) staining but the levels of cleaved caspase/caspase 3 and cleaved PARP remained unchanged. Berberine depleted ATP, glutathione (GSH), and reduced nicotinamide adenine dinucleotide phosphate (NADPH), and decreased levels of ERK1/2 in a concentration-dependent manner (Table 1).[37] LN229 and U251 cells treated with 5, 10, 20, 40, 80, 160, and 320 μM berberine had decreased cell proliferation with IC_{50} values of 40 and 30 μM, respectively. Treatment with berberine also increased apoptotic cell death, reduced Bcl-2 expression, elevated Wnt inhibitory factor (Wif)-1 transcription, and suppressed β-catenin/T-cell factor (TCF)-4 transcription and Wnt/β-catenin signaling.[38] Treatment of U87MG cells with 10, 25, 100, and 250 μM berberine for 24, 48, and 72 h decreased cell viability and proliferation in a concentration- and time-dependent fashion and stimulated apoptosis independent of AMPK activity, but the levels of total caspase 3 and p-p53 remained unchanged (Table 1). Berberine increased oxidative stress in U87MG cells by increasing reactive oxygen species, thiobarbituric acid reactive substance, and protein carbonylation.[39]

Head and neck cancer

Exposure of oral squamous carcinoma KB cells to 1, 10, and 100 μM or 0.01, 0.1, and 1 μg/mL berberine caused a time- and concentration-dependent decline in cell viability and survival, increased levels of apoptosis and caspase 3, and inhibited the expression of cyclooxygenase (COX)-2, myeloid-cell leukemia 1 (Mcl-1), and Akt (Table 1).[40] Additionally, berberine elevated DNA fragmentation and upregulated expression of Fas ligand (FasL), proapoptotic Bax, Bcl-2 agonist of cell death (Bad), and apoptotic protease activating factor 1 (Apaf-1), increased activated caspase 3/7/8/9 and PARP, and downregulated anti-apoptotic Bcl-2 and Bcl-xL in KB cells. Berberine increased phosphorylation of p38 MAPK and decreased MMP-2 and MMP-9 expression, and suppressed cell migration (Table 1).[41] Treatment of HSC-3 cells with 5, 10, 25, 50 and 75 μM berberine resulted in a concentration- and time-dependent decline in cell growth and DNA synthesis, and increased apoptosis indicated by PI and annexin V staining and caspase 3 activation. Berberine arrested HSC-3 cells in the G_0/G_1 phase of the cell cycle, triggered ROS formation, increased cytochrome c and Ca^{2+} release, increased p53 expression, and reduced $\Delta\psi m$ and Bcl2 in HSC-3 cells (Table 1).[42]

Exposure of SCC-4 tongue carcinoma cells to 65.2 and 125 μM berberine for 24 and 48 h decreased cell viability, cell migration, and invasion by attenuating the expression of urokinase-type plasminogen activator (u-PA), focal adhesion kinase (FAK), phosphorylated Janus kinase (pJNK), pERK, inhibitor of NF-κB kinase (IKK), nuclear factor kappa B (NF-κB), and MMP-2/9 (Table 1). Berberine increased ROS formation, Ca^{2+} release in the cytosol, DNA damage (comet assay), and apoptosis depending on its concentration. Berberine reduced $\Delta\psi m$, caused the release of cytochrome c, increased the proapoptotic proteins Bax, Bad and Bak, Apaf-1, Fas, Fas-associated death domain (FADD), and activated caspase 8/9 and downregulated antiapoptotic Bcl2 and Bcl-xL in SCC-4 cells. Berberine treatment also upregulated the mRNA levels of apoptosis inducing factor (AIF), caspase 8/9/3 and endonuclease G (EndoG; Table 1).[43,44]

Nasopharyngeal carcinoma (NPC) 5-8F cells treated with 2.5, 5, 10, 20, 40, 80, and 100 μM berberine had a concentration-dependent reduced cell viability and increased LDH release, especially after 40 μM. Berberine inhibited the invasion and motility of 5-8F cells in a time and concentration-dependent manner by inhibiting filopodia formation and downregulating Ezrin phosphorylation at Thr^{567} (Table 1).[45,46] NPC CNE-1 cells treated with 2.5, 5, 10, 20 or 40 μg/mL berberine hydrochloride had reduced cell viability in a concentration- and time-dependent manner and diminished cell migration, invasion, and EMT through decreased Twist expression. Berberine triggered apoptosis and activation of caspase 3 in CNE-1 cells (Table 1).[47] NPC HONE1 cells treated with 12.5, 25, 75, 150, and 300 μM berberine reduced cell proliferation in a concentration-dependent manner and its distribution in the cells was also dependent on the concentration of berberine treated cells. Berberine also reduced cell migration, invasion, RhoGTPases, formation of stress fibers (at low concentrations), and arrested cells in the G_2/M phase of the cell cycle by activating Cdc2 (p-Cdc2; Tyr^{15}) in addition to reducing the expression of p-histone 3. Berberine triggered apoptosis and the activation of PARP and caspase 3/9.[48] HONE1 and HK1-EBV cells treated with 25, 50, 100, and 200 μM berberine exhibited a reduction in cell viability in a concentration-dependent manner and downregulation of Epstein–Barr nuclear antigen 1 (*EBNA1*) and signal transducer and activator of transcription 3 (*STAT3)* at the mRNA level (Table 1).[49]

The exposure of esophageal carcinoma cells KYSE-30 to 1, 2,

4, 8, 16, 32, 64, 128, or 256 µM berberine decreased cell viability and cell migration in a time- and concentration-dependent way by significantly reducing the expression of chemokine receptor 7 (CCR7) and C-X-C chemokine receptor type 4 (CXCR4; Table 1).[50] Likewise, KYSE-70 and SKGT4 cells treated with 20, 40, 60, 80, and 100 µM berberine suppressed cell growth in a concentration- and time-dependent manner, increased apoptosis, and arrested cells in the G_2/M phase of the cell cycle. Berberine treatment repressed Akt (Ser^{47}), mTOR (Ser^{2448}) and p70S6K (Thr^{389}) phosphorylation but increased phosphorylation of AMPK at Thr^{172} (Table 1).[51] Berberine suppressed the microRNA-212-induced cell migration, invasion, and EMT in KYSe-450, TE-1, and Eca109 cells (Table 1).[52]

Exposure of head and neck squamous cell carcinoma (HNSC) FaDu cells to 12.5 or 25 µM berberine induced cytotoxicity, whereas the viability of primary human normal oral keratinocytes was unaffected. Berberine elevated nuclear condensation, apoptosis, FasL and TNF-related apoptosis-inducing ligand (TRAIL), activation of caspase 8/7/3, and PARP in FaDu cells. Berberine triggered the mitochondria-dependent apoptotic signaling pathway by elevating p53 and proapoptotic factors including Bax, Bad, Apaf-1, caspase-9, and downregulating Bcl-2 and Bcl-xL. Berberine inhibited FadU cell migration by downmodulating vascular endothelial growth factor (VEGF), MMP-2/9, and suppressing the MAPK pathway (Table 1).[53] HNSC SSC-15 and SSC-4 cells treated with 200, 250 or 300 µM berberine had significantly reduced cell viability depending on the concentration, with IC_{50} values of 235 and 242 µM for SSC-4 and SSC-15 cells, respectively. SSC-15 cells exposed to 100, 150, 200, and 250 µM berberine had significantly reduced clonogenic potential that was concentration-dependent, and autophagy was stimulated by the conversation of microtubule-associated protein 1 light chain 3β-I (LC3-I) to microtubule-associated protein 1 light chain 3β-II (LC3-II), a distinctive hallmark of autophagosome maturation. Berberine drastically reduced SQSTM1/p62 expression and triggered apoptosis by activating caspase 3 and PARP1 cleavage at higher concentrations. Berberine upregulated tumor suppressor microRNA (miRNA)-155 and downregulated oncogenic miR-21 in SSC-15 cells (Table 1).[54]

Gastrointestinal cancer

Berberine at 5, 10, 25, and 50 µM reduced cell viability and increased apoptosis in a concentration-dependent manner in SW620 cells, and 50 µM berberine activated caspase 3/8, PARP cleavage, and increased cytochrome c release with a subsequent decline in BH3 interacting domain death agonist (Bid), and antiapoptotic factors cellular inhibitor of apoptosis 1 (c-IAP1), Bcl-2, and Bcl-xL expression. Berberine increased ROS generation, phosphorylation of JNK and p38 MAPK, and increased levels of phospho-c-Jun, FasL and t-Bid levels due to JNK and p38 MAPK signaling (Table 1).[55] Treatment of HCT-116 and SW480 cells with 1, 10, and 50 µM berberine for 1, 2, and 4 days reduced cell proliferation concentration-dependently and SW480 cells responded more quickly compared to HCT-116 cells. Berberine elevated the expression of nested antisense gene 1 (NAG-1) protein in HCT and CaCo-2 cells, and activating transcription factor 3 (ATF-3) in HCT-116 and SW480 cells depending on p53 activation. Berberine increased apoptosis and caspase 3/7 activity in HCT-116 cells due to activation of NAG-1 and ATF-3 genes (Table 1).[56] SNU5 cells treated with 25, 50, 75, and 100 µM berberine showed a reduction in cell viability and cell invasion in a concentration-dependent manner. Berberine treatment also enhanced ROS formation up to 6 h and downregulated the protein expression of NF-κB and MMP-1/2/9, but not MMP-7, which remained unaltered at the mRNA level (Table 1).[57]

Berberine treatment (1, 2, 5, 10, 20, 50, or 100 µM) reduced cell viability and cell migration and increased ROS generation, activated AMPK, and significantly decreased integrin β1 levels, phosphorylation of Src, FAK, and p130Cas in SW480 and HCT116 cells (Table 1).[58] Exposure of SW480 cells to 0.5, 1, 2.5, 5, 10, 25, and 50 µM berberine decreased cell proliferation depending on the concentration and length of treatment time, and did not induce cytotoxicity in normal CCD-CoN112 colon cells up to 200 µM. Berberine arrested cells in the G_0/G_1 phase of the cell cycle, depleted Δψm, and decreased the expression of p21 (CDK), cytochrome c, and Bax/Bcl2. Berberine activated caspase 9/3/8, AIF, and cleaved PARP, and reduced VEGF, NF-κB, and COX-2 expression; however, survivin and TRAIL expression remained unaffected (Table 1).[59]

HCT116 cells treated with berberine (5, 10, 20, 40, and 80 µM) showed reduced proliferation and elevated apoptosis in a concentration-dependent fashion, cells were arrested in the G_1 phase of the cell cycle, and mRNA expression of β-catenin was inhibited in both the nucleus and cytoplasm (Table 1).[60] Exposure of HCT-8 cells to 0.03, 0.06, 0.12, 0.24, or 0.47 mM berberine for 12, 24, 48, and 72 h resulted in reduced cell proliferation in a concentration and time-dependent manner and cells were arrested in the S-phase of the cell cycle. Berberine increased tumor necrosis factor alpha (TNF-α), alkaline phosphatase, acid phosphatase, the LDH levels, the expression of FasL, p53, and prohibitin (PHB), and mRNA levels of Fas, FasL, and Bax, as well as the activation of caspase-3. Berberine reduced the expression of Bcl-2, procaspase-3, and vimentin (Table 1).[61] Treatment of HCT116 and KM12C cells with 6.25, 12.5, 25, and 50 µM berberine reduced cell proliferation and colony formation in a concentration-dependent manner by inhibiting glucose uptake as indicated by the mRNA suppression of glucose transporter 1 (GLUT1), lactate dehydrogenase A, and hexokinases 2 (HK2), in addition to the expression of hypoxia inducible factor 1 (HIF1) protein and mTOR signaling; however, analysis by reverse transcription-polymerase chain reaction (RT-PCR) did not show any change in HIF1 mRNA (Table 1).[62] Similarly, 1, 10, and 100 µM berberine depleted cell viability, increased levels of apoptosis, activated caspase-3, integrin β4 (ITGβ4), and programmed cell death 4 (PDCD4) protein expression, and inhibited miR-21 mRNA expression in HCT116 cells (Table 1).[63]

Berberine (6.25, 12.5, 25, and 50 µM) concentration-dependently reduced cell proliferation and colony formation and arrested DLD-1 and Caco-2 cells in the G_0/G_1 phase of the cell cycle and also depleted the S-phase fraction. Berberine also decreased glucose-induced lipogenesis in these cells and inhibited the mRNA expression of acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACL), and fatty acid synthase (FASN), and decreased sterol regulatory element-binding protein-1 (SREBP-1) activation, SREBP cleavage-activating protein (SCAP) expression, and β-catenin signaling (Table 1).[64]

Exposure of BGC-823 and SGC7901 cells to 10, 25, 50, 75, and 100 µM berberine slowed cell proliferation in a time- and concentration-dependent manner, and also elevated apoptosis, expression of PARP, and caspase-3 while reducing Δψm. The BCG-823 cells were more sensitive to berberine than SGC7901 cells. Berberine downregulated the Akt/mTOR/p70S6/S6 pathway in BGC-823 cells indicating that the Akt-related mitochondrial pathway may be involved in berberine-induced apoptosis (Table 1).[65] Exposure of BGC-823 cells to 14, 21, 32, 48, 72, and 108 µM berberine for 6, 12, 24, 36, and 48 h attenuated cell proliferation depending on the length of treatment time and concentration with an IC_{50} value

of 24.16 ± 1.03 μM (48 h). Berberine (25 μM) induced autophagy, increased the number of autolysosomes, increased the expression of Beclin-1, LC3-II, and p-ULK1, and attenuated the phosphorylation of Akt, ERK, JNK, and p38 depending on the treatment duration. (Table 1).[66]

Treatment of SNU-1 neoplastic and GES-1 non-cancerous cells with 6.25, 12.5, 25, 50, 100, and 200 μM berberine induced cytotoxicity and inhibited cell migration and invasion in a concentration-dependent manner, and its effect was more pronounced in SNU-1 cells (IC_{50} of 30 μM) than in GES-1 cells (IC_{50} of 120 μM). Berberine triggered apoptosis, activation of caspase-3/8/9, and repressed the activation of NF-κB depending on its concentration in SNU-1 cells (Table 1).[67] Exposure of Caco-2 and LoVo cells to 10, 20, 40, 60, and 80 μM berberine concentration-dependently reduced cell viability and colony formation with IC_{50} values of 39.87 and 23.27 μM for Caco-2 and LoVo cells, respectively. Berberine increased levels of cleaved-PARP and activated caspase 3 but did not decrease cyclin D1 expression. Proteomic profiling revealed that 503 and 277 proteins were differentially expressed (DEPs) in Caco-2 and LoVo cells, out of 8051 identified proteins, and there was an overlap of 83 downregulated DEPs. Analysis of citrate synthase (CS), Tu translation elongation factor (TUFM), pentatricopeptide repeat domain 3 (PTCD3), and mitochondrial ribosomal protein L48 (MRPL 48) showed a decline, whereas CS protein expression was greater in Caco-2 and LoVo cells than in normal specimens (Table 1).[68] Treatment of LoVo cells with 1.25, 2.5, 5, 10, 20, 40, 80, or 160 μM berberine for 24, 48, and 72 attenuated cell growth and colony formation in a concentration-dependent manner with an IC_{50} of 40.8 ± 4.1 μM. Berberine arrested the cells in the G_2/M phase of the cell cycle and inhibited the protein expression of cyclin B1, Cdc2, and Cdc25c in addition to DNA and protein synthesis (Table 1).[69]

Liver cancer

Human hepatocellular carcinoma (HCC) HepG2 cells treated with 1–50 μM or 5, 25, 50, 100, and 200 μM or 10, 50 and 100 μM berberine for 12, 24, and 48 h berberine have shown a concentration-dependent decline in cell growth through the stimulation of apoptosis, and cells were arrested in the S- and G_2/M phases of the cell cycle. There was also reduced glucocorticoid receptor expression, α-fetoprotein secretion, and decreased levels of specificity protein 1 (SP1), cyclin D1, Bcl-2 and NF-κB (Table 1).[70–72] Berberine (3.125, 6.25, 12.5, 25, 50, and 100 μM for 24 or 48 h) depleted cell viability depending on the length of treatment time and concentration in HepG2, SMMC-7721, and Bel-7402 HCC cells when compared to normal hepatocytes (HL-7702 cells). Berberine increased apoptosis, the ratio of Bax/Bcl-2, activation of caspase 9/3, phosphorylation of AMPK and Akt and cytochrome c released from the mitochondria (Table 1).[73] Berberine stimulated the expression of miR-23a in MHCC97L and PLC/PRF/5 HCC cells depending on its concentration and it transcriptionally activated p21 and GADD45 leading to p53 activation (Table 1).[74] Human HepG2 and Bel-7404 and H22 (murine) hepatoma cells and normal hepatic embryo HL-7702 cells treated with 0, 12.5, 25, 50, or 100 μM berberine for 24 h showed a concentration-dependent decline in cell proliferation, and increased apoptosis and activation of caspase 9/3 in HepG2 cells. Berberine suppressed cytosolic phospholipase A_2 (cPLA2) and COX-2 expression in H22 and HepG2 cells (Table 1).[75]

