Glycyrrhetinic Acid in Liver Cancer Therapy

Subjects: Others

Contributor: Antonio Speciale, Claudia Muscarà, Maria Sofia Molonia, Cristani Mariateresa, Francesco Cimino, Antonina Saija

Liver cancer is one of the most common causes of cancer mortality worldwide. Chemotherapy and radiotherapy are the conventional therapies generally employed in patients with liver tumors. The major issue associated with the administration of chemotherapeutics is their high toxicity and lack of selectivity, leading to systemic toxicity that can be detrimental to the patient's quality of life. An important approach to the development of original liver-targeted therapeutic products takes advantage of the employment of biologically active ligands able to bind specific receptors on the cytoplasmatic membranes of liver cells. In this perspective, glycyrrhetinic acid (GA), a pentacyclic triterpenoid present in roots and rhizomes of licorice, has been used as a ligand for targeting the liver due to the expression of GA receptors on the sinusoidal surface of mammalian hepatocytes, so it may be employed to modify drug delivery systems (DDSs) and obtain better liver or hepatocyte drug uptake and efficacy.

Keywords: glycyrrhetinic acid ; liver cancer ; liver targeting ; drug delivery systems ; biomaterials

1. Introduction

Liver cancer is one of the most common cancers and among the most common causes of cancer mortality worldwide [1]. The highest percentage of primary liver cancers is represented by hepatocellular carcinoma (HCC) [2]. Since surgical resection is feasible in only a few patients, chemotherapy and radiotherapy are the conventional therapies generally employed in patients with liver tumors. Various chemotherapeutic agents, including doxorubicin (DOX), mitoxantrone, gemcitabine, irinotecan, sorafenib, etc., used both as single or as combinational agents, are available for the treatment of HCC [3][4]. Furthermore, a number of novel therapeutic strategies, such as treatments targeting cancer stem cells, molecular targeted therapy, and immunotherapy, are going to be developed [5][6]. However, poor prognosis is found in the majority of patients with HCC due to characteristics intrinsic to the tumor (in particular drug resistance) and mainly due to poor drug bioavailability, non-selective biodistribution, low specificity, and systemic toxicity.

An enormously important approach to the development of original therapeutic products actively targeting liver cancer takes advantage of the employment of biologically active ligands able to bind specific receptors on cytoplasmatic membranes of liver cells. In fact, these ligands may be used to synthesize hybrid compounds based on traditional chemotherapy drugs or to functionalize the surface of micro- and nano-drug delivery systems (DDSs) since they can allow a specific and efficient drug internalization into tumoral cells [7].

In particular, regarding DDSs, more extensive angiogenesis is typical of tumors since more oxygen and nutrients are needed for their rapid growth [8]. However, tumor vasculature is dramatically different from that in normal tissue, with the presence of discontinuous endothelial lining and fenestrations and a lack of smooth muscle cells and pericytes [9]. A much higher DDS accumulation in tumor mass than in normal tissues is an effect known as the enhanced permeability and retention (EPR) effect, which mainly depends on the size of the DDS. On the one hand, the fenestrations in the liver sinusoidal endothelium facilitate substrate transfer into space of Disse between the liver sinusoid and hepatocytes in a normal liver. On the other hand, a longer circulation time of the nanosystems, achieved by opportune surface functionalization strategies, is also extremely important for their accumulation into tumor tissues [10][11]. Since DDSs accumulated into tumor interstitial fluid may be specifically internalized into tumor cells due to specific cell surface interactions, receptor-mediated endocytosis is an approach to active drug delivery targeted to liver cancer cells. In fact, some proteins and molecules are overexpressed on the surface of hepatoma cells or tumoral vessels, and their ligands (polysaccharides, vitamins, peptides, aptamers, transferrin, growth factors, etc.) may be utilized to functionalize DDSs to be specifically recognized by the tumor cells. Following cell internalization through receptor-mediated endocytosis, the drugs loaded in targeted DDSs (TDDSs) are released into the cytoplasm.

