International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Pharmacology Progresses and Applications of Chloroquine in Cancer Therapy

Yanqing Liu[1,*], Yuqing Meng[1,*], Junzhe Zhang[1], Liwei Gu[1], Shengnan Shen[1], Yongping Zhu[1], Jigang Wang[1,2]

[1]State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-Di Herbs, Artemisinin Research Center, and Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, People's Republic of China; [2]Department of Pharmacological Sciences, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 117600, Singapore

*These authors contributed equally to this work

Correspondence: Jigang Wang; Yongping Zhu, State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, Artemisinin Research Center, and Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, People's Republic of China, Email jgwang@icmm.ac.cn; ypzhu@icmm.ac.cn

Abstract: Chloroquine is a common antimalarial drug and is listed in the World Health Organization Standard List of Essential Medicines because of its safety, low cost and ease of use. Besides its antimalarial property, chloroquine also was used in anti-inflammatory and antivirus, especially in antitumor therapy. A mount of data showed that chloroquine mainly relied on autophagy inhibition to exert its antitumor effects. However, recently, more and more researches have revealed that chloroquine acts through other mechanisms that are autophagy-independent. Nevertheless, the current reviews lacked a comprehensive summary of the antitumor mechanism and combined pharmacotherapy of chloroquine. So here we focused on the antitumor properties of chloroquine, summarized the pharmacological mechanisms of antitumor progression of chloroquine dependent or independent of autophagy inhibition. Moreover, we also discussed the side effects and possible application developments of chloroquine. This review provided a more systematic and cutting-edge knowledge involved in the anti-tumor mechanisms and combined pharmacotherapy of chloroquine in hope of carrying out more in-depth exploration of chloroquine and obtaining more clinical applications.

Keywords: Chloroquine, pharmacology, applications, combined pharmacotherapy

Introduction

Chloroquine (CQ, 4-N-(7-chloroquinolin-4-yl)-1-N,1-N-diethylpentane-1,4-diamine) is recognized as a common anti-malarial drug and features in the World Health Organization Standard List of Essential Medicines due to its safety, affordability, and user-friendliness. Beyond its application in malaria treatment,[1,2] CQ is also utilized for lupus erythematosus,[3] autoimmune disorders such as rheumatoid arthritis,[4,5] and is being evaluated for its antiviral properties against HIV infection,[6] chikungunya fever,[7] and the novel coronavirus SARS-CoV-2.[8–10] Additionally, CQ has also been widely reported as a potential anticancer agent due to its ability to block autophagy.

Recently, modulating autophagy has emerged as a promising therapeutic method for cancer. CQ and its derivative hydroxychloroquine (HCQ) both are recognized autophagy inhibitors that remain the only autophagy inhibitors approved by the Food and Drug Administration (FDA).[11] CQ diffuses freely and rapidly across cell and organelle membranes. In lysosomes CQ was protonated and "trapped" in which CQ no longer freely diffused out.[12] It was reported that CQ suppressed autophagy through entering the lysosome and increased its pH which prevented autolysosomal degradation,[13] while others confirmed that CQ impaired autophagosome-lysosome fusion rather than inhibiting lysosomal degradation to achieve the effect of inhibiting autophagy, resulting in an inability to provide energy through the autophagy pathway,[14] and then inhibited tumor cell growth or induced tumor cell death.[15] Due to its autophagy inhibition, CQ has been used as a supplement agent in clinical trials against cancer. For example, the combination of CQ and cisplatin effectively enhanced the apoptosis of tumor cells induced by cisplatin,[16] and pterostilbene combined with CQ significantly improved its inhibition of autophagy and reduced the activity of tumor cells by down-regulating RAGE/STAT3 and AKT/mTOR

Received: 10 January 2024
Accepted: 7 May 2024
Published: 5 July 2024

signaling pathways.[17] In addition, CQ also effectively improved the inhibition of gemcitabine in gallbladder carcinoma cells and promoted the apoptosis of cancer cells.[18]

In tumor suppression, CQ also has activation of apoptosis and necroptosis of cancer cells that are independent of autophagy inhibition. Data have shown that CQ induces lysosome membrane permeability and mitochondrial membrane permeability, triggering caspases cascade and inducing apoptosis of cancer cells, which cannot be achieved by the addition of autophagy inhibitors.[19,20]

In this review, we focused on the anticancer properties of CQ, summarized the tumor progression dependent or independent of autophagy inhibition of CQ, and discussed combined pharmacotherapy in cancer therapy. At the end of the review, we also discussed the side effects of CQ, challenges and possible application development in the future hoping to help researchers carry out more in-depth exploration of CQ.

CQ and Its Analogues in Cancer Suppression

CQ was synthesized based on the structure of the natural product quinine. In the 16th century, malaria was widespread in Europe but no effective cure had yet been found. It was not until the 1630s that Indians discovered that drinking water from the bark of the tree which grows in the Andes could cure fever, then the bark was taken to Europe to treat malaria. In 1742, Swedish botanist Carl Linnaeus officially named the tree as "Cinchona tree". In 1820, French pharmacists Pierre Pelletier and Joseph Caventou isolated the antimalarial ingredient quinine from the "Cinchona tree", and it was later used to treat malaria. But quinine could not completely cure the malaria, and the side effects were obvious, so scientists had worked to synthesize new antimalarial drugs, and then quinacrine (QC) and CQ had been introduced successively.[21,22] CQ was more effective and less toxic than quinine and QC. However, with the widespread use of CQ, the drug resistance of malaria parasite to it began to appear, and the anti-malaria effect of CQ became worse, especially in *Plasmodium falciparum*.[23] Since then, scientists have synthesized many other new antimalarial drugs, such as HCQ and mefloquine (MQ).

Originally developed as a treatment for malaria, CQ and its analogues (Figure 1) such as HCQ, Lys05, MQ and QC are now used to treat a variety of other diseases, including cancer, because of their suppression on autophagy or enhancing the sensitivity of chemoradiotherapy drugs.[2,24] Here, we briefly summarized the anticancer types and mechanisms of each analogue above (Table 1).

Hydroxychloroquine (HCQ)

While high concentrations of CQ can lead to significant toxicity, its derivative, HCQ, exhibits similar pharmacological actions and mechanisms but is notably less toxic. Recently, HCQ has been clinically evaluated for its efficacy against

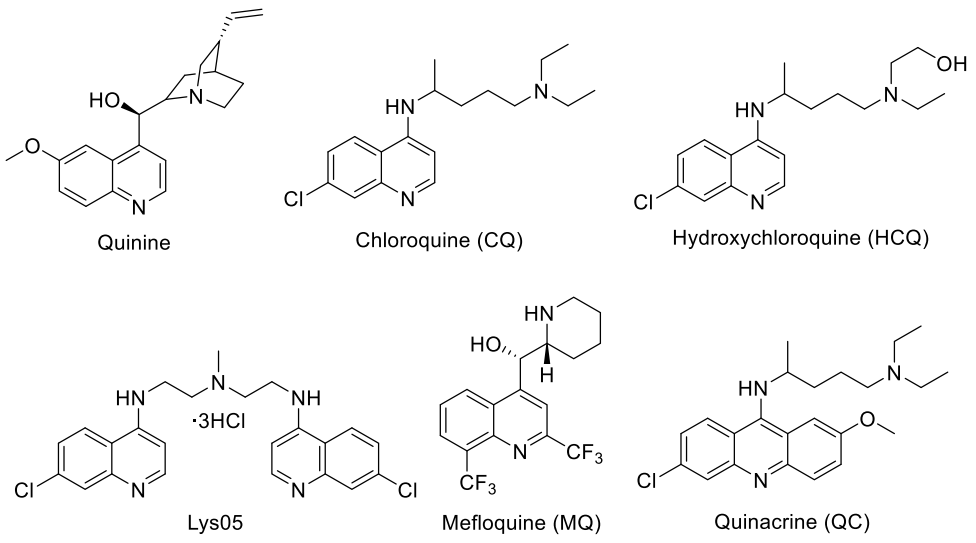

Figure 1 Chemical structures of CQ and its analogues.

Table 1 Summary of Chloroquine Analogues and Their Antitumor Types Mentioned in This Article

Analogues	Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Mechanism of Action	Ref.
HCQ	Bladder cancer	RT4, 5637, T24	In vitro	20 μM	Inhibited cell proliferation via autophagy inhibition and apoptosis induction.	[25]
HCQ	Breast Cancer	MDA-MB-231, MDA-MB-468, MCF-7	In vivo	I.p. 60 mg/kg daily	Autophagy inhibition.	[26]
HCQ	Lung cancer	A549/Lewis	In vitro	5 μM	Promoted the transition of M2 TAMs into M1-like macrophages, leading to $CD8^+$ T cell infiltration into the tumour microenvironment	[27]
		A549/Lewis	In vivo	10 mg/kg (lung perfusion) every two days		
HCQ	Ehrlich ascites carcinoma	EAC cells	In vivo	I.p. 60 mg/kg daily	Targeted autophagy and apoptosis	[28]
HCQ	Cholangiocarcinoma	HuCCT-1, CCLP-1	In vitro	IC_{50} (24h): HuCCT-1: 168.4 ± 23.4 μM, CCLP-1: 113.36 ± 14.06 μM	Inhibited cell proliferation and induced apoptosis by triggering ROS accumulation via autophagy inhibition	[29]
		CCLP-1	In vivo	I.p. 100 mg/kg daily		
HCQ	Lung adenocarcinoma	A549, PC-9	In vitro	IC_{50}: A549: 115.23±5.0 μM (24h), 91.95±9.86 μM (48h), 71.01±8.38 μM (72h) PC-9: 98.98±8.29 μM (24h), 78.71±8.01 μM (48h), 56.79±8.05 μM (72h)	Increase the expression of FoxO3a, diminished the phosphorylation of STAT3.	[30]
		PC-9	In vivo	I.p. 80mg/kg daily		
Lys05	Glioblastoma	U251, LN229	In vitro	IC_{50}: U251: 9.1 μM LN229: 6.0 μM	Induced lysosomal membrane permeabilization (LMP), caused mitochondrial depolarization.	[31]

(*Continued*)

https://doi.org/10.2147/IJN.S458910 DovePress

https://doi.org/10.2147/IJN.S458910 DovePress

Table 1 (Continued).

Analogues	Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Mechanism of Action	Ref.
Lys05	Clear cell ovarian carcinoma	OVMANA, TOV21G, OVTOKO	In vitro	10 μM	Autophagy inhibition.	[32]
		TOV21G or OVTOKO	In vivo	I.p.20 mg/kg daily		
Lys05	Myeloproliferative neoplasms	HEL, HEL, HL-60	In vitro	5 μM	Inhibited autophagy	[33]
		HEL	In vivo	I.p 32 mg/kg daily		
MQ	Colorectal cancer	HT-29, HCT116, RKO, SW620, Lovo	In vitro	0 ~ 45 μM	Inhibited NF-κB, and weaken the activity of IκBα kinase, induced cell growth arrest and apoptosis.	[34]
		HCT116	In vivo	30 mg/kg orally and daily		
MQ	Liver cancer	HepG2, CD133+ HepG2	In vitro	1, 2, 4, 8 and 10 μM	Inhibited self-renewal and proliferation of cells through targeting β-catenin pathway.	[35]
		HepG2, CD133+ HepG2	In vivo	I.p 10 mg/kg daily		
MQ	Chronic myeloid leukemia	Human:K562, KU812 Murine: 32Dp210, 32Dp210 T315I	In vitro	IC_{50} (72h): K562:10.5 μM, KU812: 8.32 μM, 32Dp210: 8.56 μM, 32Dp210 T315I:7.68 μM	Augmented the effects of tyrosine kinase inhibitors, induceD oxidative stress, lysosomal lipid damage and functional impairment.	[36]
MQ	Breast cancer	MDA-MB-231, MDA-MB-468, T47D, MCF7, MCF7/Dox	In vitro	3 ~ 12 μM	Inhibited autophagy, triggered endoplasmic reticulum stress, and caused cell death.	[37]
MQ	Glioblastoma	T98G, A172, U251 N, U87, U251MG, U373	In vitro	IC_{50} (72h): U251MG: 10 μM, U373: >30 μM	Disrupted lysosomal integrity and function, leaded to oxidative stress and lysosomal lipid damage.	[38]
		U373	In vivo	I.p 2 mg/kg daily		
MQ	Gastric cancer	SNU1, SNU16, AGS, Hs746T, NCI-N87, MKN45, MKN74, YCC1, YCC10, YCC11	In vitro	IC_{50} (72h): 0.5 ~ 0.7 μM	Inhibited PI3K/AKT/mTOR signaling pathway.	[39]
		YCC1 or SNU1	In vivo	I.p.20 mg/kg daily		

MQ	Prostate cancer	PC3	In vitro	40 μM	Induced cell growth arrest, caused ROS and induced cell death.	[40]
			In vivo	I.p.8 mg/kg Every 3 ~ 4 days		
MQ	Prostate cancer	Hs68, PC3, DU145	In vitro	IC_{50} (24h): PC3: 10 μM Hs68: NA DU145: NA	Caused ROS, downregulated Akt phosphorylation and activated ERK, JNK and AMPK pathway.	[41]
MQ	Cervical cancer	HeLa, SiHa, and C-33A	In vitro	0 ~ 50 μM	Impaired mitochondrial function, increased ROS and decreased ATP level; Inhibited of mTOR signaling pathway.	[42]
		HeLa	In vivo	I.p 100 mg/kg daily		
MQ	Esophageal squamous cell carcinoma	KYSE150 and KYSE450	In vitro	10 μM	Induced mitochondrial autophagy.	[43]
		ESCC tissues	In vivo	50 or 200 mg/kg oral gavage, daily		
MQ	Chronic lymphocytic leukemia (CLL)	CLL cells HS-5	In vitro	5, 10, 15, 20 μM	Destroyed lysosome membrane and induce cell death.	[44]
QC	Colorectal cancer	HCT 116, INT 407	In vitro	IC_{50}: HCT116: >20 μM (24h), 15 μM (48h) INT407: 10 ~ 15 μM (24h, 48h)	Disturbed the expression of small-GTPases and caspases.	[45]
QC	Breast cancer	MCF-10A-Tr	In vitro	5 μM	Enhanced the cellular apoptosis.	[46]
			In vivo	40 mg/kg, oral gavage, daily		
QC	Ovarian Cancers	SKOV3, C13, OV2008, OVCAR 3	In vitro	5 and 10 μM	Induced apoptosis via Increasing the expression of cell cycle inhibitor p21 and decreased the Skp2.	[47]

(*Continued*)

https://doi.org/10.2147/IJN.S458910

Table 1 (Continued).

Analogues	Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Mechanism of Action	Ref.
QC	Ovarian cancer	HeyA8-MDR, OVCAR5, OVCAR7, C13, OV2008	In vitro	2.5 ~ 5 μM	Induced LMP and MMP, resulting in the release of cytochrome c and induced cell death.	[48]
		HeyA8-MDR	In vivo	150 mg/kg oral gavage, every other day		
QC	Non-small cell lung cancer	A549, NCI H520	In vitro	A549:15 μM NCI H520: 12μM	Caused cell cycle G1/S arrest and leaded to cell death.	[49]
QC	Endometrial cancer	Ishikawa, Hec-1B, KLE, ARK-2, SPEC-2	In vitro	4 ~ 24 μM	Sensitive tumor cells to chemotherapy drugs via downregulating the expression of anti-apoptotic proteins.	[50]
		Hec-1B	In vivo	100 mg/kg oral gavage, every other day		
QC	Colorectal cancer	HT-29, HCT-15, RKO, DLD-1, SW-480, SW-620, HCT-116, HCT-116 p53-/-, HCT-116 bax-/-	In vitro	IC_{50} (72h): HT-29: 3.80 μM, HCT-15: 4.13 μM, RKO: 0.34 μM, DLD-1: 2.78 μM, SW-480: 2.95 μM, SW-620: 2.04 μM, HCT-116: 4.71 μM, HCT-116 p53-/-:4.37 μM, HCT-116 bax-/-:5.04 μM	Stabilized p53 and lowered anti-apoptotic protein levels.	[51]
		HT-29-*luc*,RKO or DLD-1	In vivo	100 mg/kg oral gavage, every other day		
QC	Ovarian cancer	HeyA8, HeyA8MDR, TX. C13, OV2008,	In vitro	2.5 ~ 4 μM (24h)	Induced autophagy by downregulating p62/SQSTM1 to sensitize chemoresistant cells to autophagic- and caspase-mediated cell death in a p53-independent manner.	[52]
		HEYA8MDR	In vivo	150 mg/kg oral gavage, every other day		

https://doi.org/10.2147/IJN.S458910 DovePress

QC	Upper gastrointestinal cancer	AGS, SNU1, MKN28, MKN45, FLO1, OE33, OE19	In vitro	IC_{50} (96h): AGS: 1.23 μM, SNU1: 0.73 μM, MKN28: 1.29 μM, MKN45: 0.89 μM, FLO1: 1.53 μM, OE33: 0.62 μM, OE19: 0.65 μM	Aggravated DNA damage, induced cancer cell death.	[53]
		OE33	In vivo	200 mg/kg, oral gavage, every other day		
QC	Anaplastic Thyroid Cancer	THJ-16T, THJ-21T and THJ-29T	In vitro	THJ-16T:18.43 μM; THJ-21T:15.39 μM; THJ-29T:12.74 μM	Suppressed expression of prosurvival MCL1and pSTAT3, inhibited NFκB signaling pathway.	[54]
QC	Non-small cell lung cancer	A549	In vitro	0.37, 0.75, and 1 μM	Developed a nanoformulation of Erlotinib and QC combination, improved the therapeutic efficiency and reduced the therapeutic dose.	[55]
QC	Breast cancer	MCF-10A, MCF-10A-Tr, MCF-10A-Tr-P-EMT, SP	In vitro	4 μM, 5 μM	Inhibited the expression of ABCG2, restrained the angiogenesis.	[56]
		Breast tumor samples	In vivo	20 mg/kg oral gavage, daily		

https://doi.org/10.2147/IJN.S458910

a variety of cancers, including glioblastoma, bladder, breast and prostate.[25,26,57,58] As an inhibitor of autophagy HCQ enhanced the chemosensitization via altering the lysosomal pH and inducing the transition of tumour-associated macrophages from M2 to the tumor-killing M1 phenotype.[27] HCQ also induced the autophagy and apoptosis, enhanced the anticancer effect of indole-3-carbinol in the Ehrlich ascites carcinoma (EAC) model, and induced cycle arrest, restraining cell proliferation in cholangiocarcinoma both in vitro and in vivo.[28,29] In clinical studies, HCQ demonstrated significant effectiveness, notably improving anti-cancer immunity and autophagy inhibition when used in combination with vorinostat, rapamycin, or other chemotherapeutic agents.[59–61] As an adjuvant, HCQ exhibited synergistic antitumor properties, and showed notable anticancer effects even as monotherapy. For instance, HCQ restrained lung adenocarcinoma via increasing the expression of FoxO3a and diminishing the phosphorylation of STAT3, affecting the JAK-STAT and FoxO signaling pathways in lung adenocarcinoma.[30] Despite HCQ's lower toxicity compared to CQ, its clinical application is constrained by the limitations associated with high-concentration autophagy inhibition.

