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Glycyrrhizic acid-based multifunctional nanoplatform for tumor microenvironment regulation

XIAO Meng[1], GUO Zhiqing[2], YANG Yating[1], HU Chuan[1], CHENG Qian[1], ZHANG Chen[1], WU Yihan[1], CHENG Yanfen[3], BENSON Wui Lau Man[4], SHAMAY Sheung Mei Ng[4], LEUNG George Pak-Heng[5], LI Jingjing[4, 6*], GAO Huile[7*], ZHANG Jinming[1*]

[1] *State Key Laboratory of Southwestern Chinese Medicine Resources, Pharmacy School, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China;*
[2] *Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 610072, China;*
[3] *School of Medicine, Chengdu University, Chengdu 610106, China;*
[4] *Department of Rehabilitation Sciences, Faculty of Health and Social Sciences, Hong Kong Polytechnic University, Hong Kong 999077, China;*
[5] *Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong 999077, China;*
[6] *The Research Centre for Chinese Medicine Innovation, Hong Kong Polytechnic University, Hong Kong 999077, China;*
[7] *Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry and Sichuan Province, Sichuan Engineering Laboratory for Plant-Sourced Drug and Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, Chengdu 610041, China*

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[ABSTRACT] Natural compounds demonstrate unique therapeutic advantages for cancer treatment, primarily through direct tumor suppression or interference with the tumor microenvironment (TME). Glycyrrhizic acid (GL), a bioactive ingredient derived from the medicinal herb *Glycyrrhiza uralensis* Fisch., and its sapogenin glycyrrhetinic acid (GA), have been recognized for their ability to inhibit angiogenesis and remodel the TME. Consequently, the combination of GL with other therapeutic agents offers superior therapeutic benefits. Given GL's amphiphilic structure, self-assembly capability, and liver cancer targeting capacity, various GL-based nanoscale drug delivery systems have been developed. These GL-based nanosystems exhibit angiogenesis suppression and TME regulation properties, synergistically enhancing anti-cancer effects. This review summarizes recent advances in GL-based nanosystems, including polymer-drug micelles, drug-drug assembly nanoparticles (NPs), liposomes, and nanogels, for cancer treatment and tumor postoperative care, providing new insights into the anti-cancer potential of natural compounds. Additionally, the review discusses existing challenges and future perspectives for translating GL-based nanosystems from bench to bedside.

[KEY WORDS] Glycyrrhizic acid; Tumor microenvironment; Nanosystem; Angiogenesis; Self-assembly
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Introduction

The rising incidence of cancer and the limited efficacy of current clinical treatments necessitate the development of highly efficient and less toxic anticancer strategies. Natural compounds have been extensively studied for their anticancer properties [1]. While various anticancer mechanisms of these compounds have been identified, including anti-proliferation effects, apoptosis promotion, metastasis inhibition, and multidrug resistance reversal, few can directly target tumor tissue growth as effectively as first-line cytotoxic agents.

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[*]**Corresponding author]** E-mails: kim07.li@polyu.edu.hk (LI Jingjing); gaohuile@scu.edu.cn (GAO Huile); cdutcmzjm@126.com (ZHANG Jinming)
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Numerous clinical and experimental studies have corroborated Stephen Paget's original "seed and soil" hypothesis, which describes the relationship between tumor cells and the tumor microenvironment (TME) [2, 3]. Recent research [4, 5] has increasingly focused on investigating how various natural products can modulate the TME. Harnessing the TME-modulating properties of natural compounds has emerged as a promising strategy to enhance the effectiveness of various anticancer treatments, including chemodynamic therapy, photothermal therapy, and chemotherapy [6].

Glycyrrhizic acid (GL), a principal bioactive component extracted from the medicinal herb *Glycyrrhiza uralensis* Fisch., has garnered significant interest in cancer treatment [7] due to its recognized anticancer properties in both monotherapy and combination therapies. GL demonstrates broad antitumor effects against various cancer types, including hepatocellular carcinoma (HCC) [8], breast cancer [9], lung cancer [10], and leukemia [11]. Notably, GL can mitigate chemotherapy-induced side effects, such as hepatotoxicity, nephrotoxicity, genotoxicity, neurotoxicity, and pulmonary toxicity [7]. Additionally, GL's potential as a vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor has been evidenced by its ability to significantly inhibit proliferation, migration, invasion, and tube formation in human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner [12]. Moreover, GL inhibited high mobility group box 1 (HMGB1)-induced proliferation and migration of tumor cells, as well as blood vessel formation [13]. GL influences TME remodeling by attenuating Tregs and myeloid-derived suppressor cells (MDSCs) and enhances antitumor immunity [14]. Consequently, given GL's direct cancer-inhibiting properties and its regulatory effect on the TME, an increasing number of studies have explored GL in combination therapies with other anticancer agents [15-17].