The exposure of HepG2, HepB3, and SNU-182 cells to 10, 20, 50, and 100 μM berberine concentration-dependently arrested cell proliferation and upregulated the expression of Krüppel-like factor 6 (KLF6), ATF3, and p21 at 100 μM in HepG2 cells, whereas no such effect was detected in HepB3 or SNU-182 cells. Berberine decreased the expression of the E2F transcription factor 1 (E2F1) and pituitary tumor transforming gene 1 (PTTG1; Table 1).[76] Berberine (30, 60, and 120 μM for 12–72 h) treatment of Huh-7 and HepG2 cells reduced cell viability and clonogenicity in a concentration -dependent manner, and Huh-7 cells were more sensitive than HepG2 cells. Berberine arrested Huh-7 and HepG2 cells in the G_0/G_1 phase of the cell cycle depending on the concentration and deactivated the Akt pathway, inhibited the S-phase kinase-associated protein 2 (Skp2) expression, and elevated the expression and translocation of Forkhead box O3a (FoxO3a) into the nucleus, which promoted the transcription of the cyclin-dependent kinase inhibitors (CDKIs) $p21^{Cip1}$and $p27^{Kip1}$ (Table 1).[77] Likewise, 50 μM berberine induced a concentration- and time-dependent reduction in β-catenin (independent of APMK activation) and suppressed p-$p70S6K^{Thr389}$ and $p4EBP1^{Thr37/46}$ levels in addition to the mTORC1 axis in Huh7 and Hep3B cells. Berberine increased $pAKT^{Ser473}$ and pGSK3βSer9 (downstream) levels due to activation of mTORC2.[78] Treatment of human cholangiocarcinoma cells KKU-213 and KKU-214 with 2, 4, 6, 8, 20, and 20 μM berberine inhibited cell growth depending on the concentration, arrested cells in the G_1 phase of the cell cycle, and suppressed the activation of STAT-3, NF-κB, and phosphorylation of ERK-1/2 (Table 1).[79]

Breast cancer

Exposure of MCF-7 and MDA-MB-231 cells to 0.001, 0.01, 0.1, 1, 10, 20, 50, and 100 or 500 μM berberine decreased cell proliferation in a concentration- and time-dependent fashion and elevated apoptosis. Berberine arrested cells in the G_0/G_1 phase of the cell cycle, attenuated cell migration and invasion, and interacted with vasodilator-stimulated phosphoprotein (VASP) to inhibit actin polymerization (Table 1).[80–82] Similarly, 0–100 μg/mL berberine reduced cell proliferation in a concentration-dependent manner (IC_{50} 36.91 μg/mL) and increased ROS generation in MCF-7 cells. MCF-7 cells treated with berberine (36.91 μg/mL) expressed 1800 well-defined proteins, out of which 96 proteins were DEPs as indicated by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. In MCF-7 cells, berberine downregulated the proteins involved in proteolysis, protein folding, cell signaling, redox regulation, electron transport, and metabolism, and upregulated the proteins involved in protein trafficking including Hsp27 (Table 1).[83]

Exposure of SKBR-3 (human epidermal growth factor receptor 2 $[HER2]^{high}$), BT-474 ($HER2^{high}$), T47D ($HER2^{low}$), and MDA-MB-231 ($HER2^{low}$) breast cancer cells to 20, 40, 60, 80, and 100 μM or 10, 25, 50, 75, and 100 μM berberine for 24, 48, and 72h reduced cell growth in a time- and concentration-dependent manner and arrested SKBR-3 cells in the G_1 phase of the cell cycle by downregulating the expression of cyclin D1/E. Treatment with berberine stimulated apoptosis and DNA fragmentation, attenuated Bcl-2 expression, activated caspase 9/3 and PARP, indicating the involvement of the mitochondrial/caspase pathway and the downregulation of HER2/PI3K/Akt signaling (Table 1).[84] Berberine (10, 25, 50, 75, or 100 μM) induced cytotoxicity, decreased cell migration, invasion, and expression of MMP 2/9 and suppressed expression of Akt (protein and mRNA), NF-κB, c-Jun, and activator protein 1 (AP-1) in MDA-MB-231 and MCF-7 cells (Table 1).[85] Liposomal berberine accumulated in MCF-7 cell memospheres and berberine liposomes (including targeted liposomes) reduced cell survival depending on berberine concentration (10, 20, 30, 40, and 50 μM) in MCF-7 and MCF-7 cancer stem cells

(CSCs), where targeted berberine liposomes were more effective. The targeted berberine liposomes crossed the CSC membrane, suppressed ATP-binding cassette (ABC) transporters (ABCC1, ABCC2, ABCC3, ABCG2), and selectively accumulated in the mitochondria. Berberine activated Bax and reduced Bcl-2 and mitochondrial permeability. Targeted berberine liposomes enhanced the release of cytochrome c and caspase 9/3 activation (Table 1).[86]

Berberine treated MCF-7 (20, 40, 80, 120, and 160 µM) and MDA-MB-231 (10, 20, 40, 80, and 120 µM) cells had decreased cell viability depending on the concentration with IC_{50} values of 106 µM and 85 µM, respectively, and decreased colony formation: MDA-MB-231 cells were more sensitive than MCF-7 cells. Berberine induced G_1 arrest in MCF-7 cells but not in MDA-MB-231 cells. Microarray analysis revealed that berberine upregulated 1318 and downregulated 2079 genes in MCF-7 cells, whereas MDA-MB-231 cells showed an upregulation of 1662 and downregulation of 1044 genes. Gene Ontology (GO) analysis indicated that berberine altered the regulation of genes related to apoptosis, cell cycle, cell migration, and drug response. Tenfold more genes were regulated in MCF-7 cells compared to MDA-MB-231 cells. Quantitative (q)PCR analysis showed that berberine upregulated cyclin G1, and downregulated angiopoietin-like 4 (ANGPTL4) and colony stimulating factor 1 receptor (CSF1R)-related genes, including at the mRNA level, in MDA-MB-231 cells but not in MCF-7 cells. The mRNA expression of cytochrome P450 family 1 subfamily A member 1 (CYP1A1) and GADD45A was significantly upregulated, whereas that of CXCR4 was downregulated in MCF-7 and MDA-MB-231 cells.[87] Berberine treated (10, 25, 50, 75, and 100 µM) MCF-7 and MDA-MB-231 cells exhibited a decline in cell viability that was time- and concentration-dependent, as well as increased apoptosis and ROS production. Berberine activated proapoptotic JNK signaling, depolarized Δψm, reduced Bcl-2 expression, and increased Bax, caspase-3 activity, cytochrome c, and AIF release from mitochondria (Table 1).[88]

Exposure of BT549 and MDA-MB-231 cells to 5, 10, 20, 40, and 80 µM berberine for different times reduced cell proliferation in a concentration- and time-dependent manner with IC_{50} values of 16.575 ± 1.219 µg/mL and 18.525 ± 6.139 µg/mL, respectively. Berberine decreased colony formation, cell migration, and Bcl-2 expression, and also increased apoptosis, DNA DSBs (γH2AX), caspase-3/9 activity, cytochrome c release, ligase 4, and Bax expression (Table 1).[89] Berberine treatment arrested cell motility by decreasing transforming growth factor beta (TGF-β)1 and MMP-2 levels, without altering TGF-β2.[90] In another study Hs578t triple-negative breast cancer cells treated with 25 and 50 µM berberine showed decreased IL8 expression, reduced invasiveness, and downregulated expression of EGFR, MEK and ERK with reduced phosphorylation of MEK and ERK (Table 1).[91]

MDA-MB-231 and MCF-7 cells treated with 20, 40, and 80 µM or 6.25, 12.5, 25, 50, and 100 µM berberine had reduced cell proliferation depending on the concentration and length of treatment, and increased apoptosis and necrosis. Berberine arrested cells in the G_1 phase of the cell cycle, upregulated $p21^{/Cip1}$ and $p27^{/Kip1}$ mRNAs and proteins, increased p53 protein, phospho-$p53^{Ser15}$, and GSK3β, and reduced expression of cyclin D1/E, CDK2/4/6, phospho-Akt^{Thr308}, total Akt, c-Myc, EGFR, Akt, ERK1/2, and p38 kinase activity and phosphorylation.[92,93] Berberine treated MDA-MB-468 (0, 3, 6, and 12 µM) and MDA-MB-231 (0, 6.25, 12.5, and 25 µM) cells showed a concentration-dependent reduction in cell proliferation. Berberine arrested MDA-MB-231 and MDA-MB-453 cells in the S- and G_2/M phases whereas MDA-MB-468 and BT-549 cells were arrested in the G_0/G_1 phase of the cell cycle. The expression of cyclin A/D and CDK1/4 were reduced in MDA-MB-468 and BT-549 cells treated with berberine (Table 1).[94]

Exposure of MCF-7 and MCF12A (non-tumorigenic epithelial cells) cells treated with 1 and 10 µM berberine had slower proliferation and cells arrested in the G_0/G_1 phase of the cell cycle; however, when the berberine concentration was increased up to 100 µM, MCF7 cells exhibited cell death, but the MCF12A (non-tumorigenic) cells did not. Berberine elevated the protein expression of p53 and p21 in a time- and concentration-dependent manner in MCF-7 cells (Table 1). Berberine accumulated in the mitochondria of both cells at the higher concentration (10 or 100 µM), and the accumulation within the nucleolus was prominent after the transition to the nucleoplasm in MCF7 cells. Berberine increased nucleolar stress in MCF7 cells, as indicated by the loss of ribosomal protein (RP)L5 from the nucleolus and the nuclear aggregation of p53 protein.[95] Treatment of MCF-7 and MDA-MB-231 cells with 25 or 50 µM berberine led to a reduction in cell survival, cell migration, invasion, and cells arrested in the G_2/M phase of the cell cycle. Berberine elevated miR-214-3p expression, Bax/Bcl-2 ratio, and secretin (SCT) expression at the mRNA and protein levels (Table 1).[96]

Cervical cancer

HeLa (cervical cancer) cells and L1210 (mouse leukemia) cells exposed to 0.1, 1, 5, 10, 50, 100, and 150 µg/mL berberine had inhibited growth depending on the concentration, with an IC_{50} value of 4.8 ± 0.2 µg/mL and IC_{100} value of 74.6 ± 5.1 µg/mL, respectively. Berberine treatment reduced the S-phase fraction and increased the G_2/M phase fraction, depending on its concentration. Berberine increased apoptosis in both time- and concentration-dependent manners as revealed by a DNA fragmentation assay (Table 1).[97]

Berberine (50, 100, and 150 µM) decreased CaSki cell viability depending on the concentration and length of treatment time, and also elevated apoptosis, p53, Bax/Bcl-2, ROS, GADD153, Ca^{2+} release, and caspase-3 activity and reduced Δψm (Table 1).[98] Treatment of SiHa and HeLa cells with 1–250 µg/mL berberine reduced cell viability and inhibited growth. There were also increased levels of apoptosis, activated caspase 3, and cleaved PARP, and reduced Δψm and AP-1 depending on the concentration and assay time, and also altered composition of DNA binding complexes. Berberine inhibited the expression of human papillomavirus (HPV) oncogenes, telomerase protein, human telomerase reverse transcriptase (hTERT), oncogenic c-Fos, E6 and E7, which was also accompanied by a rise in p53 and retinoblastoma (Rb) expression in SiHa and HeLa cells (Table 1).[99]

HeLa cells treated with 150, 175, 200, 225, 250, 275, and 300 µM berberine had reduced cell viability depending on the concentration with an IC_{50} of 283 µM, whereas in normal HPV-negative C33a cervical cancer cells the growth inhibition was comparatively less (2–20%). The uptake of berberine by HeLa cells reached its peak at 250 µM and then declined. Berberine triggered apoptosis, disrupted the tubulin network (confocal microscopy), induced cracks and groves holes, and negatively altered membrane potential, cell migration, and wound healing in HeLa cells. Berberine bound to plasmid DNA and fragmented HeLa cell DNA, and decreased the expression of histone deacetylase 1 and 2 (HDAC1/2; possible DNA binding sites). It upregulated p53 expression and reduced the expression of HPV-18 E7, CDKs, cyclin, NF-κB, and SMAD4 (Table 1).[100] Berberine treatment (1, 2, 4, 6, and 8 µg/ml) reduced cell viability and colony formation, glutathione transferase activity, and increased LDH release in a time- and concentration-dependent manner in HeLa cells. Berberine also induced DNA strand breaks (comet assay) in HeLa cells (Table 1).[101,102]

SiHa, HeLa, and CaSki cells treated with 5, 10, 15, and 20 µM

berberine had reduced cell viability and motility according to the concentration of berberine, and 20 μM berberine had the maximum effect on cell invasion inhibition in SiHa cells. Berberine reduced the activities of u-PA and MMP-2 and increased TIMP-2 expression in SiHa cells. Berberine treatment reduced TGF-β1, EMT, phosphorylation of p38, FAK, paxillin, NF-κB, and Src, as well as the expression of Snail-1, C23, and β-catenin (Table 1).[103] Exposure of SiHa and CaSki cells to 150, 200, and 250 μM berberine concentration-dependently decreased cell viability, migration, and invasion. Berberine increased apoptosis, expression of Bax and caspase 3 and reduced Bcl-2 expression. Berberine repressed EMT in cells by reducing MMP-9, N-cadherin, and vimentin expression and increasing E-cadherin and keratin 17 (KRT17) expression (Table 1).[104]

Leukemia

Exposure of promyelocytic leukemia HL-60 cells to 5, 10, 25, and 50 μg/mL berberine reduced cell viability and elevated apoptosis and internucleosomal DNA fragmentation in a concentration-dependent fashion, arrested cells in the G_2/M phase up to 24 h, and downregulated nucleophosmin/*B23* mRNA and telomerase. *In vitro* studies on calf thymus DNA revealed that berberine complexed with DNA to form berberine DNA-complexes (Table 1).[105,106]

Treatment of HL-60 cells with 10, 50, and 100 μM berberine inhibited N-acetyltransferase (NAT) activity, 2-aminofluorene (AF)-DNA adduct formation, and downregulated NAT mRNA depending on berberine concentration (Table 1).[107,108] Exposure of HL-60 and mouse leukemia WEHI-3 cells to 0, 5, 15, 30, and 60 μM berberine resulted in a concentration-dependent rise in cytotoxicity, apoptosis, cytochrome c release, Ca^{+2} release, caspase-3 activity, and *Bax* levels and a reduction in Δψm and Bcl-2 levels. Berberine arrested these cells not only in the G_0/G_1-phase but also in the G_2/M-phase of the cell cycle in a concentration-dependent manner, which was followed by a rise in Wee1 and 14-3-3σ and reduction in Cdc25c, CDK1, and Src levels (Table 1).[109,110]

HL-60 cells exposed to 2.5, 5, 10, 20, 40, 80, and 100 μM berberine did not show differences in cell viability up to 40 μM, but a significant decline was detected with 80 and 100 μM berberine. Berberine inhibited cell migration in a concentration-dependent (2–5 to 40 μM) manner in HL-60 cells. However, treatment of HL-60 cells with 20 μM berberine for 24, 48, and 72 h did not alter CXCR-4 expression (Table 1).[111] HL-60 cells treated with 20, 40, 60, 80, and 100 μM berberine showed decreased cell viability and proliferation due to its prompt localization into the cell nucleus within 15 min of treatment. Berberine induced apoptosis, chromatin condensation, DNA fragmentation, activation of PARP and caspase-3/8, and phosphorylation of ERK and p38 within 15 min of treatment (Table 1).[112]

The effect of 1, 10, 50, and 100 μM berberine treatment was studied in p53-null EU4 acute lymphoblastic leukemia cells, where berberine was able to induce cytotoxicity in a concentration-dependent manner, along with increased apoptosis, activation of caspase 3/9 and PARP, increased Bax, and reduced expression of Bcl-xL and XIAP. This downregulation of XIAP protein by berberine was due to an inhibition of mouse double minute 2 (MDM2) expression and a proteasome-dependent pathway (Table 1).[113] In another study, berberine downregulated *XIAP* mRNA and enhanced miR-24-3p and Pim-2 proto-oncogene (PIM-2), in p53-null EU-4 and EU-6 cells.[114] EU-6 cells exposed to 12.5, 25, 50, and 100 μM berberine chloride had reduced cell viability, autophagy, and inactivated Akt/mTORC1 signaling as indicated by the attenuated expression of p-S6 and pAkt, that was dependent on berberine concentration (Table 1).[115]

Berberine concentration-dependently reduced cell viability in human multiple myeloma MM.1S, RPMI-8266, U266, NCI-H929, and OPM2 and mouse Sp2/0 cells after treatment with 25, 50, 75, and 100 μM berberine. Berberine also significantly reduced colony formation in MM.1S and RPMI-8266 cells. However, the viability of normal human peripheral blood mononuclear cells (hPBMCs) remained unaffected at an IC_{50} of 15–25 μM for MM.1S cells. Berberine promoted the ubiquitin-like with PHD and RING finger domains 1 (UHRF1) protein degradation through the ubiquitin-proteasome pathway, but did not have any effect on *UHRF1* mRNA, and reactivated $p16^{INK4A}$ and p73 (Table 1).[116]

Prostate cancer

Berberine (10–100 μM) treated DU145 and PC-3 (androgen-insensitive) and LNCaP (androgen-sensitive) prostate cancer cells had reduced cell proliferation and increased cell death depending on the concentration and length of exposure to berberine, whereas non-neoplastic PWR-1E human prostate cells remained almost unaffected. Berberine arrested DU145 cells in the G_1-phase of the cell cycle, downregulated the expression of cyclins D1/2/E and CDK 2/4/6, and increased the expression of $p21^{/Cip1}$ and $p27^{/Kip1}$. Berberine induced apoptosis and fragmented cell DNA in DU145 and LNCaP cells, and increased Bax/Bcl-2 ratio, caspase 9/3 and PARP activation, and depolarized Δψm.[117] Similar observations were reported in PC-3 cells exposed to 25, 50, 75, and 100 μM berberine, except it also increased ROS formation, the release of cytochrome c, and second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis-binding protein with low pI (DIABLO) from mitochondria (Table 1).[118] PC-3 and LNCaP cells treated with 5, 10, 20, 50, and 100 μM berberine experienced suppressed cell growth depending on length of treatment time and concentration, but no such effect was detected in normal human prostate epithelial PWR-1E cells. Berberine arrested the cells in the G_0/G_1 phase and induced apoptotic cell death by increasing Bax and caspase 3 activation (Table 1). LNCaP ($p53^+$) cells were more sensitive than PC-3 ($p53^-$) cells to berberine treatment.[119]

