Quercetin in Hepatocellular Carcinoma

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Quercetin is a flavonoid present in fruits, vegetables and plants with beneficial effects in several human disorders, including liver cancer. The antioxidant and anti-inflammatory properties make quercetin an interesting drug to be evaluated in hepatocarcinoma (HCC), the major primary liver tumor with a high mortality rate. Moreover, increasing number of studies reported a high variety of antitumor actions which places quercetin as a promising antitumor agent, not only as single treatment but also improving current therapeutic options against advanced HCC.

Keywords: combined treatments ; encapsulation ; flavonoid ; hepatocarcinoma ; quercetin ; quercetin derivative

1. Introduction

Quercetin (3,3',4',5,7-pentahydroxy flavone) is one of the main components of the polyphenol family of flavonoids [1] and it is mostly present in fruits, vegetables and some plant-derived beverages, such as wine or tea [2]. This flavonoid has many beneficial properties on human health [2], being associated its biological activity with the presence of five hydroxyl groups on the ring structure [1]. A number of studies have investigated quercetin effects on cellular processes involved in different human pathologies [3][4]. Anti-inflammatory, antioxidant and anticancer activities are some of the mainly described quercetin mechanisms of action [1][2][5]. Besides, therapeutic potential of this flavonoid has been evaluated in a broad variety of human disorders, including diabetes, cardiovascular [3], neurodegenerative [3][4][6] and Alzheimer's diseases [6]; and positive actions on blood vessel pressure, intestinal microbiota and kidney disfunction [5], among others, were also related to quercetin efficacy.

Liver injury is largely caused by obesity or metabolic syndrome, in addition to high alcohol consumption [5][7]. Hepatocyte damage eventually contributes to the development of liver disorders including steatosis, alcoholic and non-alcoholic steatohepatitis which could cause non-alcoholic fatty liver disease (NAFLD), liver inflammation and hepatic fibrosis [5][7]. Hepatic chronic damage often leads to progression to liver cirrhosis and, in most cases, to hepatocarcinoma (HCC) [5][7]. In addition to the aforementioned beneficial effects, quercetin has been shown to exert multiple hepatoprotective actions through lipid biogenesis modulation, mitochondrial biogenesis activation [8] and the increase of cellular antioxidants and insulin sensitivity [5]. As part of its hepatoprotective ability, this flavonoid has demonstrated to reduce oxidative stress and inflammatory response in liver damage caused by alcohol and different toxic compounds (e.g. ethanol, metals and pesticides) [9]. Generation of an inflammatory and fibrotic microenvironment are key mechanisms produced in chronic-injured liver by hepatic stellate cells, and quercetin is able to abrogate its activation and modulate its polarization, restraining liver cells alteration [10]. Along with this, regulation of liver cell pathways involved in cell proliferation, differentiation and extracellular matrix synthesis is associated with quercetin-derived positive effects in the prevention of NAFLD [11][12] and liver fibrosis [7]. Some studies have also proved its beneficial activities against liver cirrhosis development and pulmonary associated complications [13][14], what makes quercetin a promising agent for the improvement of the outcomes in liver pathologies therapy [9].

HCC is the most common primary liver cancer and the sixth tumor with higher incidence, ranking as the fourth deadliest neoplasm worldwide [15]. Liver damage caused by different etiologic agents, mainly hepatitis C and B virus (HCV and HBV, respectively), contributes to HCC development through the stages of liver fibrosis and cirrhosis, which can take from years to decades [15]. Its complex pathogenesis and molecular heterogeneity hinder HCC early diagnosis making curative treatments not possible [15]. In these cases, systemic therapy is used, being available two tyrosine kinase inhibitors (TKIs), sorafenib and lenvatinib, in the first-line setting for advanced HCC [16]. Regardless its effectiveness, liver cancer cells are able to develop sorafenib resistance after sustained administration [17], where several TKIs (regorafenib and cabozantinib) and monoclonal antibodies (nivolumab, pembrolizumab and ramucirumab) have been recently approved [16]. Considering toxicity and adverse reactions caused by these chemotherapeutic agents, some investigations have focused on the study of antitumor effects of natural compounds against HCC, such as resveratrol, curcumin and melatonin [18][19][20].

2. Antitumor properties of quercetin as single agent against HCC

2.1. Antiproliferative activity of quercetin

Quercetin antitumor effects have been described in different cancer types, including HCC [1], being its antiproliferative effect widely observed in several researches [21][22][23][24][25][26][27][28][29][30][31][32][33][34][35][36][37][38][39]. It has been described that quercetin-derived inhibition of liver cancer cell growth could be mediated by the disruption of different pathways, including protein kinase B (Akt)/mammalian target of rapamycin (mTOR) [22][23][28], mitogen-activated protein kinase kinase 1 (MEK1)/extracellular signal-regulated kinase 1/2 (ERK1/2) [26][32] and Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling routes [21][34]. Another lesser known molecular pathways have also been altered by quercetin, including Src homology domain 2 containing tyrosine phosphatase-1/2 (SHP-1/2), transcription factor specificity protein 1 (Sp1), phosphatidyl inositol 3 kinase (PI3K) and protein kinase C (PKC) routes [28][30][34]. These findings evidence the high number of mechanisms of actions that are associated with the antiproliferative activity of quercetin.