The *Glycyrrhiza* genus (Fabaceae family), also known as licorice, is extensively spread in the Mediterranean basin of Africa, Europe, and Asia, and *Glycyrrhiza glabra* L. is commercially the most important species belonging to this genus, largely employed as food and for medicinal purposes. Glycyrrhetinic acid (GA), also known as enoxolone, is a triterpenoid

derivative of beta-amyrin and is the aglycone derived from intestinal hydrolysis of glycyrrhizin, a pentacyclic triterpenoid present in roots and rhizomes of licorice [12]. GA occurs naturally as 18β-GA, derived from 18β-glycyrrhizin, and may be isomerized into the α-isoform under alkaline conditions [13]. It is reported to have hepatoprotective activity but also demonstrates anticancer ability against HCC by multiple mechanisms, including inhibition of cell proliferation, invasion and metastasis, cell cycle arrest, induction of autophagy and apoptosis, and reduction of immunosuppression [14].

GA has been used as a ligand for liver targeting due to the expression of GA receptors on the sinusoidal surface of mammalian hepatocytes, so it may be employed to modify DDSs and obtain better liver or hepatocyte drug uptake and efficacy [15][16]. Previous studies have demonstrated that GA could bind with high affinity to cytomembrane-localized receptors in hepatocytes, and these proteins were named “GA receptors” [15][17]. Recently, Sun and colleagues further confirmed the competitive binding of fluorescein isothiocyanate-GA (FITC-GA) and GA to these receptors in HCC cells [18]. It has been demonstrated that the C11-carbonyl and C3-hydroxyl groups of GA have limited influence on the targeting action of GA to HCC cells and that the β-configuration hydrogen atom at the C18 position of GA contributes the most targeting effect [19]. It is believed that potential GA receptor-mediated hepatic targeting of GA is critical for the anti-HCC effects of GA. GA receptors are predominantly expressed in the liver but not in other organs [15][20], and liver tumor tissue possesses 1.5- to 5-fold more GA receptors than normal tissues. Some other kinds of receptors could be useful to project active hepatic-TDDSs (such as liposomes, micelles, and nanoparticles), including glycyrrhizin receptor (GL-R), asialoglycoprotein receptor (ASGP-R), hyaluronan receptor (HA-R), folate receptor (FA-R), and epidermal growth factor receptor (EGF-R). However, ASGP-R is normally expressed in hepatoma cells and normal hepatocytes, while data on FA-R expression in HCC are controversial. In addition, targeting transferrin receptor, HA-R, and EGF-R gives unexpected immunogenicity to protein ligands [21][22]. Finally, the GA binding sites bind to more than glycyrrhizin [20]. Among the GA-based biomaterials recently developed for liver cancer targeting treatment, particular interest is aimed at the development of those designed for use in gene therapy and photodynamic therapy (PDT).

2. GA-Functionalized Systems for Gene Therapy

Today, there is a great interest in next-generation therapies that use biological macromolecules, such as plasmid DNA, short interfering RNA (siRNA) or antisense nucleotides, so that new DDS techniques able to improve therapeutic efficacy by taking into account the molecular mechanisms of these new therapeutic agents are required. However, since these products are biological macromolecules, they cannot simply pass cell membranes, so it is essential to develop new technologies to protect them from nuclease degradation and allow them to be easily introduced into cells.

Chitosan (CS) has been shown to protect siRNA from serum degradation and deliver it to tumor cells. Zheng and coworkers [23] tested a dual receptor-targeted CS nanosystem controlled by the ligands of galactose of lactobionic acid (LA) and GA (GCGA).). In fact, LA is known to bind ASGP-R. This system was loaded with siPAK1, a siRNA targeting P21-activated kinase 1 (PAK1), a downstream effector of a wide variety of mitogenic factors implicated in HCC progression and metastasis. In vitro data confirmed that GA and LA exhibited a superior targeting capacity, as demonstrated by free GA or LA competitive inhibition assay. Hep3B-xenografted BALB/c nude mice were injected with siPAK1-loaded nanoparticles (NPs) that tended to accumulate in the tumor foci rather than in normal tissues. The findings led the the group to hypothesize that GCGA-siPAK1 promotes endogenous cell apoptosis through the PAK1/MEK/ERK pathway.