LnCaP and PC-3 cells exposed to 20, 100, and 200 μM berberine for 24, 48, and 72 h experienced attenuated cell growth depending on the length of treatment and concentration, along with increased apoptosis and arrested cells in the G_1 phase. Berberine blocked the expression of prostate-specific antigen and the activation of EGFR (Table 1).[120] Treatment of LNCaP, LAPC-4, 22Rv1, C4-2B, and PC-3 cells with 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 μM berberine reduced cell proliferation, and androgen receptor (AR)-positive cells (LNCaP and LAPC-4) were more sensitive than AR-negative cells. Berberine triggered apoptotic cell death in LNCaP cells depending on the concentration, and inhibited the transactivation of AR in AR-dependent and AR-independent cells to the same extent (Table 1).[121]

Berberine (5, 10, 20, and 50 μM) treated murine prostate cancer RM-1 cells exhibited a concentration-dependent reduced cell proliferation, increased DNA DSBs and apoptosis, and arrested cells in the G_1 phase of the cell cycle. Berberine activated the p53-p21 cascade at a low concentration and arrested cells in the G_2/M phase at a higher concentration (50 μM for 24 h) due to increased phosphorylation of ataxia-telangiectasia mutated (ATM)/checkpoint kinase 1 (Chk1).[122] Treatment of PC3 human and RM-1 mouse prostate cancer cells with 5, 10, 20 or 50 μM berberine resulted in reduced cell viability depending on the concentration, and the arrest of PC3 cells in the G_0/G_1 (10 μM) or G_2/M (50 μM) phase of the cell cycle.[123] Treatment of LNCaP and PC-82 cells with 1,

5, 25, 50, and 100 µM berberine reduced cell proliferation and decreased cell viability in a concentration-dependent manner. Berberine increased apoptosis and programmed necrosis by increasing the release of cyclophilin-D (Cyp-D) from mitochondria and the translocation of p53 into the mitochondria in these cells, ultimately causing cytotoxicity (Table 1).[124] PC-3, DU145 and LNCaP cells treated with 10, 25, 50, and 75 µM berberine showed a concentration-dependent reduction in cell proliferation. The studies on cell motility revealed that PC3 cells were highly motile with the greatest migratory potential in comparison to DU145 and LNCaP cells. Berberine treatment suppressed the motility and migration of PC3 cells by decreasing vimentin and E-cadherin expression. Berberine suppressed the expression of EMT genes, platelet-derived growth factor receptor-beta (PDGFRβ), collagen type I alpha 2 (COL1A2), bone morphogenetic protein 7 (BMP7), and TGF-β responsive genes. Nodal growth differentiation factor (NODAL) and Wnt1 were also downregulated at the level of mRNA in PC3 and DU145 cells. Berberine also downregulated the expression of Snail (*SNAI1*) mRNA, indicating its ability to inhibit metastatic potential (Table 1).[125]

22Rv1 cells treated with 12.5, 25, and 50 µM or 1, 2.5, 5, 10, 20, and 50 µM berberine had reduced cell proliferation, cellular testosterone formation, and C3 (aldo-keto reductase family 1) enzyme activity with no difference in mRNA levels, but also had elevated levels of apoptosis (Table 1).[126,127]

Ovarian cancer

OVCAR-3 and SKOV-3 cells exposed to 1, 10, and 100 µM berberine had reduced cell proliferation with IC_{50} values of 10 µM and 100 µM for OVCAR-3 and SKOV-3 cells, respectively. Cell cycle analysis showed that berberine accumulated the OVCAR-3 cells in the G_2/M phase and SKOV-3 cells in the S- phase of the cell cycle and upregulated p27 in these cells but did not induce apoptosis (Table 1).[128]

FTE187, A2780, HEY, and HO8910 cells treated with 5, 10, and 20 µM berberine had elevated levels of ROS dependent on the concentration of berberine, and this increase was comparatively low in normal FTE187 cells. It also reduced cell proliferation and clonogenicity, and triggered apoptosis in A2780 and HO8910 cells. Berberine (10, and 20 µM) induced DNA DSBs and downregulated RAD51 and homologous recombination DNA repair in A2780, HEY, and HO8910 cells, whereas FTE187 cells remained unaffected. Berberine increased PARP, ATM, and p53 activation in A2780 and HO8910 cells (Table 1).[129] SKOV3 cells treated with 5, 10, 30, 50 and 100 µM berberine had decreased levels of proliferation depending on the length of treatment time and concentration, with IC_{50} values of 15.2 µM (24 h), 9.8 µM (48 h), and 9.2 µM (72 h). Berberine also suppressed the expression of hERG1 protein and mRNA concentration-dependently and inhibited cell migration and invasion (Table 1).[130]

Berberine (12.5, 25, 50, and 100 µM) treatment of SKOV-3, TOV-21G, and MDAH-2774 cells reduced cell viability and colony-forming ability (SKOV-3 and TOV-21G cells) in soft agar, and increased cytotoxicity (concentration-dependent) while reducing cell migration and invasiveness. The IC_{50} values of 50, 25, and 32 µM (72 h) were reported for SKOV, TOV-21G, and MDAH-2774 cells, respectively. Berberine reduced the expression of EGFR, ErbB2, cyclin D1, MMP-2/9, and VEGF in all cell lines except ErbB2 in MDAH-2774 cells (Table 1).[131]

SKOV3 and 3AO cells exposed to 2.5, 5, 10, 20, 40, 80, 160, and 320 µM berberine had decreased levels of cell proliferation depending on the concentration, with IC_{50} values of 78.52 µM and 125.8 µM, respectively. Berberine attenuated cell migration and invasion by promoting miR-145 expression and reducing MMP16, a target of miR-145 (Table 1).[132] Treatment of SKOV3 (40 µM) and 3AO (80 µM) cells with berberine reduced the consumption of glucose and lactate production (Warburg effect), which was due to the upregulation of miR-145, and miR-145 targeted HK2 directly (Table 1). The elevation in miR-145 by berberine was due to increased expression of tet methylcytosine dioxygenase 3 (TET3) and decreased methylation in the promoter region of the miR-145 precursor gene.[133]

Osteosarcoma

Osteosarcoma cells, including U2OS, Saos-2, and HOS, treated with 1, 5, 10, 20, and 50 µg/ml berberine had a concentration- and time-dependent reduction in cell proliferation. Berberine arrested cells in the G_1 phase of the cell cycle, which was dependent on p53 expression and an elevation in p21 and p27, whereas p53 did not have any effect on G_2/M cell cycle arrest. Berberine also reduced the levels of cyclin E depending on the concentration of berberine, whereas cyclin D1 was attenuated only at 50 µg/mL. Berberine triggered apoptosis in a concentration-dependent manner due to elevated levels of p53, Bax, p53 upregulated modulator of apoptosis (PUMA), and Fas in these cells. Berberine treatment induced DNA DSBs, as indicated by a concentration-dependent rise in γ-H2AX (Table 1).[134]

U2OS cells treated with 12.5, 25, and 50 µg/mL berberine had inhibited cell proliferation and colony formation, and increased apoptosis that was concentration-dependent. Berberine suppressed PI3K/Akt, Bcl2, and procaspase-3, and upregulated PARP and Bax in U2OS cells.[135] Saos-2 and MG-63 cells exposed to 10, 20, 40, 60, 80, and 100 µg/mL berberine led to a concentration and time-dependent decline in cell proliferation and an induction of apoptosis. Berberine downregulated the mRNA and protein expression of caspase 1 and IL-1β.[136]

Berberine (5, 20, 40, 60, and 80 µM) treated MG-63 cells showed reduced colony formation and concentration-dependent rise in cytotoxicity, apoptosis (DNA fragmentation analysis by flow cytometry), and DNA DSBs measured by γ-H2AX foci. Berberine also reduced EMT and MMP-2 activity (did not change MMP-9), as well as mRNA expression of N-cadherin, vimentin, fibronectin, β-catenin, and Snail, in addition to inhibiting histone-methylation via decreased expression of enhancer of zeste homolog 2 (EZH2) at the protein and mRNA levels (Table 1).[137,138]

Lung cancer

A549 human lung cancer cells treated with 2.5, 5.0, 10, 20, 40, 80, and 100 µM berberine resulted in a concentration and time-dependent suppression of cell proliferation, migration, and invasion, and cells arrested in the G_1 phase of the cell cycle. Berberine inhibited the expression of cyclin B1, cAMP response element-binding protein (CREB), MAPK, MMP-2, uPA, NF-κB, cFos, cJun, and phosphorylation of Akt. Berberine treatment reduced the transcription of MMP-2 mRNA but upregulated TIMP-2 mRNA and protein expression.[139,140] Berberine treated A549 and H1299 cells showed a concentration- (25, 50, 75, and 100 µM) and time-dependent reduction in cell proliferation and increase in apoptosis and DNA fragmentation. Berberine disrupted Δψm, attenuated Bcl-2 and Bcl-xL expression, and increased Bax, Bak, and caspase 3 activation (Table 1).[141] H460 cells treated with 0.1, 1, 5, and 10 µM berberine showed a concentration-dependent reduction in cell

growth with an IC_{50} of 5 µM and cells were arrested in the G_0/G_1 phase of the cell cycle.[142] A549 cells treated with 20, 40, 80, and 160 µM berberine showed repressed cell invasion, migration, and EMT, as well as an inhibition of vimentin, Snail-1 and Slug with an increase in the expression of E-cadherin.[143] A549 cells treated with 6.25, 12.5, 25, 50, and 100 µM berberine had reduced cell viability, increased ROS and apoptosis, and cells were arrested in the G_0/G_1 phase of the cell cycle in a concentration-dependent manner. Berberine increased the expression of p21 and reduced cyclin D1.[144] Berberine (3.125, 6.25, 12.50, 25 or 50 µM) increased $p21^{WAF1}$ (at low concentrations), IL6, IL1β, TNF-α, p-NF-κB, and mTOR (at higher concentrations) and decreased NF-κB and cyclin A1/2/B1 expression, but had no effect on cyclin D1 expression (Table 1).[145]

The accumulation of berberine in cells is important for its action, and one study has shown a two-to-threefold accumulation of berberine in H1650 and H1975 cells and cell organelles compared to normal BEAS-2 lung cells.[146] A549 cells treated with 20, 40, 80, 100, 120, 140, 160, 180, and 200 µmol/mL berberine had reduced cell viability, increased ROS generation, and cells were arrested in the G_0/G_1 phase of the cell cycle. Berberine reduced Δψm and increased nuclear fragmentation along with mRNA and protein levels of p21, p53 and Bax, increased cytochrome c release and activated caspase 8/9/3, and decreased Bcl-xL, Bcl-2, TNF-α, COX-2, MMP-2, MMP-9 and HDAC 1/2/4 (Table 1).[147] A549 cells exposed to 30, 60, 90, 150, and 200 µM berberine had a concentration and time-dependent reduction in cell proliferation and an increase in cell apoptosis. Berberine downregulated MMP-2, increased Bcl2/Bax signaling, and inhibited Janus kinase-2 (Jak-2), VEGF, NF-κB, and AP-1 proteins in A549 cells.[148] A549 and PC9 cells treated with 20, 40, 60, 80, 100, 120, 140, and 160 µM berberine had reduced cell proliferation and colony formation through increased apoptosis. Berberine reduced Bcl2 and increased the expression of Bax and TF mRNA, which was followed by the downregulation of miR-19a in a concentration-dependent manner. This was followed by increased phosphorylation of JNK and p38MAPK (Table 1).[149] NCI-H460, A549 and NCI-H1299 cells treated with 10, 20, 40 and 80 µM berberine had reduced cell proliferation and colony formation with IC_{50} values of 30.3 µM, 44.5 µM, and 43.8 µM, respectively. Berberine induced DNA DSBs and apoptosis, and downregulated DNA topoisomerase 2-beta (TOP2B) and SIN3 transcription regulator family member A (Sin3A) expression, and shortened the half-life of Sin3A in human NSCLC cells (Table 1).[150]

Pancreatic cancer

Exposure of human pancreatic cancer cells BxPC-3 and pancreatic duct HPDEE6E7c7 cells to 10, 50, 100, 150, and 200 µM berberine resulted in a concentration and time-dependent decline in cell survival with an IC_{50} of 62.8 µM for the former, but the IC_{50} could not be determined for the latter, indicating that BxPC-3 cells were more sensitive to berberine than HPDEE6E7c7 cells (Table 1). Berberine (150 and 200 µM) increased apoptosis, activated caspase 3/7, and stimulated the release of AIF. Microarray analysis showed that berberine treatment upregulated 234 genes and downregulated 33 genes, which were related to BRCA1-mediated DNA damage response, G_1/S and G_2/M cell cycle checkpoint regulation, and p53 signaling.[151] Berberine treatment of pancreatic cancer stem cells PANC-1 and MiaPaCa-2 resulted in IC_{50} values of 15 and 10 µM, respectively, and it also decreased the side population of cells. Berberine downregulated Sox2, POU class 5 homeobox 1 (*POU5F1*), and Nanog homeobox (*NANOG*) genes in both cells, but the *NOTCH1* gene remained undetectable.[152] PANC-1 and MiaPaCa-2 cells exposed to 1, 5, 7, 10, and 15 µM berberine had reduced cell proliferation, and cells were arrested in the G_1 phase of the cell cycle, and apoptosis was triggered by ROS production (Table 1).[153]

Pancreatic duct cells PANC-1 and MiaPaCa-2 treated with 0.15, 0.3, 1.5, 3, and 6 µM berberine showed inhibited cell proliferation and DNA synthesis, and delayed progression through the G_1 phase of the cell cycle. Berberine reduced Δψm and intracellular ATP levels and increased the phosphorylation of AMPK at Thr^{172} and acetyl-CoA carboxylase (ACC) at Ser^{79}. Berberine inhibited mTORC1 (phosphorylation of S6K at Thr^{389} and S6 at $Ser^{240/244}$) and ERK activation (Table 1).[154] PANC-1 cells treated with 1, 5, 7.5, 10, 15, and 30 µM of berberine had reduced cell proliferation and increased apoptosis in a concentration-dependent manner. Berberine also inhibited cell migration and decreased TNF-α expression (Table 1). RNA sequencing detected 7368 differentially-expressed genes, out of which 3726 genes were downregulated and 3642 genes were upregulated after berberine treatment. Berberine downregulated K-ras genes and upregulated the tumour suppressor *CDKN2A* gene. Berberine treatment also increased amino acids, nucleotides metabolism and glycolysis, but reduced citric acid cycle metabolites and damaged the mitochondria (Table 1).[155]

Renal cancer

Treatment of ACHN human renal cancer cells with 10 and 20 µmol/L berberine significantly inhibited cell proliferation and expression of c-Fos (Table 1). However, berberine did not induce the cleavage of caspase proteins, indicating that berberine did not trigger apoptosis.[156] G401 Wilms' tumor cells treated with 5, 10, 20, and 50 µM berberine had reduced cell proliferation that was concentration-dependent, and upregulated mRNA and protein expression of p21 and p27. Berberine downregulated mRNA and protein expression of cyclin E, indicating that it interferes with the cell cycle. Berberine activated the phosphorylation of AMPK and T-ACC (a downstream target of AMPK) and increased the phosphorylation of mTOR and S6 kinase, as well as increased the expression of the tumor suppressor gene Wilms tumor gene on X chromosome (WTX) in G401 cells (Table 1).[157]

Bladder cancer

The treatment of human bladder cancer T24 cells with 0.8, 8, 80, 800, and 1,600 µM berberine resulted in decreased arylamine N-acetyltransferase activity (overactivated in tumor cells) in a concentration-dependent manner (Table 1).[158] BIU-87 and T24 cells exposed to 1, 5, 10, 25, 50, 75, and 100 µM berberine for 24, 48, and 72 showed inhibition of cell viability in a concentration- and time-dependent manner. Berberine arrested cells in the G_0/G_1 phase of the cell cycle and induced apoptosis by activating cleaved caspase-3/9 depending on the concentration. Similarly, berberine caused concentration- and time-dependent inhibition of H-Ras and c-fos mRNA and protein expression (Table 1).[159] Treatment of T24 cells with 10, 25, and 50 µg/ml berberine concentration-dependently attenuated cell migration and invasion, in addition to causing the downregulation of both mRNA and protein levels of heparanase, which is linked to tumor cell migration and invasion (Table 1).[160]

Thyroid cancer

Treating the thyroid cancer cells 8505C and TPC1 with 1, 10, and

100 µM berberine caused a concentration-dependent growth inhibition with an IC_{50} of 10 µM for both cell types, and cells were arrested in the G_0/G_1 phase of the cell cycle. Berberine upregulated p-27 and the effect was more pronounced in TPC1 than 8505 cells.[161] FTC-133 and 8305C cells treated with 10, 25, 50, and 100 µM berberine for 24, 48, and 72 h resulted in a concentration- and time-related reduction in cell viability. Berberine induced apoptosis, DNA fragmentation, and activation of cleaved caspase 3. Berberine treatment resulted in cell cycle arrest and overexpression of p53 and p27 in both 8505 and TPC1 cells, and the effect was more pronounced in TPC1 cells (Table 1).[162] TT cells treated with 0.4, 0.8. 1.6, 3.2 and 6.4 µg/mL berberine had decreased cell viability depending on the concentration with an IC_{50} of 1 µg/mL. Berberine decreased the S- and G_2 phase fractions, and arrested cells in the G_1 phase of the cell cycle. Berberine attenuated the phosphorylation of Akt, Rb, E2F1, and cyclin E in TT cells but did not affect the phosphorylation of MEK/ERK. Berberine reduced Bcl2 expression and 1 µg/ml increased apoptosis and caspase 3 activation. Berberine reduced the expression of the *RET* gene in TT cells and inhibited its promoter activity through G-quadruplex stabilization in the isogenic cell lines HEK293-WT and HEK293-MT1 (Table 1).[163]

