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Design and Synthesis of Galactose-Biotin Lipid Materials for Liposomes to Promote the Hepatoma Cell–Targeting Effect

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ABSTRACT

A series of novel low-toxic hepatoma cell–targeting lipid materials were designed and synthesized, in which monogalactose, digalactose, and galactose-biotin were used as targeting moieties and hydrophilic heads while stearate was used as hydrophobic tail (Mono-Gal-ST, Di-Gal-ST, and Gal-Biotin-ST). The corresponding galactose-biotin-modified liposomes (Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs) and conventional liposomes (LPs) were prepared. These galactose-biotin-modified liposomes can distinguish hepatoma cells from other tissue cells owing to the recognition of asialoglycoprotein receptor by galactose group. Moreover, the ability of liposomes to distinguish hepatoma cells from normal hepatocytes follows a trend of LPs < Mono-Gal-LPs < Di-Gal-LPs < Gal-Biotin-LPs, which is attributed to the cluster glycoside effect and the synergistic effect of galactose and biotin. In addition, the endocytosis of these galactose-biotin-modified liposomes were competitively inhibited by galactose, further confirming these liposomes entered hepatoma cells via asialoglycoprotein receptor–mediated pathway.

Introduction

Hepatocellular carcinoma (HCC) annually results in more than 1 million deaths in the world, being the third place of malignant tumor mortality.[1,2] 10-Hydroxycamptothecin (HCPT) shows high cytotoxicity against hepatoma cells through inhibiting topoisomerase I and inducing cell apoptosis.[3,4] However, the widespread clinical application of HCPT as liver cancer chemotherapeutics is limited by its poor solubility, low bioavailability, poor selectivity, and significant toxic side effects.[5,6]

As we known, nanoparticular, micellar, and liposomal formulations can help to improve the bioavailability of chemotherapeutics and reduce the systemic toxicity of chemotherapeutics to some extent.[7-14] For example, the pectin nanoparticles[15] can improved the water solubility of HCPT and the chitosan micelles can reduce the systemic toxicity of doxorubicin,[16] but the materials of most nanoparticles and polymeric micelles are short of enough safety for their clinical application.[17,18] By contrast, liposomes are mainly composed of natural phospholipids which are biologically safe. Therefore, liposomes were the first nanocarriers used in clinical applications. To date, some liposomes encapsulated with antitumor drugs (doxorubicin, paclitaxel, mitoxantrone, etc) have been in clinical trials or approved into markets.[18,19] However, these conventional liposomes are still unsatisfactory in the targeting effect to tumor cells, particularly to hepatoma cells.

To improve the hepatoma-cell selectivity and reduce systemic toxicity of anticancer drugs, some liver-targeting drug delivery systems have been developed through the modification with galactose, glycyrrhetinic acid, cholic acid, and so on.[20-25] Among these liver-targeting moieties, galactose can specially recognize the asialoglycoprotein receptor (ASGPR) overexpressed on the surfaces of hepatocytes, being one of the efficient liver-targeting group. For example, arabinogalactan-modified doxorubicin liposomes exhibited higher uptakes in the mice liver than the conventional liposomes.[26] The galactosyl modification promoted polymer microbubbles to accumulate in the hepatoma cells.[27] Doxorubicin nanoparticle modified with galactosamine showed higher

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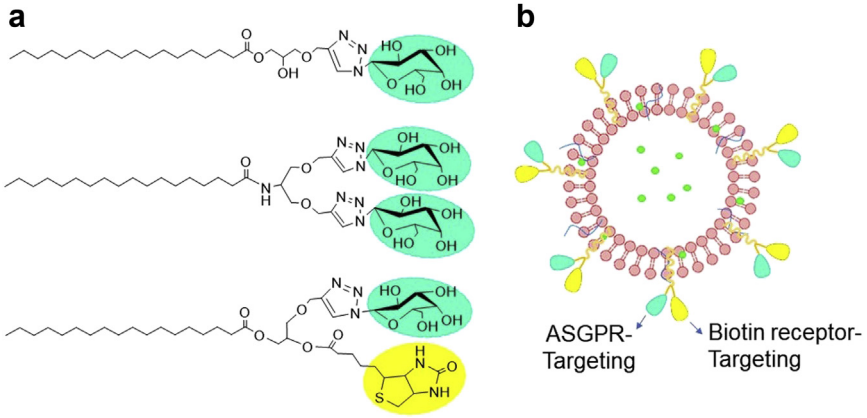


Figure 1. (a) Chemical structures of targeting lipid materials. (b) Schematic illustration for the liposomes modified with targeting lipid materials.

therapeutic effect in xenograft-bearing nude mice than non-targeted nanoparticles and free doxorubicin.[28]