Furthermore, the co-delivery of chemotherapeutic drugs and siRNA can improve antitumor efficacy compared to a single administration. Some siRNAs, such as B-cell lymphoma 2 (Bcl-2) siRNA, can target the Bcl-2 gene, inhibit Bcl-2 protein synthesis, and induce apoptosis of tumor cells. Hybrid systems based on CS and polyethylenimine (PEI) have been prepared to enhance the transfection efficiency of genomic medicines. Yan and coworkers [24] tested the chemotherapeutic effects of nanomicelles based on the prodrug polymer GA-CS-PEI-HBA-DOX [DOX attached to CS-PEI through a pH-sensitive linker 4-hydrazinobenzoic (HBA), thus obtaining a bond hydrolysable at acidic pH] and Bcl-2 siRNA (GA-CS-PEI-HBA-DOX@siRNA). PEI is a polycationic vector largely employed as a gene delivery system for cancer therapy due to its cationic charge and buffering capacity, which renders it suitable to condense large negatively charged molecules, to protect DNA from degradation, and to induce endosomal escape of the gene payload. This system, GA-CS-PEI-HBA-DOX@siRNA, demonstrated superior co-delivery and anticancer targeting abilities based on the pH-responsive drug release, surface charge conversion as a function of pH values, and receptor-mediated endocytosis. In fact, positively charged NPs could be easily uptaken by cancer cells via “electrostatic attraction-mediated targeting”, since the surface of most cancer cells is maintained negative. The competitive inhibition of GA-CS-PEI-HBA-DOX@siRNA by free GA over GA receptors on HepG2 cells confirmed the active targeting properties of this DDS on hepatoma cells. In addition, the GA-CS-PEI-HBA-DOX@siRNA showed much stronger cytotoxicity to HepG2 compared to siRNA or DOX or siRNA/DOX in combination. In vivo data on HepG2-bearing BALB/c nude mice demonstrated that GA-CS-PEI-HBA-

DOX@siRNA exhibited the highest therapeutic effect when compared to moderate antitumor properties of free DOX and limited inhibitory effects of siRNA. Furthermore, no effects on normal tissues were reported for the nanoformulations when compared to the serious side effects of free DOX.

The same strategy has been tested using liver-targeted NPs composed of distearoyl-phosphatidylethanolamine (DSPE), polyethylene glycol (PEG) and PEI (DPP) with GA-modified HA (GH) for the co-delivery of DOX and Bcl-2 siRNA (siRNA/DOX/GH-DPP) [25]. The half-maximal inhibitory concentrations (IC_{50} value) of siRNA/DOX/DPP and siRNA/DOX/GH-DPP NPs against HepG2 cell viability was 1.02 and 0.76 DOX μg/mL, respectively, which were lower than that of free DOX (1.86 DOX μg/mL), supporting an improved cellular uptake of DOX and siRNA via GA receptor-mediated endocytosis and the better sensitivity of HepG2 cells to DOX owing to down-regulation of Bcl-2 by RNA interference. In vivo data confirmed liver-targeting delivery and decreased uptake by normal cells of siRNA/DOX/GH-DPP in H22 tumor-bearing mice, resulting in higher anti-hepatoma efficacy than siRNA/DOX/DPP NPs and less systemic toxicity compared to free DOX.

Since HCC is associated with the activation of the PI3K/Akt/mTOR signaling pathway, facilitating the development of tumor cell proliferation, angiogenesis, metastasis, and invasion, Wang and coworkers [26] tested a nanosystem self-assembled from a PEI-GA amphiphilic copolymer as a versatile gene/drug dual delivery nanoplatform using DOX and a short hairpin RNA silencing Akt1 (shAkt1). The IC_{50} of free DOX, PEI-GA/DOX, and PEI−GA/DOX/shAkt1 NPs in HepG2 was estimated to be approximately 4.56, 2.07, and 0.99 μg/mL, respectively, confirming the higher drug delivery and the synergic activity of combination therapy. In addition, after treatment with PEI-GA/DOX/shAkt1 NPs, the reduction of Akt1 protein level in HepG2 cells induced autophagy, as an alternative pathway to cell death (type II cell death), via LC3B−II protein upregulation. Furthermore, in vivo treatment with PEI-GA/DOX/shAkt1 NPs indicated improved tumor growth inhibition in Hepa-1.6 cell grafted tumor-bearing C57BL-6J mice.

However, low-molecular-weight PEIs (LMWH, below 2000 Da) are known for their low toxicity but poor transfection. Cao and coworkers designed GA-modified LMWH PEIs to demonstrate that modification of LMWH PEIs with GA could give high transfection efficiency and allow liver targeting [27].