2.2. Cell cycle modulation by quercetin

Otherwise, though cell cycle regulation is a common mechanism found to be altered in HCC cells, publications that have evaluated the effects of quercetin in this process show contradictory results. As common feature, quercetin was able to arrest cell cycle, either in G0/G1 or G2/M phases; however, modulation of cell cycle markers such as p53, p21, p27 and cyclin D1 by quercetin differs among the investigations performed [21][24][27][30][35][36][37][38].

2.3. Apoptosis induction by quercetin

Apoptosis has been clearly established as one of the mechanisms of quercetin-induced cell death in HCC [21][23][27][28][29][30][36][37][38][39][40], as it was demonstrated by the increase in proapoptotic proteins expression, such as Bax and cleaved caspases-3 and -9 [21][23][26][28][29][30][37][38][39][40], and the opposite trend in the levels of antiapoptotic proteins, for instance Bcl-2 and Mcl-1 [23][26][29][30][36][37][40]. Autophagy is a self-recycle process by which damaged cell components are degraded, and it has been associated with either prosurvival or antitumor role depending on the tumor cell context [21][41]. In this case, two studies observed that quercetin treatment promoted autophagy in HCC cells, being related to apoptosis induction and suppression of tumor progression [21][23].

2.4. Antiangiogenic effects of quercetin

Angiogenesis and metastasis are tightly associated with HCC progression and represent very common targeting processes in cancer treatment [15]; however, there is a lack of studies evaluating quercetin effects on cell migration and invasion ability [21]. It was reported that this flavonoid regulated the expression of epithelial and mesenchymal markers in favor of first ones, abrogating epithelial-to-mesenchymal transition (EMT) and invasiveness of HCC cells [21]. Similarly, another study revealed a greater antiangiogenic activity by combination of quercetin and sorafenib in an *in vivo* HCC model [42], which suggest a promising role of quercetin not only as cytostatic agent but also in preventing recurrence-associated processes of angiogenesis and invasion.

2.5. Other antitumor actions of quercetin

In addition to the described properties, quercetin has shown to act as an antioxidant agent in human HCC models [24][28][43], although some investigations denoted contradictory results [29]. In addition to the already mentioned processes, several studies have determined effects of quercetin in different specific mechanisms [26][32][44][45][46]. These include inhibition of chymotrypsin-like activity of the proteasome, involved in proteasomal regulation of cancer signaling pathways [26][32]; rise of intracellular labile zinc, which has second messenger molecule activities in tumor cells [46]; and modulation of microRNAs expression [45]. Curiously, another study published in 2018 employed quercetin to analyze adequacy of the cellular antioxidant (CAA) assay in HepG2 cells in order to determine the antioxidant activity of extracts from tree nuts [44]. Besides, beneficial effects of quercetin against HCC were evaluated focusing on the sensitization of chemoresistant liver cancer cells and its hepatoprotective effects [25][31][47][48].

Altogether, the wide variety of antitumor effects of quercetin along with its demonstrated efficacy against HCC cells, set this flavonoid as a promising therapeutic agent in the treatment of HCC.

3. Encapsulation strategy for the improvement of quercetin effects in HCC

Drug delivery systems have emerged as a novel mechanism of targeting cancer progression, enhancing drug efficacy through its encapsulation [49]. In this line, nanomedicine has developed numerous nanoparticles employing either organic- or inorganic-based nanocarriers [50]. Mesoporous silica nanoparticles (MSNs) conjugated with folic acid (FA) were designed to improve antitumor activity of quercetin. As inorganic-based carriers, gold-nanoparticles are drug delivery systems commonly chosen in nanomedicine [50]. Different publications synthesized poly(DL-lactide-co-glycolide) (PLGA)-loaded gold-quercetin nanoparticles leading to higher antiproliferative actions of quercetin in different HCC cell lines and in a xenograft tumor mouse model [49][51]. Otherwise, changes in cell cycle modulation were also observed after quercetin-nanocapsules administration and apoptosis induction was incremented in both *in vitro* and *in vivo* experiments [49][51]. In addition to cell proliferation and apoptosis, PLGA nanoparticles carrying quercetin generated morphologic alterations and inhibited migration ability of HCC cells [49][51]. Likewise, another group of researchers demonstrated greater cell death stimulation by encapsulating quercetin into PLGA nano-prototypes decorated with chitosan (CS) and polyethylene glycol (PEG) in HepG2 cells [52].

Some studies have also evaluated lipid-based formulations as quercetin-encapsulation strategies [53][54]. Solid lipid nanoparticles (SLNs) containing three sterol variables (cholesterol, stigmastanol and stigmasterol) were designed and evaluated, rising quercetin-derived cell viability reduction [54]. Similarly, the employment of methoxy-poly(ethylene glycol)-b-oligo(e-caprolactone), mPEG750-b-OCL-Bz micelles to co-encapsulate quercetin and superparamagnetic iron oxide nanoparticles (SPIONs) denoted lower proliferative capacity, morphological changes and cell cycle arrest in G0/G1 phase in an HCC model [53].

Even though few studies analyzed quercetin nanoencapsulation as a drug delivery system in liver cancer cells, it has arisen as a novel therapeutic approach which could improve quercetin properties against HCC progression by specifically targeting tumor and increasing drug cellular uptake.