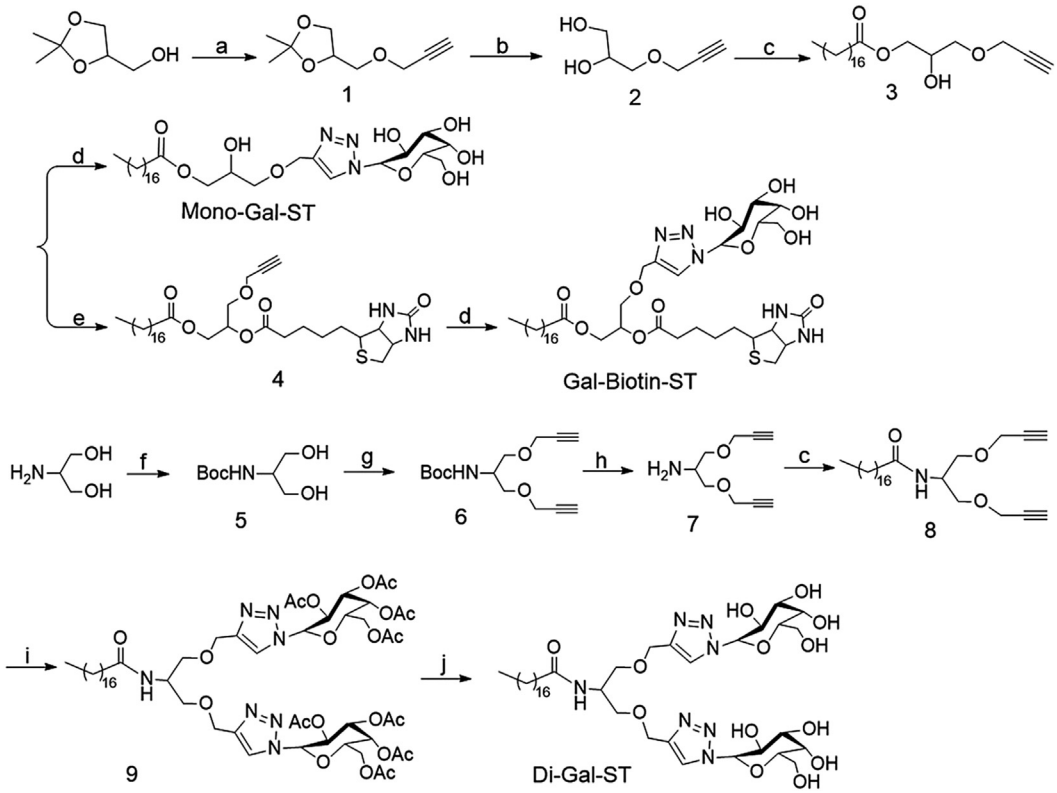
The modification of drug delivery systems with tumor-targeting group is another strategy to improve the hepatoma cell—targeting effect. For instance, a novel prodrug modified by biotin, which can specifically recognized the biotin receptors overexpressed on the surface of hepatoma cells, was developed.[29] The biotin-modified doxorubicin liposomes can obviously enhance tumor accumulation and decrease the undesired distribution in other tissues.[30] Biotin-modified rhodamine-triphenylphosphonium probes showed an excellent tumor-targeting effect.[31] Clearly, galactose- or biotin-modified materials can improve the hepatoma cell—targeting capability of drug delivery systems. However, the influences of glycocluster-modified or galactose-biotin comodified lipid materials on the hepatoma cell—targeting potential of the corresponding liposomes are still in the shadow.

To address these issues, several novel hepatoma cell—targeting lipid materials (Mono-Gal-ST, Di-Gal-ST, and Gal-Biotin-ST) are designed (Fig. 1) and synthesized (Scheme 1). Then, the HCPT-loaded liposomes assembled by these targeting lipid materials were constructed and characterized.

Experimental

Materials and Instruments

The chemicals and solvents were commercially available and used without further purification. 10-Hydroxycamptothecine was



Scheme 1. Synthetic Routes of targeting lipid materials. Reagents and conditions: a: NaH, 3-bromo-1-propyne; b: CH_3OH, ion-exchange resin; c: stearic acid, EDCI, DMAP; d: Gal-N_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate; e: biotin, EDCI, DMAP; f: $(BOC)_2O$, CH_3OH g: KOH, 3-bromo-1-propyne; h: dry CH_2Cl_2, CF_3COOH; i: acetyl-Gal-N_3, $CuSO_4 \cdot 5H_2O$, sodium ascorbate; j: CH_3OH, CH_3ONa.

Table 1
Lipid Formulation, Particle Size, Zeta Potential, Encapsulation Efficiency, and Drug Loading of Liposomes

Liposomes	SPC/CHOL/TLM/HCPT (molar Ratio)	Particle Size (nm)	Zeta (mV)	EE (%)	DL (%)
LPs	10:1:1:1	213.5 ± 1.4	−18.2 ± 1.6	67.2 ± 3.5	7.1 ± 1.1
Mono-Gal-LPs	10:1:1:1	230.3 ± 1.8	−36.9 ± 2.2	65.4 ± 3.7	6.7 ± 1.3
Di-Gal-LPs	10:1:1:1	218.7 ± 3.4	−17.0 ± 2.8	71.7 ± 3.6	7.9 ± 1.3
Gal-Biotin-LPs	10:1:1:1	213.6 ± 2.9	−38.7 ± 2.3	68.2 ± 4.2	7.2 ± 1.7

TLM, targeting lipid materials; EE%, encapsulation efficiency; DL%, drug loading; SPC, soy phosphatidylcholine; CHOL, cholesterol; TLM, targeting lipid materials.

purchased from Chengdu Yuancheng Bio-Tech co., Ltd. (Sichuan, China). Galactose was obtained from Xilong chemical co., Ltd. (Guangdong, China). Biotin was purchased from Bide Pharmatech Ltd. (Shanghai, China). Stearic acid was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 MHz (Switzerland) at 25°C, with Tetramethylsilane (Shanghai, China) as the internal standard in $CDCl_3$, and the solvent peak as the internal reference in CD_3OD. MS analysis was performed with a Thermo Fisher Scientific LTQ FT Ultra. The cytotoxicity assays were conducted using EL×800 absorbance microplate reader. Cell image assays were performed by multiphoton laser confocal scanning microscopy (Leica-TCS-SP8MP, Germany).

Synthesis of Targeting Lipid Materials

As shown in Scheme 1, DL-1,2-isopropylidene-glycerol or 2-amino-1,3-propanediol scaffold, which was used as starting material, reacted with propargyl bromide to give the alkynyl compounds. The alkynyl compounds and stearic acid then undergo an esterification reaction or an acylation reaction to give the alkynyl chain compound. Finally, the galactose or biotin was introduced by a click reaction or an esterification reaction to give the aimed materials. The structures of these compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and MS (Supporting Information).