Lys05

Lys05, a dimeric CQ analogue, has shown high potential for autophagy suppression at lower working concentrations compared to HCQ, and has efficient antitumor activity in diverse human tumor xenograft models including melanoma, glioblastoma and colon cancer.[62] Lys05 was recognized as a sensitizer and enhanced radiosensitivity in the treatment of cancer. In recent years, Lys05 has been reported to exert a sensitizing role in many kinds of cancers, such as glioblastoma in which Lys05 induced lysosomal membrane permeabilization (LMP), caused mitochondrial depolarization, and increased radiosensitivity in antiglioma activity,[31] clear cell ovarian carcinoma (CCOC) in which Lys05 inhibited the autophagy and potentiated the anticancer property of sunitinib.[32] Lys05 also had a great synergistic effect in myeloproliferative neoplasms, which effectively improved the efficiency of ruxolitinib via the inhibition of autophagy.[33] Consequently, as an autophagy inhibitor, Lys05 may be a promising compound in cancer therapy, and more researches need to be displayed to promote its application and development.

Mefloquine (MQ)

MQ is also a derivative of CQ and an FDA-approved agent with highly effective against malaria. A growing body of research showed that MQ significantly counteracted multiple cancers, including glioblastoma, breast cancer, hepatocarcinoma, colorectal cancer and liver cancer.[34–38] MQ exerted its antitumor effects through diverse molecular mechanisms. For example, studies have shown that MQ could inhibit cell proliferation and the PI3K/AKT/mTOR signaling pathway, induced reactive oxygen species (ROS) and trigger apoptosis in human gastric tumor cells.[39] The NF-κB signaling pathway has been reported to be activated in many cancers. Nowadays, researchers found that MQ could serve as an inhibitor of NF-κB in colorectal cancer, weaken the activity of IκBα kinase, and induced cell cycle arrest and apoptosis of tumor cells both in vitro and in vivo.[34] Additionally, MQ has been reported to arrest the growth of prostate cancer cells, caused ROS and induced non-apoptotic cell death, and these influences may involve the AKT, ERK, JNK and AMPK signaling pathways.[40,41] For the inhibition of cervical cancer, MQ destroyed the mitochondrial function through restraining mitochondrial respiration, abolishing membrane potential and reducing ATP levels, meanwhile inhibiting the mTOR signaling pathway in vitro and in vivo.[42] Furthermore, MQ has been found to trigger mitochondrial autophagy and lysosome disruption in the treatment of esophageal squamous cell carcinoma and chronic lymphocytic leukemia, respectively.[43,44] All the findings above extended the application of MQ, more clinical anti-tumor trials for MQ need to be performed and its clinical application is expected.

Quinacrine (QC)

QC is an acridine derivative and has cytotoxic potential in multiple kind of cancers but with limited toxicity to normal cells.[45–47,63–66] Studies have demonstrated that QC notably upregulates the expression and activity of cathepsin L, a lysosomal protease, inducing lysosomal membrane permeability and mitochondrial membrane permeability, resulting in the release of cytochrome c and inducing ovarian cancer cell death.[48] Nowadays, researchers also explained the possible mechanisms of QC anti-ovarian cancer from the transcriptome level, suggesting that QC could restrain the ribosomal biogenesis pathway, trigger nucleolar stress and intensify DNA damage both in vitro and in vivo, which extended the application of QC and the treatment strategy of ovarian cancer.[67] QC also induced nuclear fragmentation, caused cell cycle G1/S arrest, leaded to cell death in non-small cell lung cancer, and promoted the sensitivity of tumor cells to chemotherapy in endometrial cancer and other types of

cancers, such as colorectal cancer, ovarian cancer, upper gastrointestinal cancer and anaplastic thyroid cancer.[49–55] Recently, there was a study that explained the mechanism of QC increased the sensitivity of chemotherapy drugs. The study indicated that QC combined with curcumin could prevent the expression of ABCG2, which was an efflux pump and transported chemotherapy agents out of cells and resulted in cell resistance. The authors also pointed out that ABCG2 increased the expression of angiogenesis factor VEGFA which in turn promoted the angiogenesis, the combination of QC and curcumin suppressed the ABCG2 and inhibited the angiogenesis and migration in breast cancer.[56] These data above indicated that QC was a promising anticancer agent. Despite QC's recognized efficacy as an anticancer agent, its rapid intestinal absorption and slow excretion lead to significant cumulative effects and potential toxic reactions. Therefore, innovative studies and breakthroughs are essential before QC can be clinically applied in cancer treatment. For example, Kulkarni et al mentioned that the construction of nanoformulation, which was used in combination with QC and other drugs, could decrease the therapeutic dose and reduce the toxicity and side effects caused by QC to a certain extent.[55]

In addition, there are other CQ analogs like verteporfin and clioquinol, also exhibit potential antitumor properties,[24] meriting further exploration of their anticancer mechanisms. While CQ and its derivatives are noted for their anticancer effects, comparative studies assessing the efficacy of these analogs in cancer treatment are sparse. It is necessary to compare the measurement and mode of action of these analogs both in vitro and in vivo simultaneously in the future.

CQ Suppressed Cancer Based on Autophagy-Related Mechanisms

Autophagy is a critical cellular process involving the lysosomal degradation of cytoplasmic proteins and organelles to meet the metabolic needs of cell itself and renew some organelles. Under basic conditions, autophagy is essential for maintaining cell homeostasis and acts as a protein/organelle quality control mechanism. Under stressful conditions such as starvation, hypoxia, and chemotherapy/radiotherapy, it is the basis for cancer cell survival and adaptation to changes in the tumor microenvironment.

There are three main types of autophagy: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy that depending on the ways of intracellular substrates entering the lysosomal cavity (Figure 2).[68] Microautophagy is a process in which lysosome membrane directly invaginates to encase and degrade cytoplasmic substances.[69] Different from micro-autophagy, CMA does not require the involvement of membrane structures. Cytoplasmic proteins with special motifs (KFERQ) are recognized by chaperones like heat shock 70 kDa (HSC70) and are unfolded by chaperones. After that substrates are delivered to the lysosomal membrane and bind to the lysosome-related membrane protein Lamp2A which is a special receptor on the lysosome membrane, then go into lysosome and enter the degradation process.[70] Different from the previous two, macroautophagy refers to the formation of autophagosome by the endoplasmic reticulum, Golgi apparatus or plasma membrane which enclose the cytoplasmic substances and then fuse with lysosome and degrade its contents.[71] Macroautophagy is commonly known as we call autophagy which occurs rarely under normal conditions, helps cells maintain normal physiological functions by specifically degrading damaged or redundant organelles. When under stressful conditions, such as nutritional or energy starvation, macroautophagy can be further induced to degrade cytoplasmic material into metabolites that can be used in biosynthetic processes or energy production, allowing cells to survive. In this sense, macroautophagy is mainly a cell protective mechanism. However, excessive self-degradation is harmful and autophagy dysfunction has been linked to a variety of human diseases, such as neurodegeneration, diabetes and cancer.[72]

CQ is probably the most widely used autophagy inhibiting agent in vitro, and its effect on cancer cell death has been attributed to its inhibition of autophagy when used together with other chemotherapy agents.[73,74] It was indicated that CQ could prevent the fusion between autophagosomes and lysosomes through interfering with the recruitment of autophagosomal SNARE protein SNAP29, and further inhibited autophagy.[14] CQ has been reported to play an antitumor role by suppressing autophagy in a variety of tumors occurring in different parts of the body, such as breast, lung, colorectal, ovary and bladder (Figure 3). In this review, we summarized the tumor types, concentrations and specific mechanisms of CQ based on autophagy inhibition published in the past ten years (Table 2), aiming to help researchers better engage in the anti-tumor research of CQ.

Breast Cancer

Breast cancer ranks as one of the most prevalent malignancies and the leading cause of cancer-related deaths among women worldwide. Recently, CQ has been reported to have a significant protective effect against breast cancer, including triple-negative breast cancer, primarily through its inhibition of autophagy.[115] According to Hu et al, a combination of 20 μM CQ

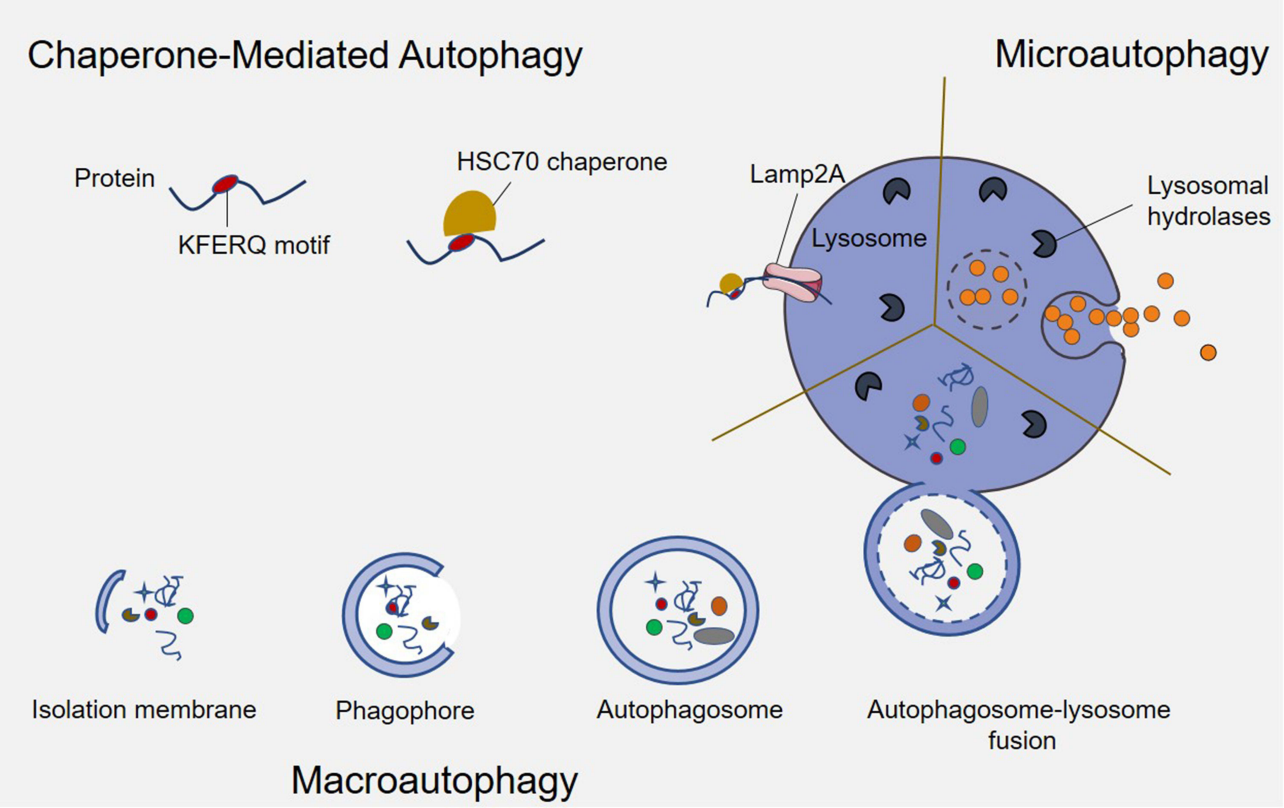

Figure 2 Classifications and mechanisms of autophagy.

and isorhamnetin (IH) decreased the MDA-MB-231 and BT549 cell viability but not MCF-7 in vitro, and 40 mg/kg CQ together with 20 mg/kg IH dramatically reduced tumor weight in vivo via inhibition of autophagy.[75] CQ also played synergistic effects on the PI3K/AKT inhibitors taselisib and ipatasertib across various breast cancer cells, including MDA-MB-231, MDA-MB-468, MCF-7 and SKBR3.[76] In addition, CQ played anti-breast cancer effect via modulating the tumor microenvironment. The research of Zhang et al revealed that only low dose of CQ (IC_{50} < 1 μM for 48 h treatment) dramatically restrained the growth and induced the apoptosis of 4T1 in vitro, and suppressed the expression of TGF-β, increased $CD8^+$ T cells and decreased macrophages in tumor microenvironment in vivo.[77] Besides, the modified CQ also displayed inhibition of breast cancer. Joshi et al used gold nanoparticles with a diameter of 7 nm as the delivery system for CQ, the pharmaceutical effect was detected in MCF-7, and the IC_{50} for 24 h treatment was 30 ± 5 μg/mL. The nanoparticle packaged CQ displayed an obvious necrosis in MCF-7 which was mediated by autophagy. This research provided new ideas for anti-tumor combination therapy, but more data such as comparing the efficacy with CQ alone and other anti-tumor drugs need to be further explored.[116] Moreover, there was data indicated that CQ blocked the potassium channels, such as Kv10.1, which abundantly expressed in MDA-MB-231 cells and controlled the migration of MDA-MB-231. The inhibition of CQ on Kv10.1 significantly reduced the migration of breast cancer cells.[78] However, some evidence suggested that CQ inhibition on autophagy in breast cancer cells related to the epithelial–mesenchymal transition (EMT) which is important for tumor cells invasion and metastasis. The treatment of CQ on MCF7 and T47D cells reduced the expression of E-cadherin, triggered the EMT-related transcription factor and caused ROS, leading to cell migration.[117] This study reminds researchers and healthcare professionals to be cautious when conducting anti-autophagy studies of CQ or targeting autophagy inhibition in breast cancer treatment and to consider the double-sided nature of CQ.

Lung Cancer

CQ inhibited the lung cancer through a variety of mechanisms as mono- or combination therapy. There was proof that when used as monotherapy CQ could control A549 cells proliferation via blocking the PI3K/AKT signaling pathway,

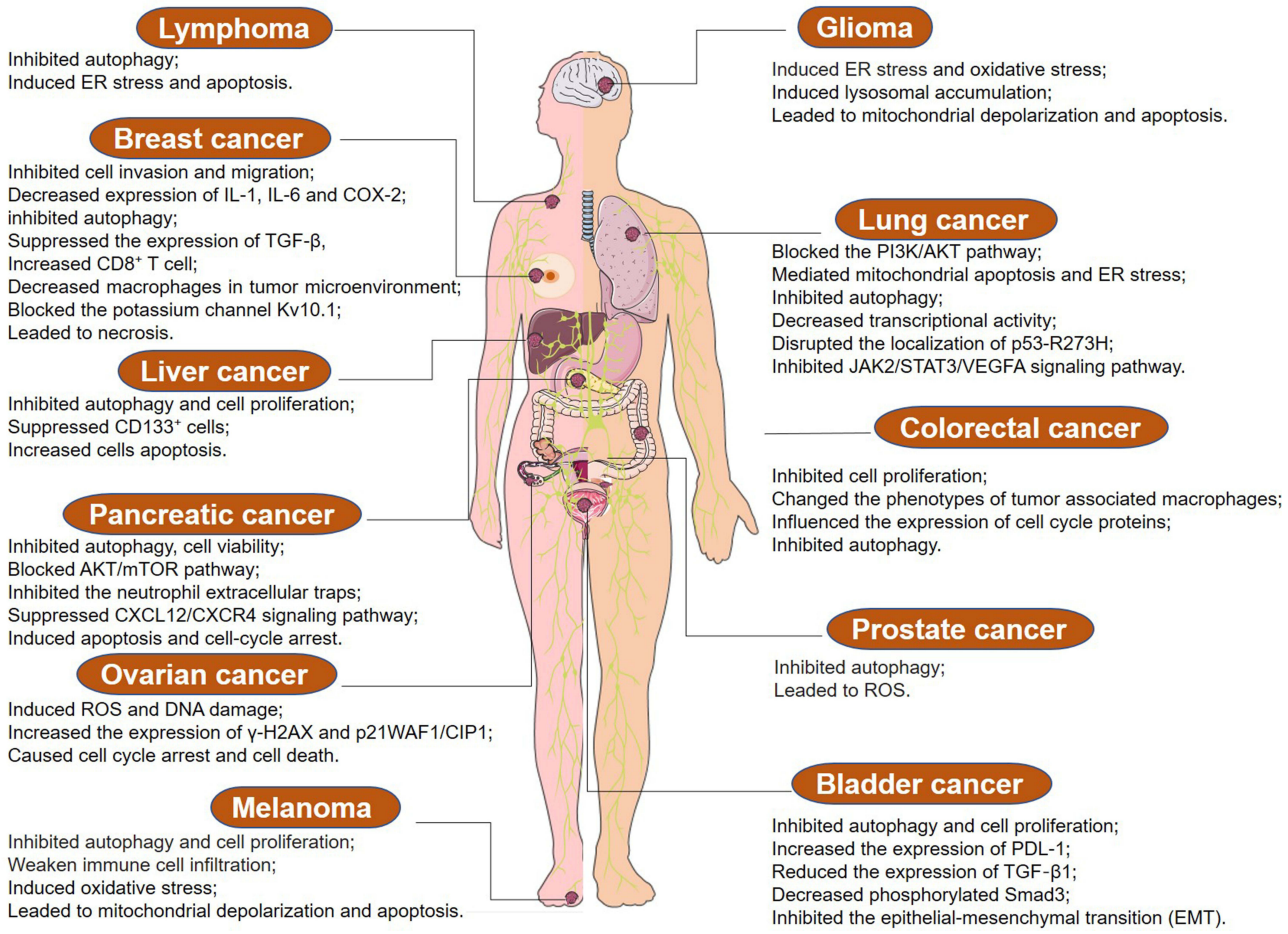

Figure 3 The distribution of CQ antitumor types in humans and its related mechanisms.

mediated mitochondrial apoptosis and inhibited autophagy.[83] For non-small cell lung carcinoma (NSCLC), CQ also did effective inhibition in H1299 cells. It was reported that most NSCLC patients harbored p53-R273H mutation which could accelerate tumorigenesis, and further analysis revealed a tight relationship exist between p53-R273H and autophagy regulation. So the authors restrained NSCLC cells with autophagy inhibitor CQ, a high dose of CQ (50 μM) decreased transcriptional activity and disrupted the localization of p53-R273H.[79] The combination of CQ and lidamycin (LDM) also had a significant effect on NSCLC. CQ enhanced the sensitivity of LDM to both H460 and A549 cells in which the IC_{50} values of CQ were 55.6 ± 12.5 and 71.3 ± 6.1 μM, respectively. The cell apoptosis was significantly increased after combined CQ with LDM and the content of key proteins such as PARP and caspase changed obviously. At the same time, cytoprotective autophagy was significantly inhibited.[84] Moreover, CQ enhanced the inhibition of anlotinib against NSCLC. Anlotinib induced Calu-1 and A549 cells apoptosis and autophagy, blocking the JAK2/STAT3/VEGFA signaling pathway, the addition of CQ suppressed autophagy and amplified these effects.[85] These findings demonstrate that CQ, whether utilized independently or in combination with other agents, exhibits a significant inhibitory impact on lung cancer, particularly NSCLC, suggesting its potential selective use in lung cancer treatment.

Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer in the world following breast cancer and lung cancer, with its incidence and mortality rates rising in recent years. CQ has emerged as a significant player in CRC treatment, attributed to its autophagy inhibition properties. There were few studies of CQ on the inhibition of CRC as monotherapy, and most studies focused on the combination with other drugs for CRC treatment. 5-Fluorouracil (5-FU) is the priority drug for

Table 2 The Classification is Summarized According to the CQ Different Antitumor Types

Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Autophagy Dependent or Not (Y/N)	Mechanism of Action	Ref.
Breast cancer	MDA-MB-231 MCF-7, BT549	In vitro	20 μM	Y	Inhibition of autophagy/mitophagy, induced apoptosis	[75]
	MDA-MB-231	In vivo	I.p. 40 mg/kg once every two days			
Breast cancer	MDAMB231, MDAMB468,MCF-7, SKBR3, MDAMB361	In vitro	10 μM	Y	Inhibited autophagy and enhanced the function of PI3K/AKT inhibitors.	[76]
	MDAMB231	In vivo	30 mg/kg oral gavage daily			
Breast cancer	4T1	In vitro	0, 0.1, 1, 10 μM	Not clear	Restrained the growth and induced the apoptosis of 4T1 in vitro, suppressed the expression of TGF-β, increased $CD8^+$ T cell and decreased macrophages in tumor microenvironment in vivo.	[77]
	4T1	In vivo	I.p. 50 mg/kg daily			
Breast cancer	MDA-MB-231	In vitro	10, 30 and 100 μM	N	Blocked the potassium channel Kv10.1.	[78]
Breast cancer	MCF10A, MCF7, T47D, MDAMB231	In vitro	10, 20, 40 μM	Y	Reduced the expression of E-cadherin, triggered the epithelial-mesenchymal transition (EMT) related transcription factor and caused ROS, leaded to tumor cell migration.	[79]
Mouse breast cancer	67NR, 4T1	In vitro	10 μM	N	Enhanced the effect of PI3K/AKT/mTOR inhibitors	[80]
Breast cancer	MDA-MB231	In vitro	10 μM	N	Interfered with the stability of lysosomes and mitochondrial membranes, lead to necrosis.	[81]
Breast cancer	D2A1, 4T1, MDA-MB-231, MC7-L1, MCF-7.	In vivo	I.p. 40 or 60 mg/kg Once every three days	Y and N	Inhibited cell invasion and migration, decreased expression of IL-1, IL-6 and COX-2. Inhibited autophagy.	[82]
Lung cancer	A549	In vitro	2.5 ~ 60 μM	Y and N	Blocked the PI3K/AKT signaling pathway, mediated mitochondrial apoptosis and inhibited autophagy.	[83]
Lung cancer	H1299	In vitro	50 μM	Y	Inhibited autophagy, decreased transcriptional activity and disrupted the localization of p53-R273H.	[79]
Lung cancer	H460, A549	In vitro	IC_{50}: H460: 55.6 ± 12.5 μM, A549: 71.3 ± 6.1 μM	Y and N	Induced apoptosis, suppressed autophagy.	[84]
	H460	In vivo	I.p. 60 mg/kg daily			

Lung cancer	A549, Calu-1	In vitro	25 μM	Y	Inhibited autophagy and JAK2/STAT3/VEGFA signaling pathway.	[85]
	Calu-1	In vivo	I.p. 60 mg/kg daily			
Lung cancer	H157 and A549	In vitro	5 ~ 80 μM	N	Induced apoptosis and ER stress.	[86]
	H157 or H549	In vivo	I.p. 50 mg/kg daily			
Lung cancer	A549, H460	In vitro	50 μM	Y and N	Inhibited autophagy and ER stress, induced apoptosis.	[16]
Colorectal cancer	HT-29	In vitro	80 μM	Y	Inhibited autophagy and influenced the expression of cell cycle proteins, such as p21Cip1, p27Kip1 and CDK2.	[87]
Colorectal cancer	SW480, HT29	In vitro	20 μM	Y	Inhibited autophagy and reinforced the apoptosis selumetinib induced.	[88]
Colorectal cancer	RKO, HCT-116, CT26	In vitro	0 ~ 480 μM	Y and N	Induced apoptosis and inhibited autophagy.	[89]
	CT26	In vivo	I.p. 50 mg/kg daily			
Colorectal cancer	HCT116, CT26	In vitro	NA	Y and N	Inhibited cell proliferation, changed the phenotypes of tumor associated macrophages.	[90]
	CT26	In vivo	NA			
Colorectal cancer	SW1116, HCT116, LOVO, HT-29, SW480	In vitro	80 μM	N	Mediated the loss of LMP and MMP.	[91]
	SW1116 and LOVO	In vivo	I.p. 60 mg/kg once every two days			
Colorectal cancer	HT-29, SW480	In vitro	20 μM	Y and N	Induced apoptosis and increased the efficiency of ionizing radiation.	[92]
Colorectal cancer	HCT-116, HT-29	In vitro	10 μM	Y	Restrained autophagy and enhanced the sensitivity radiotherapy.	[93]
Ovarian cancer	IGROV-1, OVCAR-8, SKOV-3, A2780	In vitro	IC_{50} (72 h): IGROV-1: 29.05 μM; OVCAR-8: 28.25 μM; SKOV-3: 22.28 μM; A2780: 12.31 μM.	Y and N	Restrained autophagy, induced ROS and DNA damage.	[94]
Ovarian cancer	UWB1.289, HEY, A2780, OVCAR3, OVCAR5, OVCAR8, ES-2, OC316, SKOV3, IGROV1	In vitro	5, 10 and 12 μM	Y	Inhibited autophagy, caused ROS and increased the expression of γ-H2AX.	[95]
	OVCAR8	In vivo	50 mg/kg, daily gavage			

(*Continued*)

https://doi.org/10.2147/IJN.S458910 DovePress

https://doi.org/10.2147/IJN.S458910 DovePress

Table 2 (Continued).

Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Autophagy Dependent or Not (Y/N)	Mechanism of Action	Ref.
Ovarian cancer	A2780, A2780-CP20	In vitro	10 ~ 30 μM	Y	Induction of γ-H2AX, and increased the expression of $p21^{WAF1/CIP1}$, causing cell cycle arrest and cell death.	[96]
	A2780-CP20	In vivo	I.p. 50 mg/kg, three times a week			
Ovarian cancer	A2780, IGROV-1, OVCAR-8, and SK-OV-3	In vitro	10 ~ 40 μM	Y	Induced DNA damage and apoptosis.	[97]
Bladder cancer	RT4, 5637, T24	In vitro	NA	Y	Inhibited autophagy, restrained cell proliferation and induced apoptosis.	[25]
Bladder cancer	RT4, 5637, T24	In vitro	25 μM	Y	Inhibited the cell proliferation through autophagy inhibition and apoptosis induction.	[25]
Bladder cancer	5637, T24	In vitro	50 μM	Y	Inhibited autophagy, increased the expression of PDL-1.	[98]
Bladder cancer	T24, HT1376, RT4	In vitro	5 μM	Y	Inhibited autophagy.	[99]
Bladder cancer	T24, 5637	In vitro	20 μM	Y	Inhibited the epithelial-mesenchymal transition (EMT), inhibited autophagy, reduced the expression of TGF-β1 and phosphorylated Smad3.	[100]
Bladder cancer	RT4, 5637, HT1376, T24	In vitro	25 or 50 μM	Y	Inhibited autophagy, reduced cell viability and induced apoptosis.	[101]
Bladder cancer	T24, J82	In vitro	10 μM	Y	Inhibited autophagy, reduced cell viability and induced apoptosis.	[102]
Bladder cancer	J82, T24, UMUC3	In vitro	10 μM	Y	Induced apoptosis, inhibit cell proliferation.	[103]
	J82	In vivo	I.p. 10 mg/kg daily			
Bladder cancer	EJ, T24	In vitro	10 μM	Y	Inhibited autophagy and activated apoptosis.	[104]
	T24	In vivo	I.p. 50 mg/kg daily			
Liver cancer	Huh7	In vitro	10 μM	Y	Inhibited autophagy, suppressed the colony-forming capacity of $CD133^+$ cells and increased cells apoptosis.	[105]
	Huh7	In vivo	I.p. 60 mg/kg twice a week			
Liver cancer	HepG2	In vitro	10 μM	Y	Inhibited autophagy and cell proliferation.	[106]

Pancreatic cancer	BxPC-3, PANC-1, MIA PaCa-2, AsPC-1	In vitro	5 or 10 μM	Y	Inhibited autophagy, cell viability and blocked AKT/mTOR pathway.	[17]
	MIA PaCa-2	In vivo	10 mg/kg oral gavage			
Pancreatic cancer	Panc02	In vivo	0.5 mg/mL Oral in the drinking water	Y	Inhibited the neutrophil extracellular traps.	[107]
Pancreatic cancer	PDAC tissues; Panc1, BxPC3, 8988 T	In vitro	10 μM	N	Suppressed CXCL12/CXCR4 signaling pathway.	[108]
	PDAC-354	In vivo	I.p. 50 mg/kg twice a week			
Pancreatic cancer	MiaPaCa-2, Panc-1	In vitro	0 ~ 700 nM	Y	Inhibited autophagy, induced apoptosis and cell-cycle arrest.	[109]
	MiaPaCa-2, Panc-1	In vivo	I.p. MiaPaCa-2: 50 mg/kg Panc-1:100 mg/kg daily			
Melanoma	A375, A2058, B16-F25	In vitro	10 μM	Y	Inhibited autophagy, cell proliferation, and weaken immune cell infiltration.	[110]
	A375	In vivo	I.p 40 mg/kg every other day			
Melanoma	WM793, 1205Lu	In vitro	50 μM	Y	Inhibited autophagy, decreased the translation of proteins.	[111]
Melanoma	B16	In vitro	20 μM	N	Induced lysosomal accumulation and oxidative stress, leading to mitochondrial depolarization and apoptosis.	[20]
	B16	In vivo	I.p. 20 mg/kg daily			
Melanoma	B16-F10 (murine), A375m (human)	In vitro	0, 5, 10, 25μM	N	Vessel normalization, inhibited tumor invasion and metastasis.	[112]
	B16-F10 (murine), A375m (human)	In vivo	50 or 100 mg/kg, daily injected subcutaneously			

(*Continued*)

https://doi.org/10.2147/IJN.S458910 DovePress

Table 2 (Continued).

Cancer Types	Cell Lines Used	Models	Concentration/IC_{50} In Vitro/Drug Dose in Vivo	Autophagy Dependent or Not (Y/N)	Mechanism of Action	Ref.
Glioma	LN229, U251, U87MG	In vitro	10 ~ 100 μM	Y	Inhibited autophagy and induced ER stress.	[113]
	U87MG	In vivo	10 mg/kg oral gavage			
Glioma	U251	In vitro	20 μM	N	Induced lysosomal accumulation and oxidative stress, leading to mitochondrial depolarization, apoptosis.	[20]
Glioma	U87MG	In vivo	I.p. 45 mg/kg three times a week	Y	Inhibited autophagy, leading to the accumulation of abnormal autophagolysosomes and ROS.	[73]
Prostate cancer	PC3	In vivo	I.p. 45 mg/kg three times a week	Y	Inhibited autophagy, leading to the accumulation of abnormal autophagolysosomes and ROS.	[73]
Gallbladder cancer	GBC-SD, NOZ, SGC-996	In vitro	10 μM	Y	Inhibited autophagy and induced apoptosis.	[114]
	SGC-996	In vivo	I.p. 60 mg/kg twice a week			
Primary effusion lymphoma	BCBL-1, BC-1, BC-3, TY-1, GTO	In vitro	0 ~ 30μM	Y	Inhibited autophagy, induced ER stress and apoptosis.	[18]
	GTO	In vivo	I.p 50 mg/kg daily			
Neuroblastoma	SH-EP, Kelly, SK-N-AS	In vitro	0 ~ 120μM	N	Induce LMP and loss of MMP, induce apoptosis	[19]
Fibrosarcoma	L929	In vitro	20 μM	N	Induced lysosomal accumulation and oxidative stress, leading to mitochondrial depolarization, apoptosis.	[20]

CRC, but resistance has emerged in recent years, researchers have found that combined with a high dose of CQ (80 μM) effectively enhanced the anti-tumor effect of 5-FU.[87] CQ combined with selumetinib which was a MEK inhibitor also exhibited significant enhancement effect in CRC and reinforced its efficiency on apoptosis induction.[88] The results of the study by Lu et al revealed that CQ treatment in CRC increased the expression of PD-1, which was a key immune checkpoint and inhibitors and siRNA based on PD-1 have played a vital role in tumor therapy. Therefore, the combination of CQ and therapies targeting PD-1 such as PD-1 siRNA-related agents is a promising option for CRC treatment.[89] Another innovative approach to enhance the effectiveness of chemotherapy involves drug-loaded nano-systems. Researchers found that combination of CQ and artesunate, a renowned antimalarial agent, exhibited significant inhibition on cancer cells proliferation, and altered the phenotype of tumor-associated macrophages. These drugs were accurately targeted to the tumor tissue by using a PLGA-based biomimetic nanoparticle drug-delivery system.[90] This method significantly improved the efficacy of drugs, marking it as a promising avenue for future tumor therapy research.

Ovarian Cancer

CQ also has been studied as a potential treatment for ovarian cancers. Low IC_{50} of CQ were obtained in four ovarian cancer cells (IGROV-1, OVCAR-8, SKOV-3 and A2780), and with combination CQ could effectively promote the anti-tumor ability of panobinostat, causing ROS and DNA damage and inducing apoptosis in vitro dependent or independent of its autophagy inhibition.[94] CQ also facilitated the inhibitors of poly(adenosine diphosphate ribose) polymerase (PARP), a key enzyme that recognizes DNA single-strand breaks, to suppress ovarian cancers. Recently, the application of PARP inhibitors against ovarian cancer has brought new benefits to patients. PARP inhibitors such as olaparib and niraparib triggered autophagy during ovarian cancer therapy, and their combination with CQ specifically inhibited autophagy and also caused ROS and increased the expression of γ-H2AX which was a biomarker of DNA strand breaks both in vitro and in vivo.[95] And because of the induction of γ-H2AX, CQ also displayed synergistic effect on the chemotherapy agent cisplatin in epithelial ovarian cancer, and increased the expression of $p21^{WAF1/CIP1}$, causing cell cycle arrest and cell death.[96] Given CQ's capability to induce DNA damage in ovarian cancer, its combination with DNA damage repair inhibitors presented a compelling treatment strategy. Ovejero-Sánchez et al revealed that CQ combined with the DNA repair inhibitor nonhomologous end joining (NHEJ) had efficiently induced DNA damage and apoptosis in multiple ovarian cancer cell lines, such as A2780, OVCAR-8, and SK-OV-3, and which provided new therapeutic regimen for ovarian cancer.[97]

Bladder Cancer

Bladder cancer represents a significant challenge in the field of oncology, with high recurrence rates and the necessity for innovative treatment strategies. Recent years have witnessed substantial researches into the use of CQ as a potential therapeutic option in bladder cancer because of its autophagy inhibition properties. There were data verified that CQ and its derivative HCQ produced alternation in LC3 flux and inhibited autophagy obviously in multiple human bladder cell lines and induced apoptosis.[25] CQ was detected to accelerate the expression of PDL-1 which is the ligand of PD-1 in bladder cancer, the underlying mechanism was realized on the ERK-JNK-c-Jun pathway, indicating that the combination of CQ and PD-1/PDL-1 inhibitors is also effectively applicable to the treatment of bladder cancer.[98] GSK-3β is an important therapeutic target in cancers but its inhibitors can cause obvious autophagy in tumor cells when used alone. By combined with CQ, GSK-3β inhibitors substantially inhibited the proliferation of bladder cancer cells, such as T24, HT1376 and RT4.[99] The results of Tong et al indicated that CQ inhibited the epithelial–mesenchymal transition (EMT) which was triggered by starvation and could promote migration and invasion of bladder cancer cells T24 and 5637. The use of CQ in this study not only inhibited autophagy but also reduced the expression of TGF-β1 and phosphorylated Smad3, both of which were induced in starvation.[100] Moreover, CQ was used in combination with other drugs, such as RAD001 (an inhibitor of mTOR signaling pathway), Lapatinib and Gefitinib (inhibitors of epithelial growth factor receptor (EGFR)), and Enzalutamide (an androgen receptor inhibitor) in treatment of advanced bladder cancer because of its autophagy inhibition properties.[25,101–103] In conclusion, recent advancements in understanding the molecular mechanisms of CQ's anti-bladder cancer properties have illuminated novel approaches in the treatment of this challenging malignancy, offering exciting prospects for combination therapies in the fight against bladder cancer. While the prospects are promising, further preclinical and clinical studies are warranted to optimize the use of CQ in bladder cancer treatment.