Graphene oxide (GO) represents a potentially useful material in gene therapy due to its 2D planar structure with the high presence of surface oxygenated functional groups and due to its ability to easily cross cell membranes. To ameliorate these properties, the GO surface may be modified by positively charged cationic polymers. Polyamidoamine (PAMAM) dendrimers are biodegradable cationic and highly branched spherical polymeric macromolecules with a peptide bond backbone, and their conjugation with GO has been explored as an innovative approach for gene delivery. Liu and coworkers [28] demonstrated efficient intracellular delivery of plasmid DNA using GA-PAMAM-GO nanohybrids with good transfection efficiency in SMMC-7721 cells. More recently, GA was employed by Qu and coworkers as a liver-targeting ligand to construct GA, PEG, PAMAM dendrimer (D) and nano-graphene oxide (NGO) conjugates (GPND) for siRNA delivery targeting vascular endothelial growth factor (VEGF), a well-known pivotal regulator of tumor angiogenesis [29]. Data demonstrated efficient cell uptake of the GPND/siRNA nanocomplex and gene silencing in HepG2 cells. The mechanism involved in the effect of this system is that PAMAM, due to its proton sponge effect, can attract hydrogen ions and thus cause penetration of chloride ions and water into lysosomes, resulting in their rupture. At the same time, PAMAM dendrimers are degraded in the acidic environment, allowing effective siRNA release. Notably, in vivo studies showed an evident siRNA accumulation in liver tumor tissue by the delivery of GPND, thus leading to significant growth inhibition of tumor tissues in HepG2-bearing NUNU mice.

3. GA-Functionalized Biomaterials for Photodynamic Therapy

Photodynamic therapy (PDT) is a promising alternative for cancer treatment, especially due to its painless and non-invasive modalities. A photosensitizer (PS), systemically or topically administered, accumulates in the target site during a predetermined duration time (drug-to-light interval), after which the target site is irradiated by the light of appropriate wavelength and energy, producing PS photo-excitation. This excited PS has to transfer its energy to surrounding intracellular oxygen, forming cytotoxic reactive oxygen species (ROS). However, the light energy absorbed by a PS can also be released through fluorescence or heat generation. The main targets of irradiated PS-induced damage are represented by mitochondria, lysosomes, plasma membranes, nuclei, and blood vessels around cancer cells [30]. To be clinically used, PSs must be able to highly and selectively accumulate in the tumor, possess only low or minimal dark toxicity, and be characterized by high bio-stability and high bio-clearance. However, despite the great progress in PS-mediated PDT, the clinical uses are still reduced due to the poor water solubility and tissue/cell specificity of conventional PS drugs. In this regard, the development of materials that incorporate PS drugs and transfer them into target tissues/cells is required.

For example, since GA can improve the uptake of PS to cancer cells, Wang and coworkers studied the properties of the amphiphilic GA-porphyrin (TPP) conjugate self-assembled into NPs (TPP-GA NPs). TPP, one of the most widely used photosensitizers, is hydrophobic, while GA is water soluble, so the amphiphilic conjugate TPP-GA can self-assemble into NPs. In vitro experiments demonstrated that TPP-GA NPs are uptaken by endocytosis into tumoral cells, and under opportune irradiation (620 nm, 12 mW cm^{-2} for 1 h), it showed light phototoxicity in HepG2 cells when compared to cells maintained in the dark, suggesting that TPP-based nanomaterials could be applied for the PDT of cancer cells [31].

Phthalocyanine has a high absorbance at 600−700 nm, high ROS generation efficiency, and is also stable from chemical and photochemical degradation. Silicon phthalocyanine (SiPC) was linked to GA using PEG and 3-(ethoxydimethylsilyl)propylamine (APDES) [32]. GA-PEG-SiPC was internalized via GA receptor, showing significant cytotoxicity when the liver cancer HepG2 and Huh7 cells were irradiated using a 671 nm light source (50 mW cm^{-2}) for 80 s. After intravenous administration in HepG2 tumor-bearing mice, GA-PEG-SiPC accompanied with PDT revealed liver cancer-targeted accumulation and anticancer properties via apoptosis and necrosis without side effects and resistance to treatment.