Preparation of Liposomes

All liposomes were prepared by thin-film evaporation.[32,33] For the preparation of the conventional liposomes (LPs), the mixture of soy phosphatidylcholine, cholesterol, and HCPT were dissolved in the mixed solvent of chloroform and methanol (5:1, v/v) with a molar ratio of about 10:1:1. The solvent was then evaporated at 45°C for 1 h to form a dry lipid film. Next, the film was hydrated for 2 h with PBS solution (pH 6.86) and ultrasonicated in an ice−water bath for 3 times (10 min each time) to decrease the particle size. The final lipid concentration was 10 mg/mL. The unentrapped HCPT was removed by dialysis. Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs were prepared using the similar protocol besides adding different targeting lipid materials, and the molar ratio of soy

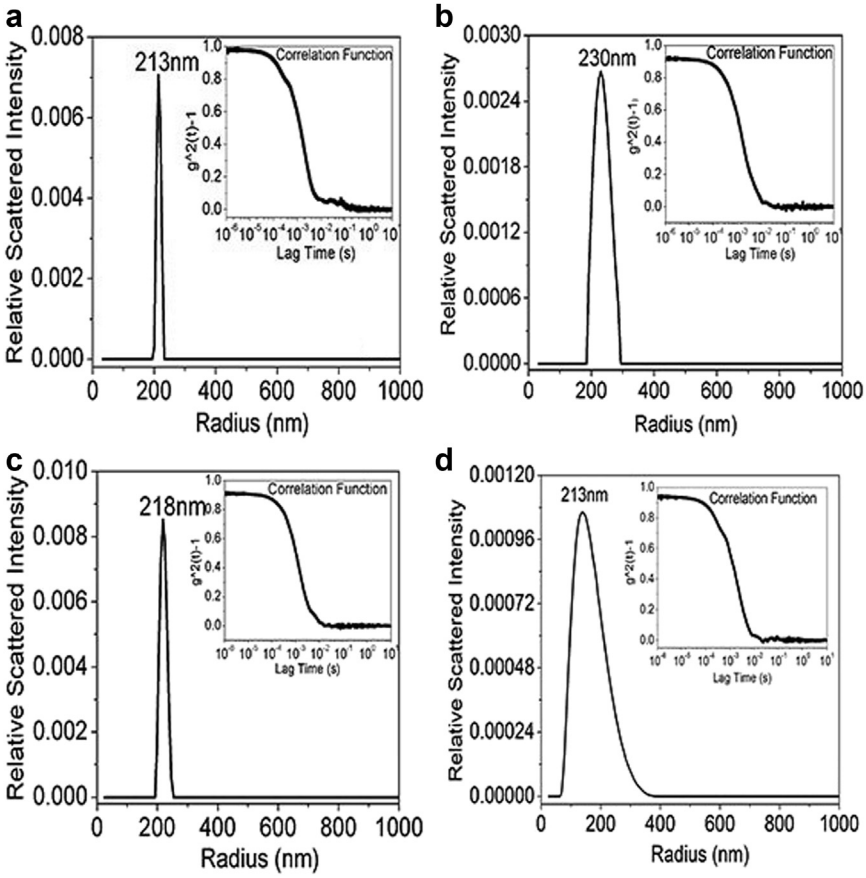


Figure 2. Size distributions of liposomes in aqueous solution. (a) LPs. (b) Mono-Gal-LPs. (c) Di-Gal-LPs. (d) Gal-Biotin-LPs.

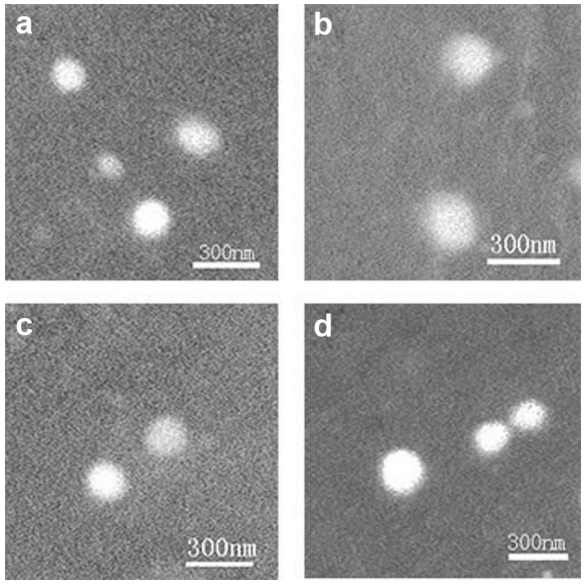


Figure 3. Scanning electron microscopic micrograph of liposomes. (a) LPs; (b) Mono-Gal-LPs; (c) Di-Gal-LPs; (d) Gal-Biotin-LPs.

phosphatidylcholine/cholesterol/targeting lipid materials/HCPT was 10:1:1:1 (Table 1).

The encapsulation efficiency and drug loading of HCPT were calculated according to the following equations.

$$EE(\%) = \frac{C_e}{C_t} \times 100\%$$

where C_t stands for the total drug amount added and C_e stands for the drug amount encapsulated in the liposomes.

$$DL(\%) = \frac{\text{weight of encapsulated HCPT}}{\text{weight of liposomes}} \times 100\%$$

Particle size and size distribution were measured by 3D DLS Spectrometer (Switzerland) and zeta potential was measured by zeta-phase analysis light scattering. Samples were diluted using water and measured in triplicate. The morphology of freeze-drying liposomes was observed by scanning electron microscopy.