C643, OCUT1, and TPC1 cells of different aberrant genotypes and Htori3 (normal) cells were treated with 10, 20, 40, 80, and 160 µM berberine for 24, 48, and 72 h, which arrested cell proliferation in a time- and concentration-dependent manner but the Htori3 cells were the least sensitive. Berberine led to a significant increase in apoptosis, loss in $\Delta\psi m$, arrest of cells in the G_0/G_1 phase of the cell cycle, and depletion of cyclin E1, CDK2, and vimentin (Table 1). Berberine treatment enhanced Bax/Bcl-2, cleaved caspase 3, and p21, and reduced p-Akt1 expression markedly. Berberine increased p-ERK, p-P38, and p-JNK in C643 cells, with no changes in p-ERK and p-p38 in OCUT1 cells; however, it significantly attenuated p-ERK and p-JNK but did not change p-p38 in TPC1 cells.[164] K1 cells treated with 10, 40 and 80 µM berberine inhibited cell proliferation depending on the concentration and repressed the expression of PI3K, p-Akt/Akt, and Nrf2 (Table 1).[165]

In vivo studies

Berberine has been tested for its anticancer activity in different animal models of cancer (Table 2). The *in vivo* anticancer activity of 2–12 mg/kg body weight berberine killed Ehrlich ascites tumor cells in tumor bearing mice in a dose-dependent manner and increased the average and mean survival time of tumor bearing mice.[166,167] BALB/c nude mice xenografted with U87 human glioblastoma cells and treated with 50 and 100 mg/kg berberine showed inhibited tumor growth, downregulated EGFR, and induced senescence.[33] Berberine (50 mg/kg) treated athymic nude mice xenografted with U87 human glioblastoma cells showed reduced tumor growth through the upregulation of p-AMPK and downregulation of p-mTOR. Histological examination showed a reduction in cell proliferation, as Ki-67 positive cells declined and LC3B levels increased.[34] Athymic nude mice transplanted with U87 human glioblastoma cells and treated with 50 mg/kg berberine had reduced tumor volume in the ectopic model and significantly decreased hemoglobin levels and CD31 mRNA, indicating reduced angiogenesis. Berberine also reduced the phosphorylation of VEGFR2, ERK, and p38 (Table 2).[168]

BALB/c nude mice with a xenograft of MCF-7CSC breast cancer cells and administered intravenously with 10 mg/kg berberine, liposomal berberine, and targeted liposomal berberine had their tumor growth inhibited by 34.31 ± 8.13%, 41.91 ± 8.8%, and 71.77 ± 8.92%, respectively, indicating the efficacy of targeted liposomal berberine, which was also non-toxic to blood cells.[86] BALB/c nude mice with a MDA-MB-231 breast cancer cell xengraft were intraperitoneally administered with 10 mg/kg berberine and showed reduced tumor volume and tumor weight.[82] Likewise, BALB/c nude mice with a MDA-MB-231 breast cancer cell xenograft were administered with 100 mg/kg berberine for 21 days and had reduced tumor growth and cell proliferation indicated by a decline in Ki-67 labeling followed by an upregulation of caspase 9 activity.[89] MDA-231-Luc cell xenografted mice given 1% berberine reduced the number of tumors and tumor volume and lung metastases.[90] The BALB/c nude mice with a xenograft of TNBC 4T1 breast cancer cells were given 0.1% berberine in their drinking water, and had inhibited tumor growth, metastasis, and cells arrested in the G_0/G_1 phase of the cell cycle (Table 2).[91]

BALB/cnu/nu mice with a BCG-823 human colon cancer cell xenograft were treated with 10 mg/kg body weight berberine, and showed a reduction in tumor growth and weight and also reduced expression of p-Akt in the tumor tissue.[65] In another study, female BALB/c nude mice with a xenograft of BGC-823 cells and injected with 5, 10, or 20 mg/kg berberine exhibited a dose-dependent slower tumor growth and reduced tumor weights. Berberine also reduced proliferating cell nuclear antigen (PCNA) labeling, indicating a reduction in tumor cell proliferation of the xenograft-derived tumor. Berberine induced autophagic cell death by markedly elevating LC3 and Beclin-1 followed by a reduction in p-mTOR, p-p70S6K, p-Akt, p-ERK, p-JNK, and p-p38 in the xenograft tumors.[66] BALB/c nu/nu mice with a human LoVo colon cancer cell xenograft were administered with 10, 30, or 50 mg kg^{-1} day^{-1} berberine by oral gavage for 10 days and experienced significantly inhibited tumor growth and reduced tumor volume.[69] Nude BALB/c mice with a xenograft of KM12C/shCtrl and KM12C/shRXRα colon cancer cells showed growth retardation after treatment with 10 mg/kg berberine in the former, whereas berberine had little effect on the latter cell type. Berberine attenuated the expression of PCNA, Ki67, Cdc2, cMyc, and β-catenin, and elevated $p21^{WAF1/CIP1}$ and retinoid X receptor alpha (RXRα) in the KM12C/shCtrl xenografts, whereas no such effect was detected in KM12C/shRXRα xenografts, indicating a direct relation between RXRα and β-catenin signaling (Table 2).[169]

Nude mice with a xenograft of HEC-1-A human endometrial carcinoma cells were administered with 50 and 100 mg/kg berberine and had retarded tumor growth that was dose-dependent, as well as inhibited cell migration and invasion in the lungs of the mice through the upregulation of miR-101 transcription via AP-1 and the suppression of COX-2/prostaglandin E2 (PGE2) signaling pathways.[170] Female athymic mice that received a xenogaft of A459 and H1299 lung cancer cells and were administered with 50, 100 and 200 mg/kg of berberine had a dose-dependent slowing of tumor growth, and reduced tumor weight. The A459 xenografts were more sensitive than the H1299 xenografted tumors.[141] A459 lung cells served as the xenograft for BALB/c nude mice, and there was an inhibition of tumor growth following treatment with 5 and 10 mg/kg berberine.[143]

BALB/c nude mice with a xenograft of RPMI-8266 multiple myeloma cells and treated with 50 mg/kg berberine showed tumor growth retardation and significantly increased survival rates.[116] Mice transplanted with B16 melanoma cells and injected with 1, 5, and 10 mg body weight berberine had reduced tumor growth in a dose-dependent manner. There was a significant reduction in tumor volume and tumor weight up to day 16 in the animals receiving 5 or 10 mg/kg body weight berberine (Table 2).[171]

BALB/c athymic nude mice with a xenograft of human PC-3

Table 2. Anticancer activity of berberine in various animal models

Mouse	Berberine	Model	Outcome	References
BALB/c	2-12 mg/kg	Ehrlich ascites carcinoma	Increased average and mean survival time	166,167
BALB/c nude	50 and 100 mg/kg	U87 human glioblastoma cells	Inhibited tumor growth, induced senescence, downregulated EGFR	33
BALB/c nude	50 and 100 mg/kg	U87 human glioblastoma cells	Reduced tumor growth, upregulated p-AMPK, downregulated p-mTOR, LC3B, reduced Ki-67 positive cells	34
Athymic nude	50 mg/kg	U87 human glioblastoma cells	Reduced tumor volume, hemoglobin level, mRNAs of CD31, VEGFR2, ERK, p38, angiogenesis	168
BALB/c nude	10 mg/kg	MCF-7CSC breast cancer cells	Inhibited tumor growth, nontoxic to blood cells	86
BALB/c nude	10 mg/kg	MDA-MB-231 breast cancer cells	Reduced tumor volume, tumor weight	82
BALB/c nude	100 mg/kg	MDA-MB-231 breast cancer cells	Reduced tumor growth, cell proliferation, Ki-67 labelling, upregulated caspase 9 activity	89
BALB/c nude	0.1% (w/v)	TNBC 4T1 breast cancer cells	Reduced tumor growth, metastasis, G_0/G_1 arrest	91
BALB/c nu/nu	10 mg/kg	BCG-823 human colon cancer cells	Reduced tumor growth, weight, p-Akt tumor tissue	65
BALB/c nu	5, 10 or 20 mg/kg	BCG-823 human colon cancer cells	Reduced tumor growth, tumor weight, tumor cell proliferation, PCNA labelling, p-mTOR, p-p70S6K, p-Akt, p-ERK, p-JNK p-p38 increased autophagic death, LC3, Beclin-1	66
BALB/c nu/nu	10, 30, or 50 mg kg^{-}	LoVo colon cancer cells	Reduced tumor growth, tumor volume	69
BALB/c nude	10 mg/kg	KM12C/shCtrl colon cancer cells	Reduced tumor growth, PCNA, Ki67, Cdc2, cMyc, β catenin, increased the $p21^{WAF1/CIP1}$, RXRα	169
Nude	50 and 100 mg/kg	HEC-1-A human endometrial carcinoma cells	Reduced tumor growth, cell migration and invasion in the mice lungs, increased transcription of miR-101 via activator protein 1, reduced COX-2/PGE2 signaling pathways	170
Athymic	50, 100 and 200 mg/kg	A459 and H1299 lung cancer cells	Reduced tumor growth	141
BALB/c nude	5 and 10 mg/kg	A459 lung cells	Reduced tumor growth	143
BALB/c nude	50 mg/kg	RPMI-8266 multiple myeloma cells	Reduced tumor growth, increased mouse survival	116
Mouse	10 mg/kg	B16 melanoma	Reduced tumor, tumor volume, tumor weight	171
BALB/c nude	5 and 10 mg/kg	PC-3 and LNCaP prostate cancer cells	Reduced tumor growth, tumor volume, tumor weight, increased cleaved caspase 3, PARP activities	119
BALB/c	12.5, 25 and 50 mg/kg	H22 mouse HCC cells	Reduced tumor growth and volume	73
BALB/c nu/nu	10 mg/kg	MHCC-97L human HCC	Reduced tumor growth, lung metastasis, HIF-1α/VEGF signaling and expression of Id-1	172
BALB/c nude	10 mg/kg	SSC-4 tongue carcinoma cells	Reduced tumor growth, tumor volume, tumor mass	173
Nude	5 and 10 mg/kg	C666-1 nasopharyngeal carcinoma cells	Reduced tumor growth, STAT-3 activation	174

AMPK, AMP-activated protein kinase; Akt, protein kinase B; Cdc, cell division cycle; COX, cyclooxygenase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; Id-1, inhibitor of differentiation/DNA binding; JNK, c-Jun N-terminal kinase; LC3, microtubule-associated protein 1A/1B-light chain 3; mTOR, mechanistic target of rapamycin; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PGE2, prostaglandin E2; STAT, signal transducer and activator of transcription VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; w/v, weight by volume.

and LNCaP prostate cancer cells and injected with 5 and 10 mg/kg body weight of berberine twice a week for four weeks experienced significantly reduced tumor growth rates, volumes, and weights. Berberine treatment significantly increased the activities of cleaved caspase 3 and PARP in the mice treated with 10 mg/kg berberine, indicating the role of apoptosis in tumor shrinkage (Table 2). The LANCaP tumors were more sensitive than PC3 tumors to berberine treatment.[119]

H22 mouse HCC cells were transplanted into BALB/c mice, and mice that received 12.5, 25 and 50 mg/kg berberine had reduced tumor growth and volume that was dose-dependent.[75] The orthotopic model of MHCC-97L human HCC in BALB/c nu/nu mice treated with 10 mg/kg berberine every two days had effectively reduced tumor growth in the liver as well as the lung metastasis. Berberine treatment suppressed HIF-1α/VEGF signaling and expression of inhibitor of differentiation/DNA binding (Id-1), a key regulatory molecule for HCC development.[172] The BALB/c nude mice with a xenograft of SSC-4 tongue carcinoma cells were treated with 10 mg/kg of berberine, which resulted in reduced tumor mass, volume, and growth.[173] The athymic nude mice with a C666-1 human NPC xenograft and administered with 5 and 10 mg/kg berberine every two days did not show tumor development on 30 and 36 days, respectively. However, tumors started to appear 31 ± 5 and 37 ± 5 days after the animals received 5 and 10 mg/kg berberine, respectively. Tumorigenic growth was detected in three out of five mice in 10 mg/kg berberine treatment group, indicating that berberine inhibited the growth of C666-1 nasopharyngeal carcinoma in nude mice. Berberine suppressed the activation of STAT-3 *in vivo* (Table 2).[174]

Clinical studies

There is only one double-blinded multicentre clinical trial that was conducted in China, where 0.3 g berberine or placebo tablets were given twice daily to colorectal cancer patients (18–75 years of age) who had more than six histologically confirmed colorectal adenomas, including tubular, tubulovillous, and villous, which were surgically removed six months prior to recruitment. The berberine group consisted of 553 patients, whereas the placebo group consisted of 555 patients. Analysis of 429 patients from the berberine group and 462 patients from the placebo group after two years revealed that 155 (36%) patients had recurrent adenomas in the berberine group, compared to 216 (47%) patients in the placebo group (unadjusted relative risk ratio for recurrence: 0.77, 95% confidence interval (CI) 0.66–0.91; p = 0.001). The patients in the berberine-treated group did not have colorectal cancers during follow-up. Six (1%) patients complained of constipation out of 446 patients in the berberine group, which was the only adverse side effect of berberine. No other serious adverse effects were reported.[175]

Toxicity studies

The mice injected with berberine through intravenous (i.v.) and intraperitoneal (i.p.) routes revealed LD_{50} values of 9.0386 and 57.6103 mg/kg, respectively. However, it was not possible to determine LD_{50} for the intragastric (i.g.) route since only 30% of deaths were recorded.[176] Berberine i.p. administration in rats revealed a LD_{50} of 205 mg/kg; however, the administration of 50 mg/kg caused diarrhea in 40% of rats. The LD_{50} in mice was 23 mg/kg following i.p. injection, whereas it was 329 mg/kg after oral administration.[177] Developmental toxicity of berberine was studied in pregnant rats at 6–20 days of gestation (GD) and mice at 6–17 GD. Rats were given 3,625, 7,250, or 14,500 ppm and mice were given 3,500, 5,250, or 7,000 ppm in their feed. The berberine did not cause the maternal death of any rats or mice, and the lowest observed adverse effect level (LOAEL) for rats was 7,250 ppm (531 mg/kg/day) and for mice was 5,250 ppm (841 mg/kg/day). Thirty-three percent of female mice died, and surviving animals drank more water. There was a reduction in fetal body weights of both rats and mice and no other adverse effects were seen.[178]

Clinically diabetic patients receiving 500 mg berberine three times a day for 13 weeks exhibited transient gastrointestinal side effects including constipation, diarrhea, abdominal pain, and flatulence with no obvious alterations in liver enzymes and creatinine levels.[179] Four out of 12 cardiac patients receiving 0.2 mg/kg berberine infusion for 30 min exhibited ventricular tachycardia with torsade de pointes as an adverse side effect of berberine.[180] Administration of berberine in infants caused kernicterus with glucose-6-phosphate-dehydrogenase (G6PD) deficiency and displacement of bilirubin from binding proteins.[181,182] Though berberine has been reported to be safe clinically, it should not be given to pregnant women, breastfeeding mothers, or G6PD-deficient neonates. Individuals with severe gastrointestinal disorders should also not be given berberine to avoid further complications.