Numerous studies have demonstrated the advantages of nanocarriers in tumor treatment [18-20]. Various nano-vehicles have been developed to co-deliver GL and other agents, including albumin nanoparticles (NPs) physically co-loaded with entecavir and GL [21], amphiphilic poly (ethylenimine)-GL nanocarriers loaded with DOX/shAkt1 complexes [22], and GL-modified polymeric prodrugs [23] loaded with doxorubicin (DOX) [24]. Notably, GL, as an HMGB1 inhibitor, has been combined with magnetic hyperthermia and immune checkpoint inhibitors to activate the immunosuppressive microenvironment, leading to complete tumor regression of poorly immunogenic melanoma in a B16-F10 melanoma mouse model [25]. Wang *et al.* [26] incorporated GL into alginate nanogel GL-ALG NGPs, which not only avoided triggering immuno-inflammatory responses of macrophages but also decreased macrophage phagocytosis. This approach reduced the rapid clearance of particles by activated macrophages and enhanced the anticancer efficacy of the combination therapy of GL and DOX. Furthermore, GL was co-delivered with chlorine6 (Ce6)-modified DNAzyme into H-MnO_2 NPs as a multiple tumor immune activation strategy, where Ce6 and glycyrrhetinic acid (GA) promoted ROS production and immunogenic cell death [27]. However, these nano-encapsulation methods, whether through physical loading or chemical conjugation, face challenges such as low drug-loading efficacy, unstable ratios of loaded drugs, and complex production processes.

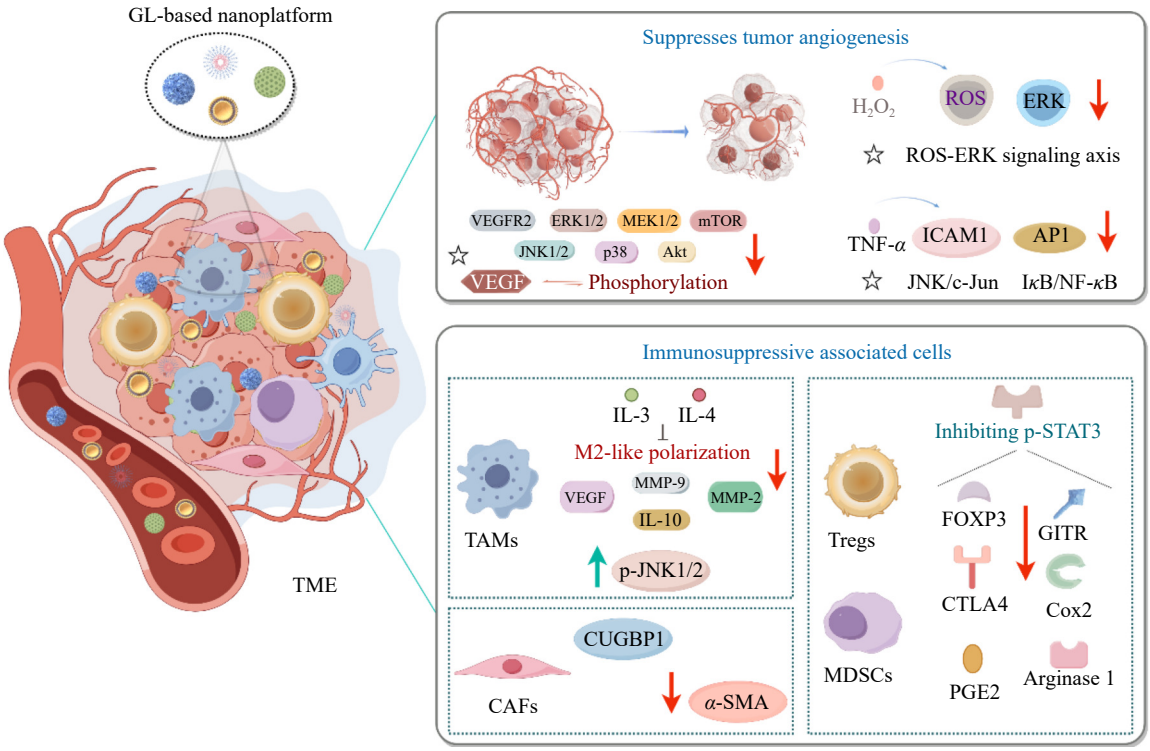
The structural characteristics of GL have recently attracted attention beyond its therapeutic applications, particularly for its potential as a carrier in drug delivery systems. Researchers have developed various amphiphilic polymer-GL conjugates incorporating TME-responsive linkages, such as hydrazone, oxime, disulfide, and double selenium bonds. Additionally, GL-based self-assembling NPs and supramolecular hydrogels have been created using facile, sustainable, and eco-friendly synthesis processes. Leveraging the structural similarity between GL, a pentacyclic triterpene, and cholesterol, novel GL-based lipid NPs were designed by substituting GL for cholesterol [28]. These GL-based lipid NPs demonstrated higher membrane stability and drug-loading efficiency compared to traditional liposomes. Moreover, the supramolecular complex of GL/hydroxypropyl-β-cyclodextrin (at a molar ratio of 1 : 2) can form nanofibers [29]. GL-based Pickering emulsions and emulsion gels exhibit ultrastability, thixotropy, and broad pH resistance, offering a novel drug delivery approach [30-32]. These GL-based nanoscale drug delivery systems enable the co-delivery of combination drugs. This article summarizes the therapeutic effects of GL related to TME regulation and its utility in nanoplatforms as a drug carrier (Scheme 1). Furthermore, GL's bioactivities extend beyond cancer treatment, including anti-hepatitis [33] and anti-colitis [34, 35] activities, wound healing [36], and weight loss [37], indicating the broad potential applications of GL-based nanosystems.