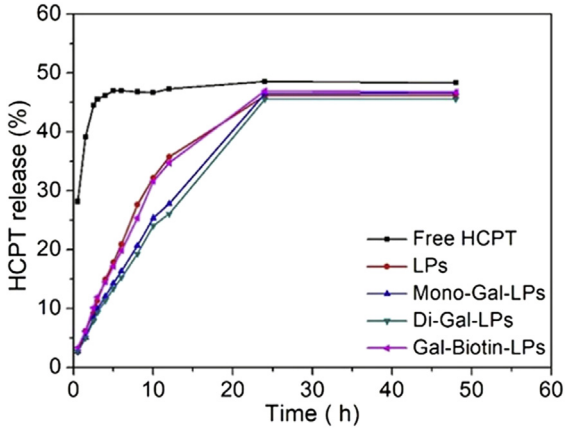


Figure 4. *In vitro* HCPT release profiles of different formulations.

In Vitro *Drug Release*

In vitro release of HCPT was investigated via dialysis. First, 2 mL of LPs, Mono-Gal-LPs, Di-Gal-LPs, Gal-Biotin-LPs, and free HCPT solution were placed in dialysis bags (molecular weight: 8000~14,000). Then, these dialysis bags were put into 100 mL of PBS buffer solution (pH 6.86) containing 30% methanol in a shaker (37°C, 120 rpm) for 48 h. Samples (2 mL) were withdrawn at different time and replaced with the same volume of fresh medium. These samples were analyzed by high-performance liquid chromatography.

Targeting Effect and Galactose or Biotin Competition Assays

LO2, HepG2, A549, Hela, and SGC-7901 cells were seeded in 24-well plates and cultured for 24 h. Then, the culture medium was replaced with fresh medium containing LPs, Mono-Gal-LPs, Di-Gal-LPs, or Gal-Biotin-LPs with equivalent HCPT concentrations (20 μg/mL). After that, the cells were incubated for another 3 h at 37°C. Subsequently, culture medium was removed, and cells were washed with PBS for 3 times. Finally, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature and rinsed with PBS for 3 times.

HepG2 cells were seeded in a 24-well plate and cultured for 24 h. After that, the cells were divided into 2 groups: control group and galactose competition group. Galactose competition group was preincubated with galactose (a competitive inhibitor of ASGPR, 40 mM) for 2 h at 37°C. Then, both control group and galactose competition group were incubated with Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs (containing 50 μg/mL of HCPT) for 2 h at 37°C. After washing with PBS for 3 times, the cells were fixed with 4% paraformaldehyde and then rinsed with PBS for 3 times.

For biotin competition assays, HepG2 cells were preincubated with 0, 50, 100, and 200 μg/mL of biotin (a competitive inhibitor of biotin receptor) for 2 h at 37°C. After that, HepG2 cells were incubated with Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs (containing 50 μg/mL of HCPT) for 2 h at 37°C. After washing with PBS for 3 times, the cells were fixed with 4% paraformaldehyde and then rinsed with PBS for 3 times.

All of the cell images were captured by multiphoton laser confocal scanning microscopy. The fluorescence quantitative analysis was performed by using ImageJ software to calculate the average fluorescence intensity of cells in each picture.

Cytotoxicity Assays of Targeting Lipid Materials and Liposomes

MTT assays[34] were carried out to evaluate the cytotoxicity of targeting lipid materials. Briefly, HepG2 cells were seeded into 96-well plates at a density of 6000 cells per well and incubated for 24 h. Subsequently, 150 μL culture medium containing different concentrations of targeting lipid materials (0-40 μM) were added in 96-well plates to incubate for 48 h. Then, 10 μL of MTT solution (5 mg/mL) was added. After 4 h coincubation at 37°C, the medium was replaced with 150 μL of dimethyl sulfoxide followed by gentle shaking for 10 min. The absorbance was measured via an EL×800 absorbance microplate reader at 490 nm.

The MTT assays were also performed to investigate the anti-tumor efficacy of liposomes and free HCPT. The HepG2 cells were treated with Mono-Gal-LPs, Di-Gal-LPs, Gal-Biotin-LPs, and free HCPT for 24 h, and then 10 μL of MTT solution (5 mg/mL) was added to coincubate for 4 h. The formed formazan was dissolved by dimethyl sulfoxide. The absorbance was measured at 490 nm.