Mechanism of action

Berberine employs multiple putative mechanisms to trigger cytotoxicity in various cancer cells (Fig. 2–4). One of the most important mechanisms of action of berberine is the acceleration of ROS formation in various cancer cells, which eventually leads to the stimulation of various pathways to kill cells.[30–32,42–44,55,57,58,83,88,98,118,145,147,161] Berberine is able to kill a variety of cancerous cells by triggering apoptosis (Fig. 2) which may be: (1) ROS-dependent, (2) Fas-dependent, (3) p53-dependent, or (4) p53-independent. The acceleration of ROS formation leads to alteration in the mitochondrial membrane permeability and increased Ca^{2+} release, which subsequently activates AIF release from the mitochondria that leads to caspase-independent apoptosis by berberine (Fig. 2).[42,44,59,88,151] The release of cytochrome c after berberine treatment also leads to apoptotic cell death by subsequent activation of Apaf-1, which causes the formation of apoptosomes and activation of caspase 9/7/3. Additionally, cytochrome c release is also mediated by the entry of Bcl2 family of proteins, especially BAX, into the mitochondria to trigger apoptosis.[30–32,35,42–44,53,55,59,71,86,88,89,118,147] The triggering of DNA damage (DNA fragmentation, DNA strand breaks) and ER stress by berberine also induces apoptotic cell death leading to suppression of Bcl2 and BclxL, and activation of tBid, BAX and BAK.[30–33,39,41,43,44,84,85,89,97,99,100,102,103,105,107,112,117,122,129,135,141,150,162] The secretion of Smac/DIABLO by the mitochondria after berberine treatment and subsequent activation of XIAP also induces cell death by apoptosis.[113,114,118] Berberine is also able to trigger the extrinsic pathway of apoptosis stimulated by TRAIL, FADD, FASL and TNFα that activates caspases 8/7/3 and PARP.[30,31,37,41,43,44,48,53,55,59,61,65,69,99,112,113,117,130,136,145,155] Berberine stimulates necrosis by elevating the release of Cyp-D from mitochondria and promoting the translocation of p53 into mitochondria (Fig. 2). Berberine induces autophagy (Fig. 2) by upregulating LC3B-II and alleviating the SQSTM1/P62 proteins, and by converting LC3-I into LC3-II in various cell types (Fig. 2).[35,37,54]

Berberine arrests cells in the G_0/G_1 phase of the cell cycle by negatively altering various cyclins and CDKs, and upregulating

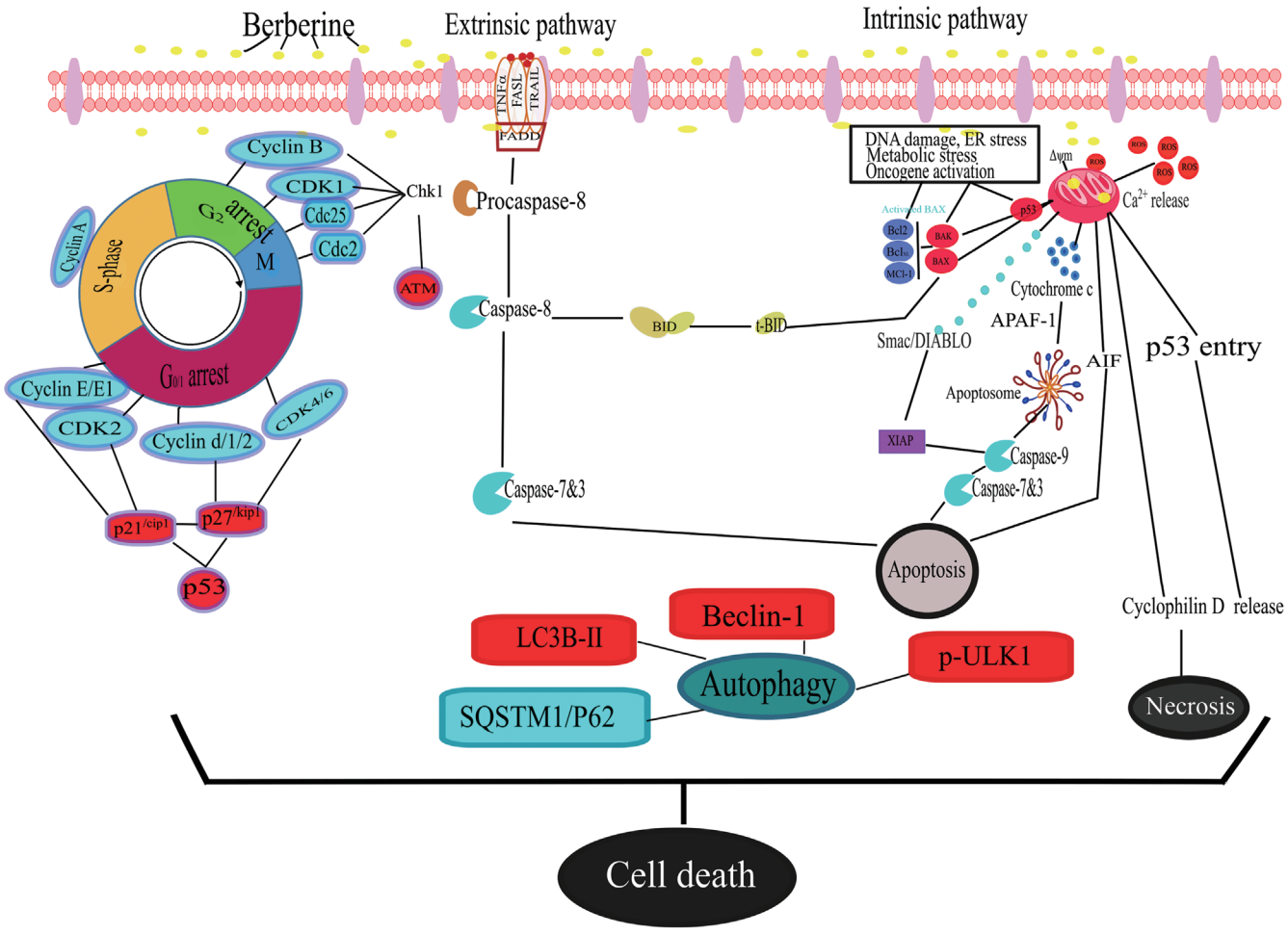

Fig. 2. Cytotoxic action of berberine (yellow shapes) through promotion of apoptosis, necrosis, autophagy, and cell cycle arrest (induces apoptosis). Red color: upregulation, except caspases and BID, which are upregulated but shown in different colours. Δψm: mitochondrial membrane potential (reduction). AIF, apoptosis-inducing factor; Apaf, apoptotic protease activating factor; ATM, ataxia telangiectasia mutated; Bad, Bcl-2 agonist of cell death; Bak, Bcl2-antagonist/killer; Bax, Bcl2-associated X apoptosis regulator; Bcl, B-cell lymphoma; BID, BH3 interacting domain death agonist; Cdc, cell division cycle; CDK, cyclin-dependent kinase; ER, endoplasmic reticulum; FADD, FAS-associated death domain; LC3, microtubule associated proteins 1A/1B light chain 3B; ROS, reactive oxygen species; SQSTM1, sequestosome-1; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand; ULK, Unc-51 like autophagy activating kinase; XIAP, X-linked inhibitor of apoptosis protein.

CDK inhibitors (Fig. 2), which would also contribute to cell death by apoptosis. Berberine also arrests cell in the G_2/M phase of the cell cycle in some of the cell lines by activating Cdc2 (p-Cdc2; Tyr15) and suppressing p-histone as well as Cdc2 and Cdc25 expression.[36,42,75–79,48,51,52,59–61,68,69,72,82,83,92–94, 103,126,132,140,142,145,152,155,161,162]

Regarding the formation of complexes with berberine and DNA, polyadenylic acid (poly-A) has a stronger affinity to bind to berberine than poly U and poly C, which may contribute to its anticancer activity and neoplastic cell death (Table 1).[105,183]

Berberine alters various cell signaling pathways to exert its anticancer activity in various neoplastic cells. EGF increases the clonogenic potential of cells by triggering cell proliferation, and the suppression of EGFR by berberine plays an important role in reducing cell proliferation by inhibiting downstream targets such as Akt, MEK, and ERK/1/2, and their phosphorylation levels.[33,35,37,43,44,65,66,79,91,93,115,120,131,154] VEGF is involved in angiogenesis, which is upregulated in different cancers due to various oncogenic stimuli including hypoxia, and berberine attenuates its expression along with VEGFR-2, reducing angiogenesis in various types of neoplasia.[53,59,131,148,168,172] PI3K/Akt and MAPK (RAF/MEK/ERK) signaling pathways play a crucial role in normal gene expression and cell proliferation, and are linked to HER-2, EGFR, and various nuclear transcription factors. Berberine downregulates HER2/PI3K/Akt, EGFR-ErbB2/PI3K/Akt, and RAF, MEK, and ERK signaling pathways to exert its anticancer effect (Tables 1, 2).[33,84,85,91,131,135,163] mTOR controls cell division, apoptosis, and autophagy by participating in multiple signaling pathways, and its activation increases cell proliferation, gene transcription, protein synthesis, and immune cell differentiation in cancer. It also plays a crucial role in the metabolism of cancer cells.[184] The suppression of mTOR activation in different cell lines by berberine is also one of its anticancer mechanisms of action.[34,51,62,65,66,78,115,145,154]

The Wnt/β-catenin signaling pathway is involved in cell adhesion and its activation is linked to cell migration and invasion (metastasis), and berberine inhibits the activation of the Wnt/β-catenin signaling and reduces cell migration and invasion. Berberine in-

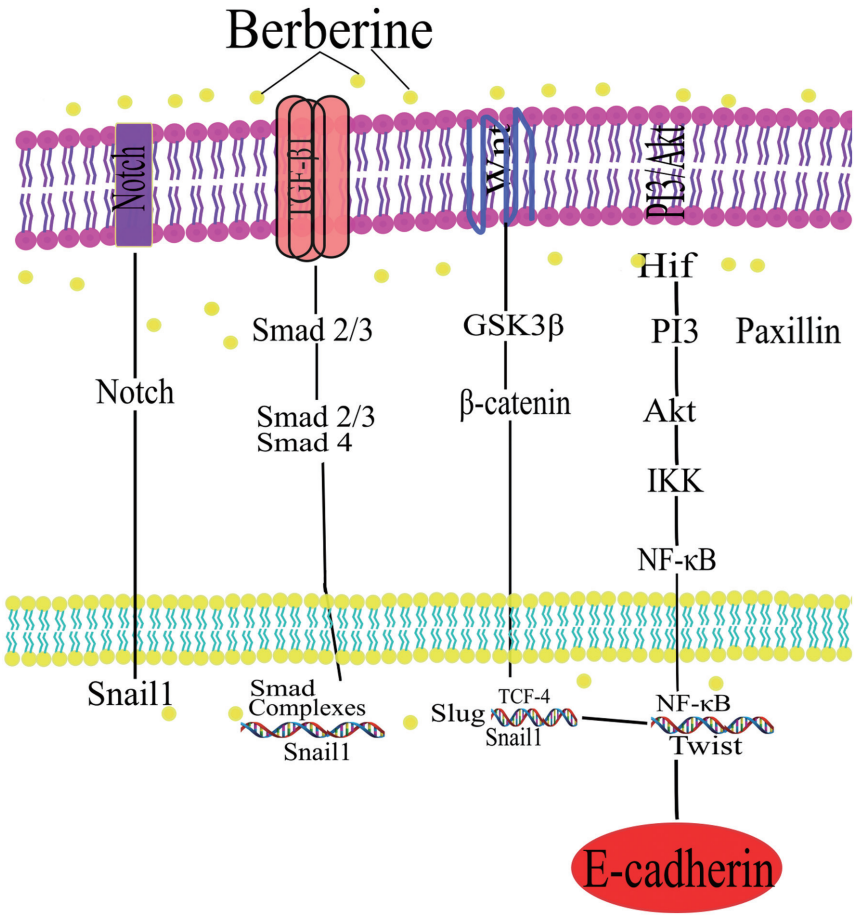

Fig. 3. Berberine treatment suppressed epithelial-to-mesenchymal transition. All molecules were downregulated except E-cadherin, which was upregulated. GSK, glycogen synthase kinase; HIF, hypoxia-inducible factor; IKK, inhibitor of nuclear factor-kappa B kinase; TCF, T-cell factor; TGF, transforming growth factor; Wnt, wingless-type MMTV integration.

creased E-cadherin and decreased N-cadherin expression, and attenuated TGF-β.[38,64,90,101,104,125,143,169] The P38/MAPK signaling pathway is crucial not only in Wnt/β-catenin signaling but also in EMT (Fig. 3). Berberine negatively alters N-cadherin, fibronectin, vimentin, ERK1/2, PI3K/Akt, Ras-Raf-ERK, MMP-9, PDGFRβ, COL1A2, Snail-1, and Slug to attenuate EMT.[35,36,104,125,143]

NF-κB and STAT-3 activation lead to an increase in cell survival, inflammation, and reduction in apoptosis, and they are overexpressed in the majority of cancerous cells. Berberine suppressed IKK/NF-κB and STAT3 activation, which seems to contribute to its anticancer effect in various cells.[49,57,59,67,72,79,100,103,139,145,174] COX-2 is also overexpressed in most cancer cells and its upregulation promotes tumour cell growth. Berberine inhibited COX-2 overexpression in different cell types to reduce their proliferation and growth rates (Tables 1, 2).[40,59,75,147,170]

p53 activates the transcription of the CDK inhibitors $p21^{Cip1}$ and $p27^{Kip1}$. Berberine elevated p53 in different cell lines and its activation is also related to the ability of berberine to trigger DNA damage and cell cycle arrest (Fig. 2).[36,39,42,53,56,61,72,92,95,98–100,113,114,119,122,124,129,134,135,147,151,162]

Additionally, berberine interacts with numerous other targets to exert its anticancer effects (Fig. 4). It has been shown to suppress Jak-2, miR-19a, MMP 1, 2 &16, CD133, n-myc, Sox2, Notch2, Nestin, IL-18, IL-1β, Mcl-1, FAK, pJNK, Nrf2, Rho GTPases, EBNA1, CCR7, CXCR4, c-IAP1, p70S6K, miR-21, ACC, ACL, FASN, SREBP-1, SCAP, PLA2, SP1, CCND1, E2F1, PTTG1, Skp2, p4EBP1, VASP, ANGPTL4, CSF1R,TGF-β1, p38 kinases, AP-1, hTERT, c-Fos, E6 and E7, HDAC1/ 2/4, HPV-18 E7, SMAD4, TIMP-2, paxillin, Src, C23, EZH2, BMP7, NODAL, RAD51, GLUT1, homologous recombination DNA repair, NANOG, POU5F1, ATP, lactate dehydrogenase A, HK2, GSH, NADPH, MRPL48, TUFM, PTCD3. Berberine has also been shown to elevate PUMA, Cyp-D, EndoG, ER kinase, ETIF-2, GRP-78, IHCBP, C/EBP, DNA damage-inducible gene 153, Rb, GADD153, GADD45α, KLF6, ATF3, FoxO3a, Wee1, 14-3-3σ, ATM/Chk1, Beclin-1, ULK-1, AMPK, Wif-1, TCF-4, miR-145, miR-101, miR-155, miR-23a, miR-214-3p, CCNG1, CYP1A1, KRT17, c-Jun, NAG-1, ITGβ4, PDCD4, CDKN2A, GSK3β and citrate synthase.[30–32,34–41,43,44,48–51,53–56,58,62–64,66,68,71,72,74,76–78,82,87,90,92,94,96,98–101,104,109,110,114,122,124,129,132,133,138,139,147–149,151–157,159,163,165,170]

Future directions

It will be purposeful to investigate various molecular targets of berberine in vitro and in vivo by cellular thermal shift assay, proteomic profiling, RNA sequencing, microarray analysis, Gene ontology analysis and MALDI-TOF in future. Future studies should also be directed to investigate the toxic profile of berberine in hu-

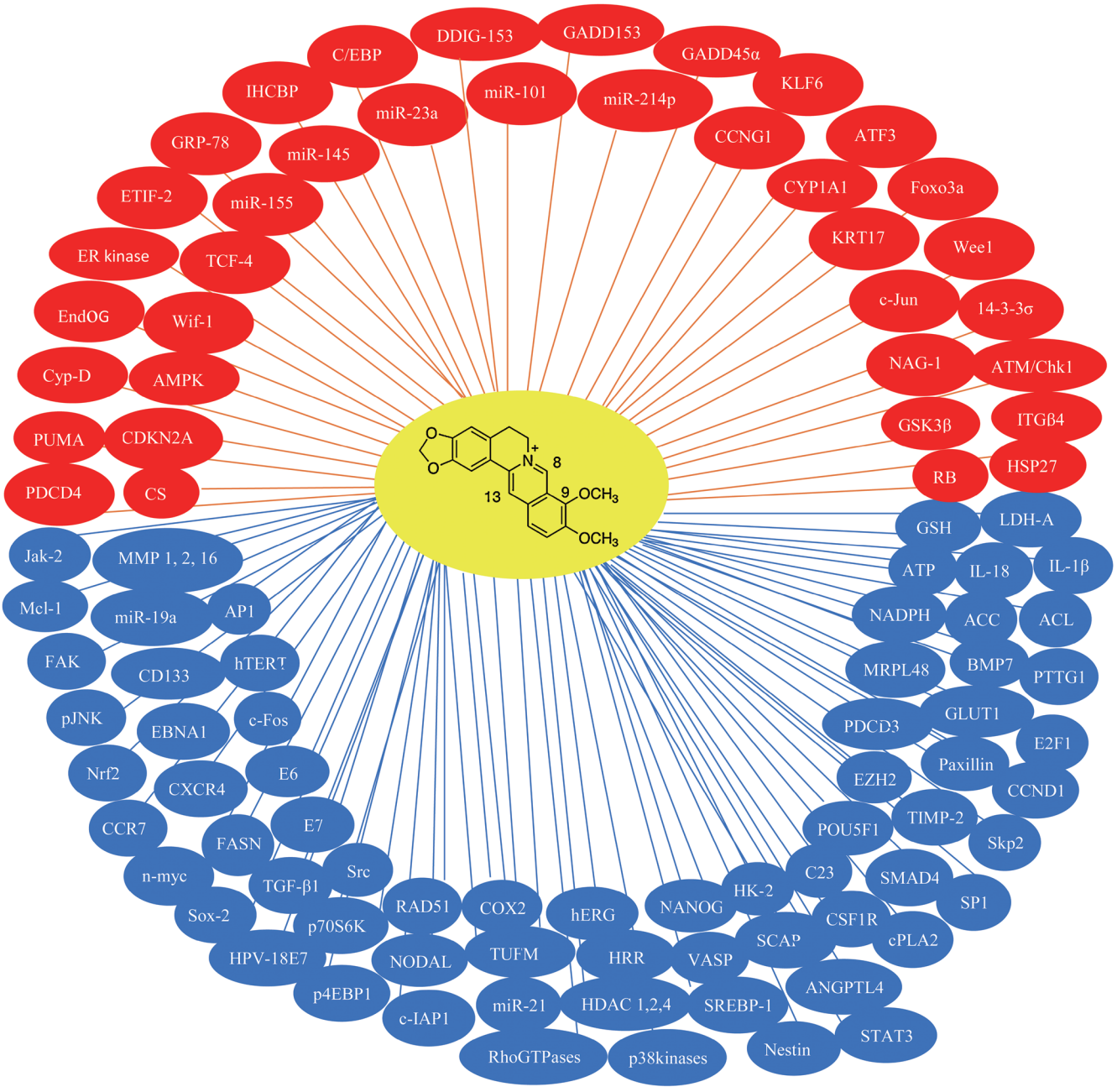

Fig. 4. Many targets participate in the cytotoxic action of berberine in various neoplastic cells. Red: upregulation. Blue: downregulation. ACC, acetyl-CoA carboxylase; ACL, adenosine triphosphate citrate lyase; AMPK, AMP-activated protein kinase; AP, activator protein; ATF, activating transcription factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; CCR, C-C chemokine receptor type; CDK, cyclin-dependent kinase; COX, cyclooxygenase; CXCR, C-X-C motif chemokine receptor; DDIG, DNA damage-inducible gene; EBNA1, Epstein–Barr nuclear antigen 1; ERK, extracellular-regulated kinases; ETIF, eukaryotic translation initiation factor; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GSH, glutathione; HDAC, histone deacetylase; hERG, human ether-à-go-go-related gene; IHCBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MMP, matrix metalloproteinase; MRP, mitochondrial ribosomal protein; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; Nestin, neuroectodermal stem cell marker; Notch, neurogenic locus notch homolog protein; PTTG, pituitary tumor transforming gene; SCAP, SREBP cleavage-activating protein; Skp, S-phase kinase-associated protein; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TCF, T-cell factor; TUFM, Tu translation elongation factor; VASP, vasodilator-stimulated phosphoprotein; Wif, Wnt inhibitory factor.

man volunteers to establish its safety after prolonged treatment. More clinical trails in different cancer types need to be conducted in future to firmly establish the chemotherapeutic potential of berberine in cancer treatment in clinical condition.