4. Synergistic effects through quercetin combination against HCC

Although a great number of antitumor properties of quercetin have been established in HCC treatment mainly in cell line but also in animal models, some researchers have focused on searching for synergistic combinations with this flavonoid with the aim of improving its effectiveness against liver cancer [33][34][35][40][36][37][38][39][55][56]. Enhanced properties with the well-stablished first-line drug sorafenib was demonstrated in several HCC cell lines by reducing its half-maximal inhibitory concentration (IC50) [36] and improving its tumor suppression activity [33]. Similarly, quercetin was able to raise antiproliferative action of several molecules, such as interferon-a (IFN-a) [34]; an oncolytic adenovirus expressing tumor necrosis factor-related apoptosis inducing ligand (ZD55-TRAIL) [56]; two derivatives of the organic compound maleic anhydride (3’5’-dimaleamylbenzoic acid and 3’5’-dimaleimylbenzoic acid) [35]; the chemotherapeutic drugs celecoxib [40], 5-fluorouracil (5-FU) [37] and cisplatin [38]; and the CDK inhibitor roscovitine [39]. Results reported quercetin-derived alterations in cell morphology when combined with roscovitine [39] as well as growth inhibition and cell cycle arrest after cisplatin and two maleic anhydride derivatives co-administration [35][38]. A higher quercetin-associated apoptosis was also observed when administered with the following compounds, ZD55-TRAIL [56], two maleic anhydride derivatives [35], celecoxib [40], cisplatin [38] and roscovitine [39].

Regulation of oxidative stress and ROS production by quercetin was also altered after addition of 3’5’-dimaleamylbenzoic acid and 3’5’-dimaleimylbenzoic acid, showing an increase in the reactive oxygen species (ROS) accumulation [35]. On the other hand, effects of quercetin and dasatinib combination in cell senescence were assessed in HCC cells, which showed inability to prevent doxorubicin-induced senescence [55].

It should be mentioned that two independent studies put together both quercetin combination and encapsulation strategies and evaluated its cytotoxic actions both *in vitro* and *in vivo* models [42][57]. Lactoferrin shell-oily core nanocapsules coupled with lactobionic acid (LA) or glycyrrhetinic acid (GA) were designed for targeted delivery of both quercetin and sorafenib, showing greater antitumor effects in HepG2 cell line and HCC-bearing mice [42]. Similar results were described with arginine-glycineaspartic acid (RGD)-modified lipid-coated nanoparticles loaded with quercetin and sorafenib using HepG2 cells and a mouse model of HCC [57].

Globally, co-treatment strategy of quercetin with different compounds may enhance its effectiveness by raising mainly its antiproliferative and proapoptotic effects and leading to improve quercetin single-therapy properties against HCC.

5. Effects of quercetin derivatives as treatment in HCC

Quercetin glycosides are one of the main quercetin naturally occurring forms, what makes them interesting compounds for cancer treatment [58]. This led several researchers to analyze effects of quercetin derivatives in different tumors, including HCC [43][58][59][60]. In this line, quercetin-3-*O*-glucoside (Q3G) and six long chain fatty acid esters of Q3G (stearic acid ester, oleic acid ester, linoleic acid ester, alpha-linoleic acid ester, eicosapentaenoic acid ester and docosahexanoic acid ester) displayed antiproliferative actions, cell cycle arrest and apoptosis induction in *in vitro* models of HCC [58][60]. On the other hand, 3,4-dihydroxyphenylacetic acid (DOPAC), a catabolite of some quercetin glycosides produced by colonic microflora, has been shown to modulate oxidative stress enzymes and to protect against acetaldehyde damage [59]. In the same way, both 3'-*O*-methyl quercetin (3'MQ) and quercetin-3-*O*-glucuronide (Q3GA) exhibited antioxidant properties by reverting ethanol-derived ROS accumulation [43].

Bioactive compounds derived from quercetin have been shown to abrogate cancer progression in liver cancer cells; nonetheless, a greater number of studies would be needed to search for more quercetin derivatives and study underlaying mechanisms of its antitumor action against HCC cells.

Main findings here described reporting quercetin effects in both *in vitro* and *in vivo* models are summarized in Table 1 and Table 2, respectively.

Table 1. Basic characteristics of *in vitro* studies using quercetin in single, encapsulated, combined or derived forms in HCC.

First author, Year of publication	Quercetin administration strategy	Cell line	General effects	Molecular alterations

Wu, 2019 [21]	Quercetin	LM3 cells	Cell viability reduction Apoptosis induction Cell cycle arrest at S and G2/M phases Autophagy induction Cell migration and invasion suppression Morphological changes	↑ Early stage apoptotic cells ↓ PCNA mRNA and protein levels ↑ Bax mRNA and protein levels ↓ Colony formation ↑ Fluorescence in TUNEL staining ↓ G0/G1 phase cells and ↓ S and G2/M phase cells ↓ Cyclin B1 protein expression ↑ E-cadherin and ↓ vimentin and MMP-9 mRNA and protein levels ↓ N-cadherin protein expression ↓ Invasiveness (Transwell invasion assay) ↓ Migrating cells (Wound-healing assay) ↑ LC3 mRNA and protein levels ↑ Beclin1 protein expression ↓ p62 mRNA and protein levels ↓ p-STAT3 protein expression ↑ LC3 protein levels decreased by IL-6 ↓ PCNA and MMP-9 protein levels enhanced by IL-6 ↓ Wound healing speed
Wu, 2019 [22]	Quercetin	SMMC-7721, BEL-7402 HCC cells LO2 normal liver cells	Proliferation suppression of HCC cell lines No cytotoxic for normal hepatic cells Glycolysis inhibition	↓ Glucose uptake and lactate production ↑ 2-DG-derived cell growth inhibition ↓ HK2 mRNA and protein expression ↓ p-Akt/Akt and p-mTOR/mTOR rates