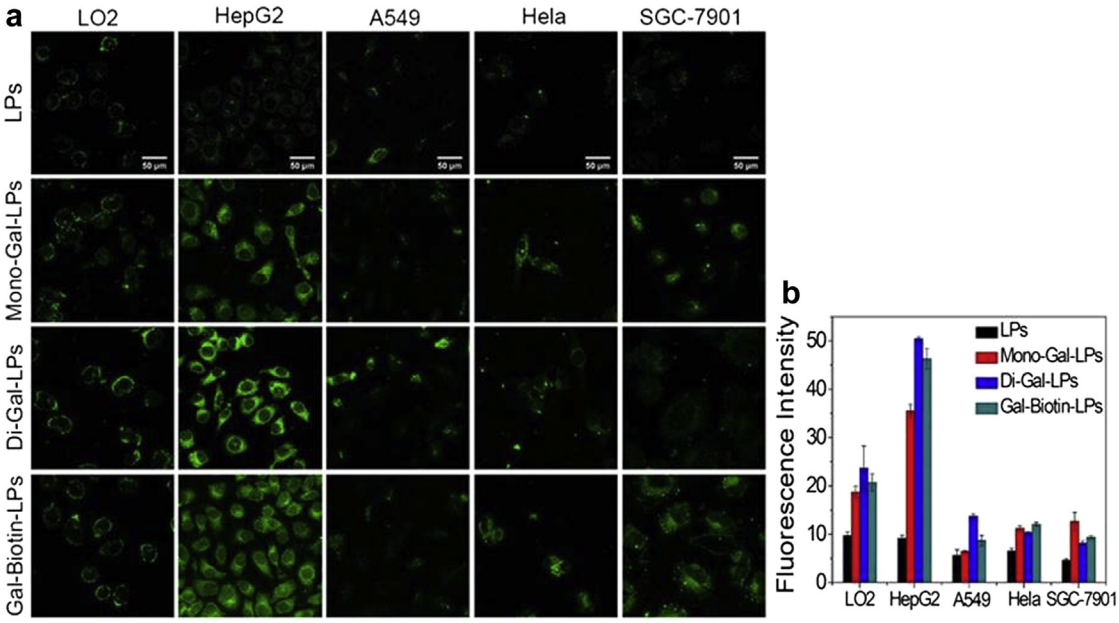


Figure 5. (a) Confocal fluorescence microscopy images of LO2, HepG2, A549, Hela, and SGC-7901 cells incubated with LPs, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs for 3 h at 37°C. (b) The average fluorescence intensity of each image was calculated using Image J software. Scale bar: 50 μm. For the fluorescence intensities of Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs in HepG2 versus those in A549, Hela, or SGC-7901, $p < 0.01$.

Results and Discussion

Characterization of Liposomes

As shown in Table 1, the average particle sizes of LPs, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs were 213, 230, 218, and 213 nm, respectively. The liposomes showed relatively narrow particle size distributions, with the order of distribution width being LPs ≈ Di-Gal-LPs < Mono-Gal-LPs < Gal-Biotin-LPs (Fig. 2). Four types of liposomes were negatively charged (−17.0 ~ −38.7 mV), suggesting that the galactose or biotin modifications did not reverse the electrical potentials of liposomes. Although the HCPT encapsulation efficiency of Mono-Gal-LPs (65.4%) is a little lower than that of LPs (67.2%), the encapsulation efficiencies of Di-Gal-LPs (71.7%) and Gal-Biotin-LPs (68.2%) are higher than LPs, implying that dual-targeting group—modified lipid material may slightly promote the encapsulation efficiencies of liposome. In addition, the drug-loading efficiencies of these liposomes were similar with the values ranging from 6.7% to 7.9%. Furthermore, all 4 types of liposomes had spherical morphology revealed by scanning electron microscopy images (Fig. 3).

In vitro release profiles of free HCPT and HCPT-loaded liposomes are shown in Figure 4. In the first hour, the free HCPT solution rapidly released about 35% HCPT, whereas HCPT-loaded liposomes released about 5% HCPT, indicating that HCPT-loaded liposomes had no burst-release phenomenon. The accumulative drug release of the free HCPT solution reached the peak after 5 h, whereas those of HCPT-loaded liposomes reached the peak after 24 h, indicating that the liposomes possess sustained-release properties. Similar release patterns were observed among LPs, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs, implying that the addition of targeting lipid materials did not apparently affect the drug release behaviors of liposomes. The final accumulative drug release of the free HCPT solution and HCPT-load liposomes were less than 50%, which is because that the concentrations on both sides of the dialysis bag reached balance.

Targeting Effects of Liposomes

To evaluate the hepatoma cell—targeting effects of galactose-biotin-modified liposomes, we investigated their cellular uptakes in HepG2 (hepatoma cells), LO2 (normal hepatocytes), A549 (lung

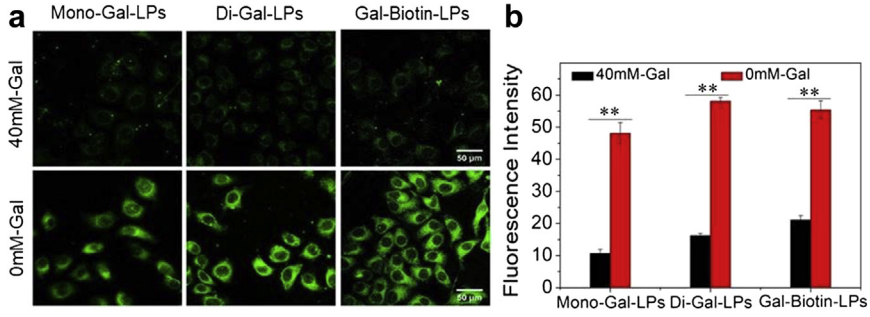


Figure 6. (a) Confocal fluorescence microscopy images of HepG2 cells pretreated with 40 mM galactose (Gal) or 0 mM Gal for 2 h at 37°C and then incubated with LPs, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs for 2 h at 37°C. (b) The average fluorescence intensity of each image was calculated using ImageJ software. Scale bar: 50 μm. $^{**}p < 0.01$.

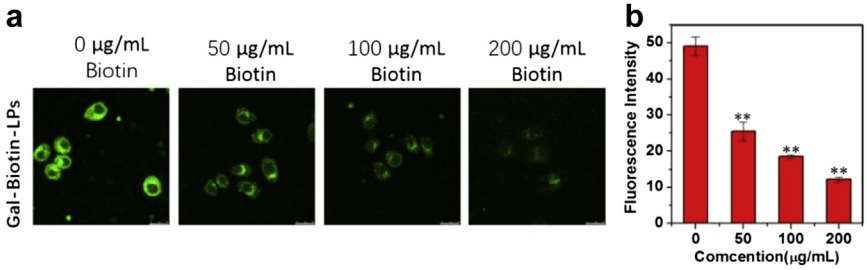


Figure 7. (a) Confocal fluorescence microscopy images of HepG2 cells pretreated with 0, 50, 100, and 200 μg/mL biotin for 2 h at 37°C and then incubated with Gal-Biotin-LPs for 2 h at 37°C. (b) The average fluorescence intensity of each image was calculated using Image J software. Data are presented as mean ± SD, $^{**}p < 0.01$.