TME Remodeling Effects of GL

GL has demonstrated a wide range of pharmacological effects in numerous preclinical studies, influencing various aspects of cancer progression, including malignant cell proliferation, differentiation, and invasion. A clinical trial investigating GL for prostate cancer is currently in progress. Several comprehensive reviews have summarized the antitumor activities of GL [38-40]. Consequently, this review primarily focuses on GL's role in modulating TME, with particular emphasis on angiogenesis suppression and the regulation of immunosuppressive-associated cells (Table 1). These cells include tumor-associated macrophages (TAMs), carcinoma-associated fibroblasts (CAFs), Treg cells, and MDSCs.

Angiogenesis suppression

Abnormal angiogenesis is a hallmark of solid tumors, crucial for supplying nutrients and oxygen to tumor cells, thereby facilitating cancer growth and progression [41]. VEGFs plays a pivotal role in tumor angiogenesis, enhancing vascular permeability and tube formation [42]. Anti-angiogenic cancer treatments predominantly target the VEGF pathway, utilizing tyrosine kinase inhibitors and monoclonal antibod-



Scheme 1 Illustration for TME regulation effects of GL-based multifunctional nanoplatform.

Table 1 GL as a potential candidate for remodeling of the TME

Classification		*In vitro*	*In vivo*	The mechanism of GL	Ref.
Suppresses tumor angiogenesis		ECV304 cells	/	Reduce ROS production and ERK activation in the endothelial cells	[46]
		TNF-α-activated HUVECs	/	Interruption of both JNK/c-Jun and IκB/NF-κB signaling pathways, which decreases activator protein-1 (AP-1) and NF-κB mediated ICAM-1 expression in HUVECs	[47]
		VEGF-stimulated HUVECs	Ovarian cancer (A2780 cells)-bearing mouse model	Inhibiting the phosphorylation of VEGFR2, mTOR, Akt, ERK1/2, MEK1/2, p38 and JNK1/2 in HUVECs	[48]
		VEGF-stimulated HUVECs	Breast cancer (4T1)-bearing mouse model		[15]
Immunosuppressive associated cells	TAMs	IL-4/IL-13-treated RAW 264.7 and THP-1 cells	Breast cancer (4T1) bearing mouse	Increase the phosphorylation of JNK1/2 in M2 macrophages	[53]
	CAFs	4T1 cells	Breast cancer (4T1)-bearing mouse model	Downregulation of CUGBP1 and α-SMA of CAFs	[58]
	Tregs	B16F10 cells	Melanoma (B16F10)-bearing mouse model	Inhibition phosphorylated STAT3 and downregulation of $FOXP_3$, GITR and CTLA4 of Tregs	[14, 27]
		Immature DCs, M2-TAMs, GL261 cells	Glioblastoma (GL261-Luc)-bearing mouse model		[62]
	MDSCs	B16F10 cells	Melanoma (B16F10)-bearing mouse model	Inhibition phosphorylated STAT3 and inhibition Cox2, PGE2 and Arginase 1 of MDSCs	[14]

ies. However, due to the complex mechanisms underlying angiogenesis, the efficacy of these drugs remains suboptimal [43, 44]. GL demonstrates potent anti-angiogenic effects by modulating various biomolecules that inhibit angiogenesis. Low levels of reactive oxygen species (ROS) in TME can promote human endothelial cell proliferation *in vitro* [45]. KIM et al. observed that GL significantly reduced hemoglobin levels in mouse tumors and inhibited microvessel sprouting in a rat aortic ring model. Another study revealed that GL mitigated H_2O_2-induced ROS elevation and inhibited extracellular signal-regulated kinase (ERK) activation in ECV304 cells, suggesting that GL exerts its anti-angiogenic effect by inhibiting the ROS-ERK signaling axis [46]. CHANG et al. demonstrated that GL could inhibit tumor necrosis factor (TNF)-α-induced ICAM-1 expression in HUVECs, thereby reducing leukocyte adhesion [47]. Our group's research determined that

GL significantly impeded VEGF-stimulated migration, invasion, and tube formation in HUVECs [48]. GL effectively inhibited the phosphorylation of key biomolecules involved in VEGF-mediated angiogenesis in a concentration-dependent manner. Notably, high levels of GL did not induce side effects, indicating its potential as an anti-angiogenic drug that may overcome the limitations of existing therapeutics. In a subsequent study, we combined GL with DOX to evaluate their anti-breast cancer effects through anti-angiogenic activity [15]. This combination enhanced intracellular DOX accumulation in MCF-7 cancer cells. Furthermore, synergistic proapoptotic effects on MCF-7 cells and anti-angiogenic effects on VEGF-activated HUVECs were observed.

