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REVIEW

Lipid Nanoparticles-Based Therapy in Liver Metastasis Management: From Tumor Cell-Directed Strategy to Liver Microenvironment-Directed Strategy

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Abstract: Metastasis to the liver, as one of the most frequent metastatic patterns, was associated with poor prognosis. Major drawbacks of conventional therapies in liver metastasis were the lack of metastatic-targeting ability, predominant systemic toxicities and incapability of tumor microenvironment modulations. Lipid nanoparticles-based strategies like galactosylated, lyso-thermosensitive or active-targeting chemotherapeutics liposomes have been explored in liver metastasis management. This review aimed to summarize the state-of-art lipid nanoparticles-based therapies in liver metastasis management. Clinical and translational studies on the lipid nanoparticles in treating liver metastasis were searched up to April, 2023 from online databases. This review focused not only on the updates in drug-encapsulated lipid nanoparticles directly targeting metastatic cancer cells in treating liver metastasis, but more importantly on research frontiers in drug-loading lipid nanoparticles targeting nonparenchymal liver tumor microenvironment components in treating liver metastasis, which showed promise for future clinical oncological practice.

Keywords: liver neoplasms, neoplasm metastasis, liposomes, tumor microenvironment

Key Points

1. Liver metastasis, which is highly prevalent in advanced malignancies, was associated with poor prognosis.
2. Contemporary therapies in liver metastasis were associated with the lack of metastatic-targeting ability, significant systemic toxicities and incapability of tumor microenvironment (TME) modulations.
3. Lipid nanoparticles (LNP)-based strategies would overcome the deficiencies of conventional approaches in liver metastasis management.
4. The switch from directly targeting metastatic tumor cells to nonparenchymal cells in liver TME has been explored using LNP-based strategies in treating liver metastasis.
5. Current challenges included the optimization of LNP properties and identifications of liver-specific ligands.

Overview of Liver Metastasis and Its Current Treatment Landscapes

Metastasis, which is the process of tumor cells evading their primary sites and spreading to other distant sites, was reportedly to cause over 90% of cancer-related death.[1] Metastasis to the liver was one of the most common metastatic patterns which would be frequently seen in all cancer types,[2] such as colorectal cancer,[3] melanoma and neuroendocrine tumors.[4,5] Also, liver metastasis was a negative factor for the treatment response and survival in various cancer types.[3,6–13] As much of tumor-related morbidity and mortality resulted from liver metastasis progression, it would be critical to generate effective treatment modalities for liver metastasis. However, at present only limited treatment approaches were used in patients with liver metastasis. Although surgery has long been regarded as the only curative modality to treat resectable hepatic metastasis, the eligibility, efficacy and safety of surgical procedures in patients with liver metastasis remained unsatisfactory.[14–17] For systemic approaches, many patients were

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ineligible for chemotherapy as their intolerance for chemotherapy-caused severe adverse effects or being unresponsive to systemic chemotherapy.[18] Strategies combining systemic therapy with locoregional therapy were thought to be effective in many cancers with distant metastasis.[19] However, its efficacy in liver metastasis was far from satisfactory.[20] Large randomized clinical trials have revealed that peri-operative chemotherapy or targeted therapeutics would not improve overall survival otherwise increasing drug-related side effects in patients with resectable colorectal liver metastasis.[16,20,21] Accordingly, conventional therapeutics were associated with considerable systemic toxicities, treatment resistance to single-target therapies and limited local drug concentrations in liver metastasis treatment. Novel chemotherapeutic formulations were urgently needed to overcome the deficiencies of conventional therapeutics. Recently, with the development of novel liposomal drug delivery systems, the efficacy, tolerability and targeting ability of contemporary systemic therapy modalities in cancer treatment have been largely improved.[22] However, the role of LNP-based therapeutic strategies in liver metastasis remained equivocal. As the liver tumor microenvironment (TME) has been regarded as a significant obstacle for pronounced treatment response,[23] the liver-TME-modulating strategy would further synergize the anti-tumor effect of conventional therapeutics in liver metastasis treatment.