cancer cells), Hela (cervical carcinoma cells), and SGC-7901 (gastric cancer cells). As shown in fluorescence images and fluorescence quantification (Fig. 5), the HepG2 cellular uptake of Mono-Gal-LPs was higher than that of LPs (fluorescence intensity ratio 3.9:1), indicating that galactose modification could promote liposomes to recognize hepatoma cells. The possible reason is that the galactosyl group of Mono-Gal-LPs can specifically recognize ASGPR overexpressed on the surface of hepatoma cells.[35] The fluorescence intensity of Di-Gal-LPs in HepG2 was 1.4 times that of Mono-Gal-LPs, indicating the hepatoma cell—targeting ability of liposomes was improved with the increasing of galactosyl groups. This is because that multivalent galactose group has stronger affinity to ASGPR of hepatoma cells (cluster glycoside effect).[36,37] In addition, the fluorescence intensity of Gal-Biotin-LPs in HepG2 was 1.3 times that of Mono-Gal-LPs as well as 5.0 times that of LPs. These data implied that the targeting lipid materials with biotin group could be favorable for liposomes to recognize hepatoma cells, and liposomes comodified with galactose and biotin groups had synergistic targeting effects.

As shown in Figure 5, the uptakes of LPs in several cell lines were less and did not show obvious differences among them. However, because of the HepG2 cellular uptakes of Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs were more than several other tissue cells (Fig. 5), further demonstrating that galactose modification or galactose-biotin comodification could promote liposomes to accurately recognize hepatoma cells via ASGPR-mediated pathway (HepG2 has high expression of ASGPR, whereas A549, Hela, and SGC-7901 have low expression of ASGPR[37-39]). Interestingly, the cellular uptakes of LPs, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs in HepG2 were 0.9, 1.9, 2.1, and 2.2 times those in LO2, respectively (Fig. 5b), indicating that the capacity of liposomes to distinguish hepatoma cells from normal hepatocytes follows a trend of LPs < Mono-Gal-LPs < Di-Gal-LPs < Gal-Biotin-LPs. The possible reason was that the expressions of ASGPR and biotin receptor on hepatoma cells were higher than those on normal hepatocytes. Obviously, Gal-Biotin-LPs are more promising for precise treatment of HCC.

To further illuminate the contribution of galactose group on the hepatoma cell—targeting capacity of these galactose-biotin-modified liposomes, we also performed galactose competition experiments (galactose is a competitive inhibitor of ASGPR). As shown in Figure 6a, after preincubation with galactose, the HepG2 cellular uptakes of Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs were significantly reduced, indicating that liposomes modified with galactose group entered hepatoma cells via ASGPR-mediated endocytosis. The fluorescence quantification also gave a similar conclusion. Interestingly, with the preincubation of 40 mM galactose, the HepG2 cellular uptakes decreased 80% for Mono-Gal-LPs, 72% for Di-Gal-LPs, and 66% for Gal-Biotin-LPs (Fig. 6b). These data imply that Mono-Gal-LPs enter HepG2 cells mainly mediated by ASGPR, Di-Gal-LPs possess higher HepG2-endocytosis capacity than Mono-Gal-LPs owing to the galactosyl cluster effect, and Gal-Biotin-LPs enter HepG2 cells not only mediated by ASGPR but also mediated by biotin receptor.

To elucidate the contribution of biotin group on the hepatoma cell—targeting capacity of the galactose-biotin-modified liposomes, we also performed biotin-blocking experiments (biotin is a competitive inhibitor of biotin receptor). As shown in Figure 7, after preincubation with 0, 50, 100, and 200 μg/mL biotin, the HepG2 cellular uptakes of Gal-Biotin-LPs were progressively decreased, indicating that liposomes modified with biotin group can enter HepG2 cells via biotin-receptors—mediated endocytosis.

Cytotoxicity of Targeting Lipid Materials and Liposomes

As shown in Figure 8, when the concentrations of galactose or biotin lipid materials reached 40 μM, the cell viabilities were still

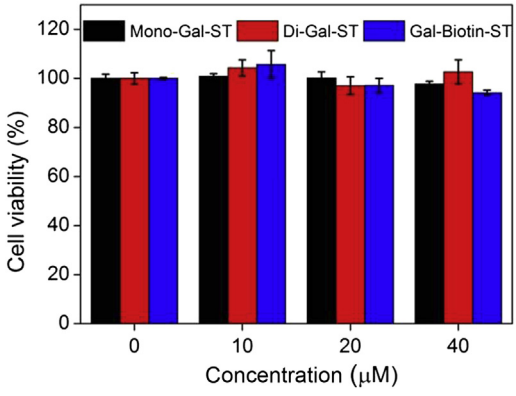


Figure 8. *In vitro* cells viability of HepG2 cells treated (for 48 h) with different concentrations of Mono-Gal-ST, Di-Gal-ST, and Gal-Biotin-ST.

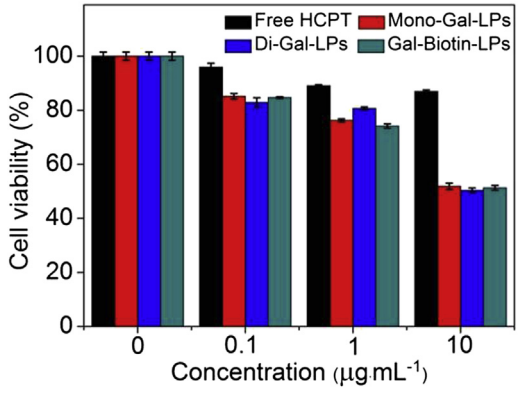


Figure 9. *In vitro* cells viability of HepG2 cells treated with different concentrations of Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs for 24 h.

above 90%, indicating that these materials have relatively high safety and can be applied to biological systems. The liposomes modified with galactose or biotin lipid materials displayed a concentration-dependent toxicity toward HepG2 cells (Fig. 9). The IC_{50} values of the HCPT-loaded liposomes against HepG2 cells were 10.16 μg/mL for Mono-Gal-LPs, 10.05 μg/mL for Di-Gal-LPs, and 10.01 μg/mL for Gal-Biotin-LPs, indicating that the inhibitory effect of liposomes against HepG2 cells followed Mono-Gal-LPs < Di-Gal-LPs < Gal-Biotin-LPs. Moreover, the IC_{50} values of these three liposomes were lower than that of free HCPT (28.17 μg/mL),[40] implying that the liposomes modified with galactose or biotin lipid materials exhibited better antitumor activity than free HCPT. However, the differences of IC_{50} values between these three liposomes were not big, which was because that HepG2 cells were incubated with these three liposomes for a long time (48 h) and HepG2 cells may uptake most of liposomes.