Regulation of immunosuppression-associated cells

The TME functions as a crucial ecological niche supporting tumor progression and metastasis. TAMs, the most prevalent component of the TME, play pivotal roles in angiogenesis, cancer cell proliferation and metastasis, immunosuppression, and resistance to cancer treatment [49, 50]. Macrophages demonstrate high plasticity and primarily polarize into two phenotypes: M1-like macrophages with antitumor capabilities and M2-like macrophages that promote tumor growth and immunosuppression. In most murine and human tumors, TAMs exhibit an M2-like phenotype [51]. Thus, strategies to shift the TAM M1/M2 ratio are gaining traction in cancer treatment. The JNK pathway plays a pivotal role in steering macrophages toward M2-like polarization. Consequently, strategies to alter the TAM M1/M2 ratio are gaining prominence in cancer treatment. The JNK pathway plays a crucial role in directing macrophages toward M2-like polarization [52]. A study conducted by our group demonstrated that GL enhanced the phosphorylation of JNK1/2, thereby significantly inhibiting the M2-like polarization of macrophages. An increased M1/M2 ratio was observed in breast-tumor-bearing mice treated with GL. Moreover, GL significantly suppressed the progression of metastatic breast cancer [53]. Additionally, our group developed a GL-lipid framework nano-vehicle loaded with triptolide. The capacity of this nano-vehicle to shift TAM polarization from M2 to M1 was thoroughly investigated both *in vivo* and *in vitro*. As anticipated, the findings revealed satisfactory anti-HCC outcomes due to the synergistic effects of inducing cancer cell apotheosis and regulating TAM polarization [28].

CAFs are a crucial component of TME and play a significant role in cancer progression [54]. CAFs can impair immune cell functions by secreting various cytokines or metabolites, shape the tumor extracellular matrix, and form barriers that hinder the penetration of therapeutic agents or immune cells into the tumor, thereby reducing therapeutic efficacy [55]. DAVIDSON et al. demonstrated that GL and its derivatives are novel inhibitors of gap-junctional intercellular communication between human fibroblasts [56]. Alpha-smooth muscle actin (*α*-SMA, also known as *α*-actin 2), expressed by many activated CAF subsets, serves as a marker of CAF-targeted therapeutic efficacy [57]. TANG et al. found that GL could significantly downregulate *α*-SMA expression. When combined with DOX in a nanosystem, GL synergistically enhanced breast cancer treatment outcomes by inhibiting the crosstalk between tumor cells and CAFs [58].

Treg cells are classified as immunosuppressive $CD4^+$ T cells. The immunosuppressive TME mediated by Treg cells is proposed as a critical mechanism of cancer immune escape [59, 60]. Additionally, MDSCs, an important type of immunosuppressive immune cell, can suppress natural killer (NK) cells and effector T cells while expanding Treg cells, also playing a pivotal role in the immunosuppressive TME [61]. JUIN et al. demonstrated that GL exhibited an immunomodulatory role by attenuating both Tregs and MDSCs through inhibiting phosphorylation of signal transducer and activator of transcription 3 (p-STAT3) [14]. DU et al. further proved that co-delivery of GL and Ce6-labeled-DNAzyme *via* a hollow-manganese dioxide (H-MnO_2) nanosystem could activate tumor immunity and weaken Treg cells, thereby amplifying the immunotherapeutic effects in melanoma-bearing mice [27]. CUI *et al.* revealed that Treg cells were significantly downregulated *via* an exosome (EXO)-coated tanshinone II A (TAN) and GL self-assembled nanomicelle, demonstrating a promising chemo-immunotherapy outcome against glioblastoma [62].

Structural Characteristics and Delivery Issues of GL

Despite these numerous bioactivities for tumor treatment, the targeted delivery of GL *in vivo* frequently encounters significant challenges due to its poor water solubility, susceptibility to degradation in the gastric acid environment, and low oral bioavailability [63]. Furthermore, considering the potential hemolysis associated with natural saponins [64], intravenous administration of GL as a free drug also presents difficulties. The solubility, surface tension, aggregate formation, biological activity, and pharmacokinetics of GL and its derivatives can be altered by modifying the pH or chemically altering carboxyl, hydroxyl, or sugar groups in the structure. GL exhibits very low water solubility in the lower pH range, with solubility gradually increasing above pH 4.5. At pH 5−6, GL can form numerous ordered molecular aggregates, specifically micelles. The distinctive pH dependence of GL and the presence of aggregates underscore its potential application not only as an emulsifier and solubilizer but also as a drug delivery vehicle to enhance bioavailability, facilitate passive or active cancer targeting, and delay drug release. Leveraging the micellar dissociation of GL in alkaline conditions, it can be further utilized as a carrier for release under specific alkaline conditions [65, 66].

Due to the different trans and cis configurations of C_{18}-H in GL's structure, it can form two isomers: 18*α*-GL and 18*β*-GL (Fig. 1A). Although 18*α*-GL exhibits higher lipophilicity, bioavailability, and liver accumulation [67] compared to 18*β*-GL, higher concentrations of 18*β*-GL are found in liquorice herbs. Consequently, 18*β*-GL is more frequently utilized. GL

comprises two molecules of hydrophilic glucuronic acid and one molecule of hydrophobic GA [68]. Based on its structural characteristics, researchers have designed a series of derivatives to enhance druggability and bioactive efficacy. These derivatives are typically obtained by introducing alternative groups on the carboxyl groups of the pentacyclic triterpene structure or conjugating residues into the carbohydrate part (Fig. 1B). Introducing carbohydrate segments, such as amino sugar molecules, by modifying the carbohydrate chain of GL can increase its solubility and cell membrane permeability while controlling its interaction with biological targets [69]. GA, the product of GL losing its glycosyl moiety through hydrolysis reaction, has low water solubility. Cyclodextrin can synthesize water-soluble triazole-bridged *β*-CD-pentacyclic triterpene conjugates with pentacyclic triterpenes, thereby improving the solubility, stability, and bioavailability of GA and its derivatives [70]. The introduction of N-acetylglucosamine into the glycosidic chain of GL can increase the anti-SARS-CoV activity of GL up to 9-fold [71]. Furthermore, given the high specific binding sites for GL on the cellular membrane of hepatocytes [72], several studies have successfully utilized GL as a special ligand to graft a series of polymers for hepatocyte-targeting by grafting with the carboxyl group in the hexatomic ring of GL [24, 73].