DDSs projected for the combinate use of chemotherapeutics and PS are an innovative strategy for cancer treatment, also because ROS produced by PS irradiation can disrupt the lysosome membrane and thus induce lysosome escape of the drugs. Then, DOX and the photosensitizer pheophorbide A (PHA) were loaded into micelles made of the poly-ε-caprolactone-cystamine-carboxymethyl CS-GA (PCL-SS-CMC-GA) polymer, where the switchable disulfide bonds (SS) were predisposed to be degraded in the high redox potential of cancer cells and trigger the release of therapeutic agents [33]. The redox-responsive release mechanism of PCL-SS-CMC-GA@DOX/PHA can allow the controlled release of drugs specifically in tumor cells. The difference can be due to the reduced glutathione (GSH) levels, since its concentration in the extracellular matrix and body fluids of normal tissues is about 2~20 μM, but it can reach 20 mM in tumor cell endosomes with important changes in redox potential. The findings obtained in HepG2 cells showed that both the functionalization with GA and the charge conversion property of this system can promote its adsorption and uptake by tumor cells, while the cytotoxicity of the PCL-SS-CMC-GA@DOX/PHA system is dependent on laser irradiation. The biodistribution in tumor-bearing BALB/c nude mice showed that PCL-SS-CMC-GA@PHA accumulated in the tumor site when compared to GA-undecorated micelles and confirmed a stronger inhibition rate (54.1%) of the subcutaneous tumors after laser irradiation compared to the controls.

4. Conclusions

Chemotherapy is the main treatment for hepatic tumors, including HCC, but it is still challenging due to several problems, including its nonselective biodistribution and effects, as well as multidrug chemoresistance. Active targeting biomaterials, which employment is based on the interaction between a ligand introduced in the biomaterial and specific receptors present on the target cells, hold enormous potential in cancer therapy for their capability to improve delivery system internalization and therapeutic agent uptake into specific cells with higher bioavailability and lower systemic toxicity.
Taking this into account, herein have described the more recent advances in the development of biomaterials based on GA projected for active drug delivery targeted to liver cancer, to be used gene therapy and PTD. Besides the capability to target hepatic cells, some of these systems can offer, due to their unique chemical characteristics, several additional advantages.

First, innovative systems aimed at the simultaneous delivery of more therapeutic agents acting on different targets or mechanisms involved in cancer growth and progression have been developed. In particular, one has to remember that GA itself has well-documented anti-cancer properties. Another important aspect involves delivering nucleic acids such as siRNA and plasmid DNA, since these therapeutic agents can enter target cells, usually via endocytosis, and their functionality strongly depends on the efficiency of endosomal escape. Particularly interesting is the development of GA-based biomaterials that incorporate PS drugs to be used in liver cancer PDT.

Special attention is focused on dual functionalization and on the design of stimuli-responsive systems. In fact, the introduction of two ligands (for example, GA together with LA) enables the delivery of more drugs to specific cells and further reduces normal tissue toxicity. There has been increased development of GA-based systems responsive to intrinsic characteristics of tumor microenvironments, in particular the lower pH.

Thus, these results highlight the potential therapeutic efficacy of rationally designed biomaterials based on GA and lead us to hypothesize that the research about these products will continue to increase in the future. However, at this phase of research, the translation of the existing data from the laboratory to the clinical field appears to still be very problematic. A particular problem is represented by the limited scientific knowledge about the hepatic GA receptors. Although several

papers have demonstrated the existence of these proteins called GA receptors on the membranes of normal and tumoral liver cells, scientists know very little about the physiological and biochemical aspects related to their functionality.

On the other hand, the efficacy of GA-based nanomaterials described by the international literature has been demonstrated in vitro on opportune cancer cell lines, and in a few cases, in vivo using experimental animals. Thus, the difficulty of extrapolating these experimental findings from isolated cell lines to complex biological systems and then to humans is evident, and only further studies can allow us to demonstrate their actual clinical efficacy, as well as their safety and biocompatibility.

One has also to point out that the increased complexity of methodologies employed to realize the described biomaterials can also introduce significant obstacles in reproducibility, scale-up/out, and quality control, and consequently significantly higher production costs, all aspects that make their applicability to the clinic more complex.

Finally, several obstacles have limited the clinical application of licorice-derived therapeutics. The metabolism of a large part of marketed drugs is regulated by cytochrome P450 (CYP) isoforms (CYP3A, CYP2C9, CYP2C19, CYP2D6, CYP2E1, etc.), which are most frequently involved in drug phase I biotransformation, and by uridine 5′-diphospho-glucuronosyltransferases (UGTs), which play a main role in phase II metabolism. Furthermore, the activity of the transmembrane ATP-binding cassette transporter P-glycoprotein, predominantly expressed in the intestinal tract, brain, liver, and kidney, is also crucial for drug metabolism and bioavailability. It has been reported that GA can significantly affect the activity of some metabolic enzymes, including several CYP450 isoforms and UGTs, as well as of P-gp, so it could mediate potential drug-drug interactions. In addition, a further complication due to licorice uptake seems to be pseudohyperaldosteronism, a clinical condition characterized by hypertension, hypokalemia, and suppression of plasma renin and aldosterone levels. This effect is due to its component, GA, which acts mainly through two different mechanisms: by blocking the enzyme 11-β-hydroxysteroid dehydrogenase type 2 (11-β-HSD2), which inactivates cortisol to cortisone, and by directly binding the mineralocorticoid receptor as an agonist [34].