Other Types of Cancers

In addition to the aforementioned cancer types, CQ also had inhibitory effect on other cancers, such as hepatocellular carcinoma (HCC), pancreatic cancer, melanoma, glioma, among others. Song et al investigated that autophagy could improve adaptability of liver cancer stem cells (LCSCs) which can drive and sustain the growth, metastasis, and recurrence of HCC under adverse conditions like hypoxia and nutrient deficiency. Their findings revealed that $CD133^+$ cells which hold LCSCs properties were significantly enriched after hypoxia and starvation, higher autophagy level, higher survival and less apoptosis were detected in $CD133^+$ cells. However, treatment with autophagy inhibitor CQ had significantly reversed these effects and dramatically impaired the colony-forming capacity of $CD133^+$ cells and increased cells apoptosis.[105] In addition, the results of Xu et al demonstrated that in HepG2 cells, inhibition of autophagy with CQ decreased the degradation of lipid droplets by inhibiting autophagic flux and reducing ATP production, thereby hindering cell proliferation.[106] For pancreatic cancer, CQ dramatically facilitated the efficacy of pterostilbene, suppressing cell viability and downregulating the AKT/mTOR signaling pathway both in vitro and in vivo.[17] Recently, CQ has been found to inhibit the neutrophil extracellular traps which was related to the hypercoagulability occurance in murine pancreatic cancer, the finding provided fresh insights into the anti-pancreatic cancer potential of CQ.[107] Autophagy also was induced in chemotherapeutics in melanoma and glioma, there was no doubt that CQ played important synergistic role in the chemotherapy of these tumors.[110,111,113] All these data affirmed the importance of autophagy for tumor cells, and autophagy inhibitor CQ was effective therapeutic strategy of human cancers.

Although numerous studies have documented that CQ induces cancer cell death via the inhibition of autophagy, the underlying mechanisms remain to be fully elucidated. Masud et al, using Primary Effusion Lymphoma (PEL) cells to identify the mechanisms of CQ-induced cancer cell death. They found that CQ could induce endoplasmic reticulum (ER) stress obviously and cause caspase-dependent apoptosis in vitro and in vivo through autophagy inhibition. The treatment of CQ did not affect the protein markers expression of NF-κB, JAK/STAT, and PI3K/AKT signaling pathways, indicating that CQ-induced cell death is independent of these pathways in PEL cells.[18] More elaborate mechanisms were discovered, for example, the discovery of palmitoyl-protein thioesterase 1 (PPT1) which was the target of CQ and its derivatives in lysosomes and was highly expressed in most cancer cell lines. CQ derivatives have been used as therapeutic agents for decades, but no protein targets have been found, so the appearance of PPT1 was a major breakthrough. Knockout of PPT1 in cancer cells abrogated the enhancement of lysosomal deacidification and autophagy regulation of CQ derivatives.[118] Recently, many new inhibitors for PPT1 have been designed, which greatly promoted the development of cancer treatment strategies.

CQ Suppressed Cancer with Autophagy-Independent Mechanisms

In tumor suppression, CQ also has activation of apoptosis and necroptosis of cancer cells that independent of autophagy inhibition. Apoptosis refers to the autonomic and orderly death of cells controlled by genes in order to maintain the stability of internal environment. Different from cell necrosis which is a passive process, apoptosis is an active process that adapts to the living environment.[119,120] Abnormal apoptosis may lead to cancer. There are two main apoptosis signaling pathways, one is the death receptor pathway, which activates the intracellular caspases through extracellular signals, and the other is the mitochondrial-mediated apoptosis pathway that activates caspases through the release of cytochrome c into cytosol.[121–123] In addition, lysosomal mediated apoptosis also plays an important role in cell death. Nowadays, more and more attentions have been paid to the role of lysosomes in regulating apoptosis. Lysosomes contain a variety of proteolytic enzymes, such as cathepsins and other hydrolases, and the release of these enzymes from the lysosomal lumen to the cytosol can promote the release of mitochondrial cytochrome c, activating caspases and eventually leading to apoptosis.[124–128]

PI3K/AKT/mTOR signaling pathway is crucial for cell survival, proliferation and differentiation, especially in tumor cells. Studies have indicated that inhibitors against this pathway have become a focus in cancer treatment research. It was reported that CQ could enhance the effects of PI3K/AKT/mTOR inhibitors in mouse breast cancer cells 67NR and 4T1 in vitro, and which could not be imitated through knockdown or deletion of key autophagy-related genes.[80] Seitz et al revealed an underlying mechanism in which CQ and the PI3K/mTOR inhibitor BEZ235 cooperated to induce apoptosis via initiating LMP and lysosome-mediated apoptosis in neuroblastoma cells. The authors investigated that treatment with CQ alone led to

the accumulation of CQ in lysosomes which initiated LMP, and inhibition of CQ in lysozymes markedly reduced LMP and apoptosis, which was independent of autophagy inhibition. The authors also found that CQ and BEZ235 cooperated to trigger Bax activation and loss of mitochondrial membrane potential (MMP), indicating that the combination treatment with CQ and BEZ235 could mediate mitochondrial outer membrane permeabilization (MOMP) and induce apoptosis. Otherwise, they further explored the correlation between LMP and MOMP through adding CA-074-Me, an inhibitor of lysosomal enzyme cathepsin B, which significantly reduced the loss of MMP and ultimately pointed that CQ and BEZ235 could synergize to trigger apoptosis via a lysosomal-mitochondrial cross-talk.[19] Moreover, Harhaji-Trajkovic et al investigated that CQ induced apoptosis in serum-starved cancer cells in vitro and in vivo, and this effect could not be mimicked by autophagy inhibitors or LC3II shRNA, indicating a mechanism independent of autophagy inhibition. The authors found that CQ mediated lysosomal dysfunction in nutrient-deprived cancer cells which brought about oxidative stress, accompanied by loss of MMP and finally led to the activation of caspase cascades and apoptosis of cancer cells.[20] In CRC treatment, CQ induced ROS and also mediated the loss of LMP and MMP, facilitating the effect of SN-38 which is a chemotherapy agent in vitro and in vivo.[91]

The property of CQ that induces LMP categorizes it as an enhancer in drugs, genes or siRNA delivery. Studies have shown that CQ can markedly enhance the transfection activity and promote the expression of exogenous genes.[86,112] Furthermore, it plays a pivotal role in the field of nanotechnology for the treatment of cancer. CQ can promote the release of drugs, genes or siRNA from lysosomes by inducing LMP, improving the efficiency of drug delivery system, which strengthens the anti-tumor effect of agents. Bhattarai et al co-loaded CQ and plasmid DNA/siRNA into polycation- and PEG-coated mesoporous silica nanoparticles and significantly improved transfection efficiency, providing a powerful tool for tumor targeted therapy.[108] CQ co-packaged with cisplatin into hyaluronan (HA) nanogel facilitated the escape of cisplatin from lysosomal and enhanced its anti-tumor efficacy in breast cancer cells. The CQ/cisplatin HA nanogel also decreased toxicity compared with the combination of free CQ and cisplatin. In conclusion, CQ can not only play an active anti-tumor role by inducing LMP itself but also exert a synergistic role via assisting other drugs delivery.

ER stress can lead to proteotoxicity and induce apoptosis, becoming another breakthrough point in cancer therapy. Lopiccolo et al revealed that the combination of CQ and nelfinavir dramatically induced ER stress and increased the cell apoptosis of NSCLC in vitro and in vivo, in an autophagy-independent pattern.[129] Furthermore, research indicated that CQ mediated tumor vessel normalization.[109] In this study, the authors investigated that CQ could improve tumor perfusion and oxygenation, reduce invasion and intravasation of cancer cells, and promote tumor vessel normalization in metastatic melanoma models. However, these events were not simulated with ATG5 (an essential autophagy factor) knockdown, even though ATG5 silence decreased autophagy in cancer cells and increased tumor cell death. Therefore, the effect of CQ on normalizing tumor vessels was not due to its inhibition of tumor autophagy but an autophagy-independent mechanism.[109] CQ was also reported to reduce cancer stem cells (CSC) in pancreatic cancer and decrease tumorigenicity in vivo, this was contributed to its suppression on the CXCL12/CXCR4 signaling pathway.[114]

These researches strongly suggested that CQ could induce cancer cell apoptosis through a pathway independent of autophagy inhibition (Figure 4). Many other researches also amply demonstrated this opinion.

CQ Combined with Chemoradiotherapy for Tumor Treatment

CQ Combined with Chemotherapy Drugs in Cancer Therapy

Deregulation of autophagy is believed to play a key pathogenic role in cancer cells. Therefore, CQ, as an autophagy inhibitor combined with other chemotherapy drugs, is widely used in the treatment of various cancers, making cancer cells more sensitive to chemotherapy. In recent years, many studies have reported on the combination with CQ. Cisplatin is widely used as one of the most effective chemotherapeutic agents for the treatment of cancers[130] and has been shown to increase apoptosis in human lung cancer cells (A549 and H460) and epithelial ovarian cancer cells (SKOV3 and hey) when combined with CQ, via autophagy inhibition.[16,131] Chen et al revealed that pterostilbene combined with CQ significantly improved autophagy inhibition, decreased cell viability and increased apoptosis in pancreatic ductal adenocarcinoma cells via the downregulation of the RAGE/STAT3 and AKT/mTOR pathways.[17] Moreover, Monma et al found CQ could enhance TRAIL-induced apoptosis in two human pancreatic cancer cell lines: MiaPaCa-2 and Panc-1. The tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which can deliver death signals via the

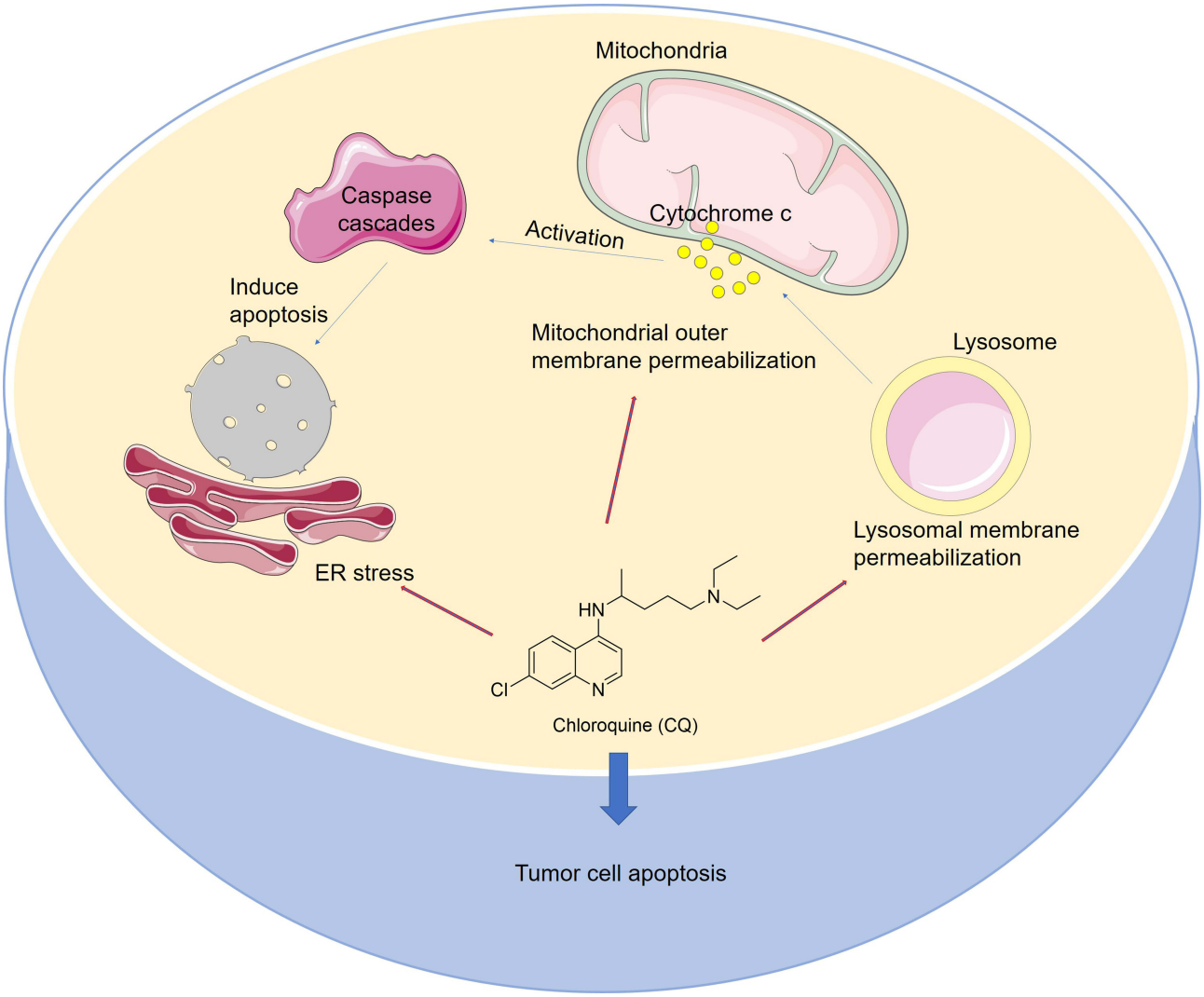

Figure 4 Mechanisms of CQ induced apoptosis in tumor cells independent of autophagy inhibition.

extrinsic apoptosis pathway and induce cancer cell death, was reported to encounter resistance in tumor cells. The authors investigated that CQ could improve the sensitivity of cancer cells to TRAIL, contributing to apoptosis via partially downregulating the anti-apoptotic proteins and inducing cell cycle arrest at the G2/M phase.[81] Therefore, the research further proved that combination with CQ in the treatment of pancreatic cancer was assuredly promising. Wang et al combined CQ with gemcitabine which is an anti-metabolic nucleoside analog, aiming at gallbladder cancer (GBC) and found that CQ significantly enhanced the inhibition of proliferation and colony formation, facilitated the induction of apoptosis and cell cycle arrest.[82] Nelfinavir is a human immunodeficiency virus protease inhibitor, currently being repositioned as an anticancer drug for its inhibition of cancer cell proliferation and induction of apoptosis.[92,129] Johnson et al revealed that CQ could promote nelfinavir-induced ER stress and cell death due to its cytotoxic action in an autophagy-independent manner.[93]

CQ Used as Sensitizer in Radiotherapy Against Cancer

In addition to chemotherapy, CQ has been shown to enhance the sensitivity of radiation therapy for cancer. Findings showed that a low dose of CQ (10 μM) was sufficient to enhance radiosensitization and induce cell death without producing significant cytotoxicity in breast cancer cells. The authors also revealed that CQ-mediated radiosensitization attributed to the destabilization of lysosomal membrane and increased cell necrosis.[104] In mouse model, a low dose of

CQ (40 mg/kg) significantly restrained the invasion and migration of triple negative breast tumor cells, which were enhanced by radiotherapy. The underlying mechanism was that CQ decreased the levels of interleukin-1β, interleukin-6, and cyclooxygenase-2, which are inflammatory factors.[132] In colorectal cancer, CQ compared with temsirolimus (a mTOR inhibitor) dramatically induced apoptosis and increased the efficiency of ionizing radiation in vitro and in vivo. CQ alone or combined with 5-FU enhanced the sensitivity of HCT-116 and HT-29 cells to radiotherapy in vitro.[133,134] CQ was also detected to enhance the sensitivity of radiation in bladder cancer cells.[135]

Clinically, CQ has shown significant sensitization to radiotherapy or chemotherapy, as verified by Briceno et al. In their research, CQ was used as an adjunct therapeutic agent to treat glioblastoma multiforme. The average survival time of patients treated with CQ was significantly longer than those treated with conventional therapy.[136] The analysis of clinical data also confirmed this view, indicating that the effect of the combined autophagy inhibitor CQ was significantly better than that of traditional therapies.[137,138] The sensitization of CQ to conventional cancer therapies, such as chemotherapy and radiotherapy, makes CQ a potential new adjuvant drug for cancer treatment. Further exploration of new properties of CQ is anticipated.