Given GL's desired interference effect on the tumor microenvironment and its poor druggability, approaches to facilitate GL's tumor-targeted accumulation are urgently needed. While some nanocarriers, such as liposomes, micelles, and NPs, are available for cancer treatment, the majority of these carriers suffer from low drug-loading efficiency. Notably, sufficient tumor suppression efficacy has been achieved through the combination of GL and other therapeutic agents. However, co-delivering multiple agents in a single vehicle would exacerbate the drug-loading disadvantages. Consequently, employing GL as the scaffold of drug delivery systems, rather than solely as the payload, would be more valuable in combination therapy for tumor treatment.

GL-based Nanosystems for Cancer Treatment

Polymer-drug micelles

To date, numerous studies have investigated the utilization of GL in nanomaterials, particularly polymer–GL conjugate therapeutics. For instance, WU et al. developed a tumor-targeting nano-sized drug delivery system by synthesizing GL-conjugated human serum albumin NPs encapsulating resveratrol. *In vitro* studies demonstrated that these NPs released drugs in a sustained and controlled manner. Furthermore, the GL-conjugated human serum albumin NPs exhibited enhanced uptake by HepG2 cells and preferential accumulation in liver tumors *in vivo* [74]. In a separate study, GL was conjugated with bovine serum albumin to form NPs for encapsulating 10-hydroxycamptothecin (HCTP). These NPs showed promise for the targeted treatment of liver tumors, suggesting a novel carrier approach for HCC therapy [75].

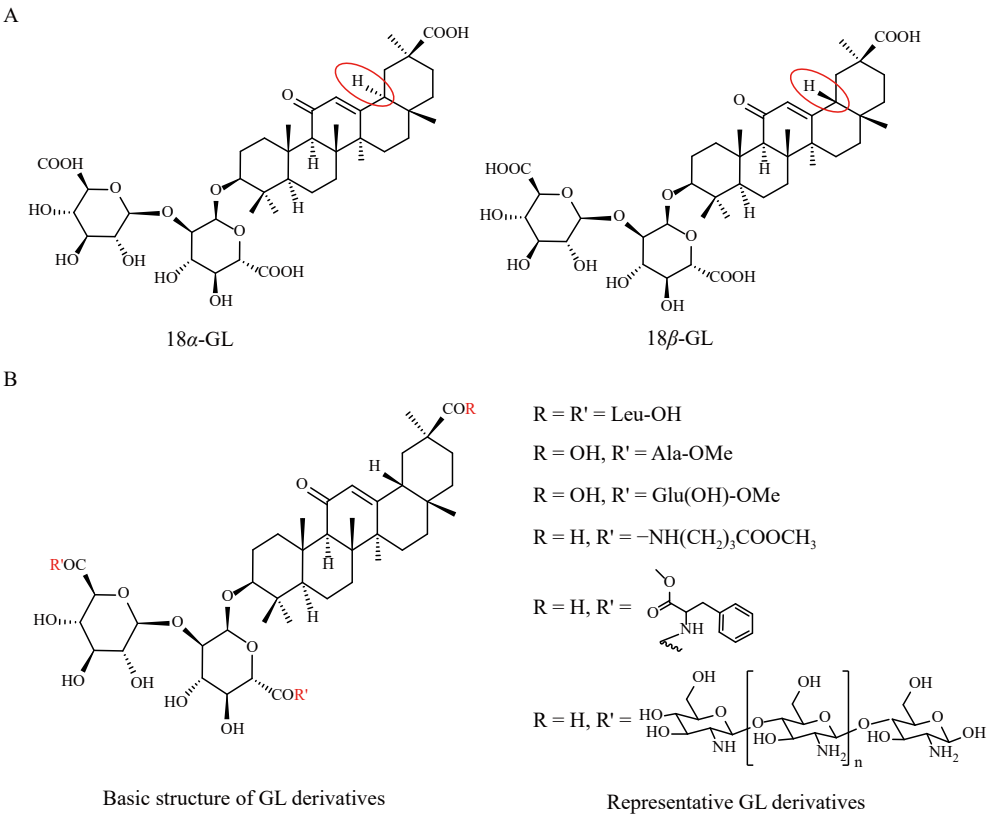


Fig. 1 Structures of (A) 18*α*-GL, 18*β*-GL, and (B) GL derivatives.