Comparison of Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs With Other Reported Drug Delivery Systems

A comparison of these liposomes (Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs) with some reported drug delivery systems is summarized in Table S1. First, the targeting lipid materials synthesized in this work were small molecular compounds which can be precisely determined molecular weight and molecular structure. In addition, Gal-Biotin-LPs were modified with 2 targeting moieties (galactose and biotin) against 2 cellular targets (ASGPR and biotin receptor), showing a synergistic hepatoma cell—targeting effect. Furthermore, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs exhibited excellent hepatoma cell—targeting effects. More importantly, Mono-Gal-LPs, Di-Gal-LPs, and Gal-Biotin-LPs showed impressive potential to distinguish hepatoma cells from normal hepatocytes.

Conclusions

In summary, hepatoma cell—targeting lipid materials (Mono-Gal-ST, Di-Gal-ST, and Gal-Biotin-ST) were designed and synthesized, and the corresponding galactose-biotin-modified liposomes were prepared. *In vitro* cellular uptake assays showed these galactose-biotin-modified liposomes can distinguish hepatoma cells from other tissue cells because of the introduction of galactose group. The capacity of liposomes to distinguish hepatoma cells from normal hepatocytes follows a trend of LPs < Mono-Gal-LPs (galactosyl effect) < Di-Gal-LPs (cluster glycoside effect) < Gal-Biotin-LPs (the synergistic effect of galactose and biotin). Galactose competition experiments demonstrated that these galactose-biotin-modified liposomes could enter hepatoma cells through ASGPR-mediated endocytosis. Furthermore, *in vitro* cytotoxicity assay demonstrated that these materials had high biosafety. Overall, this work developed a series of novel hepatoma cell—targeting lipid materials for liposomes and opened new avenues for the precise treatment of HCC.

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References

1. Jemal A, Center MM, Desantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*. 2010;19:1893-1907.
2. Nowak AK, Chow PK, Findlay M. Systemic therapy for advanced hepatocellular carcinoma: a review. *Eur J Cancer*. 2004;40:1474-1484.
3. Dai L, Liu K, Si C, He J, Lei J, Guo L. A novel self-assembled targeted nanoparticle platform based on carboxymethylcellulose Co-delivery of anticancer drugs. *J Mater Chem B*. 2015;3:6605-6617.
4. Du Y, Wei Z, He R, et al. Dual 7-Ethyl-10-hydroxycamptothecin conjugated phospholipid prodrug assembled liposomes with in vitro anticancer effects. *Bioorg Med Chem*. 2017;25:3247-3258.
5. Zhang Y, Wu X, Mi Y, Li H, Hou W. Engineering of (10-hydroxycampto-thecin intercalated layered double Hydroxide)@ liposome nanocomposites with excellent water dispersity. *J Phys Chem Sol*. 2017;108:125-132.
6. Li Y, Lin J, Huang Y, et al. Self-targeted, shape-assisted, and controlled-release self-delivery nanodrug for synergistic targeting/anticancer effect of cytoplasm and nucleus of cancer cells. *ACS Appl Mater Interfaces*. 2015;7:25553-25559.
7. Zhang P, Hu L, Yin Q, Feng L, Li Y. Transferrin-modified c[RGDfK]-paclitaxel loaded hybrid micelle for sequential blood-brain barrier penetration and glioma targeting therapy. *Mol Pharm*. 2012;9:1590-1598.
8. Chantarasrivong C, Ueki A, Ohyama R, et al. Synthesis and functional characterization of novel sialyl LewisX mimic-decorated liposomes for E-selectin-mediated targeting to inflamed endothelial cells. *Mol Pharm*. 2017;14:1528-1537.
9. Liu X, Jia K, Wang Y, et al. Dual-responsive bola-type supra-amphiphile constructed from water-soluble pillar[5]arene and naphthalimide-containing amphiphile for intracellular drug delivery. *ACS Appl Mater Interfaces*. 2017;9:4843-4850.
10. Rajora MA, Ding L, Valic M, et al. Tailored theranostic apolipoprotein E3 porphyrin-lipid nanoparticles target glioblastoma. *Chem Sci*. 2017;8:5371-5384.
11. Shen J, Kim HC, Wolfram J, et al. A liposome encapsulated ruthenium polypyridine complex as a theranostic platform for triple-negative breast cancer. *Nano Lett*. 2017;17:2913-2920.
12. He R, Du Y, Ling L, et al. Nanoformulation of dual bexarotene-tailed phospholipid conjugate with high drug loading. *Eur J Pharm Sci*. 2017;100:197-204.
13. Ling L, Du Y, Ismail M, et al. Self-assembled liposomes of dual paclitaxel-phospholipid prodrug for anticancer therapy. *Int J Pharm*. 2017;526:11-22.
14. Lee J, Kim J, Jeong M, et al. Liposome-Based engineering of cells to package hydrophobic compounds in membrane vesicles for tumor penetration. *Nano Lett*. 2015;15:2938-2944.
15. Liu Y, Qi Q, Li X, et al. Self-assembled pectin-conjugated eight-arm polyethylene glycol—dihydroartemisinin nanoparticles for anticancer combination therapy. *ACS Sustain Chem Eng*. 2017;5:8097-8107.
16. Yang S, Ren Z, Chen M, et al. Nucleolin-targeting AS1411-aptamer-modified graft polymeric micelle with dual pH/redox sensitivity designed to enhance tumor therapy through the codelivery of doxorubicin/TLR4 siRNA and suppression of invasion. *Mol Pharm*. 2018;15:314-325.
17. Wu J, You X, Gu Z, et al. Polymeric nanoparticles for colon cancer therapy: overview and perspectives. *J Mater Chem B*. 2016;4:7779-7792.
18. Shukla SK, Shukla SK, Govender PP, Giri NG. Biodegradable polymeric nanostructures in therapeutic applications: opportunities and challenges. *RSC Adv*. 2016;6:94325-94351.
19. Sobot D, Mura S, Couvreur P. How can nanomedicines overcome cellular-based anticancer drug resistance? *J Mater Chem B*. 2016;4:5078-5100.
20. Cao S, Pei Z, Xu Y, Pei Y. Glyco-nanovesicles with activatable near-infrared probes for real-time monitoring of drug release and targeted delivery. *Chem Mater*. 2016;28:4501-4506.
21. Latxague L, Ziane S, Chassande O, Patwa A, Dalila MJ, Barthélémy P. Glycosylated nucleoside lipid promotes the liposome internalization in stem cells. *Chem Commun*. 2011;47:12598-12600.
22. Zhang LJ, Kuang Y, Liu J, Liu Z, Huang SW. Long circulating anionic liposomes for hepatic targeted delivery of cisplatin. *RSC Adv*. 2016;6:76905-76914.
23. Chen J, Jiang H, Wu Y, Li Y, Gao Y. A novel glycyrrhetinic acid-modified oxaliplatin liposome for liver-targeting and in vitro/vivo evaluation. *Drug Des Devel Ther*. 2015;9:2265-2275.
24. Qi WW, Yu HY, Guo H, et al. Doxorubicin-loaded glycyrrhetinic acid-modified recombinant human serum albumin nanoparticles for targeting liver tumor chemotherapy. *Mol Pharm*. 2015;12:675-683.
25. Leamon CP, Cooper SR, Hardee GE. Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: evaluation in vitro and in vivo. *Bioconjug Chem*. 2003;14:738-747.
26. Shah SM, Goel PN, Jain AS, et al. Liposomes for targeting hepatocellular carcinoma: use of conjugated arabinogalactan as targeting ligand. *Int J Pharm*. 2014;477:128-139.
27. Fu F, Wu Y, Zhu J, Wen S, Shen M, Shi X. Multifunctional lactobionic acid-modified dendrimers for targeted drug delivery to liver cancer cells: investigating the role played by PEG spacer. *ACS Appl Mater Interfaces*. 2014;6:16416-16425.