Clinical Trials and Adverse Reactions of CQ and Its Analogues

Autophagy is activated in tumor patients during chemoradiotherapy, serving as a survival mechanism for tumor cells. Therefore, as autophagy inhibitors, CQ and its analogues are widely used as adjuvant agents in chemoradiotherapy. Among the analogues, mainly CQ and HCQ are used in clinical trials (Table 3). CQ has been used in glioblastoma multiforme treatment accompanied with chemotherapy or radiotherapy of all patients. The results showed that patients receiving CQ displayed lower death rate compared to those in the control group, which may be due to the ability of CQ to enhance the cytotoxicity caused by conventional therapy or prevent the mutagenicity of tumor cells.[136,139] A Phase II trial was carried out to assess the efficacy of CQ combined with taxane or taxane-like drugs in treatment of patients with advanced or metastatic breast cancer who are resistant to anthracycline chemotherapy drugs. The results indicated that the combination was more effective than chemotherapy alone and without significant toxicity.[140] The trial of Horne et al presented an obvious enhancement of the combination of HCQ and imatinib compared with imatinib used alone in chronic-phase chronic myeloid leukaemia, but a common diarrhea was happened with one case displayed cardiac rhythm disorder, dyspnoea and heart failure.[141] High dose HCQ (1800 mg/d) combined with gemcitabine/nab-paclitaxel revealed improved pathological tumor response and serum biomarker response in pancreatic cancer, and HCQ did not increase the toxicity of chemotherapy.[142] However, the other study displayed a dose-limiting toxicity of HCQ (the maximum tolerated dose (MTD) was 600 mg/d), and at the MTD dose, HCQ did not show obvious improvement in the survival of glioblastoma patients, which remind us that more low-toxicity autophagy inhibitors need to be developed in tumor adjuvant therapy.[143] In addition, the combinations of HCQ in clinical trials of other tumor types, such as non-small cell lung cancer, melanoma, colorectal cancer and prostate, have also demonstrated that it can significantly improve treatment response of chemotherapy agents and possess great anti-tumor potential.[144–150]

Although CQ and its analogues can improve the efficacy of chemotherapy or radiotherapy, safety concerns have arisen, with severe side effects observed in some cases. Rustogi et al noted in their medical work that CQ could cause skin desquamation when used alongside radiotherapy.[151] Because of the double-edged properties of autophagy, CQ's inhibition of autophagy sensitized not only cancer cells but also normal cells to chemotherapy, potentially causing acute kidney damage while increasing chemotherapy sensitivity in cancer cells.[153,154] Furthermore, combination with CQ in chemotherapy also caused damage to other organs, such as the liver, heart, brain and hematopoietic cells, due to the critical role of autophagy in these organs.[155–159] Additionally, Angel et al evaluated the effect of CQ alone in breast tumor patients before their surgery and found that compared with placebo, treatment with CQ had no significant effects on breast cancer cellular proliferation but was associated with toxicity.[152] These findings highlight the side effects of CQ, underscoring the need for more clinical trials to further verify its pharmacological properties.

Nanotechnology Improve the Safety of CQ

The side effects of CQ limit its usage, but the emergence of nanotechnology, a novel technique that has already contributing to a huge impact in the biomedical sciences, particularly in the fields of cancer treatment and diagnosis, has effectively reduced the side effects of CQ and greatly improved its efficacy in cancer therapy. CQ wrapped in gold nanoparticles displayed an

Table 3 Clinical Trials and Adverse Reactions of CQ and Its Analogues

Analogues	Accompanied Agents	Cancer Types	Concentration	Mechanism of Action	Adverse Events	Ref.
CQ	Carmustine	Glioblastoma multiforme	CQ: 150 mg/d, orally, for 12 months; Carmustine: 200 mg/m^2, one given every 5 weeks, 4 courses	Enhanced the cytotoxicity caused by conventional therapy or prevented the mutagenicity of tumor cells.	NA	[139]
CQ	Carmustine	Glioblastoma multiforme	CQ: 150 mg/d, orally; Carmustine: 200 mg/m^2, i.v, once every 6 weeks	Antimutagenic effect.	Seizures	[136]
CQ	Docetaxel, Paclitaxel, Nab-paclitaxel, Ixabepilone	Breast cancer	CQ: 250 mg/d, orally, for 18 weeks; Paclitaxel: 175 mg/m^2, Docetaxel: 75 mg/m^2, Nab-paclitaxel: 260 mg/m^2, Ixabepilone: 40 mg/m^2, i.v every 3 weeks, for 18 weeks	Inhibited cancer stem cells in breast cancer.	NA	[140]
CQ	Radiotherapy	Glioma	An immediate dose of 500 mg, followed by 250 mg given after 6 h, and 250 mg given once per day for the next 2 days.	NA	Localised brisk bullous eruptions, fulminant moist desquamation	[151]
CQ	NA	Breast cancer	500 mg/d, orally, for 2 to 6 weeks	Autophagy inhibition and cell cycle disruption	Muscle weakness, dry mouth, nausea, diarrhea, dizziness, visual symptoms	[152]
HCQ	Imatinib	Chronic myeloid leukaemia	HCQ: 400 mg twice, daily, orally; Imatinib: <400 mg, 400 to <600 mg, 600–800 mg	Autophagy inhibition	Diarrhea was common; one case: cardiac rhythm disorder, dyspnoea and heart failure.	[141]
HCQ	Gemcitabine and Nab-paclitaxel	Pancreatic adenocarcinoma	HCQ: 1200 mg, 600 mg twice daily; Gemcitabine: 1000 mg/m^2, Nab-paclitaxel: 125 mg/m^2, on days 1, 8, and 15 of each monthly cycle, 2 cycles	Autophagy inhibition	Abdominal pain, anemia, dehydration, fatigue, and hyponatremia	[142]
HCQ	Temozolomide	Glioblastoma multiforme	HCQ: 200 to 800 mg, orally, daily; TMZ: 75 mg/m^2/d, orally, 6 weeks, 150 mg/m^2/d for 5 consecutive days every month, for 6 months	Autophagy inhibition	Nausea, fatigue, constipation, and diarrhea	[143]

HCQ	Temsirolimus	Melanoma; Colorectal cancer, Head and neck, Breast, Gastric/esophageal, Prostate, Pancreas Non-small cell lung, and Pheo/adrenocortical cancer	HCQ: 200 to 1200 mg, daily orally, Temsirolimus: 25mg, i.v, weekly 4 weeks	Autophagy inhibition	Anorexia, fatigue, nausea, fatigue, anorexia, nausea, stomatitis, rash, and weight loss	[144]
HCQ	Gemcitabine	Pancreatic adenocarcinoma	HCQ: 1200 mg/day, one month; 600 mg, twice daily, 2 months; Gemcitabine: NA	Autophagy inhibition	NA	[145]
HCQ	Carboplatin, Paclitaxel, Bevacizumab	Non-small cell lung cancer	HCQ: orally (200 mg BID) on Days 1–21 Paclitaxel: 200 mg/m^2, i.v, over 3 h on Day 1; Carboplatin: AUC = 6, i.v, over 15–30 min on Day 1; Bevacizumab: 15 mg/kg, i.v, 90 min on Day 1. 6 cycles.	Autophagy inhibition	Neutropenia, neuropathy, and anemia.	[146]
HCQ	Gemcitabine	Pancreatic adenocarcinoma	HCQ: 200 mg/day to 1200 mg/day, orally; Gemcitabine: 1500 mg/m^2, for 31 days.	Autophagy inhibition	Neutropenia, lymphopenia, rash, and hypoalbuminemia	[147,148]
HCQ	Bortezomib	Myeloma	HCQ: 200, 400, 600 mg twice daily, orally, for 2 weeks; Bortezomib: 1.3 mg/m^2, i.v on d 1, 4, 8, and 11 of each 21-d cycle.	Autophagy inhibition	Bone marrow suppression and fatigue, gastrointestinal toxicity	[149]
HCQ	Everolimus	Clear cell renal carcinoma	HCQ: 600 mg twice daily, orally; Everolimus: 10 mg daily, orally; For 35 days + 28 days	Autophagy inhibition	Nausea, vomiting	[150]

https://doi.org/10.2147/IJN.S458910

obvious necrosis in MCF-7 and reduced its side effects compared with CQ along.[116] Drug delivery systems play a vital role in inhibiting CSCs. CQ was reported to reduce CSCs in pancreatic cancer and decrease tumorigenicity in vivo, nanoparticles carrying CQ accurately and efficiently delivered CQ to the tumor site, suppressing tumor development.[114,160,161] Sun et al encapsulated CQ and other chemotherapeutic agents, such as DOX, DTXL into poly(ethylene glycol)-block-poly(D, L-lactide) (PEG-b-PLA) along or together, forming nanoparticles about 110 nm in diameter, which significantly increased drug delivery efficiency. The nanoparticles contained CQ and other drugs effectively enhanced autophagy inhibition, reduced CSCs and improved the efficacy of chemotherapeutic drugs against breast cancer in vitro.[162] These studies indicate that nanoparticle formulations of CQ are a promising strategy with strong prospects for application.

Application of CQ in Other Diseases

In addition to its anti-malaria and anti-tumor properties, CQ also exerts inhibitory effects on rheumatic autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren's syndrome. Moreover, it possesses antiviral activity, including the inhibition of HIV-1 and the severe acute respiratory syndrome (SARS) coronavirus. Here, we provide a brief overview and discussion of these properties of CQ (Figure 5).

Inhibition of Rheumatic Autoimmune Diseases

Rheumatic autoimmune diseases, such as RA, SLE and Sjögren's syndrome, are types of diseases caused by the immune system mistakenly attacking its own tissues, including joints.[163] CQ and its analogue HCQ have been reported to be effective in treatment of rheumatic autoimmune diseases because of their anti-inflammatory and immunomodulatory properties.[164] The possible mechanisms are related to the inhibition of autophagy, lysosomal acidification, suppression of toll-like-receptors (TLRs), prevention of the interaction between TLRs and their ligands, and restraint of calcium allocation.[163] TLRs are

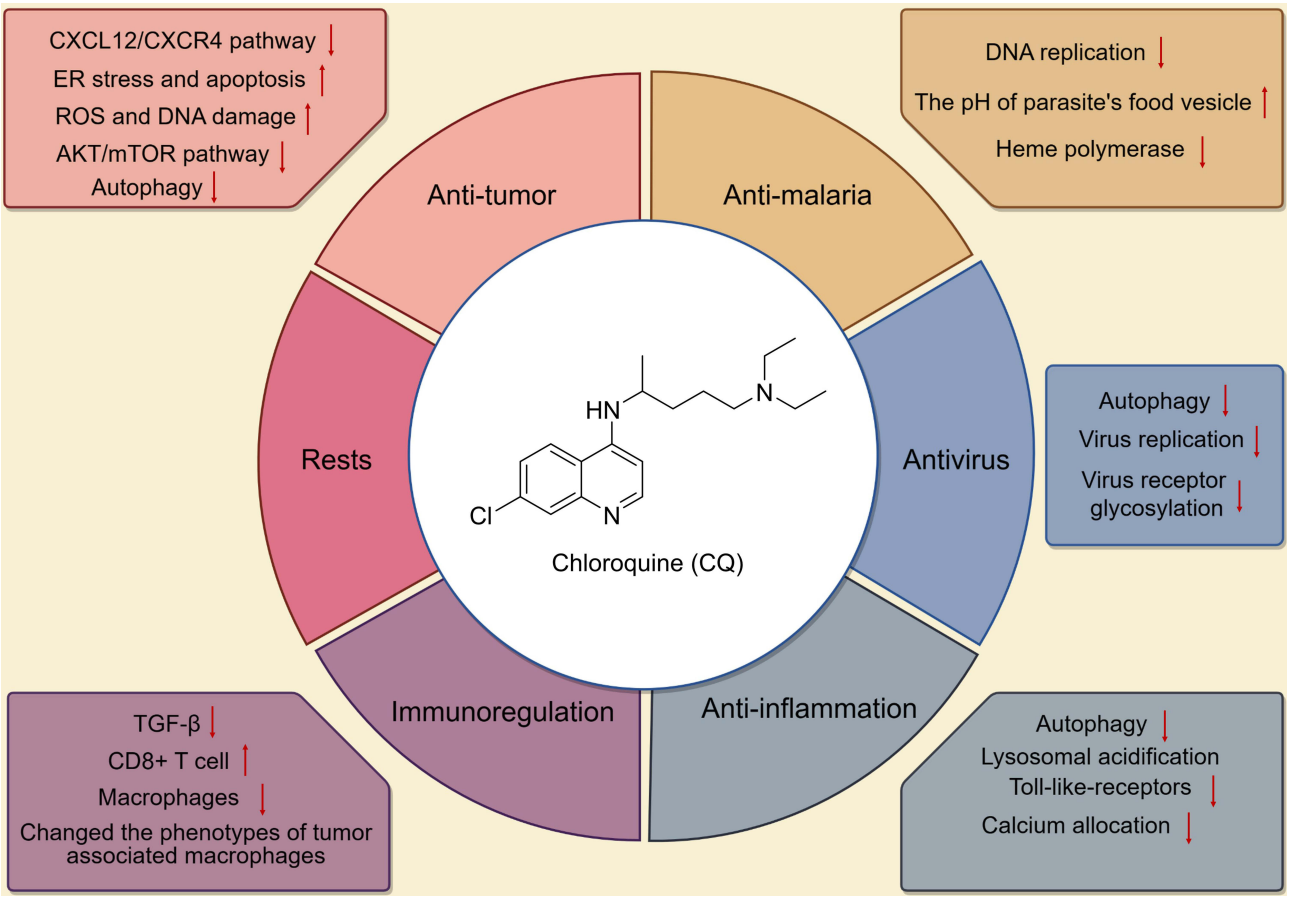

Figure 5 Functional classifications of CQ.

transmembrane proteins and expressed in various types of cells, like macrophages, monocytes, T/B lymphocytes, and dendritic cells, act as the host's innate immune defense sensors and are associated with the activation of adaptive immunity. TLRs can recognize pathogenic microorganisms or endogenous second messengers (such as nucleic acids), inducing the production of pro-inflammatory cytokines, causing immune response and even inflammatory. Pro-inflammatory cytokines like TNF-α, IL-1 and IL-6 are essential in mediating the inflammatory response in RA, SLE and Sjögren's syndrome.[165–167] More than 10 TLRs have been identified in mammals and humans, with TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10 expressed on the plasma membrane, and TLR3, TLR7, TLR8 and TLR9 expressed in endosomes and involved in identifying nucleic acid components of microorganisms.[168] Studies have shown that CQ can inhibit TLR3, TLR7 and TLR9, impairing the TLR signaling via blocking the binding of TLR to ligands, disturbing endosomal acidification or other mechanisms as described above, thus suppressing the secretion of pro-inflammatory cytokines.[163] Therefore, the inhibition of the TLR signaling pathway by CQ plays an important role in the treatment of rheumatic autoimmune diseases.

Antiviral Activity

Apart from its anti-inflammatory activity, CQ also possesses antiviral properties with a broad spectrum of virus types.[169,170] Studies have shown that CQ can inhibit HIV-1/AIDS virus in vitro no matter the treatment is executed before or after the infection of cells with the virus, and this effect may be attributed to the ability of CQ to inhibit the glycosylation of the virus receptor on the cell, thereby reducing HIV-1/AIDS infection.[171–173] CQ also had a braking effect on the SARS coronavirus, where it suppressed the replication of the SARS coronavirus and decreased the glycosylation of SARS coronavirus receptor ACE2 that expressed on the human cell surface.[174,175] Moreover, Yiwu et al have found that the avian influenza A H5N1 virus infection leads to acute lung injury through triggering autophagic cell death of alveolar epithelial. As an autophagy inhibitor, CQ significantly reduced lung injury and prolonged the lifespan of mice infected with the avian influenza A H5N1 virus.[176] The massive outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019 claimed many lives, there was no definitive treatment in the early phase, but scientists have found that the old drug CQ and its derivative HCQ have an inhibitory effect on this novel coronavirus which are similar to the SARS coronavirus and use ACE2 as a receptor to enter cells.[177,178] However, some people are skeptical about the conclusion that CQ is effective in treating the SARS-CoV-2 and note its significant side effects.[179] But the research on CQ against SARS-CoV-2 is continuing and its potential prophylactic and therapeutic effects remain to be determined.

Existing researches have shown CQ has a broad spectrum antiviral activity or can be used to treat complications caused by certain vital infections. More studies on the antiviral mechanism of CQ are expected to expand its medicinal value and scope of application.

Conclusion

CQ is a widely used antimalarial drug that has received much attentions for its potential use in cancer therapy recently. Early studies have found that CQ can induce apoptosis of tumor cells by inhibiting autophagy, thus inhibiting tumor growth and diffusion.[99,105,106] Studies also have shown that CQ enhances the efficacy of certain chemotherapeutic drugs by blocking autophagy, thus increasing the accumulation of damaged proteins and organelles, and sensitizing cancer cells to chemotherapy-induced cell death.

While autophagy is a well-accepted survival mechanism during cancer treatment with different chemotherapy agents, and inhibiting autophagy with CQ increases the sensitivity of anticancer drugs, there are still people challenge this view and test it. Maycotte et al suggested that combination treatment with CQ in cancers should consider the possibility that CQ may act through mechanisms other than inhibition autophagy, since in their research they found that CQ could decrease the viability of cancer cells treated with chemotherapy but this effect could not be mimicked with autophagy associated proteins knockdown or autophagy inhibitors treatment. Hence, the authors concluded that CQ mediated chemotherapy sensitization was an autophagy-independent event in tumor cells.[80] Beyond the autophagy-independent mechanisms above CQ was always thought to induce tumor cell apoptosis via activating the p53 pathway. Kim et al found that CQ treatment stabilized p53 protein prominently and increased the expression of p53 target genes simultaneously in the glioma lines expressing wild type p53, compared with the cells lacking functional p53. They also reported a mitochondrial dysfunction apoptosis pathway that is independent of p53 effect which may be consistent with those

described above.[180] In summary, we draw a conclusion that CQ has many other autophagy-independent mechanisms in tumor therapy and one should put the mechanisms independent of autophagy inhibition into account when treated with CQ in their studies (Figure 6).