In conclusion, active targeted biomaterials based on GA represent a great promise for the development of an innovative therapeutic strategy in the treatment of liver cancer, although there is an imperative need for further studies to demonstrate their efficacy and safety.

Abbreviations

ΔΨm	**membrane potential**
APDES	**3-(ethoxydimethylsilyl)propylamine**
ASGP-R	**asialoglycoprotein receptor**
ATP	**adenosine triphosphate**
Bcl-2	**B-cell lymphoma 2**
CMC	**carboxymethyl chitosan**
CS	**chitosan**
CYP	**cytochrome P450**
DDS	**drug delivery system**
DOX	**doxorubicin**
DSPE	**distearoyl-phosphatidylethanolamine**
EGF-R	**epidermal growth factor receptor**
EPR	**enhanced permeation and retention effect**
ERK	**extracellular signal-regulated kinase**
FA-R	**folate receptor**
FITC	**fluorescein isothiocyanate**
GA	**glycyrrhetinic acid**
GH	**GA modified HA**

GL-R	**glycyrrhizin receptor**
GO	**graphene oxide**
GR	**galactose receptor**
HA	**hyaluronic acid**
HA-R	**hyaluronan receptor**
HBA	**4-hydrazinobenzoic**
HCC	**hepatocellular carcinoma**
IV	**intravenous**
LA	**lactobionic acid**
LMWH	**low molecular weight**
MDR	**multidrug resistant**
MEK	**mitogen-activated protein/extracellular signal-regulated kinase**
mPEG	**polyethylene glycol methyl ether**
mTOR	**mechanistic target of rapamycin**
MR	**mannose receptor**
NGO	**nano-graphene oxide**
NP	**nanoparticle**
OX	**oxaliplatin**
PAK1	**P21-activated kinase 1**
PAMAM	**poly(amidoamine)**
PCL	**poly-ε-caprolactone**
PDT	**photodynamic therapy**
PEG	**polyethylene glycol**
PEI	**polyethylenimine**
PHA	**pheophorbide A**
PI3K	**phosphatidyl Inositol 3-kinase**
PS	**photosensitizers**
ROS	**reactive oxygen species**
SC	**subcutaneous**
SH	**sulfhydryl group**
sHA	**sulfated hyaluronic acid**
shRNA	**short/small hairpin RNA**
siPAK1	**siRNA-targeting P21-activated kinase 1**
SiPC	**silicon phthalocyanine**
siRNA	**short interfering RNA**
SP	**substance P**
SS	**disulfide**
TDDS	**targeted drug delivery system**
TPP	**tetraphenylporphyrin**