Overview of Liposomal Delivery Systems

Liposomal nanoparticles (LNPs) were unilamellar or multilamellar lipid bilayer vesicles encapsulating drugs.[24] Liposomal delivery systems were composed of LNPs which embedded chemotherapy or gene therapy agents. During the COVID-19 pandemic, the applications of various LNP-based, mRNA-loading SARS-CoV-2 vaccines such as the BNT162b2 have demonstrated tremendous efficacy in preventing the infection of coronavirus.[25] In vivo studies have also demonstrated that lipid nanoparticles would improve tumor-targeting ability while decreasing the accumulation of drugs in the non-tumor area, thus reducing drug-induced systemic adverse events. The high density and permeability of tumor vessels enabled drug-encapsulated lipid nanoparticles to accumulate in neoplasm lesions, which led to high drug concentrations and selective anti-cancer activity.[22,26] The first FDA-approved liposomal chemotherapy nanoparticle, namely Doxil, has achieved influential anti-tumor effects in clinical practice.[27] Doxil, which was made of polyethylene glycol-modified doxorubicin-encapsulated liposomes, has been observed to specifically accumulate in breast cancer lesions.[28] Compared with conventional intravenous doxorubicin formulations, Doxil showed better pharmacokinetics as a lower half-life, 250-fold reduced clearance rate and nearly 300-fold greater Area Under Curve (AUC) after a dose of 50 mg/m^2.[29] Correspondingly, doxorubicin-induced cardiotoxicity has been drastically reduced in patients treated with Doxil.[29] A Phase I clinical study[30] demonstrated the benefit of Doxil + vinorelbine in four breast cancer patients with liver metastasis. Besides drug-loading liposomes, recent advances have been achieved in LNP-based gene therapy. Liposome-coated small interfering RNAs (siRNA) would effectively silence the expression of the target gene ex vivo. Tabernero et al[31] reported results from a phase I clinical trial which evaluated the anti-tumor efficacy and safety profile of ALN-VSP, an LNP formulation of siRNAs targeting VEGF and kinesin spindle protein in treating the liver metastasis of various cancer types. Results from both pre-clinical and clinical experiments showed that the ALN-VSP was well-tolerated and exhibited complete response in an endometrial cancer patient with multiple liver metastasis. However, the lack of liver-targeting limited its application in contemporary liver metastasis management. Recently, Onpattro, a clinically approved non-viral LNP-based siRNA delivery system with liver-targeting ability, has been successfully used intravenously to improve neuropathy in patients with hereditary transthyretin amyloidosis.[32] As low-density lipoprotein receptor (LDL-r) was heavily expressed on the surface of hepatocytes, researchers coated Onpattro with LDL-r ligand apolipoprotein E (Apo-E) as its surface marker. The binding of Apo-E and LDL-r enabled the endocytosis of siRNA-loading lipid particles, which led to efficient target gene knockdown.[33] Once delivered intracellularly, Onpattro would bind the 3' UTR of transthyretin mRNA and further silence its expression, which led to the reduction of pathological transthyretin protein deposit in hepatocytes.[33] Although not applicable in malignancies, Onpattro has opened the door to future explorations of LNP-based, liver-targeting gene therapies in liver metastasis management. In that, LNP-based delivery systems appeared to be theoretically selective and well-tolerated formulations that would enhance the efficacy while reducing the systemic toxicities of encapsulated agents. This review highlighted the advantages of LNP-based strategies in liver metastasis management such as liver-targeting ability, systemic side effects reductions and modulatory effect to liver TME based on evidences from both preclinical and clinical studies, which would be promising in future real-world clinical oncology practice.