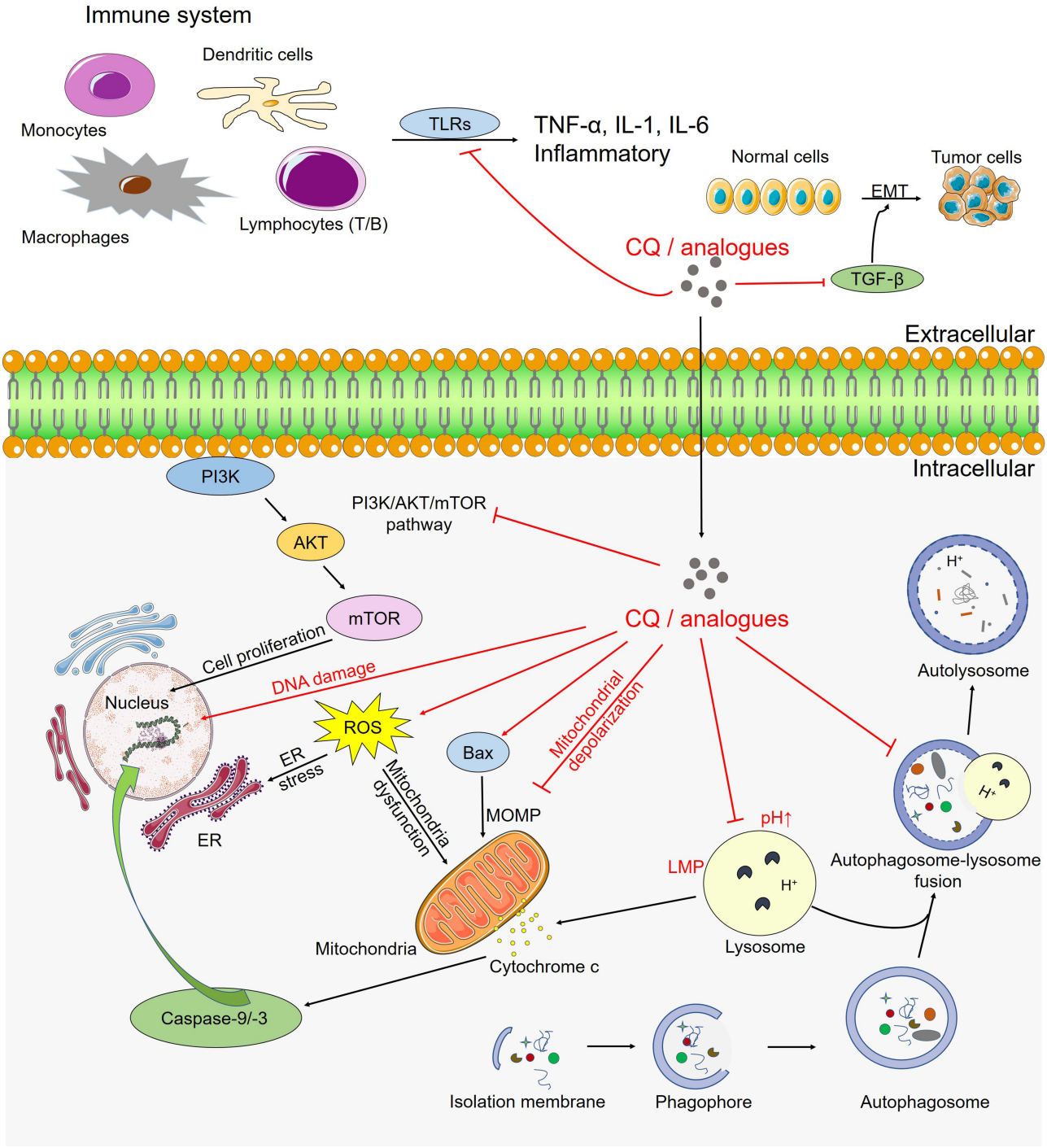

Figure 6 The pharmacological mechanisms of CQ with autophagy dependent or independent in cancer therapy. CQ inhibited autophagy and induced ROS, leading to the loss of LMP. CQ also promoted the activation of Bax, which further led to the release of cytochrome c by mitochondria and mediated the apoptosis of tumor cells. CQ also blocked the PI3K/AKT/mTOR signaling pathway and inhibited tumor cell proliferation. In addition, CQ decreased the expression of TGF-β and inhibited EMT. CQ also inhibited the TLRs and thus played its anti-inflammatory properties on rheumatic autoimmune diseases.

Abbreviations: CQ, Chloroquine; ER, Endoplasmic reticulum; ROS, Reactive oxygen species; LMP, Lysosomal membrane permeabilization; MOMP, Mitochondrial outer membrane permeabilization; EMT, Epithelial–mesenchymal transition; TLRs, Toll-like-receptors.

In addition, CQ can inhibit tumor growth through various mechanisms, including inducing tumor cell necrosis and preventing angiogenesis. In recent years, more and more studies have shown that CQ not only has anti-autophagy effect but also can affect tumor immunity and remodel tumor microenvironment. Research showed that CQ could act as an antitumor immunomodulator to the tumor-associated macrophages (TAMs), which could be switched from M2 phenotype to tumor killing M1 phenotype. The specific mechanism was that CQ induced the activation of p38 and NF-κB via increasing the pH of macrophage lysosomes and leading to the release of Ca^{2+}, thus polarizing TAMs into the M1 phenotype. Resetting macrophages to improve the tumor immune microenvironment raised anti-tumor T cell immunity and enhanced the body's ability to attack cancer cells.[181,182] Therefore, CQ has extensive antitumor activity and potential therapeutic application value.

Except its anti-malaria and anti-tumor properties, CQ also has inhibitory effects on rheumatic autoimmune diseases, such as RA, SLE and Sjögren's syndrome, and also possesses antiviral activity, like the inhibition of HIV-1 virus and SARS coronavirus. More studies on these properties and its underlying mechanisms are expected to expand the medicinal value and scope of application of CQ.

Although CQ has shown promise in antitumor research, there are still many problems to be solved. For example, the antitumor mechanism of CQ is not fully understood, necessitating further investigation. Clinical trials are still ongoing to determine its safety and efficacy in humans. In addition, there are certain side effects associated with clinical use of CQ, which need to be better understood and managed. Moreover, the exploration of CQ's potential in combination with other antitumor agents and its applicability across various tumor types also warrants further study.

Looking ahead, the antitumor research of CQ is poised for further development, including exploring more therapeutic application scenarios, improving dosage forms and medication regimens, and developing new CQ analogues. In general, the antitumor research of CQ holds broad application prospects and potential therapeutic value, and future research endeavors will continue to advance its development and clinical therapy applications.

Abbreviations

CQ, Chloroquine; HCQ, Hydroxychloroquine; FDA, Food and Drug Administration; QC, Quinacrine; MQ, Mefloquine; EAC, Ehrlich ascites carcinoma; LMP, Lysosomal membrane permeabilization; CCOC, Clear cell ovarian carcinoma; ROS, Reactive oxygen species; CMA, Chaperone-mediated autophagy; HSC70, Heat shock 70kDa; IH, Isorhamnetin; EMT, Epithelial–mesenchymal transition; NSCLC, Non-small cell lung carcinoma; LDM, Lidamycin; CRC, Colorectal cancer; 5-FU, 5-Fluorouracil; PARP, Poly(adenosine diphosphate ribose) polymerase; NHEJ, Nonhomologous end joining; EGFR, Epithelial growth factor receptor; AR, Androgen receptor; LCSCs, Liver cancer stem cells; HCC, Hepatocellular carcinoma; PEL, Primary Effusion Lymphoma; ER, Endoplasmic reticulum; PPT1, Palmitoyl-protein thioesterase 1; MMP, Mitochondrial membrane potential; MOMP, Mitochondrial outer membrane permeabilization; CSC, Cancer stem cells; TRAIL, Tumor necrosis factor (TNF)-related apoptosis-inducing ligand; GBC, Gallbladder cancer; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; SARS, Severe acute respiratory syndrome; TLRs, Toll-like-receptors; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; TAMs, Tumor-associated macrophages.

Acknowledgments

The authors appreciate all the members of the Jigang Wang Lab for helpful discussions and comments. We also thank the FigDraw platform for providing us some elements of our figures.

Funding

This work was supported by the Fundamental Research Funds for the National Natural Science Foundation (No.82204672, 82204322 and 82104480), the Central Public Welfare Research Institutes (ZZ13-YQ-103, ZZ13−YQ −105, ZZ14-YQ-058, ZZ15−YQ−065).

Disclosure

The authors declare that they have no competing interests.

References

1. Homewood CA, Warhurst DC, Peters W, Baggaley VC. Lysosomes, pH and the anti-malarial action of chloroquine. *Nature*. 1972;235 (5332):50–52. doi:10.1038/235050a0
2. Al-Bari MA. Chloroquine analogues in drug discovery: new directions of uses, mechanisms of actions and toxic manifestations from malaria to multifarious diseases. *J Antimicrob Chemother*. 2015;70(6):1608–1621. doi:10.1093/jac/dkv018
3. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. *Inflammopharmacology*. 2015;23(5):231–269. doi:10.1007/s10787-015-0239-y
4. Kremer JM. Rational use of new and existing disease-modifying agents in rheumatoid arthritis. *Ann Intern Med*. 2001;134(8):695–706. doi:10.7326/0003-4819-134-8-200104170-00013
5. Townsend KN, Hughson LR, Schlie K, Poon VI, Westerback A, Lum JJ. Autophagy inhibition in cancer therapy: metabolic considerations for antitumor immunity. *Immunol Rev*. 2012;249(1):176–194. doi:10.1111/j.1600-065X.2012.01141.x
6. Romanelli F, Smith KM, Hoven AD. Chloroquine and hydroxychloroquine as inhibitors of human immunodeficiency virus (HIV-1) activity. *Curr Pharm Des*. 2004;10(21):2643–2648. doi:10.2174/1381612043383791
7. Savarino A, Cauda R, Cassone A. On the use of chloroquine for chikungunya. *Lancet Infect Dis*. 2007;7(10):633. doi:10.1016/S1473-3099(07)70217-7
8. Devaux CA, Rolain JM, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? *Int J Antimicrob Agents*. 2020;55(5):105938. doi:10.1016/j.ijantimicag.2020.105938
9. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020;30(3):269–271. doi:10.1038/s41422-020-0282-0
10. Zhou W, Wang H, Yang Y, Chen ZS, Zou C, Zhang J. Chloroquine against malaria, cancers and viral diseases. *Drug Discov Today*. 2020;25 (11):2012–2022. doi:10.1016/j.drudis.2020.09.010
11. Manic G, Obrist F, Kroemer G, Vitale I, Galluzzi L. Chloroquine and hydroxychloroquine for cancer therapy. *Mol Cell Oncol*. 2014;1(1):e29911. doi:10.4161/mco.29911
12. Solomon VR, Lee H. Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *Eur J Pharmacol*. 2009;625(1–3):220–233. doi:10.1016/j.ejphar.2009.06.063
13. Poole B, Ohkuma S. Effect of weak bases on the intralysosomal pH in mouse peritoneal macrophages. *J Cell Biol*. 1981;90(3):665–669. doi:10.1083/jcb.90.3.665
14. Mauthe M, Orhon I, Rocchi C, et al. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy*. 2018;14 (8):1435–1455. doi:10.1080/15548627.2018.1474314
15. Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat Rev Cancer*. 2017;17(9):528–542. doi:10.1038/nrc.2017.53
16. Shi S, Tan P, Yan B, et al. ER stress and autophagy are involved in the apoptosis induced by cisplatin in human lung cancer cells. *Oncol Rep*. 2016;35(5):2606–2614. doi:10.3892/or.2016.4680
17. Chen RJ, Lyu YJ, Chen YY, et al. Chloroquine potentiates the anticancer effect of pterostilbene on pancreatic cancer by inhibiting autophagy and downregulating the RAGE/STAT3 pathway. *Molecules*. 2021;26(21):6741. doi:10.3390/molecules26216741
18. Masud Alam M, Kariya R, Kawaguchi A, Matsuda K, Kudo E, Okada S. Inhibition of autophagy by chloroquine induces apoptosis in primary effusion lymphoma in vitro and in vivo through induction of endoplasmic reticulum stress. *Apoptosis*. 2016;21(10):1191–1201. doi:10.1007/s10495-016-1277-7
19. Seitz C, Hugle M, Cristofanon S, Tchoghandjian A, Fulda S. The dual PI3K/mTOR inhibitor NVP-BEZ235 and chloroquine synergize to trigger apoptosis via mitochondrial-lysosomal cross-talk. *Int, J, Cancer*. 2013;132(11):2682–2693. doi:10.1002/ijc.27935
20. Harhaji-Trajkovic L, Arsikin K, Kravic-Stevovic T, et al. Chloroquine-mediated lysosomal dysfunction enhances the anticancer effect of nutrient deprivation. *Pharm Res*. 2012;29(8):2249–2263.
21. Krafts K, Hempelmann E, Skórska-Stania A. From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy. *Parasitol Res*. 2012;111(1):1–6. doi:10.1007/s00436-012-2886-x
22. Schlitzer M. Malaria chemotherapeutics part I: history of antimalarial drug development, currently used therapeutics, and drugs in clinical development. *ChemMedChem*. 2007;2(7):944–986.
23. Coban C. The host targeting effect of chloroquine in malaria. *Curr Opin Immunol*. 2020;66:98–107. doi:10.1016/j.coi.2020.07.005
24. Al-Bari MAA. Co-targeting of lysosome and mitophagy in cancer stem cells with chloroquine analogues and antibiotics. *J Cell Mol Med*. 2020;24(20):11667–11679. doi:10.1111/jcmm.15879
25. Lin YC, Lin JF, Wen SI, et al. Chloroquine and hydroxychloroquine inhibit bladder cancer cell growth by targeting basal autophagy and enhancing apoptosis. *Kaohsiung J Med Sci*. 2017;33(5):215–223. doi:10.1016/j.kjms.2017.01.004
26. Van Eaton KM, Gustafson DL. Pharmacokinetic and Pharmacodynamic Assessment of Hydroxychloroquine in Breast Cancer. *J Pharmacol Exp Ther*. 2021;379(3):331–342. doi:10.1124/jpet.121.000730
27. Li Y, Cao F, Li M, et al. Hydroxychloroquine induced lung cancer suppression by enhancing chemo-sensitization and promoting the transition of M2-TAMs to M1-like macrophages. *J Exp Clin Cancer Res*. 2018;37(1):259. doi:10.1186/s13046-018-0938-5
28. Donia T, Gerges MN, Mohamed TM. Anticancer Effects of Combination of Indole-3-Carbinol and Hydroxychloroquine on Ehrlich Ascites Carcinoma via Targeting Autophagy and Apoptosis. *Nutr Cancer*. 2022;74(5):1802–1818. doi:10.1080/01635581.2021.1960388
29. Chen J, Pan Q, Bai Y, Chen X, Zhou Y. Hydroxychloroquine Induces Apoptosis in Cholangiocarcinoma *via* Reactive Oxygen Species Accumulation Induced by Autophagy Inhibition. *Front Mol Biosci*. 2021;8:720370. doi:10.3389/fmolb.2021.720370
30. Lyu X, Zeng L, Zhang H, et al. Hydroxychloroquine suppresses lung tumorigenesis via inducing FoxO3a nuclear translocation through STAT3 inactivation. *Life Sci*. 2020;246:117366. doi:10.1016/j.lfs.2020.117366
31. Zhou W, Guo Y, Zhang X, Jiang Z. Lys05 induces lysosomal membrane permeabilization and increases radiosensitivity in glioblastoma. *J Cell Biochem*. 2020;121(2):2027–2037. doi:10.1002/jcb.29437
32. DeVorkin L, Hattersley M, Kim P, et al. Autophagy inhibition enhances sunitinib efficacy in clear cell ovarian carcinoma. *Mol Cancer Res*. 2017;15(3):250–258. doi:10.1158/1541-7786.MCR-16-0132