TIAN et al. developed liver-targeted NPs (siRNA/DOX/DPP) using 1,2-distearoyl-snglycero-3-phosphoethanolamine-polyethyleneglycol-polyetherimide (DSPE-PEG-PEI) and GL-modified hyaluronic acid (GL-HA) for the co-delivery of DOX and Bcl-2 siRNA. These NPs were approximately spherical and displayed dose-dependent cytotoxicity against HepG2 cells. Additionally, the siRNA/DOX/GH-DPP NPs induced greater apoptosis, demonstrating superior antitumor effects compared to their counterparts without GL (siRNA/DOX/DPP) and GH-DPP NPs delivering DOX or Bcl-2 siRNA alone. These findings underscore the potential of GH-DPP NPs to deliver both chemotherapeutic drugs and siRNAs to tumors, indicating their significant potential in antitumor therapy [76]. TONG Yang developed a novel polymeric prodrug micellar carrier based on polyethylene GL (PEG-Fmoc-GL) for the co-delivery of DOX as part of a combined anticancer treatment. The synthesized PEG-Fmoc-GL polymeric prodrugs could self-assemble to form prodrug micelles and serve as carriers for further physical encapsulation of DOX. Following intravenous injection, the micelles accumulated in tumor tissues through the enhanced permeability and retention effect. The PEG-Fmoc-GL-conjugated micelles significantly enhanced the intracellular uptake of DOX in HepG2 cells compared to the use of DOX alone.

Subsequently, these micelles underwent endocytosis into HepG2 cells, where they decomposed and released DOX and GL into the cytoplasm, inhibiting cell proliferation enhancement and inducing apoptosis, thus achieving a synergistic antitumor effect [24]. To enhance the oral bioavailability of paclitaxel (PTX), GL was utilized as a carrier. PTX-loaded GL micelles were prepared, exhibiting small particle sizes and spherical shapes. The micelles demonstrated high encapsulation efficiency (90%) and drug-loading rate (7.90%). *In vitro* release experiments showed delayed drug release compared to taxol. Pharmacokinetic analysis in rats revealed a significantly higher area under the plasma concentration-time curve (AUC0→24 h) for PTX-loaded GL micelles than for taxol, indicating enhanced oral absorption, potentially due to increased uptake in the jejunum and colon [77]. These findings suggest GL's potential as a carrier for oral PTX delivery. In another study, researchers developed nanocomplexes using GL for efficient curcumin (CUR) delivery. Sonication of an amphiphilic GL solution with hydrophobic CUR yielded nano-sized complexes (164.8 ± 51.7 nm), significantly enhancing CUR solubility in aqueous solutions. Furthermore, GL/CUR nanocomplexes exhibited high intracellular uptake in both human breast cancer cells and macrophages (RAW264.7 cells). Consequently, these GL/CUR nanocomplexes demonstrated stronger anticancer effects than free CUR, effectively reducing the release of the pro-inflammatory cytokine TNF-α [78]. LI *et al.* utilized the self-assembly properties of GL and polymer-PLGA to encapsulate piperine and modified it with transferrin to create transferrin-modified NPs (Tf-PIP-NPs). These NPs enhanced antitumor drug efficacy through rapid internalization into tumor cells *via* transferrin receptor-mediated endocytosis, inducing apoptosis and mitochondrial membrane potential loss. Additionally, the Tf-PIP-NPs significantly upregulated proapoptotic protein levels and induced tumor necrosis, indicating their potential to enhance piperine's antitumor efficacy [37]. CAO *et al.* developed an effective gene delivery system for HCC treatment by covalently conjugating GL or GL with polyethyleneimine. These conjugates demonstrated enhanced transfection efficiency and targeted delivery to HepG2 cells, offering a promising approach for HCC therapy. By utilizing a non-caveolae- and non-clathrin-mediated endocytosis pathway, the authors bypassed lysosomal entry and achieved high gene expression levels in an HCC tumor model, highlighting their potential for *in vivo* applications [79]. To address the limitations of HCTP as a DNA topoisomerase I inhibitor, researchers developed injectable GL-HCPT micelles. These micelles significantly improved HCPT's solubility and stability, facilitating rapid internalization by HepG2 cells and increasing intracellular HCPT accumulation. Compared to conventional HCPT formulations, GL-HCPT micelles exhibited enhanced antitumor activity against liver cancer cells and effectively suppressed tumor growth in a mouse model. Moreover, these micelles showed preferential accumulation in the liver while minimizing cytotoxicity to normal liver cells [80]. GL, serving as a nanocarrier, can also be applied to treat brain disorders. A study demonstrated that chitosan-coated-Poly-ε-caprolactone (PCL)-NPs prepared using the double emulsion solvent evaporation method improved drug brain bioavailability in rats. In middle cerebral artery (MCA)-occluded rats, the administration of these NPs resulted in enhanced neurological behavior and a simultaneous reduction in proinflammatory cytokine levels (TNF-α and IL-1β) [81].