LNP-Based Strategy in Liver Metastasis Treatment

Systemic LNP-Based Therapy in Liver Metastasis Treatment

Systemic Chemotherapy

Systemic chemotherapy has long been considered the first-line treatment approach for patients with liver metastasis,[34,35] however the systemic toxicity and limited efficacy were major obstacles to achieving optimal survival benefits. Irinotecan, which acted by causing DNA damage to kill tumor cells, has been widely used as cancer chemotherapeutics.[36] Liposomal irinotecan would increase both the circulation time and the intratumoral level of irinotecan and its active metabolite (SN-38). Pre-clinical HT-29 xenograft animal models showed that liposomal irinotecan with a 5-fold lower dose than conventional irinotecan would achieve the same anti-colon cancer effect.[36] A Phase III open-label randomized trial NAPOLI-1 evaluated the survival benefits of liposomal irinotecan monotherapy or combing fluorouracil and folinic acid in gemcitabine-refractory metastatic pancreatic cancer.[37] Median overall survival in patients using liposomal irinotecan plus fluorouracil and folinic acid was 6.1 months [95% Confidence Interval(CI): 4.8–8.9], which was much longer than the survival (3.2 months) in patients with unencapsulated irinotecan-based regimens.[38] Based on these findings, the NCCN guideline for pancreatic cancer has recommended using liposomal irinotecan for metastatic disease including liver metastasis instead of unencapsulated formulations.[39]

Oxaliplatin was a third-generation platinum chemotherapy drug, with an enhanced anti-tumor activity compared to cisplatin.[40] Oxaliplatin-based chemotherapy like CAPEOX (oxaliplatin + capecitabine) regimen has been recommended by NCCN guidelines as standard therapy for patients with unresectable colorectal cancer liver metastasis (CRLM).[35] However, oxaliplatin-induced neuropathy was hardly tolerable, which was dose-limited involving over 80% of patients.[41] To improve the oxaliplatin-induced off-target systemic adverse effect, Gogineni et al[42] developed a heat-sensitive-Fe_3O_4-based liposomal oxaliplatin formulation (L-NIR-Fe_3O_4/OX). Under magnetic field stimulation, the release of oxaliplatin from L-NIR-Fe_3O_4/OX was improved (18%) and the biodistribution experiment in animal models showed increased accumulation of this liposomal formulation in the liver compared to lung and gut ($p<0.001$). Predominant necrosis was observed in CRLM lesions of rat models. Survival analysis demonstrated that L-NIR-Fe_3O_4/OX injected through mesenteric veins under magnetic field stimulation was associated with extra survival benefits. These results above revealed the superiority of liposomal chemotherapeutics especially the magnitude-triggered liposomes in liver metastasis.

Combined chemotherapy has been accepted as a clinically effective strategy in the management of liver metastatic cancer.[43] Researchers have explored the LNP-based co-delivery of chemotherapeutics with different physicochemical properties. Lin et al[44] developed a novel Pluronic® P123-coated liposomal drug carrier (ITZ/DOX-Plip) which would deliver both hydrophilic doxorubicin and hydrophobic itraconazole simultaneously. Pharmacological experiments showed ITZ/DOX-Plip exhibited satisfactory loading efficacy and prolonged circulation time. Cell viability experiments revealed ITZ/DOX-Plip was more cytotoxic compared with free doxorubicin and liposomal doxorubicin formulations. In vivo study demonstrated ITZ/DOX-Plip would significantly suppress the growth of liver metastasis in tumor-bearing mice. Regarding the doxorubicin-related cardiotoxicity, in vivo biodistribution assay found a decreased accumulation of ITZ/DOX-Plip in mice's heart tissue compared with free or liposomal doxorubicin ($p < 0.01$). In light of the findings above, it would be promising to conduct a liposomal co-delivery chemotherapy system in the treatment of liver metastasis. However, no clinical validations existed.[45]

Gene Therapeutics

RNA interference (RNAi) liposomes have been widely used in preclinical studies to selectively silence the expression of oncogenes. However, one major limitation was the entrapment of siRNA liposomes in the lung caused by the non-specific agglutination between positively charged lipoplex and negatively charged erythrocytes.[46] Hattori et al[47] developed an approach of sequential injection of anionic polymer and cationic lipoplex into mice with liver metastasis. This approach was associated with a significantly decreased accumulation of lipoplex in the lung and increased delivery to liver lesions. Additionally, the accumulation of siRNA liposomes in the liver was found to successfully silence the expression of target gene. The results above indicated the potential therapeutic value of liposome-based siRNA gene therapy in the management of liver metastasis.

The clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9 (CRISPR/Cas9) has been considered a more effective genomic editing technique compared to the RNAi. Wang et al[48] fabricated a novel liposomal vector PS@Lip/pCas9 which delivers CRISPR/Cas9 plasmid targeting mutT homolog 1 (MTH) gene to inhibit the

growth of tumor cells. Coated with protamine sulfate (PS) containing nuclei-directing sequence, PS@Lip/pCas9 would effectively deliver CRISPR-Cas9 plasmid into the nuclei of cancer cells and induce MTH gene deletion. Furthermore, the liposomal coating would protect the plasmid from circulatory nuclease degradation. In vivo assay revealed that PS@Lip/pCas9 inhibited the progression of liver metastasis in non-small cell lung cancer mice models by disrupting MTH-induced pro-metastatic effect. However, liposomal formulations did not resolve the inevitable off-target effect of CRISPR/Cas9 gene editing, which would limit its potential application in clinical settings.

The lack of site-specific targeting in liposomal gene therapy would cause severe side effects by non-specific gene silencing in normal cells. Thus, liposomal vectors with specific targets were urgently needed. The transferrin receptor (TR) was reported to be highly over-expressed in pancreatic cancer cells relative to normal pancreatic cells.[49] This differential expression pattern made TR a possible target for liposomal drug carriers. As the cell-senescence-modulating strategy like Tp53-targeting agents has demonstrated suppressive effect of the cell malignant behavior in pre-clinical studies of gastrointestinal malignancies,[50,51] the incorporation of senescence-targeted-Tp53-modulations and TR-targeted LNP would further enhance its anti-tumor efficacy. Camp et al[52] constructed a pancreatic-targeted liposome coated with a single-chain antibody fragment to the transferrin receptor (TfRscFv). The "cargo" of this liposome was wild-type human p53 for its senescence-restoring effect on p53-mutated tumor cells and the synergic effect on concomitant chemotherapy.[53] TfRscFv-liposomes were found to increasingly accumulate in pancreatic cancer lesions compared to non-specific liposomes. Survival analysis showed an increased median survival observed in the liposomes plus gemcitabine group compared with the liposomes or the gemcitabine monotherapy group (37 days versus 29 days versus 30 days). These experiments suggested a successful strategy to specifically deliver gene therapeutics to tumor cells in liver metastasis.

Drug-Free Liposomes

Interestingly, drug-free liposomes would also exhibit anti-tumor effects in liver metastasis. Ichihara et al[54] found that drug-free HL-25 liposomes composed of L-a-dimyristoyl-phosphatidylcholine and polyoxyethylene dodecyl would also have therapeutic effects in the CRLM mice model. The therapeutic effect of this drug-free liposome was due to its pro-apoptotic effect on tumor cells. These findings revealed that the liposome itself may have some tumor-suppressing effects to synergize encapsulated chemotherapy drugs.

Locoregional Use of LNP-Based Therapy in Liver Metastasis

Transarterial Chemoembolization

Transarterial chemoembolization (TACE) has achieved promising survival benefits in patients with liver metastasis and has been included in current practice guidelines.[34] To date, there was no consensus on the recommended chemotherapy regimen for TACE in patients with liver metastasis. A retrospective study compared the efficacy and safety of TACE with raltitrexed plus liposomal doxorubicin (R+PGLD) with tegafur plus pirarubicin (T+P).[55] Compared with T+P, patients receiving TACE with R+PGLD had significantly better efficacy (Objective Response Rate: 64.9% vs 45.9%, p=0.031; Disease Control Rate: 89.2% vs 74.3%, p=0.032; respectively) and fewer severe adverse events like myelosuppression and cardiotoxicity (p=0.011 and p=0.037, respectively). This finding revealed the feasibility of using liposomal chemotherapeutics with TACE procedure in treating liver metastasis. Recently progress in novel nano-flexible liposomes has made TACE more targeted and efficient. Li et al[56] reported a novel liposomal drug carrier which was made by Bletilla striata polysaccharide (BSP) polymer. The BSP polymer would incorporate into a dimensional porous network microsphere, which can inhibit the neovascularization in post-embolized ischemic regions. Additionally, the binary progressive structure of this novel liposomal carrier enhanced its site-specific targeting ability to liver tissue. The abovementioned characteristics of this novel LNP would make it a promising chemotherapeutics delivery system in TACE settings.