Ji, 2019 [23]	Quercetin	SMMC-7721 and HepG2 HCC cells LO2 normal hepatic cells	Cell growth inhibition in HCC cell lines Absence of antiproliferation effect in LO2 cells Induction of autophagy Apoptosis increase	↑ In both tumor cell lines: ↑ LC3A/LC3B-II and Beclin1 protein levels ↓ p62 protein expression In SMMC-7721: ↑ Autophagosomes and autolysosomes In all cell lines: ↓ p-Akt, p-mTOR, p-p70S6K and p-4EBP1 protein levels ↑ p-JNK, p-ERK1/2 and p-p38 MAPK protein expression ↑ Apoptotic cells percentage ↑ Bax and cleaved caspase-3 protein levels ↓ Bcl-2 protein expression
Jeon, 2019 [24]	Quercetin	HepG2, HuH7, PLC/PRF-5 and Hep3B cells	Proliferation inhibition (in HepG2, PLC/PRF-5 and Hep3B cells) ROS levels reduction (in HepG2 cells) Morphological alterations	Only in HepG2 cell line: ↑ p53 and HO-1 protein expression ↓ Cyclin A and CHK1 protein levels No variation in cyclin E and SOD1 protein expression
Chen, 2018 [25]	Quercetin	BEL-7402 HCC cells Multidrug resistant cell line BEL/5-FU	Increase of 5-FU, MMC and ADR chemosensitivity in BEL/5-FU cells	Only in BEL/5-FU cell line: ↓ IC50 of 5-FU, MMC and ADR ↓ ABCB1, ABCC1 and ABCC2 mRNA levels ↑ Rh123 accumulation Inhibition of ABCC2 function ↑ ADR accumulation In both cell lines: ↓ FZD7, b-catenin (nuclear and cytoplasmic), ABCB1, ABCC1 and ABCC2 mRNA and protein expression

Ding, 2018 [26]	Quercetin	HepG2 HCC cell line	Decrease of cell viability Apoptosis induction Inhibition of chymotrypsin-like activity	↑ TUNEL-positive cells ↑ Cleaved caspase-3, cleaved PARP and Bax protein expression ↓ Bcl-2 protein levels ↓ Chymotrypsin-like activity No changes in trypsin-like and caspase-like activities ↑ p-p38 MAPK and JNK protein expression ↓ p-ERK1/2 protein levels ↓ Protein expression of b1, b2 and b5 proteasomal subunits
Kellet, 2018 [44]	Quercetin	HepG2 cells	Antioxidant activity	↑ CAA unit dose dependent
Shaalan, 2018 [45]	Quercetin	HuH7 cell line	-	↑ miR-1275 mRNA levels ↓ IGF2BP1 and IGF2BP3 mRNA expression
Pi, 2016 [27]	Quercetin	HepG2 cells	Suppression of cell proliferation Cell cycle arrest at G2/M phase Apoptosis increase Disruption of mitochondrial membrane potential Morphological alterations Changes in surface ultrastructure	↑ G2/M cell population ↑ Early apoptotic, late apoptotic and necrotic cells ↓ Fluorescence signal of Rh123 ↑ F-actin filaments aggregation in apoptotic cells ↑ Particle size on HepG2 membrane ↑ Surface root-mean-squared and surface average roughness ↑ Cell stiffness and Young's modulus
Maurya, 2015 [28]	Quercetin	HepG2 cells	Antiproliferative activity Morphological changes	↓ ROS generation and PKC activity ↓ p-p85α, p-PKC, PKCα, COX-2 protein levels ↑ p53 protein expression and Bax mRNA levels

Zhang, 2015 [29]	Quercetin	HepG2 cells	Cell viability inhibition Induction of apoptosis	↑ Chromatin condensation and nuclei fragmentation into oligonucleosomes ↑ PIG3 mRNA and protein expression ↑ Early apoptotic cells ↑ ROS accumulation ↓ Mitochondrial membrane potential ↓ Mitochondrial cytochrome c and Bcl-2 protein expression ↑ Cytosolic cytochrome c, Bax and activated caspases -9 and -3
Lee, 2015 [30]	Quercetin	HepG2 cells	Decrease in cell viability Apoptosis induction	↑ Nuclear condensation and fragmentation ↑ Early and late apoptotic cells ↓ Sp1 mRNA and protein levels ↑ p21, p27, Bax, cleaved caspase-3 and cleaved PARP protein expression ↓ Cyclin D1, Mcl-1, survivin and Bcl-xL protein levels
Dabbagh-Bazarbachi, 2014 [46]	Quercetin	Mouse hepatoma Hepa 1-6 cell line	Augmented cytoplasmic labile zinc High ionophore activity	↑ FluoZin-3-detectable zinc ↑ Fluorescence signal of FluoZin-3
Kozics, 2011 [31]	Quercetin	HepG2 cells	Reduction of cell proliferation	↓ B(a)P-induced micronuclei formation and DNA damage
Oliva, 2011 [47]	Quercetin	Cederbaum's CYP2E1 overexpressing HepG2 cell line	Decrease of ethanol-derived oxidative stress	↓ MDA, 4-HNE and carbonyl protein levels augmented by ethanol ↓ Ethanol-induced glutathione peroxidase 4 and SOD2 mRNA expression ↓ Gadd45b mRNA levels ↑ Nrf2 protein levels reduced by ethanol
Choi, 2010 [48]	Quercetin	HepG2 cells	Reduction of the AFB_1 antiproliferative effect	↓ ROS accumulation generated by AFB_1 ↑ AFB_1-reduced GSH levels