UGTs	**uridine 5’-diphospho-glucuronosyltransferases**

References

1. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D.M.; Piñeros, M.; Znaor, A.; Bray, F. Cancer statistics for the year 2020: An overview. Int. J. Cancer 2021, 149, 778–789.
2. Llovet, J.M.; Kelley, R.K.; Villanueva, A.; Singal, A.G.; Pikarsky, E.; Roayaie, S.; Lencioni, R.; Koike, K.; Zucman-Rossi, J.; Finn, R.S. Hepatocellular carcinoma. Nat. Rev. Dis. Primers 2021, 7, 6.
3. Kim, D.W.; Talati, C.; Kim, R. Hepatocellular carcinoma (HCC): Beyond sorafenib—chemotherapy. J. Gastrointest. Oncol. 2017, 8, 256–265.
4. Alqahtani, S.A.; Colombo, M.G. Current status of first-line therapy, anti-angiogenic therapy and its combinations of other agents for unresectable hepatocellular carcinoma. World J. Gastrointest. Oncol. 2021, 13, 2038–2049.
5. Llovet, J.M.; Castet, F.; Heikenwalder, M.; Maini, M.K.; Mazzaferro, V.; Pinato, D.J.; Pikarsky, E.; Zhu, A.X.; Finn, R.S. Immunotherapies for hepatocellular carcinoma. Nat. Rev. Clin. Oncol. 2021, 19, 151–172.
6. Zhang, X.; Li, N.; Zhu, Y.; Wen, W. The role of mesenchymal stem cells in the occurrence, development, and therapy of hepatocellular carcinoma. Cancer Med. 2022, 11, 931–943.
7. Li, M.; Zhang, W.; Wang, B.; Gao, Y.; Song, Z.; Zheng, Q.C. Ligand-based targeted therapy: A novel strategy for hepatocellular carcinoma. Int. J. Nanomed. 2016, 11, 5645–5669.
8. McDougall, S.R.; Anderson, A.R.; Chaplain, M.A. Mathematical modelling of dynamic adaptive tumour-induced angiogenesis: Clinical implications and therapeutic targeting strategies. J. Theor. Biol. 2006, 241, 564–589.
9. Maeda, H. The enhanced permeability and retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. Adv. Enzym. Regul. 2001, 41, 189–207.
10. Cho, K.J.; Wang, X.; Nie, S.; Chen, Z.; Shin, D.M. Therapeutic Nanoparticles for Drug Delivery in Cancer. Clin. Cancer Res. 2008, 14, 1310–1316.
11. Byrne, J.D.; Betancourt, T.; Brannon-Peppas, L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv. Drug Deliv. Rev. 2008, 60, 1615–1626.
12. Sharifi-Rad, J.; Quispe, C.; Herrera-Bravo, J.; Belén, L.H.; Kaur, R.; Kregiel, D.; Uprety, Y.; Beyatli, A.; Yeskaliyeva, B.; Kırkın, C.; et al. Glycyrrhiza Genus: Enlightening Phytochemical Components for Pharmacological and Health-Promoting Abilities. Oxidative Med. Cell. Longev. 2021, 2021, 7571132.
13. Pastorino, G.; Cornara, L.; Soares, S.; Rodrigues, F.; Oliveira, M.B.P.P. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother. Res. 2018, 32, 2323–2339.
14. Graebin, C.S. The Pharmacological Activities of Glycyrrhizinic Acid (“Glycyrrhizin”) and Glycyrrhetinic Acid. In Sweeteners: Pharmacology, Biotechnology, and Applications; Mérillon, J.-M., Ramawat, K.G., Eds.; Springer International Publishing: Cham, Switzerland, 2018; pp. 245–261.
15. Cai, Y.; Xu, Y.; Chan, H.F.; Fang, X.; He, C.; Chen, M. Glycyrrhetinic Acid Mediated Drug Delivery Carriers for Hepatocellular Carcinoma Therapy. Mol. Pharm. 2016, 13, 699–709.
16. Wu, F.; Li, X.; Jiang, B.; Yan, J.; Zhang, Z.; Qin, J.; Yu, W.; Gao, Z. Glycyrrhetinic Acid Functionalized Nanoparticles for Drug Delivery to Liver Cancer. J. Biomed. Nanotechnol. 2018, 14, 1837–1852.
17. Li, X.; Sun, R.; Liu, R. Natural products in licorice for the therapy of liver diseases: Progress and future opportunities. Pharmacol. Res. 2019, 144, 210–226.
18. Sun, Y.; Lu, J.; Yan, D.; Shen, L.; Hu, H.; Chen, D. Cellular uptake mechanism and clearance kinetics of fluorescence-labeled glycyrrhetinic acid and glycyrrhetinic acid–modified liposome in hepatocellular carcinoma cells. Environ. Toxicol. Pharmacol. 2017, 53, 46–56.
19. Sun, Y.; Dai, C.; Yin, M.; Lu, J.; Hu, H.; Chen, D. Hepatocellular carcinoma-targeted effect of configurations and groups of glycyrrhetinic acid by evaluation of its derivative-modified liposomes. Int. J. Nanomed. 2018, 13, 1621–1632.
20. Negishi, M.; Irie, A.; Nagata, N.; Ichikawa, A. Specific binding of glycyrrhetinic acid to the rat liver membrane. Biochim. Biophys. Acta 1991, 1066, 77–82.
21. Parker, N.; Turk, M.J.; Westrick, E.; Lewis, J.D.; Low, P.; Leamon, C.P. Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. Anal. Biochem. 2005, 338, 284–293.