Conclusions

Berberine triggers a cytotoxic effect in various cancer cells of different tissue origins as well as mouse/xenograft human tumor models, indicating its potential as an anticancer agent. Berberine is able to upregulate or downregulate several cellular proteins to kill various cancer cells. Berberine accelerates ROS formation in tumor cells by triggering both Fas and mitochondrial-mediated caspase-dependent and caspase-independent apoptosis, necrosis, and autophagy to kill cells. Berberine arrests cells in G_0/G_1, S- and G_2/M phases, indicating that it can act at any stage of the cell cycle by suppressing cyclins and CDKs, and upregulating p53, $p21^{/Cip,}$ and $p27^{/Kip}$. The cytotoxic effect of berberine is also due to its ability to modulate various cell signaling pathways including Wnt/β-catenin, mTOR, Ras-Raf-ERK, HER2/PI3K/Akt, EGFR-ErbB2/PI3K/Akt, JNK, ATM/Chk1, p53, NF-κB, and COX-2/PGE2. A single clinical trial has shown improvement in gastric cancer patients with berberine. Clinically, berberine has exerted adverse effects in the form of constipation, diarrhea, abdominal pain, flatulence, and ventricular tachycardia with torsade de pointes in humans. Pregnant mothers should not be given berberine as it has shown adverse effects in preclinical models. There is a need to study the toxic profile of berberine more thoroughly in preclinical and clinical conditions to prove its safety after long term use in humans.

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Conflict of interest

The author has no conflict of interest statement to declare.

Author contributions

This study is the sole work of GC Jagetia.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021;71(1):7–33. doi:10.3322/caac.21654.
[2] Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, *et al*. Cancer Statistics, 2020: Report From National Cancer Registry Programme, India. JCO Glob Oncol 2020;6:1063–1075. doi:10.1200/go.20.00122.
[3] WHO. WHO report on cancer: setting priorities, investing wisely and providing care for all. Geneva: World Health Organization; 2020. Available from: https://apps.who.int/iris/handle/10665/330745. Accessed March 05, 2021.
[4] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021. doi:10.3322/caac.21660.
[5] Simoens S, van Harten W, Lopes G, Vulto A, Meier K, Wilking N. What happens when the cost of cancer care becomes unsustainable? Eur Oncol Haematol 2017;13:108–113. doi:10.17925/EOH.2017.13.02.108.
[6] Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod 2016;79(3):629–661. doi:10.1021/acs.jnatprod.5b01055.
[7] Sun Y, Xun K, Wang Y, Chen X. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anticancer Drugs 2009;20(9):757–769. doi:10.1097/CAD.0b013e328330d95b.
[8] Chen XW, Di YM, Zhang J, Zhou ZW, Li CG, Zhou SF. Interaction of herbalcompounds with biological targets: A case study with berberine. Sci World J 2012;2012:708292. doi:10.1100/2012/708292.
[9] Singh HB, Bharati KA. Handbook of Natural Dyes and Pigments. New Delhi: Woodhead Publishing India Pvt Limited; 2015. doi:10.1016/C2014-0-02803-1.
[10] Pavelka S, Smékal E. The fluorescence properties of protoberberine and tetrahydroprotoberberine alkaloids. Collect Czech Chem Commun 1976;41(10):3157–3169. doi:10.1135/cccc19763157.
[11] Domingo MP, Pardo J, Cebolla V, Galvez EM. Berberine: a fluorescent alkaloid with a variety of applications from medicine to chemistry. Mini Rev Org Chem 2010;7(4):335–340. doi:10.2174/157019310792246445.
[12] Nadkarni KM. Indian Materia Medica. 3rd edition. Bombay: Popular Prakashan; 1982.
[13] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: National Institute of Science Communication and Information Resources; 2002.
[14] Chander V, Aswal JS, Dobhal R, Uniyal DP. A review on Pharmacological potential of Berberine; an active component of Himalayan *Berberis* Glossary of Indian Medicinal Plants.*aristata*. J Phytopharm 2017;6(1):53–58.
[15] Li Z, Geng YN, Jiang JD, Kong WJ. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evid Based Complement Altern Med 2014;2014:289264. doi:10.1155/2014/289264.
[16] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008;57(5):712–717. doi:10.1016/j.metabol.2008.01.013.
[17] Singh A, Duggal S, Kaur N, Singh J. Berberine: Alkaloid with wide spectrum of pharmacological activities. J Nat Prod 2010;3:64–75.
[18] Peng WH, Lo KL, Lee YH, Hung TH, Lin YC. Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sci 2007;81(11):933–938. doi:10.1016/j.lfs.2007.08.003.
[19] Peng L, Kang S, Yin Z, Jia R, Song X, Li L, *et al*. Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Int J Clin Exp Pathol 2015;8(5):5217–5223.
[20] Hu PF, Chen WP, Tang JL, Bao JP, Wu LD. Protective effects of berberine in an experimental rat osteoarthritis model. Phytother Res 2011;25(6):878–885. doi:10.1002/ptr.3359.
[21] Chang W, Li K, Guan F, Yao F, Yu Y, Zhang M, *et al*. Berberine pretreatment confers cardioprotection against ischemia-reperfusion injury in a rat model of type 2 diabetes. J Cardiovasc Pharmacol Ther 2016;21(5):486–494. doi:10.1177/1074248415627873.
[22] Cai Z, Wang C, Yang W. Role of berberine in Alzheimer's disease. Neuropsychiatr Dis Treat 2016;12:2509–2520. doi:10.2147/NDT.S114846.
[23] Zhao GL, Yu LM, Gao WL, Duan WX, Jiang B, Liu XD, *et al*. Berberine protects rat heart from ischemia/reperfusion injury via activating JAK2/STAT3 signaling and attenuating endoplasmic reticulum stress. Acta Pharmacol Sin 2016;37(3):354–367. doi:10.1038/aps.2015.136.
[24] Liu DQ, Chen SP, Sun J, Wang XM, Chen N, Zhou YQ, *et al*. Berberine protects against ischemia-reperfusion injury: A review of evidence from animal models and clinical studies. Pharmacol Res 2019;148:104385. doi:10.1016/j.phrs.2019.104385.
[25] Hu Y, Ehli EA, Kittelsrud J, Ronan PJ, Munger K, Downey T, *et al*. Lipid-lowering effect of berberine in human subjects and rats. Phytomedi-

cine 2012;19(10):861–867. doi:10.1016/j.phymed.2012.05.009.
[26] Huang M, Chen S, Liang Y, Guo Y. The role of berberine in the multi-target treatment of senile dementia. Curr Top Med Chem 2015; 16(8):867–873. doi:10.2174/1568026615666150827095433.
[27] Koppen LM, Whitaker A, Rosene A, Beckett RD. Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: A systematic review. J Evid Based Complement Altern Med 2017; 22(4):956–968. doi:10.1177/2156587216687695.
[28] Di Pierro F, Putignano P, Villanova N. Retrospective analysis of the effects of a highly standardized mixture of berberis Aristata, Silybum Marianum, and Monacolins K and KA in diabetic patients with dyslipidemia. Acta Biomed 2017;88(4):462–469. doi:10.23750/abm.v88i4.5851.
[29] Yang Y, Wang Q, Xie M, Liu P, Qi X, Liu X, *et al*. Berberine exerts an anti-inflammatory role in ocular Behcet's disease. Mol Med Rep 2017;15(1): 97–102. doi:10.3892/mmr.2016.5980.
[30] Eom KS, Hong JM, Youn MJ, So HS, Park R, Kim JM, *et al*. Berberine induces G1 arrest and apoptosis in human glioblastoma T98G cells through mitochondrial/caspases pathway. Biol Pharm Bull 2008;31(4): 558–562. doi:10.1248/bpb.31.558.
[31] Eom KS, Kim HJ, So HS, Park R, Kim TY. Berberine-induced apoptosis in human glioblastoma T98G Cells Is mediated by endoplasmic reticulum stress accompanying reactive oxygen species and mitochondrial dysfunction. Biol Pharm Bull 2010;33(10):1644–1649. doi:10.1248/bpb.33.1644.
[32] Chen TC, Lai KC, Yang JS, Liao CL, Hsia TC, Chen GW, *et al*. Involvement of reactive oxygen species and caspase-dependent pathway in berberine-induced cell cycle arrest and apoptosis in C6 rat glioma cells. Int J Oncol 2009;34(6):1681–1690. doi:10.3892/ijo_00000299.
[33] Liu Q, Xu X, Zhao M, Wei Z, Li X, Zhang X, *et al*. Berberine induces senescence of human glioblastoma cells by downregulating the EGFR-MEK-ERK signaling pathway. Mol Cancer Ther 2015;14(2):355–363. doi:10.1158/1535-7163.MCT-14-0634.
[34] Wang J, Qi Q, Feng Z, Zhang X, Huang B, Chen A, *et al*. Berberine induces autophagy in glioblastoma by targeting the AMPK/mTOR/ULK1-pathway. Oncotarget 2016;7(41):66944–66958. doi:10.18632/oncotarget.11396.
[35] Tong L, Xie C, Wei Y, Qu Y, Liang H, Zhang Y, *et al*. Antitumor effects of berberine on gliomas via inactivation of caspase-1mediated IL-1β and IL-18 release. Front Oncol 2019;9:364. doi:10.3389/fonc.2019.00364.
[36] Naveen CR, Gaikwad S, Agrawal-Rajput R. Berberine induces neuronal differentiation through inhibition of cancer stemness and epithelial-mesenchymal transition in neuroblastoma cells. Phytomedicine 2016;23(7):736–44. doi:10.1016/j.phymed.2016.03.013.
[37] Sun Y, Yu J, Liu X, Zhang C, Cao J, Li G, *et al*. Oncosis-like cell death is induced by berberine through ERK1/2-mediated impairment of mitochondrial aerobic respiration in gliomas. Biomed Pharmacother 2018;102:699–710. doi:10.1016/j.biopha.2018.03.132.
[38] Xie D, Li X, Qi X, Wan Y, Zhu Y, Yang S. Berberine inhibits cell growth via Wnt/beta-catenin signaling in glioma. Int J Clin Exp Med 2019;12(1): 652–657.
[39] Palma TV, Lenz LS, Bottari NB, Pereira A, Schetinger MRC, Morsch VM, *et al*. Berberine induces apoptosis in glioblastoma multiforme U87MG cells via oxidative stress and independent of AMPK activity. Mol Biol Rep 2020;47(6):4393–4400. doi:10.1007/s11033-020-05500-9.
[40] Kuo CL, Chi CW, Liu TY. Modulation of apoptosis by berberine through inhibition of cyclooxygenase-2 and Mcl-1 expression in oral cancer cells. In Vivo 2005;19(1):247–252.
[41] Kim JS, Oh D, Yim MJ, Park JJ, Kang KR, Cho IA, *et al*. Berberine induces FasL-related apoptosis through p38 activation in KB human oral cancer cells. Oncol Rep 2015;33(4):1775–1782. doi:10.3892/or.2015.3768.
[42] Lin CC, Yang JS, Chen JT, Fan S, Yu FS, Yang JL, *et al*. Berberine induces apoptosis in human HSC-3 oral cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. Anticancer Res 2007;27(5A):3371–3378.
[43] Ho YT, Lu CC, Yang JS, Chiang JH, Li TC, Ip SW, *et al*. Berberine induced apoptosis via promoting the expression of caspase-8,-9 and-3, apoptosis-inducing factor and endonuclease G in SCC-4 human tongue squamous carcinoma cancer cells. Anticancer Res 2009;29(10):4063–4070.
[44] Ho YT, Yang JS, Li TC, Lin JJ, Lin JG, Lai KC, *et al*. Berberine suppresses in vitro migration and invasion of human SCC-4 tongue squamous cancer cells through the inhibitions of FAK, IKK, NF-κB, u-PA and MMP-2 and -9. Cancer Lett 2009;279(2):155–162. doi:10.1016/j.canlet.2009.01.033.
[45] Tang F, Wang D, Duan C, Huang D, Wu Y, Chen Y, *et al*. Berberine inhibits metastasis of nasopharyngeal carcinoma 5-8F cells by targeting rho kinase-mediated ezrin phosphorylation at threonine 567. J Biol Chem 2009;284(40):27456–27466. doi:10.1074/jbc.M109.033795.
[46] Huang D, Wang W, Feng Z, Wang L, Chen Y, Xie C, *et al*. Berberine inhibits the invasion and metastasis of nasopharyngeal carcinoma cells through Ezrin phosphorylation. J Cent South Univ (Medical Sci) 2011;36(7):616–623. doi:10.3969/j.issn.1672-7347.2011.07.006.
[47] Li CH, Wu DF, Ding H, Zhao Y, Zhou KY, Xu DF. Berberine hydrochloride impact on physiological processes and modulation of twist levels in nasopharyngeal carcinoma CNE-1 cells. Asian Pacific J Cancer Prev 2014;15(4):1851–1857. doi:10.7314/APJCP.2014.15.4.1851.
[48] Tsang CM, Lau EPW, Di K, Cheung PY, Hau PM, Ching YP, *et al*. Berberine inhibits Rho GTPases and cell migration at low doses but induces G2 arrest and apoptosis at high doses in human cancer cells. Int J Mol Med 2009;24(1):131–138. doi:10.3892/ijmm_00000216.
[49] Wang C, Wang H, Zhang Y, Guo W, Long C, Wang J, *et al*. Berberine inhibits the proliferation of human nasopharyngeal carcinoma cells via an Epstein-Barr virus nuclear antigen 1-dependent mechanism. Oncol Rep 2017;37(4):2109–2120. doi:10.3892/or.2017.5489.
[50] Mishan MA, Ahmadiankia N, Matin MM, Heirani-Tabasi A, Shahriyari M, Bidkhori HR, *et al*. Role of berberine on molecular markers involved in migration of esophageal cancer cells. Cell Mol Biol 2015;61(8): 37–43. doi:10.14715/cmb/2015.61.8.6.
[51] Jiang SX, Qi B, Yao WJ, Gu CW, Wei XF, Zhao Y, *et al*. Berberine displays antitumor activity in esophageal cancer cells in vitro. World J Gastroenterol 2017;23(14):2511–2518. doi:10.3748/wjg.v23.i14.2511.
[52] Chen Z, Liu Y, Qi B, Gu C, Wei X, Guo L, *et al*. MicroRNA-212 facilitates the motility and invasiveness of esophageal squamous carcinoma cells. Mol Med Rep 2019;20(4):3633–3641. doi:10.3892/mmr.2019.10647.
[53] Seo YS, Yim MJ, Kim BH, Kang KR, Lee SY, Oh JS, *et al*. Berberine-induced anticancer activities in FaDu head and neck squamous cell carcinoma cells. Oncol Rep 2015;34(6):3025–3034. doi:10.3892/or.2015.4312.
[54] Zhang B, He J, Xue K. Berberine induces autophagy, apoptosis and modulates miR-155 in head and neck squamous carcinoma cells. Acta Pol Pharm Drug Res 2020;77(3):485–494. doi:10.32383/appdr/123018.
[55] Hsu WH, Hsieh YS, Kuo HC, Teng CY, Huang HI, Wang CJ, *et al*. Berberine induces apoptosis in SW620 human colonic carcinoma cells through generation of reactive oxygen species and activation of JNK/p38 MAPK and FasL. Arch Toxicol 2007;81(10):719–728. doi:10.1007/s00204-006-0169-y.
[56] Piyanuch R, Sukhthankar M, Wandee G, Baek SJ. Berberine, a natural isoquinoline alkaloid, induces NAG-1 and ATF3 expression in human colorectal cancer cells. Cancer Lett 2007;258(2):230–240. doi:10.1016/j.canlet.2007.09.007.
[57] Lin JP, Yang JS, Wu CC, Lin SS, Hsieh WT, Lin ML, *et al*. Berberine induced down-regulation of matrix metalloproteinase-1, -2 and -9 in human gastric cancer cells (SNU-5) in vitro. In Vivo 2008;22(2):223–230.
[58] Park JJ, Seo SM, Kim EJ, Lee YJ, Ko YG, Ha J, *et al*. Berberine inhibits human colon cancer cell migration via AMP-activated protein kinase-mediated downregulation of integrin β1 signaling. Biochem Biophys Res Commun 2012;426(4):461–467. doi:10.1016/j.bbrc.2012.08.091.
[59] Chidambara Murthy KN, Jayaprakasha GK, Patil BS. The natural alkaloid berberine targets multiple pathways to induce cell death in cultured human colon cancer cells. Eur J Pharmacol 2012;688(1-3):14–21. doi:10.1016/j.ejphar.2012.05.004.
[60] Wu K, Yang Q, Mu Y, Zhou L, Liu Y, Zhou Q, *et al*. Berberine inhibits the proliferation of colon cancer cells by inactivating Wnt/β-catenin signaling. Int J Oncol 2012;41(1):292–298. doi:10.3892/ijo.2012.1423.
[61] Xu LN, Lu BN, Hu MM, Xu YW, Han X, Qi Y, *et al*. Mechanisms involved in the cytotoxic effects of berberine on human colon cancer HCT-8 cells. Biocell 2012;36(3):113–120.
[62] Mao L, Chen Q, Gong K, Xu X, Xie Y, Zhang W, *et al*. Berberine decelerates glucose metabolism via suppression of mTOR-dependent HIF-1α protein synthesis in colon cancer cells. Oncol Rep 2018;39(5):2436–2442. doi:10.3892/or.2018.6318.