Granado-Serrano, 2010 [32]	Quercetin	HepG2 cells	Cell proliferation suppression	↓ NF-kB and p65 nuclear translocation, NF-kB DNA-binding activity ↓ p-IkBa and IKKa protein expression ↓ Chymotrypsin-like activity No changes in trypsin-like activity ↑ DNA-binding activity of AP-1 ↑ Nuclear c-Jun levels
Srisa-nga, 2019 [53]	Quercetin encapsulation (Quercetin-SPION-loaded micelles)	HepG2.2.15 cell line	Suppression of cell growth Morphological alterations	↑ G0/G1 and ↓ G2/M phase cells
AbouAitah, 2018 [50]	Quercetin encapsulation (FA-conjugated MSNs)	HepG2 cells	Increased antiproliferative activity	↑ Antioxidant effect ↑ Inhibition of ABTS.+ radical formation
Abd-Rabou, 2017 [52]	Quercetin encapsulation (CS and PEG-decorated PLGA nano-prototypes)	HepG2 cells	Cell viability reduction Apoptosis induction	↓ Quercetin IC50 ↑ Late apoptotic and necrotic cells

Ren, 2017 [49]	Quercetin encapsulation (PLGA-loaded gold-quercetin nanoparticles)	MHCC97H, Hep3B, HCCLM3 and BEL-7402 HCC cell lines	Decreased cell proliferation Only in MHCC97H cell line: Morphological alterations Reduction of cell migration ability Apoptosis increase	Only in MHCC97H line: ↓ Colony formation ↑ Cell-to-cell adhesions and ↓ filopodia generation and cell spreading ↓ Migrating cells ↑ P-27 protein levels ↓ c-Myc, cyclin D1, CDK1, MMP-7 and b-catenin protein expression ↑ Apoptotic cell number ↑ Cleaved caspases -9 and -3 protein levels ↑ Cytochrome c release to cytoplasm ↓ hTERT and AP-2β mRNA and protein expression ↓ hTERT promoter-binding activity of AP-2β ↓ COX-2 protein expression ↓ Binding activity of p50 on COX-2 promoter ↓ p-IKKa and p-IkBa protein levels ↑ NF-kB and p50 cytoplasm translocation from nuclei ↓ p-Akt and p-ERK1/2 protein levels

Bishayee, 2015 [51]	Quercetin encapsulation (PLGA-loaded gold-quercetin nanoparticles)	HepG2 cells	Inhibition of cell proliferation Growth rate reduction Apoptosis stimulation Morphological changes	Alteration of B-conformation of DNA ↓ p-Akt protein expression ↑ sub G-phase cells and ↓ S-phase cells ↑ p21 protein levels ↓ CDK1 and cyclin D1 protein expression ↓ HDAC activity and HDAC1/2 protein levels ↑ ROS formation ↑ rac-1 activity and later returned to basal levels ↑ Depolarization of mitochondrial membrane ↑ Bax translocation to the mitochondrial outer membrane ↑ Cytochrome c release to cytosol ↑ Generation of DNA damage ↓ Mcl-1, Bcl-2 and Bcl-xL protein levels ↑ Apaf1, caspases -9 and -3, and cleaved PARP protein expression
Varshosaz, 2013 [54]	Quercetin encapsulation (SLNs containing cholesterol, stigmastanol or stigmasterol)	HepG2 cells	Cell growth inhibition (the highest with cholesterol)	-
Kovacovicova, 2018 [55]	Quercetin combined with dasatinib	HepG2 and HuH7 cell lines	No senolytic activity exhibited	No effects in β-galactosidase activity No protein expression alteration of the senescence markers p16 and γH2A.X
Bahman, 2018 [33]	Quercetin	HepG2 and Hep3B cells	Antiproliferative effect	-
	Quercetin combined with sorafenib		Suppression of cell proliferation	-

Zou, 2018 [56]	Quercetin combined with ZD55-TRAIL	SMMC-7721, HepG2 and HuH7 cell lines	Decrease of cell proliferation Apoptosis induction	↑ Apoptotic bodies, nuclear fragmentation and chromatin condensation ↑ Cleaved caspases -9 and -3, cleaved PARP, Bid and Bax protein expression ↓ Bcl-2 and FLIP protein levels ↓ IκBα, p65 and p50 protein expression
Igbe, 2017 [34]	Quercetin	HepG2 and HuH7 HCC cell lines	Inhibition of cell viability	↓ SHP-1 and SHP-2 protein expression in HepG2 cells
	Quercetin combined with IFN-a		Increased cell growth inhibition in both HCC cell lines	Only in HepG2 cell line: ↓ SHP-2 protein expression ↑ p-STAT1, p-Jak1 and p-Tyk2 protein levels ↑ ISRE reporter expression ↑ 2’,5’-OAS and PKR mRNA levels ↓ Colony formation ↓ Cyclin D1 protein expression