22. Yan, J.-J.; Liao, J.-Z.; Lin, J.-S.; He, X.-X. Active radar guides missile to its target: Receptor-based targeted treatment of hepatocellular carcinoma by nanoparticulate systems. Tumor Biol. 2015, 36, 55–67.
23. Zheng, Q.C.; Jiang, S.; Wu, Y.Z.; Shang, D.; Zhang, Y.; Hu, S.B.; Cheng, X.; Zhang, C.; Sun, P.; Gao, Y.; et al. Dual-Targeting Nanoparticle-Mediated Gene Therapy Strategy for Hepatocellular Carcinoma by Delivering Small Interfering RNA. Front. Bioeng. Biotechnol. 2020, 8, 512.
24. Yana, T.; Zhua, S.; Huia, W.; Heb, J.; Liua, Z.; Chengac, J. Chitosan based pH-responsive polymeric prodrug vector for enhanced tumor targeted co-delivery of doxorubicin and siRNA. Carbohydr. Polym. 2020, 250, 116781.
25. Tian, G.; Pan, R.; Zhang, B.; Qu, M.; Lian, B.; Jiang, H.; Gao, Z.; Wu, J. Liver-Targeted Combination Therapy Basing on Glycyrrhizic Acid-Modified DSPE-PEG-PEI Nanoparticles for Co-delivery of Doxorubicin and Bcl-2 siRNA. Front. Pharmacol. 2019, 10, 4.
26. Wang, F.-Z.; Xing, L.; Tang, Z.-H.; Lu, J.-J.; Cui, P.-F.; Qiao, J.-B.; Jiang, L.; Jiang, H.-L.; Zong, L. Codelivery of Doxorubicin and shAkt1 by Poly(ethylenimine)–Glycyrrhetinic Acid Nanoparticles to Induce Autophagy-Mediated Liver Cancer Combination Therapy. Mol. Pharm. 2016, 13, 1298–1307.
27. Cao, M.; Gao, Y.; Zhan, M.; Qiu, N.; Piao, Y.; Zhou, Z.; Shen, Y. Glycyrrhizin Acid and Glycyrrhetinic Acid Modified Polyethyleneimine for Targeted DNA Delivery to Hepatocellular Carcinoma. Int. J. Mol. Sci. 2019, 20, 5074.
28. Liu, F.; Yang, D.; Liu, Y.; Cao, Q.; Sun, Y.; Wang, Q.; Tang, H. Improving dispersive property, biocompatibility and targeting gene transfection of graphene oxide by covalent attachment of polyamidoamine dendrimer and glycyrrhetinic acid. Colloids Surfaces B Biointerfaces 2018, 171, 622–628.
29. Qu, Y.; Sun, F.; He, F.; Yu, C.; Lv, J.; Zhang, Q.; Liang, D.; Yu, C.; Wang, J.; Zhang, X.; et al. Glycyrrhetinic acid-modified graphene oxide mediated siRNA delivery for enhanced liver-cancer targeting therapy. Eur. J. Pharm. Sci. 2019, 139, 105036.
30. Gunaydin, G.; Gedik, M.E.; Ayan, S. Photodynamic Therapy for the Treatment and Diagnosis of Cancer–A Review of the Current Clinical Status. Front. Chem. 2021, 9, 686303.
31. Wang, X.; Wang, P.; Xue, S.; Zheng, X.; Xie, Z.; Chen, G.; Sun, T. Nanoparticles based on glycyrrhetinic acid modified porphyrin for photodynamic therapy of cancer. Org. Biomol. Chem. 2018, 16, 1591–1597.
32. Kim, J.; Lee, S.; Na, K. Glycyrrhetinic Acid-Modified Silicon Phthalocyanine for Liver Cancer-Targeted Photodynamic Therapy. Biomacromolecules 2020, 22, 811–822.
33. Yan, T.; Hui, W.; Zhu, S.; He, J.; Liu, Z.; Cheng, J. Carboxymethyl chitosan based redox-responsive micelle for near-infrared fluorescence image-guided photo-chemotherapy of liver cancer. Carbohydr. Polym. 2020, 253, 117284.
34. Sabbadin, C.; Bordin, L.; Donà, G.; Manso, J.; Avruscio, G.; Armanini, D. Licorice: From Pseudohyperaldosteronism to Therapeutic Uses. Front. Endocrinol. 2019, 10, 484.