[63] Lü Y, Han B, Yu H, Cui Z, Li Z, Wang J. Berberine regulates the microRNA-21-ITGB4-PDCD4 axis and inhibits colon cancer viability. Oncol Lett 2018;15(4):5971–5976. doi:10.3892/ol.2018.7997.
[64] Liu Y, Hua W, Li Y, Xian X, Zhao Z, Liu C, *et al*. Berberine suppresses colon cancer cell proliferation by inhibiting the SCAP/SREBP-1 signaling pathway-mediated lipogenesis. Biochem Pharmacol 2020;174:113776. doi:10.1016/j.bcp.2019.113776.
[65] Yi T, Zhuang L, Song G, Zhang B, Li G, Hu T. Akt signaling is associated with the berberine-induced apoptosis of human gastric cancer cells. Nutr Cancer 2015;67(3):523–531. doi:10.1080/01635581.2015.1004733.
[66] Zhang Q, Wang X, Cao S, Sun Y, He X, Jiang B, *et al*. Berberine represses human gastric cancer cell growth in vitro and in vivo by inducing cytostatic autophagy via inhibition of MAPK/mTOR/p70S6K and Akt signaling pathways. Biomed Pharmacother 2020;128:110245. doi:10.1016/j.biopha.2020.110245.
[67] Wang Y, Zhou M, Shang D. Berberine inhibits human gastric cancer cell growth via deactivation of p38/JNK pathway, induction of mitochondrial-mediated apoptosis, caspase activation and NF-κB inhibition. J BUON 2020;25(1):314–318.
[68] Tong M, Liu H, Hao J, Fan D. Comparative pharmacoproteomics reveals potential targets for berberine, a promising therapy for colorectal cancer. Biochem Biophys Res Commun 2020;525(1):244–250. doi:10.1016/j.bbrc.2020.02.052.
[69] Cai Y, Xia Q, Luo R, Huang P, Sun Y, Shi Y, *et al*. Berberine inhibits the growth of human colorectal adenocarcinoma in vitro and in vivo. J Nat Med 2014;68(1):53–62. doi:10.1007/s11418-013-0766-z.
[70] Chi CW, Chang YF, Chao TW, Chiang SH, P'eng FK, Lui WY, *et al*. Flow-cytometric analysis of the effect of berberine on the expression of glucocorticoid receptors in human hepatoma HepG2 cells. Life Sci 1994;54(26):2099–2107. doi:10.1016/0024-3205(94)00719-5.
[71] Chen J, Wu FX, Luo HL, Liu JJ, Luo T, Bai T, *et al*. Berberine upregulates miR-22-3p to suppress hepatocellular carcinoma cell proliferation by targeting Sp1. Am J Transl Res 2016;8(11):4932–4941.
[72] Li M, Zhang M, Zhang ZL, Liu N, Han XY, Liu QC, *et al*. Induction of apoptosis by berberine in hepatocellular carcinoma HepG2 cells via downregulation of NF-κB. Oncol Res 2017;25(2):233–239. doi:10.3727/096504016X14742891049073.
[73] Yang X, Huang N. Berberine induces selective apoptosis through the AMPK-mediated mitochondrial/caspase pathway in hepatocellular carcinoma. Mol Med Rep 2013;8(2):505–510. doi:10.3892/mmr.2013.1506.
[74] Wang N, Zhu M, Wang X, Tan HY, Tsao S wah, Feng Y. Berberine-induced tumor suppressor p53 up-regulation gets involved in the regulatory network of MIR-23a in hepatocellular carcinoma. Biochim Biophys Acta 2014;1839(9):849–857. doi:10.1016/j.bbagrm.2014.05.027.
[75] Li J, Li O, Kan M, Zhang M, Shao D, Pan Y, *et al*. Berberine induces apoptosis by suppressing the arachidonic acid metabolic pathway in hepatocellular carcinoma. Mol Med Rep 2015;12(3):4572–4577. doi:10.3892/mmr.2015.3926.
[76] Chuang TY, Wu HL, Min J, Diamond M, Azziz R, Chen YH. Berberine regulates the protein expression of multiple tumorigenesis-related genes in hepatocellular carcinoma cell lines. Cancer Cell Int 2017;17:59. doi:10.1186/s12935-017-0429-3.
[77] Li F, Dong X, Lin P, Jiang J. Regulation of Akt/FoxO3a/Skp2 axis is critically involved in berberine-induced cell cycle arrest in hepatocellular carcinoma cells. Int J Mol Sci 2018;19(2):327. doi:10.3390/ijms19020327.
[78] Vishnoi K, Ke R, Saini KS, Viswakarma N, Nair RS, Das S, *et al*. Berberine represses β-catenin translation involving 4E-BPs in hepatocellular carcinoma cells. Mol Pharmacol 2021;99(1):1–16. doi:10.1124/molpharm.120.000029.
[79] Puthdee N, Seubwai W, Vaeteewoottacharn K, Boonmars T, Cha'on U, Phoomak C, *et al*. Berberine induces cell cycle arrest in cholangiocarcinoma cell lines via inhibition of NF-κB and STAT3 pathways. Biol Pharm Bull 2017;40(6):751–757. doi:10.1248/bpb.b16-00428.
[80] Kim JB, Lee KM, Ko E, Han W, Lee JE, Shin I, *et al*. Berberine inhibits growth of the breast cancer cell lines MCF-7 and MDA-MB-231. Planta Med 2008;74(1):39–42. doi:10.1055/s-2007-993779.
[81] Kim JB, Yu JH, Ko E, Lee KW, Song AK, Park SY, *et al*. The alkaloid Berberine inhibits the growth of Anoikis-resistant MCF-7 and MDA-MB-231 breast cancer cell lines by inducing cell cycle arrest. Phytomedicine 2010;17(6):436–440. doi:10.1016/j.phymed.2009.08.012.
[82] Su K, Hu P, Wang X, Kuang C, Xiang Q, Yang F, *et al*. Tumor suppressor berberine binds VASP to inhibit cell migration in basal-like breast cancer. Oncotarget 2016;7(29):45849–45862. doi:10.18632/oncotarget.9968.
[83] Chou HC, Lu YC, Cheng CS, Chen YW, Lyu PC, Lin CW, *et al*. Proteomic and redox-proteomic analysis of berberine-induced cytotoxicity in breast cancer cells. J Proteomics 2012;75(11):3158–3176. doi:10.1016/j.jprot.2012.03.010.
[84] Kuo HP, Chuang TC, Yeh MH, Hsu SC, Way TD, Chen PY, *et al*. Growth suppression of HER2-overexpressing breast cancer cells by berberine via modulation of the HER2/PI3K/Akt signaling pathway. J Agric Food Chem 2011;59(15):8216–8224. doi:10.1021/jf2012584.
[85] Kuo HP, Chuang TC, Tsai SC, Tseng HH, Hsu SC, Chen YC, *et al*. Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. J Agric Food Chem 2012;60(38):9649–9658. doi:10.1021/jf302832n.
[86] Ma X, Zhou J, Zhang CX, Li XY, Li N, Ju RJ, *et al*. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. Biomaterials 2013;34(18):4452–4465. doi:10.1016/j.biomaterials.2013.02.066.
[87] Wen CJ, Wu LX, Fu LJ, Yu J, Zhang YW, Zhang X, *et al*. Genomic screening for targets regulated by berberine in breast cancer cells. Asian Pacific J Cancer Prev 2013;14(10):6089–6094. doi:10.7314/APJCP.2013.14.10.6089.
[88] Xie J, Xu Y, Huang X, Chen Y, Fu J, Xi M, *et al*. Berberine-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species generation and mitochondrial-related apoptotic pathway. Tumor Biol 2015;36(2):1279–1288. doi:10.1007/s13277-014-2754-7.
[89] Zhao Y, Jing Z, Lv J, Zhang Z, Lin J, Cao X, *et al*. Berberine activates caspase-9/cytochrome c-mediated apoptosis to suppress triple-negative breast cancer cells in vitro and in vivo. Biomed Pharmacother 2017;95:18–24. doi:10.1016/j.biopha.2017.08.045.
[90] Kim S, Lee J, You D, Jeong Y, Jeon M, Yu J, *et al*. Berberine suppresses cell motility through downregulation of TGF-β1 in triple negative breast cancer cells. Cell Physiol Biochem 2018;45(2):795–807. doi:10.1159/000487171.
[91] Kim S, You D, Jeong Y, Yu J, Kim SW, Nam SJ, *et al*. Berberine down-regulates IL-8 expression through inhibition of the EGFR/MEK/ERK pathway in triple-negative breast cancer cells. Phytomedicine 2018;50:43–49. doi:10.1016/j.phymed.2018.08.004.
[92] Tak J, Sabarwal A, Shyanti RK, Singh RP. Berberine enhances posttranslational protein stability of p21/cip1 in breast cancer cells via down-regulation of Akt. Mol Cell Biochem 2019;458(1-2):49–59. doi:10.1007/s11010-019-03529-4.
[93] Jabbarzadeh Kaboli P, Leong MPY, Ismail P, Ling KH. Antitumor effects of berberine against EGFR, ERK1/2, P38 and AKT in MDA-MB231 and MCF-7 breast cancer cells using molecular modelling and in vitro study. Pharmacol Rep 2019;71(1):13–23. doi:10.1016/j.pharep.2018.07.005.
[94] Lin YS, Chiu YC, Tsai YH, Tsai YF, Wang JY, Tseng LM, *et al*. Different mechanisms involved in the berberine-induced antiproliferation effects in triple-negative breast cancer cell lines. J Cell Biochem 2019;120(8):13531–13544. doi:10.1002/jcb.28628.
[95] Sakaguchi M, Kitaguchi D, Morinami S, Kurashiki Y, Hashida H, Miyata S, *et al*. Berberine-induced nucleolar stress response in a human breast cancer cell line. Biochem Biophys Res Commun 2020;528(1):227–233. doi:10.1016/j.bbrc.2020.05.020.
[96] Zhu C, Li J, Hua Y, Wang J, Wang K, Sun J. Berberine inhibits the expression of SCT through miR-214-3p stimulation in breast cancer cells. Evidence-Based Complement Altern Med 2020;2020:2817147. doi:10.1155/2020/2817147.
[97] Jantová S, Čipák L, Čerňáková M, Košt'álová D. Effect of berberine on proliferation, cell cycle and apoptosis in HeLa and L1210 cells. J Pharm Pharmacol 2003;55(8):1143–1149. doi:10.1211/0022357703322277186.
[98] Lin JP, Yang JS, Chang NW, Chiu TH, Su CC, Lu KW, *et al*. GADD153 mediates berberine-induced apoptosis in human cervical cancer Ca ski cells. Anticancer Res 2007;27(5A):3379–3386.
[99] Mahata S, Bharti AC, Shukla S, Tyagi A, Husain SA, Das BC. Berberine modulates AP-1 activity to suppress HPV transcription and down-

stream signaling to induce growth arrest and apoptosis in cervical cancer cells. Mol Cancer 2011;10:39. doi:10.1186/1476-4598-10-39.
[100] Saha SK, Khuda-Bukhsh AR. Berberine alters epigenetic modifications, disrupts microtubule network, and modulates HPV-18 E6-E7 oncoproteins by targeting p53 in cervical cancer cell HeLa: A mechanistic study including molecular docking. Eur J Pharmacol 2015;744: 132–146. doi:10.1016/j.ejphar.2014.09.048.
[101] Jagetia GC, Rao SK. Isoquinoline alkaloid berberine exerts its antineoplastic activity by inducing molecular DNA damage in hela cells: A comet assay study. Biol Med 2015;7(1):1000223. doi:10.4172/0974-8369.1000223.
[102] Jagetia GC, Rao SK. Berberine chloride, an isoquinoline alkaloid induces cytotoxicity in cultured Hela cells. Adv Biotechnol Biochem 2017;2:120. doi:10.29011/2574-7258.000020.
[103] Chu SC, Yu CC, Hsu LS, Chen KS, Su MY, Chen PN. Berberine reverses epithelial-to-Mesenchymal transition and inhibits metastasis and tumor-induced angiogenesis in human cervical cancer cells. Mol Pharmacol 2014;86(6):609–623. doi:10.1124/mol.114.094037.
[104] Liu L, Sun L, Zheng J, Cui L. Berberine modulates Keratin 17 to inhibit cervical cancer cell viability and metastasis. J Recept Signal Transduct Res 2020:1–11. doi:10.1080/10799893.2020.1830110.
[105] Kuo CL, Chou CC, Yung BY. Berberine complexes with DNA in the berberine-induced apoptosis in human leukemic HL-60 cells. Cancer Lett 1995;93(2):193–200. doi:10.1016/0304-3835(95)03809-B.
[106] Wu HL, Hsu CY, Liu WH, Yung BY. Berberine-induced apoptosis of human leukemia HL-60 cells is associated with down-regulation of nucleophosmin/B23 and telomerase activity. Int J Cancer 1999;81(6): 923–929. doi:10.1002/(SICI)1097-0215(19990611)81:6<923::AID-IJC 14>3.0.CO;2-D.
[107] Chung JG, Chen GW, Hung CF, Lee JH, Ho CC, Ho HC, *et al*. Effects of berberine on arylamine N-acetyltransferase activity and 2-aminofluorene-DNA adduct formation in human leukemia cells. Am J Chin Med 2000;28(2):227–238. doi:10.1142/S0192415X00000271.
[108] Lin CC, Kao ST, Chen GW, Chung JG. Berberine decreased N-acetylation of 2-aminofluorene through inhibition of N-acetyltransferase gene expression in human leukemia HL-60 cells. Anticancer Res 2005;25(6B):4149–4155.
[109] Lin CC, Kao ST, Chen GW, Ho HC, Chung JG. Apoptosis of human leukemia HL-60 cells and murine leukemia WEHI-3 cells induced by berberine through the activation of caspase-3. Anticancer Res 2006; 26(1A):227–242.
[110] Lin CC, Lin SY, Chung JG, Lin JP, Chen GW, Kao ST. Down-regulation of cyclin B1 and up-regulation of Wee1 by berberine promotes entry of leukemia cells into the G2/M-phase of the cell cycle. Anticancer Res 2006;26(2A):1097–1104.
[111] Li H, Guo L, Jie S, Liu W, Zhu J, Du W, *et al*. Berberine inhibits SDF-1-induced AML cells and leukemic stem cells migration via regulation of SDF-1 level in bone marrow stromal cells. Biomed Pharmacother 2008;62(9):573–578. doi:10.1016/j.biopha.2008.08.003.
[112] Okubo S, Uto T, Goto A, Tanaka H, Nishioku T, Yamada K, *et al*. Berberine induces apoptotic cell death via activation of caspase-3 and-8 in HL-60 human leukemia cells: nuclear localization and structure–activity relationships. Am J Chin Med 2017;45(7):1497–1511. doi:10.1142/S0192415X17500811.
[113] Liu J, Zhang X, Liu A, Liu S, Zhang L, Wu B, *et al*. Berberine induces apoptosis in p53-null leukemia cells by down-regulating XIAP at the post-transcriptional level. Cell Physiol Biochem 2013;32(5):1213–1224. doi:10.1159/000354520.
[114] Liu J, Chen Z, Cui Y, Wei H, Zhu Z, Mao F, *et al*. Berberine promotes xiap-mediated cells apoptosis by upregulation of miR-24-3p in acute lymphoblastic leukemia. Aging (Albany NY) 2020;12(4):3298–3311. doi:10.18632/aging.102813.
[115] Liu J, Liu P, Xu T, Chen Z, Kong H, Chu W, *et al*. Berberine induces autophagic cell death in acute lymphoblastic leukemia by inactivating AKT/mTORC1 signaling. Drug Des Devel Ther 2020;14:1813–1823. doi:10.2147/DDDT.S239247.
[116] Gu C, Yin Z, Nie H, Liu Y, Yang J, Huang G, *et al*. Identification of berberine as a novel drug for the treatment of multiple myeloma via targeting UHRF1. BMC Biol 2020;18(1):33. doi:10.1186/s12915-020-00766-8.
[117] Mantena SK, Sharma SD, Katiyar SK. Berberine, a natural product, induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells. Mol Cancer Ther 2006;5(2):296–308. doi:10.1158/1535-7163.MCT-05-0448.
[118] Meeran SM, Katiyar S, Katiyar SK. Berberine-induced apoptosis in human prostate cancer cells is initiated by reactive oxygen species generation. Toxicol Appl Pharmacol 2008;229(1):33–43. doi:10.1016/j.taap.2007.12.027.
[119] Choi MS, Oh JH, Kim SM, Jung HY, Yoo HS, Lee YM, *et al*. Berberine inhibits p53-dependent cell growth through induction of apoptosis of prostate cancer cells. Int J Oncol 2009;34(5):1221–1230.
[120] Huang ZH, Zheng HF, Wang WL, Wang Y, Zhong LF, Wu JL, *et al*. Berberine targets epidermal growth factor receptor signaling to suppress prostate cancer proliferation in vitro. Mol Med Rep 2015;11(3): 2125–2128. doi:10.3892/mmr.2014.2929.
[121] Li J, Cao B, Liu X, Fu X, Xiong Z, Chen L, *et al*. Berberine suppresses androgen receptor signaling in prostate cancer. Mol Cancer Ther 2011;10(8):1346–1356. doi:10.1158/1535-7163.MCT-10-0985.
[122] Wang Y, Liu Q, Liu Z, Li B, Sun Z, Zhou H, *et al*. Berberine, a genotoxic alkaloid, induces ATM-Chk1 mediated G2 arrest in prostate cancer cells. Mutat Res 2012;734(1-2):20–29. doi:10.1016/j.mrfmmm.2012.04.005.
[123] Lu W, Du S, Wang J. Berberine inhibits the proliferation of prostate cancer cells and induces G0/G1 or G2/M phase arrest at different concentrations. Mol Med Rep 2015;11:3920–4. doi:10.3892/mmr.2014.3139.
[124] Zhang LY, Wu YL, Gao XH, Guo F. Mitochondrial protein cyclophilin-D-mediated programmed necrosis attributes to berberine-induced cytotoxicity in cultured prostate cancer cells. Biochem Biophys Res Commun 2014;450(1):697–703. doi:10.1016/j.bbrc.2014.06.039.
[125] Liu CH, Tang WC, Sia P, Huang CC, Yang PM, Wu MH, *et al*. Berberine inhibits the metastatic ability of prostate cancer cells by suppressing epithelial-to-mesenchymal transition (EMT)-associated genes with predictive and prognostic relevance. Int J Med Sci 2015;12(1):63–71. doi:10.7150/ijms.9982.
[126] Tian Y, Zhao L, Wang Y, Zhang H, Xu D, Zhao X, *et al*. Berberine inhibits androgen synthesis by interaction with aldo-keto reductase 1C3 in 22Rv1 prostate cancer cells. Asian J Androl 2016;18(4):607–612. doi:10.4103/1008-682X.169997.
[127] Li X, Zhang A, Sun H, Liu Z, Zhang T, Qiu S, *et al*. Metabolic characterization and pathway analysis of berberine protects against prostate cancer. Oncotarget 2017;8(39):65022–65041. doi:10.18632/oncotarget.17531.
[128] Park KS, Kim JB, Lee SJ, Bae J. Berberine-induced growth inhibition of epithelial ovarian carcinoma cell lines. J Obstet Gynaecol Res 2012;38(3):535–540. doi:10.1111/j.1447-0756.2011.01743.x.
[129] Hou D, Xu G, Zhang C, Li B, Qin J, Hao X, *et al*. Berberine induces oxidative DNA damage and impairs homologous recombination repair in ovarian cancer cells to confer increased sensitivity to PARP inhibition. Cell Death Dis 2017;8(10):e3070. doi:10.1038/cddis.2017.471.
[130] Zhi D, Zhou K, Yu D, Fan X, Zhang J, Li X, *et al*. HERG1 is involved in the pathophysiological process and inhibited by berberine in SKOV3 cells. Oncol Lett 2019;17:5653–5661. doi:10.3892/ol.2019.10263.
[131] Chuang TC, Wu K, Lin YY, Kuo HP, Kao MC, Wang V, *et al*. Dual down-regulation of EGFR and ErbB2 by berberine contributes to suppression of migration and invasion of human ovarian cancer cells. Environ Toxicol 2021;36(5):737–747. doi:10.1002/tox.23076.
[132] Li J, Zhang S, Wu L, Pei M, Jiang Y. Berberine inhibited metastasis through miR-145/MMP16 axis in vitro. J Ovarian Res 2021;14(1):4. doi:10.1186/s13048-020-00752-2.
[133] Li J, Zou Y, Pei M, Zhang Y, Jiang Y. Berberine inhibits the Warburg effect through TET3/miR-145/HK2 pathways in ovarian cancer cells. J Cancer 2021;12(1):207–216. doi:10.7150/JCA.48896.
[134] Liu Z, Liu Q, Xu B, Wu J, Guo C, Zhu F, *et al*. Berberine induces p53-dependent cell cycle arrest and apoptosis of human osteosarcoma cells by inflicting DNA damage. Mutat Res 2009;662(1-2):75–83. doi:10.1016/j.mrfmmm.2008.12.009.
[135] Chen ZZ. Berberine induced apoptosis of human osteosarcoma cells by inhibiting phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) signal pathway activation. Iran J Public Health 2016;45(5):578–585.
[136] Hao J, Jin X, Cao B, Wenbo W. Berberine affects osteosarcoma via downregulating the caspase-1/IL-1β signaling axis. Oncol Rep 2017;