28. Wei M, Guo X, Tu L, et al. Lactoferrin-modified PEGylated liposomes loaded with doxorubicin for targeting delivery to hepatocellular carcinoma. *Int J Nanomedicine*. 2015;10:5123-5137.
29. Park S, Kim E, Kim WY, Kang C, Kim JS. Biotin-guided anticancer drug delivery with acidity-triggered drug release. *Chem Commun*. 2015;51:9343-9345.
30. Chiang YT, Cheng YT, Lu CY, et al. Polymer−liposome complexes with a functional hydrogen-bond cross-linker for preventing protein adsorption and improving tumor accumulation. *Chem Mater*. 2013;25:4364-4372.
31. Li K, Hou JT, Yang J, Yu XQ. A tumor-specific and mitochondria-targeted fluorescent probe for real-time sensing of hypochlorite in living cells. *Chem Commun (Camb)*. 2017;53:5539-5541.
32. Belhadj Z, Ying M, Cao X, et al. Design of Y-shaped targeting material for liposome-based multifunctional glioblastoma-targeted drug delivery. *J Control Release*. 2017;255:132-141.
33. Liu Y, Lu Z, Ling M, et al. Tandem peptide based on structural modification of poly-arginine for enhancing tumor targeting efficiency and therapeutic effect. *ACS Appl Mater Interfaces*. 2017;9:2083-2092.
34. Min HL, Kim EJ, Lee H, et al. Liposomal texaphyrin theranostics for metastatic liver cancer. *J Am Chem Soc*. 2016;138:16380-16387.
35. Sliedregt LA, Rensen PC, Rump ET, et al. Design and synthesis of novel amphiphilic dendritic galactosides for selective targeting of liposomes to the hepatic asialoglycoprotein receptor. *J Med Chem*. 1999;42:609-618.
36. Contribution JE, Zhang Y, Zang Y, et al. Receptor-targeting fluorescence imaging and theranostics using a graphene oxide based supramolecular glycocomposite. *J Mater Chem B*. 2015;3:9182-9185.
37. Ji DK, Zhang Y, Zang Y, et al. Targeted intracellular production of reactive oxygen species by a 2D molybdenum disulfide glycosheet. *Adv Mater*. 2016;28:9356-9363.
38. Li KB, Li N, Zang Y, et al. Foldable glycoprobes capable of fluorogenic cross-linking of bio-macromolecules. *Chem Sci*. 2016;7:6325-6329.
39. Ma Y, Chen H, Su S, et al. Galactose as broad ligand for multiple tumor imaging and therapy. *J Cancer*. 2015;6:658-670.
40. Zhang Y, Wu X, Mi Y, et al. Engineering of (10-hydroxycamptothecin intercalated layered double hydroxide)@liposome nanocomposites with excellent water dispersity. *J Phys Chem Sol*. 2017;108:125-132.