Carrasco-Torres, 2017 [35]	Quercetin	HuH7 and HepG2 HCC cells	Antiproliferative effect Cell cycle arrest at G0/G1 phase	↑ G0/G1 cell population ↓ ROS levels and oxidized glutathione levels ↑ Reduced glutathione and GSH/GSSG index ↑ Nuclear condensation ↑ Pro-caspase-9 and cleaved caspases -9 and -3 protein expression
	Quercetin combined with 3’5’-dimaleamylbenzoic acid or 3’5’-dimaleimylbenzoic acid		Cell viability reduction Cell cycle arrest at S phase Antioxidant activity Apoptosis induction	In both cell lines: ↓ G2/M-phase and ↑ S-phase populations ↓ Reduced and oxidized glutathione levels and GSH/GSSG index in both cell lines (maleic anhydride derivative + quercetin) ↑ Nuclear condensation, degradation of actin and DNA ↑ Pyknotic nuclei number and TUNEL-positive cells ↑ Pro-caspase-9 and cleaved caspases -9 and -3 protein expression In HuH7 line: ↓ ROS levels In HepG2 line: ↓ ROS levels (quercetin + maleic anhydride derivative) ↑ ROS levels (maleic anhydride derivative + quercetin) ↑ Reduced glutathione levels and de novo glutathione synthesis (quercetin + maleic anhydride derivative)
Yu, 2017 [40]	Quercetin combined with celecoxib	HepG2 and HuH7 cell lines	Antiproliferative effect Apoptosis induction	↑ DNA fragmentation ↑ Bax protein expression ↓ Bcl-2 protein levels

Brito, 2016 [36]	Quercetin	HepG2, HuH7 and Hep3B2.1-7 HCC cell lines	Inhibition of cell growth and survival Apoptosis increase Cell cycle arrest	↑ Apoptotic and necrotic cells ↑ Bax/Bcl-2 ratio ↑ G0/G1 and G2/M cell population in HepG2 and HuH7 ↓ S phase cells in all cell lines ↓ p53 protein expression in HepG2 and HuH7 cells ↑ DNA damage ↑ Membrane expression of GLUT-1 ↓ Cytoplasmic expression of GLUT-1 in HepG2 and HuH7 cells ↓ ^{18}F-FDG uptake
	Quercetin combined with sorafenib		Decrease in sorafenib IC50	-
Dai, 2016 [37]	Quercetin	HepG2 and SMMC-7721 HCC cells	Suppression of cell proliferation Cell cycle arrest at G0/G1 phase Apoptosis increase	↑ G0/G1 phase and ↓ S phase cell population ↑ Bax and Bad protein expression ↓ Bcl-2 and survivin protein levels
	Quercetin combined with 5-FU		Rise of 5-FU antiproliferative effects Higher apoptotic activity	-
Zhao, 2014 [38]	Quercetin	HepG2 cells	Inhibition of cell survival Apoptosis induction G1-phase arrest of cell cycle	↑ Cleaved caspase-3 and cleaved PARP protein levels ↑ p21, p53 and p16 protein expression ↑ G1-phase cells and ↓ S-phase cells ↑ sub-G1 cell population
	Quercetin combined with cisplatin		Increased growth inhibitory action Greater apoptotic effects	↑ Cleaved caspase-3 and cleaved PARP protein levels ↑ p21 and p53 protein levels

Sharma, 2011 [39]	Quercetin	HepG2 and Hep3B cell lines	Reduced cell survival Morphological changes Apoptosis induction	↑ Apoptotic bodies ↑ p53 protein expression in HepG2 cells ↓ Pro-caspase-9 and ↑ caspase-9 protein levels in HepG2 cells
	Quercetin combined with roscovitine		Augmented cell proliferation inhibition Morphological alterations Apoptosis stimulation	↓ Cell density ↑ Floating cells number and apoptotic bodies ↓ p-Akt, Bcl-2 and pro-caspases -9 and -3 protein expression ↓ Bcl-2/Bax ratio and ↑ Caspases -9 and -3 protein levels
Abdelmoneem, 2019 [42]	Co-encapsulated quercetin and sorafenib (LF-coated, LA/LF-coated or GA/LF-coated nanocapsules)	HepG2 cells	Higher antitumoral efficacy of quercetin and sorafenib Cell viability suppression	↓ IC50 of quercetin and sorafenib ↓ Combination index ↑ Dose reduction index of quercetin and sorafenib ↑ Cellular uptake of both drugs
Wang, 2016 [57]	Co-encapsulated quercetin and sorafenib (RGD-modified lipid-coated nanoparticles)	HepG2 cells	Reduced cell proliferation	↓ IC50 of quercetin and sorafenib

Lee, 2017 [43]	Quercetin	HepG2 cells	Reduced antiproliferative action of ethanol Antioxidant activity	Reversal of ethanol effects: ↓ ROS formation ↓ MDA levels ↑ GSH, SOD and CAT levels ↑ HO-1 and nuclear Nrf2 protein expression ↑ AP-1 activity
	3'MQ		Lower ethanol-induced cell viability inhibition Antioxidant activity	Reversal of ethanol effects: ↓ ROS formation ↑ SOD and CAT levels ↑ HO-1 and nuclear Nrf2 protein expression ↑ AP-1 activity
	Q3GA		Reversion of proliferation suppression induced by ethanol Antioxidant activity	Reversal of ethanol effects: ↓ ROS formation and GSH, SOD and CAT levels ↑ HO-1 and nuclear Nrf2 protein expression ↑ AP-1 activity
Liu, 2017 [59]	DOPAC	Mouse hepatoma Hepa1c1c7 cell line	Reduced acetaldehyde-derived cell growth inhibition	↑ ALDH activity ↑ ALDH1A1, ALDH2 and ALDH3A1 mRNA and protein levels ↑ Nrf2 and AhR total and nuclear protein expression ↓ NF-kB nuclear expression