33. Courdy C, Platteeuw L, Ducau C, et al. Targeting PP2A-dependent autophagy enhances sensitivity to ruxolitinib in JAK2^{V617F} myeloproliferative neoplasms. *Blood Cancer J*. 2023;13(1):106. doi:10.1038/s41408-023-00875-x
34. Xu X, Wang J, Han K, Li S, Xu F, Yang Y. Antimalarial drug mefloquine inhibits nuclear factor kappa B signaling and induces apoptosis in colorectal cancer cells. *Cancer Sci*. 2018;109(4):1220–1229. doi:10.1111/cas.13540
35. Li YH, Yang SL, Zhang GF, et al. Mefloquine targets β-catenin pathway and thus can play a role in the treatment of liver cancer. *Microb Pathog*. 2018;118:357–360. doi:10.1016/j.micpath.2018.03.042
36. Lam Yi H, Than H, Sng C, Cheong MA, Chuah C, Xiang W. Lysosome Inhibition by Mefloquine Preferentially Enhances the Cytotoxic Effects of Tyrosine Kinase Inhibitors in Blast Phase Chronic Myeloid Leukemia. *Transl Oncol*. 2019;12(9):1221–1228. doi:10.1016/j.tranon.2019.06.001
37. Sharma N, Thomas S, Golden EB, et al. Inhibition of autophagy and induction of breast cancer cell death by mefloquine, an antimalarial agent. *Cancer Lett*. 2012;326(2):143–154. doi:10.1016/j.canlet.2012.07.029
38. Wan B, Wu Z, Zhang X, Huang B. Mefloquine as a dual inhibitor of glioblastoma angiogenesis and glioblastoma via disrupting lysosomal function. *Biochem Biophys Res Commun*. 2021;580:7–13. doi:10.1016/j.bbrc.2021.09.069
39. Liu Y, Chen S, Xue R, Zhao J, Di M. Mefloquine effectively targets gastric cancer cells through phosphatase-dependent inhibition of PI3K/Akt/mTOR signaling pathway. *Biochem Biophys Res Commun*. 2016;470(2):350–355. doi:10.1016/j.bbrc.2016.01.046
40. Yan KH, Lin YW, Hsiao CH, et al. Mefloquine induces cell death in prostate cancer cells and provides a potential novel treatment strategy in vivo. *Oncol Lett*. 2013;5(5):1567–1571. doi:10.3892/ol.2013.1259
41. Yan KH, Yao CJ, Hsiao CH, et al. Mefloquine exerts anticancer activity in prostate cancer cells via ROS-mediated modulation of Akt, ERK, JNK and AMPK signaling. *Oncol Lett*. 2013;5(5):1541–1545. doi:10.3892/ol.2013.1211
42. Li H, Jiao S, Li X, Banu H, Hamal S, Wang X. Therapeutic effects of antibiotic drug mefloquine against cervical cancer through impairing mitochondrial function and inhibiting mTOR pathway. *Can J Physiol Pharmacol*. 2017;95(1):43–50. doi:10.1139/cjpp-2016-0124
43. Xie Y, Zhang J, Lu B, et al. Mefloquine Inhibits Esophageal Squamous Cell Carcinoma Tumor Growth by Inducing Mitochondrial Autophagy. *Front Oncol*. 2020;10:1217. doi:10.3389/fonc.2020.01217
44. Das S, Dielschneider R, Chanas-LaRue A, Johnston JB, Gibson SB. Antimalarial drugs trigger lysosome-mediated cell death in chronic lymphocytic leukemia (CLL) cells. *Leuk Res*. 2018;70:79–86. doi:10.1016/j.leukres.2018.06.005
45. Samanta A, Ravindran G, Sarkar A. Quinacrine causes apoptosis in human cancer cell lines through caspase-mediated pathway and regulation of small-GTPase. *J Biosci*. 2020;45(1):43. doi:10.1007/s12038-020-0011-3
46. Das S, Tripathi N, Preet R, et al. Quinacrine induces apoptosis in cancer cells by forming a functional bridge between TRAIL-DR5 complex and modulating the mitochondrial intrinsic cascade. *Oncotarget*. 2017;8(1):248–267. doi:10.18632/oncotarget.11335
47. Jung D, Khurana A, Roy D, et al. Quinacrine upregulates p21/p27 independent of p53 through autophagy-mediated downregulation of p62-Skp2 axis in ovarian cancer. *Sci Rep*. 2018;8(1):2487. doi:10.1038/s41598-018-20531-w
48. Thirusangu P, Pathoulas CL, Ray U, et al. Quinacrine-Induced Autophagy in Ovarian Cancer Triggers Cathepsin-L Mediated Lysosomal/Mitochondrial Membrane Permeabilization and Cell Death. *Cancers*. 2021;13(9):2004. doi:10.3390/cancers13092004
49. Kumar M, Martin A, Nirgude S, Chaudhary B, Mondal S, Sarkar A. Quinacrine inhibits GSTA1 activity and induces apoptosis through G_1/S arrest and generation of ROS in human non-small cell lung cancer cell lines. *Oncotarget*. 2020;11(18):1603–1617. doi:10.18632/oncotarget.27558
50. Kalogera E, Roy D, Khurana A, et al. Quinacrine in endometrial cancer: repurposing an old antimalarial drug. *Gynecol Oncol*. 2017;146(1):187–195. doi:10.1016/j.ygyno.2017.04.022
51. Gallant JN, Allen JE, Smith CD, et al. Quinacrine synergizes with 5-fluorouracil and other therapies in colorectal cancer. *Cancer Biol Ther*. 2011;12(3):239–251. doi:10.4161/cbt.12.3.17034
52. Khurana A, Roy D, Kalogera E, et al. Quinacrine promotes autophagic cell death and chemosensitivity in ovarian cancer and attenuates tumor growth. *Oncotarget*. 2015;6(34):36354–36369. doi:10.18632/oncotarget.5632
53. Zhu S, Chen Z, Wang L, et al. A combination of SAHA and quinacrine is effective in inducing cancer cell death in upper gastrointestinal cancers. *Clin Cancer Res*. 2018;24(8):1905–1916. doi:10.1158/1078-0432.CCR-17-1716
54. Abdulghani J, Gokare P, Gallant JN, et al. Sorafenib and quinacrine target anti-apoptotic protein MCL1: a poor prognostic marker in anaplastic thyroid cancer (ATC). *Clin Cancer Res*. 2016;22(24):6192–6203. doi:10.1158/1078-0432.CCR-15-2792
55. Kulkarni NS, Vaidya B, Gupta V. Nano-synergistic combination of Erlotinib and Quinacrine for non-small cell lung cancer (NSCLC) therapeutics - Evaluation in biologically relevant in-vitro models. *Mater Sci Eng C Mater Biol Appl*. 2021;121:111891. doi:10.1016/j.msec.2021.111891
56. Nayak D, Paul S, Das C, Bhal S, Kundu CN. Quinacrine and Curcumin in combination decreased the breast cancer angiogenesis by modulating ABCG2 via VEGF A. *J Cell Commun Signal*. 2023;17(3):609–626. doi:10.1007/s12079-022-00692-0
57. Amaravadi RK, Lippincott-Schwartz J, Yin XM, et al. Principles and current strategies for targeting autophagy for cancer treatment. *Clin Cancer Res*. 2011;17(4):654–666. doi:10.1158/1078-0432.CCR-10-2634
58. Lorente J, Velandia C, Leal JA, et al. The interplay between autophagy and tumorigenesis: exploiting autophagy as a means of anticancer therapy. *Biol Rev Camb Philos Soc*. 2018;93(1):152–165. doi:10.1111/brv.12337
59. Patel S, Hurez V, Nawrocki ST, et al. Vorinostat and hydroxychloroquine improve immunity and inhibit autophagy in metastatic colorectal cancer. *Oncotarget*. 2016;7(37):59087–59097. doi:10.18632/oncotarget.10824
60. Chi K-H, Ko H-L, Yang K-L, Lee C-Y, Chi M-S, Kao S-J. Addition of rapamycin and hydroxychloroquine to metronomic chemotherapy as a second line treatment results in high salvage rates for refractory metastatic solid tumors: a pilot safety and effectiveness analysis in a small patient cohort. *Oncotarget*. 2015;6(18):16735–16745. doi:10.18632/oncotarget.3793
61. Mahalingam D, Mita M, Sarantopoulos J, et al. Combined autophagy and HDAC inhibition: a Phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. *Autophagy*. 2014;10(8):1403–1414. doi:10.4161/auto.29231
62. McAfee Q, Zhang Z, Samanta A, et al. Autophagy inhibitor Lys05 has single-agent antitumor activity and reproduces the phenotype of a genetic autophagy deficiency. *Proc Natl Acad Sci U S A*. 2012;109(21):8253–8258. doi:10.1073/pnas.1118193109

63. de Souza PL, Castillo M, Myers CE. Enhancement of paclitaxel activity against hormone-refractory prostate cancer cells in vitro and in vivo by quinacrine. *Br J Cancer*. 1997;75(11):1593–1600. doi:10.1038/bjc.1997.272
64. Gurova KV, Hill JE, Guo C, et al. Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors. *Proc Natl Acad Sci U S A*. 2005;102(48):17448–17453. doi:10.1073/pnas.0508888102
65. Eriksson A, Österroos A, Hassan S, et al. Drug screen in patient cells suggests quinacrine to be repositioned for treatment of acute myeloid leukemia. *Blood Cancer J*. 2015;5(4):e307. doi:10.1038/bcj.2015.31
66. Dermawan JK, Gurova K, Pink J, et al. Quinacrine overcomes resistance to erlotinib by inhibiting FACT, NF-κB, and cell-cycle progression in non-small cell lung cancer. *Mol Cancer Ther*. 2014;13(9):2203–2214. doi:10.1158/1535-7163.MCT-14-0013
67. Oien DB, Ray U, Pathoulas CL, et al. Quinacrine induces nucleolar stress in treatment-refractory ovarian cancer cell lines. *Cancers*. 2021;13(18):4645. doi:10.3390/cancers13184645
68. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. *Antioxid Redox Signal*. 2014;20(3):460–473. doi:10.1089/ars.2013.5371
69. Mijaljica D, Prescott M, Devenish RJ. Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. *Autophagy*. 2011;7(7):673–682. doi:10.4161/auto.7.7.14733
70. Massey A, Kiffin R, Cuervo AM. Pathophysiology of chaperone-mediated autophagy. *Int J Biochem Cell Biol*. 2004;36(12):2420–2434. doi:10.1016/j.biocel.2004.04.010
71. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ*. 2005;12(Suppl 2):1542–1552. doi:10.1038/sj.cdd.4401765
72. Wirawan E, Vanden Berghe T, Lippens S, Agostinis P, Vandenabeele P. Autophagy: for better or for worse. *Cell Res*. 2012;22(1):43–61. doi:10.1038/cr.2011.152
73. Degtyarev M, De Mazière A, Orr C, et al. Akt inhibition promotes autophagy and sensitizes PTEN-null tumors to lysosomotropic agents. *J Cell Biol*. 2008;183(1):101–116. doi:10.1083/jcb.200801099
74. Fan QW, Cheng C, Hackett C, et al. Akt and autophagy cooperate to promote survival of drug-resistant glioma. *Sci Signal*. 2010;3(147):ra81. doi:10.1126/scisignal.2001017
75. Hu J, Zhang Y, Jiang X, et al. ROS-mediated activation and mitochondrial translocation of CaMKII contributes to Drp1-dependent mitochondrial fission and apoptosis in triple-negative breast cancer cells by isorhamnetin and chloroquine. *J Exp Clin Cancer Res*. 2019;38(1):225. doi:10.1186/s13046-019-1201-4
76. Cocco S, Leone A, Roca MS, et al. Inhibition of autophagy by chloroquine prevents resistance to PI3K/AKT inhibitors and potentiates their antitumor effect in combination with paclitaxel in triple negative breast cancer models. *J Transl Med*. 2022;20(1):290. doi:10.1186/s12967-022-03462-z
77. Zhang Y, Cao Y, Sun X, et al. Chloroquine (CQ) exerts anti-breast cancer through modulating microenvironment and inducing apoptosis. *Int Immunopharmacol*. 2017;42:100–107. doi:10.1016/j.intimp.2016.11.027
78. Valdés-Abadía B, Morán-Zendejas R, Rangel-Flores JM, Rodríguez-Menchaca AA. Chloroquine inhibits tumor-related Kv10.1 channel and decreases migration of MDA-MB-231 breast cancer cells in vitro. *Eur J Pharmacol*. 2019;855:262–266. doi:10.1016/j.ejphar.2019.05.017
79. Saini H, Choudhary M, Sharma H, Chowdhury S, Mukherjee S, Chowdhury R. Chloroquine induces transitory attenuation of proliferation of human lung cancer cells through regulation of mutant P53 and YAP. *Mol Biol Rep*. 2023;50(2):1045–1058. doi:10.1007/s11033-022-08072-y
80. Maycotte P, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. *Autophagy*. 2012;8(2):200–212. doi:10.4161/auto.8.2.18554
81. Monma H, Iida Y, Moritani T, et al. Chloroquine augments TRAIL-induced apoptosis and induces G2/M phase arrest in human pancreatic cancer cells. *PLoS One*. 2018;13(3):e0193990. doi:10.1371/journal.pone.0193990
82. Wang FT, Wang H, Wang QW, et al. Inhibition of autophagy by chloroquine enhances the antitumor activity of gemcitabine for gallbladder cancer. *Cancer Chemother Pharmacol*. 2020;86(2):221–232. doi:10.1007/s00280-020-04100-5
83. Liu L, Han C, Yu H, et al. Chloroquine inhibits cell growth in human A549 lung cancer cells by blocking autophagy and inducing mitochondrial-mediated apoptosis. *Oncol Rep*. 2018;39(6):2807–2816. doi:10.3892/or.2018.6363
84. Liu F, Shang Y, Chen SZ. Chloroquine potentiates the anti-cancer effect of lidamycin on non-small cell lung cancer cells in vitro. *Acta Pharmacol Sin*. 2014;35(5):645–652. doi:10.1038/aps.2014.3
85. Liang L, Hui K, Hu C, et al. Autophagy inhibition potentiates the anti-angiogenic property of multikinase inhibitor anlotinib through JAK2/STAT3/VEGFA signaling in non-small cell lung cancer cells. *J Exp Clin Cancer Res*. 2019;38(1):71. doi:10.1186/s13046-019-1093-3
86. Luthman H, Magnusson G. HIgh efficiency polyoma DNA transfection of chloroquine treated cells. *Nucleic Acids Res*. 1983;11(5):1295–1308. doi:10.1093/nar/11.5.1295
87. Sasaki K, Tsuno NH, Sunami E, et al. Chloroquine potentiates the anti-cancer effect of 5-fluorouracil on colon cancer cells. *BMC Cancer*. 2010;10(1):370. doi:10.1186/1471-2407-10-370
88. Grasso S, Pereira GJS, Palmeira-Dos-Santos C, et al. Autophagy regulates Selumetinib (AZD6244) induced-apoptosis in colorectal cancer cells. *Eur J Med Chem*. 2016;122:611–618. doi:10.1016/j.ejmech.2016.06.043
89. Lu S, Gao J, Jia H, et al. PD-1-siRNA delivered by attenuated *Salmonella* enhances the antitumor effect of chloroquine in colon cancer. *Front Immunol*. 2021;12:707991. doi:10.3389/fimmu.2021.707991
90. Peng J, Zhou J, Sun R, et al. Dual-targeting of artesunate and chloroquine to tumor cells and tumor-associated macrophages by a biomimetic PLGA nanoparticle for colorectal cancer treatment. *Int J Biol Macromol*. 2023;244:125163. doi:10.1016/j.ijbiomac.2023.125163
91. Chen P, Luo X, Nie P, et al. CQ synergistically sensitizes human colorectal cancer cells to SN-38/CPT-11 through lysosomal and mitochondrial apoptotic pathway via p53-ROS cross-talk. *Free Radic Biol Med*. 2017;104:280–297. doi:10.1016/j.freeradbiomed.2017.01.033
92. Gills JJ, Lopiccolo J, Tsurutani J, et al. Nelfinavir, a lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo. *Clin Cancer Res*. 2007;13(17):5183–5194. doi:10.1158/1078-0432.CCR-07-0161
93. Johnson CE, Hunt DK, Wiltshire M, et al. Endoplasmic reticulum stress and cell death in mTORC1-overactive cells is induced by nelfinavir and enhanced by chloroquine. *Mol Oncol*. 2015;9(3):675–688. doi:10.1016/j.molonc.2014.11.005