Drug-drug assembly system

TME maintains an immunosuppressive state, which impedes drug identification of lesion locations and accurate release. This challenge accounts for the difficulty in delivering ordinary nanomedicines and their unsatisfactory therapeutic efficacy. GL receptors, expressed on the sinusoidal surface of hepatocytes, have been widely utilized as a targeting ligand for liver therapy, enhancing liver or hepatocyte drug uptake and therapeutic efficacy. SUN et al. developed an amphiphilic GL-porphyrin conjugate for photodynamic cancer therapy. The hydrophilic GL improved the solubility of tetraphenylporphyrin (TPP) in water, facilitating efficient endocytosis by various cancer cells and enabling precise photodynamic therapy effects post-irradiation [82]. Another approach involves GL co-assembling with oleanolic acid (OA) through non-covalent interactions, forming nanocarriers for anticancer drug delivery. A notable example is the co-assembled OA-GL nanocarrier, exhibiting excellent stability, high drug-loading efficacy, and sustained release properties. When loaded with paclitaxel, it enhances the antitumor effect and reduces liver damage through upregulated antioxidant pathways induced by OA and GL [83]. TAN, a major lipophilic non-quinone compound extracted from *Salvia miltiorrhiza* root, demon-

strates anti-oxidation, anti-inflammatory, anti-thrombosis, blood pressure-lowering, vascular endothelial dysfunction-regulating, anti-liver fibrosis, and antitumor activities. Pu's group [84] developed TAN-GL co-loaded self-assemblies as a delivery system to enhance the chemical stability and solubility of TAN and GL, producing a synergetic anti-hepatoma effect. WANG *et al.* [62] utilized TAN and GL self-assembled to form targeted glycol chitosan micelles (TGM) nanomicelles. They employed endogenous serum EXOs to decorate the TGM nanomicelles' surface and anchored CpG oligonucleotides on the EXO membrane to obtain CpG-EXO/TGM. Both *in vitro* and *in vivo* results demonstrated that CpG-EXO/TGM could prolong blood circulation and increase targeting and cellular uptake of glioblastoma cells, inducing tumor cell apoptosis. CpG-EXO/TGM also activated dendritic cells and polarized TAMs, resulting in improved efficacy. QIU et al. combined GL and DOX to prepare M-DOX-GL NPs for breast cancer treatment. These NPs effectively targeted and released the drug within breast tumors, demonstrating good tumor selectivity and substantial therapeutic effect, with a tumor inhibition rate of 77.37%. Immunofluorescence detection and western blotting analysis verified GL's ability to remodel the TME [58]. Collectively, these findings indicate that GL is a crucial targeting ligand in liver-targeted drug delivery systems, demonstrating the ability to regulate the immunosuppressive TME.

GL-based lipid NPs

Liposomes have been extensively utilized for anticancer drug delivery due to their significant enhancements in delivering anticancer agents to tumor tissues compared to their corresponding free forms [85]. Cholesterol, a crucial component of liposomes, maintains the stability of the liposome phospholipid bilayer membrane. However, cholesterol is vulnerable to external factors such as oxygen, light, and metals, potentially leading to the formation of cholesterol oxidation products during processing and storage. These products may exhibit cytotoxicity, mutagenicity, and carcinogenicity [86]. Consequently, it is essential to identify alternative compounds with structures similar to cholesterol for liposome construction. GL, with its steroid structure similar to cholesterol, demonstrates favorable pharmacological activity and can serve as a cholesterol substitute. GL not only maintains the drug-loading performance of liposomes but also confers additional therapeutic or targeting functions, embodying a "drug and adjuvant integration" approach. Zhang's group utilized GL to construct the lipid membrane backbone of GL-based lipid NPs and incorporated TP into the lipid bilayer. The prepared GL-based NPs exhibited drug-loading efficiency, particle size, and stable storage capacity comparable to traditional cholesterol-based liposomes. Moreover, GL-based NPs demonstrated superior safety profiles, higher membrane fluidity, and lower opsonin protein absorption rates compared to conventional liposomes. GL-based NPs also showed enhanced uptake and improved cytotoxicity in HepG2 cells, as well as improved retention and accumulation in the tumor area compared to TP-loaded conventional liposomes. Additionally, GL-based NPs could ameliorate tumor immunosuppression by promoting TAM polarization toward the M1 phenotype. Treatment with GL-based NPs led to reductions in the expression levels of tumor recombinant Ki-67 protein and VEGF, resulting in synergistic antitumor efficacy [28]. Similarly, HAN *et al.* developed GL-based liposomes loaded with cantharidin [GL–CTD–loaded polymeric micelles (LP)] using GL as a cholesterol alternative. These GL-CTD-LP selectively targeted tumor cells and significantly improved cellular absorption through GL-receptor-mediated endocytosis. The tumor inhibitory rate of GL–CTD–LP reached 74.79%, which was 1.33, 2.12, and 1.36 times higher than that of CTD−LP (56.42%), free CTD (35.34%), and Cerebrotendinous xanthomatosis (CTX) (54.95%), respectively. These findings highlight the potential of GL-based liposomes as novel nanocarriers, offering an innovative platform for efficient anticancer drug delivery [87].

GL-based hydrogel

GL exhibits amphiphilic properties that enable its self-assembly into hydrogels through non-covalent interactions, including hydrogen bonding, hydrophobic interactions, and π-π stacking. These interactions facilitate the close association of GL molecules, initiating the formation of fibrous or micellar structures that interweave to create a three-dimensional network, ultimately resulting in hydrogel formation [88, 89]. The gelatinization process of GL is influenced by various factors, such as pH, temperature, concentration, and solvent conditions. These hydrogels demonstrate excellent biocompatibility and biodegradability, as well as responsive characteristics to environmental stimuli, highlighting their potential in drug delivery systems and other therapeutic applications [90, 91].