Sudan, 2015 [60]	Six Q3G esters: Stearic acid ester Oleic acid ester Linoleic acid ester Alpha-linoleic acid ester Eicosapentaenoic acid ester Docosahexanoic acid ester	HepG2 HCC cells and normal hepatocytes	Higher cell viability of normal hepatocytes In HepG2 cells: Cell proliferation decrease Morphology changes Apoptosis induction Activity as catalytic inhibitor by DNA relaxation activity blockade	In HepG2 cells: ↓ HepG2 cell number ↑ DNA fragmentation ↑ Caspase-3 activity ↑ S and G2/M cell population ↓ G0/G1-phase cells No stabilization of topoisomerase II cleavage complexes and no formation of single linear DNA ↑ Supercoiled DNA intensity
Sudan, 2014 [58]	Q3G	HepG2 cell line	Cell growth suppression S-phase arrest of cell cycle Morphology alterations Apoptosis induction Catalytic inhibitor action by DNA relaxation activity inhibition	↑ S-phase and ↓ G0/G1 cell percentage ↑ DNA fragmentation ↑ Caspase-3 activity ↑ Apoptotic and necrotic cells No stabilization of topoisomerase II cleavage complexes and no formation of single linear DNA ↑ Supercoiled DNA intensity

^{18}F-FDG: fluorine-18 fluorodeoxy-glucose; 2’5’-OAS: 2’5’ oligoadenylate synthetase; 2-DG: 2-deoxy-D-glucose; 3’MQ: 3’-*O*-methyl quercetin; 4-HNE: 4-hydroxynonenal; 4EBP1: eukaryotic translation initiation factor 4E-binding protein 1; 5-FU: 5-fluorouracil; ABCB1: ATP-binding cassette subfamily B member 1; ABCC1: ATP-binding cassette subfamily C member 1; ABCC2: ATP-binding cassette subfamily C member 2; ABTS.+: radical cations of 2,2’-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) diammonium salt; ADR: doxorubicin; AFB_1: aflatoxin B_1; AhR: aryl hydrocarbon receptor; Akt: protein kinase B; ALDH: aldehyde dehydrogenase; ALDH1A1: aldehyde dehydrogenase 1 member A1; ALDH2: aldehyde dehydrogenase 2; ALDH3A1: aldehyde dehydrogenase 3 member A1; AP-1: transcription factor AP-1; Apaf1: apoptotic protease-activating factor 1; B(a)P: benzo[a]pyrene; Bad: Bcl-2-associated agonist of cell death; Bax: Bcl-2-associated X; Bcl-xL: Bcl-2-like protein 1; Bid: BH3-interacting domain death agonist; CAA: cellular antioxidant activity; CAT: catalase; CDK1: cyclin-dependent kinase 1; CHK1: checkpoint kinase 1; COX-2: cyclooxygenase-2; CS: chitosan; DOPAC: 3,4-dihydroxyphenylacetic acid; ERK1/2: extracellular signal-regulated kinase 1/2; FA: folic acid; FLIP: FLICE-like inhibitory protein; FZD7: Frizzled homolog protein 7; GA: glycyrrhetinic acid; Gadd45b: growth arrest and DNA damage-inducible protein GADD45 beta; GLUT-1: glucose transporter type 1; GSH: glutathione; GSSG: oxidized glutathione; HCC: hepatocarcinoma; HDAC: histone deacetylase; HK2: hexokinase-2; HO-1: heme oxygenase-1; hTERT: telomerase reverse transcriptase; IC50: half-maximal inhibitory concentration; IFN-a: interferon-a; IGF2BP1: insulin-like growth factor-2 binding protein 1; IGF2BP3: insulin-like growth factor-2 binding protein 3; IkBa: nuclear factor-kB inhibitor a; IKKa: inhibitor of nuclear factor-kB kinase subunit a; IL-6: interleukin 6; ISRE: interferon-sensitive response element; Jak1: Janus kinase 1; JNK: c-Jun N-terminal kinase; LA: lactobionic acid; LC3: microtubule-associated protein 1 light chain 3; LC3A: microtubule-associated protein 1A/1B light chain 3A; LC3B-II: microtubule-associated protein 1A/1B light chain 3B; LF: lactoferrin; MDA: malondialdehyde; Mcl-1: induced myeloid leukemia cell differentiation protein; MMC: mitomycin; MMP-7: matrix metalloproteinase-7; MMP-9: matrix metalloproteinase-9; MSNs: mesoporous silica nanoparticles; mTOR: mammalian target of rapamycin; NF-kB: nuclear factor-kB; Nrf2: nuclear factor erythroid 2-related factor 2; p38 MAPK:

mitogen-activated protein kinase p38; p62: sequestosome-1; p70S6K: ribosomal protein S6 kinase beta-1; PARP: poly(ADP-ribose) polymerase; PCNA: proliferating cell nuclear antigen; PEG: polyethylene glycol; PIG3: p53-inducible gene 3; PKC: protein kinase C; PKR: RNA-activated protein kinase; PLGA: poly(DL-lactide-co-glycolide); Q3G: quercetin-3-*O*-glucoside; Q3GA: quercetin-3-*O*-glucuronide; RGD: arginine-glycineaspartic acid; Rh123: rhodamine 123; ROS: reactive oxygen species; SHP-1: Src homology domain 2 tyrosine phosphatase-1; SHP-2: Src homology domain 2 containing tyrosine phosphatase-2; SLNs: solid lipid nanoparticles; SOD: superoxide dismutase; SOD1: superoxide dismutase 1; SOD2: superoxide dismutase 2; Sp1: specificity protein 1; SPION: superparamagnetic iron oxide nanoparticles; STAT1: signal transducer and activator of transcription 1; STAT3: signal transducer and activator of transcription 3; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling; Tyk2: non-receptor tyrosine-protein kinase 2; ZD55-TRAIL: oncolytic adenovirus expressing tumor necrosis factor-related apoptosis inducing ligand.