94. Ovejero-Sánchez M, González-Sarmiento R, Herrero AB. Synergistic effect of Chloroquine and Panobinostat in ovarian cancer through induction of DNA damage and inhibition of DNA repair. *Neoplasia*. 2021;23(5):515–528. doi:10.1016/j.neo.2021.04.003
95. Santiago-O'Farrill JM, Weroha SJ, Hou X, et al. Poly(adenosine diphosphate ribose) polymerase inhibitors induce autophagy-mediated drug resistance in ovarian cancer cells, xenografts, and patient-derived xenograft models. *Cancer*. 2020;126(4):894–907. doi:10.1002/cncr.32600
96. Hwang JR, Kim WY, Cho YJ, et al. Chloroquine reverses chemoresistance via upregulation of $p21^{WAF1/CIP1}$ and autophagy inhibition in ovarian cancer. *Cell Death Dis*. 2020;11(12):1034. doi:10.1038/s41419-020-03242-x
97. Ovejero-Sánchez M, Rubio-Heras J, Vicente de la Peña MDC, et al. Chloroquine-induced DNA damage synergizes with nonhomologous end joining inhibition to cause ovarian cancer cell cytotoxicity. *Int J Mol Sci*. 2022;23(14):7518. doi:10.3390/ijms23147518
98. Tsai TF, Chang AC, Chen PC, et al. Autophagy blockade potentiates cancer-associated immunosuppression through programmed death ligand-1 upregulation in bladder cancer. *J Cell Physiol*. 2022;237(9):3587–3597. doi:10.1002/jcp.30817
99. Shirono Y, Bilim V, Anraku T, et al. Targeting pro-survival autophagy enhanced GSK-3β inhibition-induced apoptosis and retarded proliferation in bladder cancer cells. *Curr Oncol*. 2023;30(6):5350–5365. doi:10.3390/curroncol30060406
100. Tong H, Yin H, Hossain MA, et al. Starvation-induced autophagy promotes the invasion and migration of human bladder cancer cells via TGF-β1/Smad3-mediated epithelial-mesenchymal transition activation. *J Cell Biochem*. 2019;120(4):5118–5127. doi:10.1002/jcb.27788
101. Lin JF, Lin YC, Yang SC, et al. Autophagy inhibition enhances RAD001-induced cytotoxicity in human bladder cancer cells. *Drug Des Devel Ther*. 2016;10:1501–1513. doi:10.2147/DDDT.S95900
102. Kang M, Lee KH, Lee HS, et al. Concurrent Autophagy Inhibition Overcomes the Resistance of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Human Bladder Cancer Cells. *Int J Mol Sci*. 2017;18(2):321. doi:10.3390/ijms18020321
103. Quan Y, Lei H, Wahafu W, Liu Y, Ping H, Zhang X. Inhibition of autophagy enhances the anticancer effect of enzalutamide on bladder cancer. *Biomed Pharmacother*. 2019;120:109490. doi:10.1016/j.biopha.2019.109490
104. Zhao H, Cai Y, Santi S, Lafrenie R, Lee H. Chloroquine-mediated radiosensitization is due to the destabilization of the lysosomal membrane and subsequent induction of cell death by necrosis. *Radiat Res*. 2005;164(3):250–257. doi:10.1667/RR3436.1
105. Song YJ, Zhang SS, Guo XL, et al. Autophagy contributes to the survival of CD133+ liver cancer stem cells in the hypoxic and nutrient-deprived tumor microenvironment. *Cancer Lett*. 2013;339(1):70–81. doi:10.1016/j.canlet.2013.07.021
106. Xu F, Tautenhahn HM, Dirsch O, Dahmen U. Blocking autophagy with chloroquine aggravates lipid accumulation and reduces intracellular energy synthesis in hepatocellular carcinoma cells, both contributing to its anti-proliferative effect. *J Cancer Res Clin Oncol*. 2022;148(12):3243–3256. doi:10.1007/s00432-022-04074-2
107. Boone BA, Murthy P, Miller-Ocuin J, et al. Chloroquine reduces hypercoagulability in pancreatic cancer through inhibition of neutrophil extracellular traps. *BMC Cancer*. 2018;18(1):678. doi:10.1186/s12885-018-4584-2
108. Bhattarai SR, Muthuswamy E, Wani A, et al. Enhanced gene and siRNA delivery by polycation-modified mesoporous silica nanoparticles loaded with chloroquine. *Pharm Res*. 2010;27(12):2556–2568. doi:10.1007/s11095-010-0245-0
109. Maes H, Kuchnio A, Peric A, et al. Tumor vessel normalization by chloroquine independent of autophagy. *Cancer Cell*. 2014;26(2):190–206. doi:10.1016/j.ccr.2014.06.025
110. Degan S, May BL, Jin YJ, et al. Co-Treatment of Chloroquine and Trametinib Inhibits Melanoma Cell Proliferation and Decreases Immune Cell Infiltration. *Front Oncol*. 2022;12:782877. doi:10.3389/fonc.2022.782877
111. Gil D, Laidler P, Zarzycka M, Dulińska-Litewka J. Inhibition Effect of Chloroquine and Integrin-Linked Kinase Knockdown on Translation in Melanoma Cells. *Int J Mol Sci*. 2021;22(7):3682. doi:10.3390/ijms22073682
112. Cheng J, Zeidan R, Mishra S, et al. Structure-function correlation of chloroquine and analogues as transgene expression enhancers in nonviral gene delivery. *J Med Chem*. 2006;49(22):6522–6531. doi:10.1021/jm060736s
113. Golden EB, Cho HY, Jahanian A, et al. Chloroquine enhances temozolomide cytotoxicity in malignant gliomas by blocking autophagy. *Neurosurg Focus*. 2014;37(6):E12. doi:10.3171/2014.9.FOCUS14504
114. Balic A, Sørensen MD, Trabulo SM, et al. Chloroquine targets pancreatic cancer stem cells via inhibition of CXCR4 and hedgehog signaling. *Mol Cancer Ther*. 2014;13(7):1758–1771. doi:10.1158/1535-7163.MCT-13-0948
115. Sun CY, Zhang QY, Zheng GJ, Feng B. Autophagy and its potent modulators from phytochemicals in cancer treatment. *Cancer Chemother Pharmacol*. 2019;83(1):17–26. doi:10.1007/s00280-018-3707-4
116. Joshi P, Chakraborti S, Ramirez-Vick JE, et al. The anticancer activity of chloroquine-gold nanoparticles against MCF-7 breast cancer cells. *Colloids Surf B Biointerfaces*. 2012;95:195–200. doi:10.1016/j.colsurfb.2012.02.039
117. Rojas-Sanchez G, García-Miranda A, Montes-Alvarado JB, et al. Chloroquine induces ROS-mediated macrophage migration inhibitory factor secretion and epithelial to mesenchymal transition in ER-positive breast cancer cell lines. *J Mammary Gland Biol Neoplasia*. 2021;26(4):341–355. doi:10.1007/s10911-021-09503-5
118. Rebecca VW, Nicastri MC, Fennelly C, et al. PPT1 Promotes Tumor Growth and Is the Molecular Target of Chloroquine Derivatives in Cancer. *Cancer Discov*. 2019;9(2):220–229. doi:10.1158/2159-8290.CD-18-0706
119. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495–516. doi:10.1080/01926230701320337
120. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biol Int*. 2019;43(6):582–592. doi:10.1002/cbin.11137
121. Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov*. 2010;9(6):447–464. doi:10.1038/nrd3137
122. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*. 2006;25(34):4798–4811. doi:10.1038/sj.onc.1209608
123. Ashkenazi A. Targeting the extrinsic apoptosis pathway in cancer. *Cytokine Growth Factor Rev*. 2008;19(3–4):325–331. doi:10.1016/j.cytogfr.2008.04.001
124. Wang F, Gómez-Sintes R, Boya P. Lysosomal membrane permeabilization and cell death. *Traffic*. 2018;19(12):918–931. doi:10.1111/tra.12613
125. Luo S, Jiang L, Li Q, et al. Acrolein-induced autophagy-dependent apoptosis via activation of the lysosomal-mitochondrial pathway in EAhy926 cells. *Toxicol In Vitro*. 2018;52:146–153. doi:10.1016/j.tiv.2018.05.018
126. Kroemer G, Jäättelä M. Lysosomes and autophagy in cell death control. *Nat Rev Cancer*. 2005;5(11):886–897. doi:10.1038/nrc1738
127. Loeffler M, Kroemer G. The mitochondrion in cell death control: certainties and incognita. *Exp Cell Res*. 2000;256(1):19–26. doi:10.1006/excr.2000.4833

128. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. *Biochim Biophys Acta*. 2006;1757(5–6):509–517. doi:10.1016/j.bbabio.2006.04.029
129. Lopiccolo J, Kawabata S, Gills JJ, Dennis PA. Combining nelfinavir with chloroquine inhibits in vivo growth of human lung cancer xenograft tumors. *Vivo*. 2021;35(1):141–145. doi:10.21873/invivo.12241
130. Lee HY, Mohammed KA, Goldberg EP, Kaye F, Nasreen N. Cisplatin loaded albumin mesospheres for lung cancer treatment. *Am J Cancer Res*. 2015;5(2):603–615.
131. Agalakova NI. Chloroquine and Chemotherapeutic Compounds in Experimental Cancer Treatment. *Int J Mol Sci*. 2024;25(2):945. doi:10.3390/ijms25020945
132. Bouchard G, Therriault H, Geha S, et al. Stimulation of triple negative breast cancer cell migration and metastases formation is prevented by chloroquine in a pre-irradiated mouse model. *BMC Cancer*. 2016;16(1):361. doi:10.1186/s12885-016-2393-z
133. Shiratori H, Kawai K, Hata K, et al. The combination of temsirolimus and chloroquine increases radiosensitivity in colorectal cancer cells. *Oncol Rep*. 2019;42(1):377–385.
134. Schonewolf CA, Mehta M, Schiff D, et al. Autophagy inhibition by chloroquine sensitizes HT-29 colorectal cancer cells to concurrent chemoradiation. *World J Gastrointest Oncol*. 2014;6(3):74–82. doi:10.4251/wjgo.v6.i3.74
135. Wang F, Tang J, Li P, et al. Chloroquine enhances the radiosensitivity of bladder cancer cells by inhibiting autophagy and activating apoptosis. *Cell Physiol Biochem*. 2018;45(1):54–66. doi:10.1159/000486222
136. Briceño E, Reyes S, Sotelo J. Therapy of glioblastoma multiforme improved by the antimutagenic chloroquine. *Neurosurg Focus*. 2003;14(2):1–6. doi:10.3171/foc.2003.14.2.4
137. Xu R, Ji Z, Xu C, Zhu J. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers: a systematic review and meta-analysis. *Medicine*. 2018;97(46):e12912. doi:10.1097/MD.0000000000012912
138. Fong W, To KKW. Repurposing chloroquine analogs as an adjuvant cancer therapy. *Recent Pat Anticancer Drug Discov*. 2021;16(2):204–221. doi:10.2174/1574892815666210106111012
139. Sotelo J, Briceño E, López-González MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 2006;144(5):337–343. doi:10.7326/0003-4819-144-5-200603070-00008
140. Anand K, Niravath P, Patel T, et al. A phase II study of the efficacy and safety of chloroquine in combination with taxanes in the treatment of patients with advanced or metastatic anthracycline-refractory breast cancer. *Clin Breast Cancer*. 2021;21(3):199–204. doi:10.1016/j.clbc.2020.09.015
141. Horne GA, Stobo J, Kelly C, et al. A randomised phase II trial of hydroxychloroquine and imatinib versus imatinib alone for patients with chronic myeloid leukaemia in major cytogenetic response with residual disease. *Leukemia*. 2020;34(7):1775–1786. doi:10.1038/s41375-019-0700-9
142. Zeh HJ, Bahary N, Boone BA, et al. A randomized phase II preoperative study of autophagy inhibition with high-dose hydroxychloroquine and gemcitabine/nab-paclitaxel in pancreatic cancer patients. *Clin Cancer Res*. 2020;26(13):3126–3134. doi:10.1158/1078-0432.CCR-19-4042
143. Rosenfeld MR, Ye X, Supko JG, et al. A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy*. 2014;10(8):1359–1368. doi:10.4161/auto.28984
144. Rangwala R, Chang YC, Hu J, et al. Combined MTOR and autophagy inhibition: phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. *Autophagy*. 2014;10(8):1391–1402. doi:10.4161/auto.29119
145. Fei N, Wen S, Ramanathan R, et al. SMAD4 loss is associated with response to neoadjuvant chemotherapy plus hydroxychloroquine in patients with pancreatic adenocarcinoma. *Clin Transl Sci*. 2021;14(5):1822–1829. doi:10.1111/cts.13029
146. Malhotra J, Jabbour S, Orlick M, et al. Phase Ib/II study of hydroxychloroquine in combination with chemotherapy in patients with metastatic non-small cell lung cancer (NSCLC). *Cancer Treat Res Commun*. 2019;21:100158. doi:10.1016/j.ctarc.2019.100158
147. Boone BA, Bahary N, Zureikat AH, et al. Safety and Biologic Response of Pre-operative Autophagy Inhibition in Combination with Gemcitabine in Patients with Pancreatic Adenocarcinoma. *Ann Surg Oncol*. 2015;22(13):4402–4410. doi:10.1245/s10434-015-4566-4
148. AlMasri SS, Zenati MS, Desilva A, et al. Encouraging long-term survival following autophagy inhibition using neoadjuvant hydroxychloroquine and gemcitabine for high-risk patients with resectable pancreatic carcinoma. *Cancer Med*. 2021;10(20):7233–7241. doi:10.1002/cam4.4211
149. Vogl DT, Stadtmauer EA, Tan KS, et al. Combined autophagy and proteasome inhibition: a Phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy*. 2014;10(8):1380–1390. doi:10.4161/auto.29264
150. Haas NB, Appleman LJ, Stein M, et al. Autophagy inhibition to augment mTOR inhibition: a Phase I/II trial of everolimus and hydroxychloroquine in patients with previously treated renal cell carcinoma. *Clin Cancer Res*. 2019;25(7):2080–2087. doi:10.1158/1078-0432.CCR-18-2204
151. Rustogi A, Munshi A, Jalali R. Unexpected skin reaction induced by radiotherapy after chloroquine use. *Lancet Oncol*. 2006;7(7):608–609. doi:10.1016/S1470-2045(06)70763-X
152. Arnaout A, Robertson SJ, Pond GR, et al. A randomized, double-blind, window of opportunity trial evaluating the effects of chloroquine in breast cancer patients. *Breast Cancer Res Treat*. 2019;178(2):327–335. doi:10.1007/s10549-019-05381-y
153. Kimura T, Takabatake Y, Takahashi A, Isaka Y. Chloroquine in cancer therapy: a double-edged sword of autophagy. *Cancer Res*. 2013;73(1):3–7. doi:10.1158/0008-5472.CAN-12-2464
154. Periyasamy-Thandavan S, Jiang M, Wei Q, Smith R, Yin XM, Dong Z. Autophagy is cytoprotective during cisplatin injury of renal proximal tubular cells. *Kidney Int*. 2008;74(5):631–640. doi:10.1038/ki.2008.214
155. Komatsu M, Waguri S, Ueno T, et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol*. 2005;169(3):425–434. doi:10.1083/jcb.200412022
156. Mortensen M, Soilleux EJ, Djordjevic G, et al. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J Exp Med*. 2011;208(3):455–467. doi:10.1084/jem.20101145
157. Komatsu M, Waguri S, Chiba T, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006;441(7095):880–884. doi:10.1038/nature04723
158. Hara T, Nakamura K, Matsui M, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006;441(7095):885–889. doi:10.1038/nature04724

159. Nakai A, Yamaguchi O, Takeda T, et al. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med*. 2007;13(5):619–624. doi:10.1038/nm1574
160. Duan H, Liu Y, Gao Z, Huang W. Recent advances in drug delivery systems for targeting cancer stem cells. *Acta Pharm Sin B*. 2021;11(1):55–70. doi:10.1016/j.apsb.2020.09.016
161. Pelt J, Busatto S, Ferrari M, Thompson EA, Mody K, Wolfram J. Chloroquine and nanoparticle drug delivery: a promising combination. *Pharmacol Ther*. 2018;191:43–49. doi:10.1016/j.pharmthera.2018.06.007
162. Sun R, Shen S, Zhang YJ, et al. Nanoparticle-facilitated autophagy inhibition promotes the efficacy of chemotherapeutics against breast cancer stem cells. *Biomaterials*. 2016;103:44–55. doi:10.1016/j.biomaterials.2016.06.038
163. Nirk EL, Reggiori F, Mauthe M. Hydroxychloroquine in rheumatic autoimmune disorders and beyond. *EMBO Mol Med*. 2020;12(8):e12476. doi:10.15252/emmm.202012476
164. Plantone D, Koudriavtseva T. Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review. *Clin Drug Investig*. 2018;38(8):653–671. doi:10.1007/s40261-018-0656-y
165. Kondo N, Kuroda T, Kobayashi D. Cytokine networks in the pathogenesis of rheumatoid arthritis. *Int J Mol Sci*. 2021;22(20):10922. doi:10.3390/ijms222010922
166. Ren Y, Cui G, Gao Y. Research progress on inflammatory mechanism of primary Sjögren syndrome. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2021;50(6):783–794. doi:10.3724/zdxbyxb-2021-0072
167. Idborg H, Oke V. Cytokines as biomarkers in systemic lupus erythematosus: value for diagnosis and drug therapy. *Int J Mol Sci*. 2021;22(21):11327. doi:10.3390/ijms222111327
168. Horton CG, Pan ZJ, Farris AD. Targeting Toll-like receptors for treatment of SLE. *Mediators Inflamm*. 2010;2010:498980. doi:10.1155/2010/498980
169. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis*. 2003;3(11):722–727. doi:10.1016/S1473-3099(03)00806-5
170. Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. *Lancet Infect Dis*. 2006;6(2):67–69. doi:10.1016/S1473-3099(06)70361-9
171. Tsai WP, Nara PL, Kung HF, Oroszlan S. Inhibition of human immunodeficiency virus infectivity by chloroquine. *AIDS Res Hum Retroviruses*. 1990;6(4):481–489. doi:10.1089/aid.1990.6.481
172. Pardridge WM, Yang J, Diagne A. Chloroquine inhibits HIV-1 replication in human peripheral blood lymphocytes. *Immunol Lett*. 1998;64(1):45–47. doi:10.1016/S0165-2478(98)00096-0
173. Savarino A, Gennero L, Chen HC, et al. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. *AIDS*. 2001;15(17):2221–2229. doi:10.1097/00002030-200111230-00002
174. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun*. 2004;323(1):264–268. doi:10.1016/j.bbrc.2004.08.085
175. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J*. 2005;2(1):69. doi:10.1186/1743-422X-2-69
176. Yan Y, Zou Z, Sun Y, et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. *Cell Res*. 2013;23(2):300–302. doi:10.1038/cr.2012.165
177. Meo SA, Klonoff DC, Akram J. Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19. *Eur Rev Med Pharmacol Sci*. 2020;24(8):4539–4547. doi:10.26355/eurrev_202004_21038
178. Lei ZN, Wu ZX, Dong S, et al. Chloroquine and hydroxychloroquine in the treatment of malaria and repurposing in treating COVID-19. *Pharmacol Ther*. 2020;216:107672. doi:10.1016/j.pharmthera.2020.107672
179. Zhao B, Yang TF, Zheng R. Theory and reality of antivirals against SARS-CoV-2. *World J Clin Cases*. 2021;9(23):6663–6673. doi:10.12998/wjcc.v9.i23.6663
180. Kim EL, Wüstenberg R, Rübsam A, et al. Chloroquine activates the p53 pathway and induces apoptosis in human glioma cells. *Neuro Oncol*. 2010;12(4):389–400. doi:10.1093/neuonc/nop046
181. Chen D, Xie J, Fiskesund R, et al. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. *Nat Commun*. 2018;9(1):873. doi:10.1038/s41467-018-03225-9
182. Mukhopadhyay S, Mahapatra KK, Praharaj PP, Patil S, Bhutia SK. Recent progress of autophagy signaling in tumor microenvironment and its targeting for possible cancer therapeutics. *Semin Cancer Biol*. 2022;85:196–208. doi:10.1016/j.semcancer.2021.09.003