Studies have examined the impact of metal ions with varying valences and concentrations on the linear and nonlinear rheological properties and network structure of GL supramolecular hydrogels. Monovalent metal ions (Na and K) demonstrate weaker binding affinity with GL, primarily relying on the electrostatic shielding effect at high concentrations (e.g., 50 mmol·L^{-1}) to enhance interactions between GL fibers, thereby forming a denser and more ordered gel network. This network exhibits distinct nonlinear rheological behavior, characterized by a typical transition from elastic to viscous response. Conversely, multivalent metal ions (Ca, Zn, and Al), owing to their larger charge density and stronger binding with GL, significantly enhance the network strength of the hydrogel at low concentrations (e.g., 5 mmol·L^{-1}). However, at high ion concentrations (e.g., 50 mmol·L^{-1}), excessive cation-carboxylate complexation leads to the formation of discrete aggregated network structures of GL-M, resulting in irregular nonlinear rheological responses and reduced resistance to large deformations. These insights enhance our understanding of the highly tunable rheological behavior and network structure of GL hydrogels, presenting new opportunities for the design and development of respons-

ive, natural supramolecular hydrogels with controlled properties through metal ion coordination strategies [92].

Drawing inspiration from effective clinical treatments and the directed self-assembly of natural saponin GL, researchers have developed a novel GL-based hybrid hydrogel to enhance full-thickness wound healing and bacterial-infected wound repair. This advancement is attributed to a layered dual network comprising a self-assembled hydrogen-bonded fiber network containing aldehydated GL (AGL) and a dynamic covalent network formed through Schiff base reactions between AGL and the biopolymer carboxymethyl cellulose (CMC). The AGL-CMC hydrogel demonstrated excellent stability, mechanical properties, and multifunctional characteristics, including injectability, shape adaptability and reshaping, self-repair, and adhesive capabilities [93]. Moreover, leveraging the amphiphilic structure of GL, GL-based micelle hydrogels were fabricated to encapsulate CUR through non-covalent interactions. These micelle hydrogels exhibited outstanding stability, remarkable biocompatibility, and superior antioxidant performance [94].

Leveraging GL's gelatinization property as a scaffold, several GL-based hydrogels for tumor treatment have been developed. LEI et al. created a natural product hydrogel based on the self-assembly of GK (a natural chemical substance from *Genkwa Flos*) and GL for breast cancer therapy. Using a one-step "green" approach, the GK-GL hydrogel significantly inhibited tumor growth and prevented lung metastasis without apparent toxicity [95]. WANG et al. also developed a natural small molecule carrier-free injectable hydrogel composed of Cu^{2+}, GL, and norcantharidin (NCTD), utilizing coordination interactions and hydrogen bonds. Under 808 nm laser irradiation, this carrier-free hydrogel generated ROS, depleted glutathione, and mitigated hypoxia in the TME, thereby synergistically modulating the TME through apoptosis, ferroptosis, and anti-inflammatory effects. The preparation process was simple, environmentally friendly, and cost-effective, requiring no additional excipients. Compared to micelles or NPs, hydrogels with three-dimensional networks function more like warehouses, continuously releasing drugs at the tumor site with minimal side effects on normal tissues and organs. These studies provided clear evidence that carrier-free hydrogels based on the self-assembly of natural compounds with GL offer a synergistic antitumor strategy [96].

Conclusion and Perspectives

GL, a recognized bioactive compound in licorice, has attracted increasing attention for its antitumor properties, particularly when combined with other therapeutic drugs to enhance antitumor effects. Notably, GL has also demonstrated potential as a promising carrier material. This review provides a comprehensive analysis of GL's anticancer effects, focusing on its role in tumor microenvironment regulation and state-of-the-art GL-based delivery strategies. While previous reviews have examined diverse GL-based carriers, including inclusion complexes, GL-modified bovine serum albumin, chitosan, and dendrimer NPs, this work uniquely summarizes novel GL-based vehicles such as cholesterol-free liposomes, self-assembly NPs, and hydrogels.

Despite the advancements in nanotechnology and immunotherapy in the field of tumor treatment, several challenges in nanotechnology continue to significantly limit the clinical success of GL-based nanosystems [97]. Primarily, the common characteristic of nanosystems, including all marketed cancer nanomedicines, relies on EPR effects for passive tumor targeting. This approach is influenced by tumor heterogeneity, leading to variations between and within patients. The mechanisms and tumor specificity of GL-mediated cancer-targeting accumulation remain unclear. Additionally, the technological repeatability during pharmaceutical scale-up of commonly used laboratory techniques for GL-based nanosystems, such as nanoprecipitation, emulsified solvent evaporation, supercritical fluid, and high-pressure homogenization, requires further consideration. Improvements are necessary to enhance drug loading capacity and reproducibility. Furthermore, considering GL's role in chemical synthesis and as a pharmaceutical adjuvant, the potential benefits of GL-based nanomedicine must be evaluated based on factors such as production cost, biosafety, and the complexity of the preparation process. Consequently, improving the clinical conversion rate remains the central challenge in applying these GL-based nanomedicines for tumor immunotherapy.

Given the extensive bioactivities of GL [98], the potential applications of these GL-based delivery systems are considerable. These systems have demonstrated significant potential in addressing representative inflammatory diseases, including colitis, neurodermatitis, psoriasis, and arthritis. While this approach offers a novel method for enhancing combination therapy, further fundamental research is necessary to substantiate the clinical application and therapeutic potential of GL-based systems.

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