Table 2. Basic characteristics of *in vivo* studies using quercetin in single, encapsulated, combined or derived forms in HCC.

Wu, 2019 [21]	Quercetin	Nude mice subcutaneously injected with LM3 HCC cells	Tumor growth inhibition	↓ Tumor volume (70% *vs* control) ↓ Mouse weight and tumor volume ↑ Necrosis ↑ TUNEL-positive cells ↓ PCNA protein levels ↑ Bax and Beclin1 protein levels
Wu, 2019 [22]	Quercetin	SMMC-7721 xenograft mouse model	Tumor growth inhibition	↓ Tumor size ↓ HK2 and Ki67 protein expression ↓ p-Akt and p-mTOR protein levels
Ji, 2019 [23]	Quercetin	Nude mice subcutaneously injected with SMMC-7221 HCC cells	Suppression of tumor growth Apoptosis and autophagy induction	↓ Tumor weight and volume ↑ LC3A/LC3B and ↓ p62 protein levels ↑ Necrosis and TUNEL staining ↑ Bax and cleaved caspase-3 protein levels ↓ Bcl-2 protein expression
Ren, 2017 [49]	Quercetin encapsulation (PLGA-loaded gold-quercetin nanoparticles)	MHCC97H xenograft mouse model	Suppression of tumor growth and progression Apoptosis increase	↓ Tumor weight and volume ↓ AP-2β and COX-2 protein levels ↑ TUNEL-positive cells ↓ Cleaved caspases -9 and -3, cytoplasmic cytochrome c, p-IKKα, p-IκBα, p-NF-kB, p50, hTERT, p-Akt, Raf, and p-ERK1/2 protein expression

Kovacovicova, 2018 [55]	Quercetin combined with dasatinib	Mice subcutaneously injected with HuH7 cells	Absence of tumor growth inhibition	-
Zou, 2018 [56]	Quercetin combined with ZD55-TRAIL	HuH7 xenograft mouse model	Tumor growth inhibition	↓ Tumor volume
Dai, 2016 [37]	Quercetin	Nude mice subcutaneously injected with HepG2 HCC cells	Decreased tumor progression	↓ Tumor volume
	Quercetin combined with 5-FU		Higher tumor growth inhibition	↓ Tumor volume
Abdelmoneem, 2019 [42]	Co-encapsulated quercetin and sorafenib (LF-coated, LA/LF-coated or GA/LF-coated nanocapsules)	DEN-induced HCC in a rat model	Antiangiogenic activity Apoptosis induction Liver weight reduction	↓ NF-kB and TNF-α mRNA expression ↓ VEGF and Ki67 protein expression ↑ Caspase-3 protein expression ↓ ALT levels by LF-coated nanocapsules ↓ ALT, AST and RLW levels by LA/LF-coated and GA/LF-coated nanoparticles Improved histological features
Wang, 2016 [57]	Co-encapsulated quercetin and sorafenib (RGD-modified lipid-coated nanoparticles)	HepG2 xenograft mouse model	Tumor progression suppression	↓ Tumor volume

5-FU: 5-fluorouracil; Akt: protein kinase B; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Bax: Bcl-2-associated X; COX-2: cyclooxygenase-2; DEN: diethylnitrosamine; ERK1/2: extracellular signal-regulated kinase 1/2; GA: glycyrrhetinic acid; HCC: hepatocarcinoma; HK2: hexokinase-2; hTERT: telomerase reverse transcriptase; IkBa: nuclear factor-kB inhibitor a; IKKa: inhibitor of nuclear factor-kB kinase subunit a; LA: lactobionic acid; LC3A: microtubule-associated protein 1A/1B light chain 3A; LC3B: microtubule-associated protein 1A/1B light chain 3B; LF: lactoferrin; mTOR: mammalian target of rapamycin; NF-kB: nuclear factor-kB; p62: sequestosome-1; PCNA: proliferating cell nuclear antigen; PLGA: poly(DL-lactide-co-glycolide); RGD: arginine-glycineaspartic acid; RLW: relative liver weight; TNF-a: tumor necrosis factor-a ; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling; VEGF: vascular endothelial growth factor; ZD55-TRAIL: oncolytic adenovirus expressing tumor necrosis factor-related apoptosis inducing ligand.

6. Conclusion

In conclusion, quercetin seems to have a clear antiproliferative and proapoptotic effect against HCC, and likely a modulating role on tumor cell cycle progression which needs to be deeper investigated. This flavonoid seems to have antitumoral efficacy through the alteration of a great variety of cellular processes and signaling pathways, though more studies are required to further elucidate its mechanisms of action against HCC. Arising strategies of combination and drug-delivery systems may improve such cancer inhibition properties and, along with emergent use of quercetin derivatives with anticancer efficacy, let enlarging therapeutic options for HCC patients.

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