37(2):729–736. doi:10.3892/or.2016.5327.
[137] Zhu Y, Ma N, Li HX, Tian L, Ba YF, Hao B. Berberine induces apoptosis and DNA damage in MG-63 human osteosarcoma cells. Mol Med Rep 2014;10(4):1734–1738. doi:10.3892/mmr.2014.2405.
[138] Mishra R, Nathani S, Varshney R, Sircar D, Roy P. Berberine reverses epithelial-mesenchymal transition and modulates histone methylation in osteosarcoma cells. Mol Biol Rep 2020;47(11):8499–8511. doi:10.1007/s11033-020-05892-8.
[139] Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP. Inhibitory effect of berberine on the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. Toxicol Appl Pharmacol 2006;214(1):8–15. doi:10.1016/j.taap.2005.11.010.
[140] James MA, Fu H, Liu Y, Chen DR, You M. Dietary administration of berberine or Phellodendron amurense extract inhibits cell cycle progression and lung tumorigenesis. Mol Carcinog 2011;50(1):1–7. doi:10.1002/mc.20690.
[141] Katiyar SK, Meeran SM, Katiyar N, Akhtar S. P53 cooperates berberine-induced growth inhibition and apoptosis of non-small cell human lung cancer cells in vitro and tumor xenograft growth in vivo. Mol Carcinog 2009;48(1):24–37. doi:10.1002/mc.20453.
[142] Sung JH, Kim JB, Park SH, Park SY, Lee JK, Lee HS, *et al*. Berberine decreases cell growth but increases the side population fraction of H460 lung cancer cells. J Korean Soc Appl Biol Chem 2012;55:491–495. doi:10.1007/s13765-012-2119-0.
[143] Qi HW, Xin LY, Xu X, Ji XX, Fan LH. Epithelial-to-mesenchymal transition markers to predict response of berberine in suppressing lung cancer invasion and metastasis. J Transl Med 2014;12:22. doi:10.1186/1479-5876-12-22.
[144] Zheng F, Tang Q, Wu J, Zhao S, Liang Z, Li L, *et al*. P38α MAPK-mediated induction and interaction of FOXO3a and p53 contribute to the inhibited-growth and induced-apoptosis of human lung adenocarcinoma cells by berberine. J Exp Clin Cancer Res 2014;33(1):36. doi:10.1186/1756-9966-33-36.
[145] Kumar R, Awasthi M, Sharma A, Padwad Y, Sharma R. Berberine induces dose-dependent quiescence and apoptosis in A549 cancer cells by modulating cell cyclins and inflammation independent of mTOR pathway. Life Sci 2020;244:117346. doi:10.1016/j.lfs.2020.117346.
[146] Yuan ZW, Leung ELH, Fan XX, Zhou H, Ma WZ, Liu L, *et al*. Quantitative evaluation of berberine subcellular distribution and cellular accumulation in non-small cell lung cancer cells by UPLC-MS/MS. Talanta 2015;144:20–28. doi:10.1016/j.talanta.2015.05.017.
[147] Kalaiarasi A, Anusha C, Sankar R, Rajasekaran S, John Marshal J, Muthusamy K, *et al*. Plant isoquinoline alkaloid berberine exhibits chromatin remodeling by modulation of histone deacetylase to Induce growth arrest and apoptosis in the A549 cell line. J Agric Food Chem 2016;64(50):9542–9550. doi:10.1021/acs.jafc.6b04453.
[148] Li J, Liu F, Jiang S, Liu J, Chen X, Zhang S, *et al*. Berberine hydrochloride inhibits cell proliferation and promotes apoptosis of non-small cell lung cancer via the suppression of the MMP2 and Bcl-2/bax signaling pathways. Oncol Lett 2018;15:7409–7414. doi:10.3892/ol.2018.8249.
[149] Chen QQ, Shi JM, Ding Z, Xia Q, Zheng TS, Ren YB, *et al*. Berberine induces apoptosis in non-small-cell lung cancer cells by upregulating miR-19a targeting tissue factor. Cancer Manag Res 2019;11:9005–9015. doi:10.2147/CMAR.S207677.
[150] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, *et al*. Berberine chloride suppresses non-small cell lung cancer by deregulating Sin3A/TOP2B pathway in vitro and in vivo. Cancer Chemother Pharmacol 2020;86(1):151–161. doi:10.1007/s00280-020-04050-y.
[151] Pinto-Garcia L, Efferth T, Torres A, Hoheisel JD, Youns M. Berberine inhibits cell growth and mediates caspase-independent cell death in human pancreatic cancer cells. Planta Med 2010;76(11):1155–1161. doi:10.1055/s-0030-1249931.
[152] Park SH, Sung JH, Chung N. Berberine diminishes side population and down-regulates stem cell-associated genes in the pancreatic cancer cell lines PANC-1 and MIA PaCa-2. Mol Cell Biochem 2014;394(1-2):209–215. doi:10.1007/s11010-014-2096-1.
[153] Park SH, Sung JH, Kim EJ, Chung N. Berberine induces apoptosis via ROS generation in PANC-1 and MIA-PaCa2 pancreatic cell lines. Brazilian J Med Biol Res 2015;48(2):111–119. doi:10.1590/1414-431X20144293.
[154] Ming M, Sinnett-Smith J, Wang J, Soares HP, Young SH, Eibl G, *et al*. Dose-dependent AMPK-dependent and independent mechanisms of berberine and metformin inhibition of mTORC1, ERK, DNA synthesis and proliferation in pancreatic cancer cells. PLoS One 2014;9(12):e114573. doi:10.1371/journal.pone.0114573.
[155] Liu J, Luo X, Guo R, Jing W, Lu H. Cell metabolomics reveals berberine-inhibited pancreatic cancer cell viability and metastasis by regulating citrate metabolism. J Proteome Res 2020;19(9):3825–3836. doi:10.1021/acs.jproteome.0c00394.
[156] Fang X, Hong X, YueRong Z, Ting J. Berberine and its molecular mechanisms in inhibiting the growth of renal carcinoma cell line ACH. Acta Acad Med Wannan 2013;32(2):104–106.
[157] Liu Y, Liu S. Berberine inhibits Wilms' tumor cell progression through upregulation of Wilms' tumor gene on the X chromosome. Mol Med Rep 2013;8(5):1537–1541. doi:10.3892/mmr.2013.1665.
[158] Chung JG, Wu LT, Chu CB, Jan JY, Ho CC, Tsou MF, *et al*. Effects of berberine on arylamine N-acetyltransferase activity in human bladder tumour cells. Food Chem Toxicol 1999;37(4):319–326. doi:10.1016/S0278-6915(99)00016-2.
[159] Yan K, Zhang C, Feng J, Hou L, Yan L, Zhou Z, *et al*. Induction of G1 cell cycle arrest and apoptosis by berberine in bladder cancer cells. Eur J Pharmacol 2011;661(1-3):1–7. doi:10.1016/j.ejphar.2011.04.021.
[160] Yan L, Yan K, Kun W, Xu L, Ma Q, Tang Y, *et al*. Berberine inhibits the migration and invasion of T24 bladder cancer cells via reducing the expression of heparanase. Tumor Biol 2013;34(1):215–221. doi:10.1007/s13277-012-0531-z.
[161] Park KS, Kim J Bin, Bae J, Park SY, Jee HG, Lee KE, *et al*. Berberine inhibited the growth of thyroid cancer cell lines 8505C and TPC1. Yonsei Med J 2012;53(2):346–351. doi:10.3349/ymj.2012.53.2.346.
[162] Scordino A, Campisi A, Grasso R, Bonfanti R, Gulino M, Iauk L, *et al*. Delayed luminescence to monitor programmed cell death induced by berberine on thyroid cancer cells. J Biomed Opt 2014;19(11):117005. doi:10.1117/1.jbo.19.11.117005.
[163] Kumarasamy VM, Shin YJ, White J, Sun D. Selective repression of RET proto-oncogene in medullary thyroid carcinoma by a natural alkaloid berberine. BMC Cancer 2015;15:599. doi:10.1186/s12885-015-1610-5.
[164] Li L, Wang X, Sharvan R, Gao J, Qu S. Berberine could inhibit thyroid carcinoma cells by inducing mitochondrial apoptosis, G0/G1 cell cycle arrest and suppressing migration via PI3K-AKT and MAPK signaling pathways. Biomed Pharmacother 2017;95:1225–1231. doi:10.1016/j.biopha.2017.09.010.
[165] Ni J, Wang F, Yue L, Xiang GD, Zhao LS, Wang Y, *et al*. The effects and mechanisms of berberine on proliferation of papillary thyroid cancer K1 cells induced by high glucose (in Chinese). Zhonghua Nei Ke Za Zhi 2017;56(7):507–511. doi:10.3760/cma.j.issn.0578-1426.2017.07.007.
[166] Jagetia GC, Baliga MS. Effect of Alstonia scholaris in enhancing the anticancer activity of berberine in the Ehrlich ascites carcinoma-bearing mice. J Med Food 2004;7(2):235–244. doi:10.1089/109662 0041224094.
[167] Jagetia GC, Rao SK. Determination of the antineoplastic activity of berberine isolated from Tinospora cordifolia in Swiss albino mice transplanted with Ehrlich ascites carcinoma. J Tumor Med Prev 2017;1(3):555561. doi:10.19080/JTMP.2017.01.555561.
[168] Jin F, Xie T, Huang X, Zhao X. Berberine inhibits angiogenesis in glioblastoma xenografts by targeting the VEGFR2/ERK pathway. Pharm Biol 2018;56(1):665–671. doi:10.1080/13880209.2018.1548627.
[169] Ruan H, Zhan YY, Hou J, Xu B, Chen B, Tian Y, *et al*. Berberine binds RXRα to suppress β-catenin signaling in colon cancer cells. Oncogene 2017;36(50):6906–6918. doi:10.1038/onc.2017.296.
[170] Wang Y, Zhang S. Berberine suppresses growth and metastasis of endometrial cancer cells via miR-101/COX-2. Biomed Pharmacother 2018;103:1287–1293. doi:10.1016/j.biopha.2018.04.161.
[171] Letasiová S, Jantová S, Múcková M, Theiszová M. Antiproliferative activity of berberine in vitro and in vivo. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2005;149(2):461–463. doi:10.5507/bp.2005.080.
[172] Tsang CM, Cheung KCP, Cheung YC, Man K, Lui VWY, Tsao SW, *et al*. Berberine suppresses Id-1 expression and inhibits the growth and development of lung metastases in hepatocellular carcinoma. Biochim Biophys Acta 2015;1852(3):541–551. doi:10.1016/j.bbadis.2014.12.004.

[173] Ho YT, Yang JS, Lu CC, Chiang JH, Li TC, Lin JJ, *et al*. Berberine inhibits human tongue squamous carcinoma cancer tumor growth in a murine xenograft model. Phytomedicine 2009;16(9):887–890. doi:10.1016/j.phymed.2009.02.015.
[174] Tsang CM, Cheung YC, Lui VWY, Yip YL, Zhang G, Lin VW, *et al*. Berberine suppresses tumorigenicity and growth of nasopharyngeal carcinoma cells by inhibiting STAT3 activation induced by tumor associated fibroblasts. BMC Cancer 2013;13:619. doi:10.1186/1471-2407-13-619.
[175] Chen YX, Gao QY, Zou TH, Wang BM, Liu S De, Sheng JQ, *et al*. Berberine versus placebo for the prevention of recurrence of colorectal adenoma: a multicentre, double-blinded, randomised controlled study. Lancet Gastroenterol Hepatol 2020;5(3):267–275. doi:10.1016/S2468-1253(19)30409-1.
[176] Kheir MM, Wang Y, Hua L, Hu J, Li L, Lei F, *et al*. Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol 2010;48(4):1105–1110. doi:10.1016/j.fct.2010.01.033.
[177] Haginiwa J, Harada M. Pharmacological studies on crude drugs. V. Comparison of berberine type alkaloid-containing plants on their components and several pharmacological actions (in Japanese). Yakugaku Zasshi 1962;82:726–731.
[178] Jahnke GD, Price CJ, Marr MC, Myers CB, George JD. Developmental toxicity evaluation of berberine in rats and mice. Birth Defects Res B Dev Reprod Toxicol 2006;77(3):195–206. doi:10.1002/bdrb.20075.
[179] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008;57(5):712–717. doi:10.1016/j.metabol.2008.01.013.
[180] Marin-Neto JA, Maciel BC, Secches AL, Gallo Júnior L. Cardiovascular effects of berberine in patients with severe congestive heart failure. Clin Cardiol 1988;11(4):253–260. doi:10.1002/clc.4960110411.
[181] Fung FY, Linn YC. Developing traditional Chinese medicine in the era of evidence-based medicine: current evidences and challenges. Evid Based Complement Alternat Med 2015;2015:425037. doi:10.1155/2015/425037.
[182] Bateman J, Chapman RD, Simpson D. Possible toxicity of herbal remedies. Scott Med J 1998;43(1):7–15. doi:10.1177/003693309804300104.
[183] Kumar SG. RNA targeting by small molecules: Binding of protoberberine, benzophenanthridine and aristolochia alkaloids to various RNA structures. J Biosci 2012;37(3):539–552. doi:10.1007/s12038-012-9217-3.
[184] Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges. Cell Biosci 2020;10:31. doi:10.1186/s13578-020-00396-1.