Hepatic Arterial Infusion Chemotherapy

The efficacy of hepatic arterial infusion chemotherapy (HAIC), which has been recommended by current guidelines together with TACE for liver metastasis, would also be improved using liposomal drug delivery systems.[34,35]

5-fluorouracil (5-FU) has been widely accepted as the cornerstone chemotherapeutics for the treatment of gastrointestinal-originated liver metastasis.[35] Pohlen et al[57] compared the efficacy of PEG (polyethylene glycol)-coated 5-FU liposomes with or without degradable starch microspheres (DSM) in CRLM mice models and found that hepatic arterial infusion with PEG 5-FU plus DSM was associated with the most survival benefit compared with other groups. Another in vivo study showed when intraarterial-infused, the intratumoral concentration of PEG-coated liposomal 5-FU was 110 times higher than conventional 5-FU, which demonstrated the superior pharmacokinetics compared to conventional formulations in HAIC settings.[58] Although LNP-based HAIC exhibited an enhanced anti-tumor effect, the lack of internal liver-targeting ability would limit its application in liver metastasis. To improve liver-targeting ability, Zhao et al[59] fabricated a galactose-modified doxorubicin-loading liposome. The binding of galactose to the asialoglycoprotein receptor on human hepatocytes enhanced the liver-targeting ability of galactose-modified liposomes.[60] In vitro experiments showed increased uptake of liposomes in human hepatocellular carcinoma cell line HepG2 compared with non-modified liposomes. Further in vivo biodistribution assay confirmed the enhanced aggregation of galactose-modified liposomes in the liver. Considering the anti-tumor ability, locoregional infusion of galactosylated doxorubicin liposomes demonstrated superior efficacy both in liver metastasis and lymph node metastasis compared with conventional formulations. The safety profile of galactosylated doxorubicin liposomes was acceptable, with similar hepatotoxicity compared with conventional formulations. Although lack of clinical validations, these pre-clinical findings suggested a possible approach to increase the liver-targeting ability of drug-loading liposomes in the locoregional management of liver metastasis.

Radionuclide Therapeutics or Radiofrequency Ablation Plus Liposomal Chemotherapeutics

Radionuclide therapeutics have been integrated into the landscape of liver malignancy management.[34] Liposomal radionuclides would improve the tumor uptake of radionuclide particles while also reducing the radiation-induced severe adverse effects. Chang et al[45] evaluated the efficacy of ^{188}Re-liposomes plus sorafenib compared with sorafenib monotherapy in CRLM mouse models. Survival analysis showed ^{188}Re-liposomes plus sorafenib was associated with a superior survival rate compared with sorafenib monotherapy or negative controlled groups (p=0.0000).

Radiofrequency ablation (RFA) has been recommended by current guidelines for patients with liver metastasis ineligible for surgical intervention.[34] However, long-term survival data showed a high local recurrence rate post-ablation.[61,62] Therefore, combining radiofrequency ablation with systemic cytotoxic therapy like liposomal doxorubicin was considered a possible strategy to improve outcomes in patients with liver metastasis. It has been observed that RFA followed by liposomal doxorubicin would increase the tumor destruction volume by 25% to 30% compared to RFA alone.[63] This synergic effect was due to ablation-induced vascular changes, which led to increased intra-tumoral liposomal accumulation.[64] Lyso-thermosensitive liposomal doxorubicin (LTLD) was one of novel liposomal formulations made of doxorubicin and its thermosensitive lipid capsules. Since ablation procedures would produce extra heat in target regions, subsequent thermosensitive doxorubicin liposomes would release more drugs in such specific microenvironments. It was reported that when the tumor region is heated up to 40 °C, the release of liposomal doxorubicin in tumor regions was 25-fold higher than the release of conventional doxorubicin formulations.[65] Also, the enhanced permeability and retention effect, which was caused by high vascular density and permeability of tumor stroma, was more predominant in post-ablation regions. Pharmacokinetic modeling revealed that using temperature-sensitive doxorubicin liposomes in post-ablation settings would improve local drug delivery compared with conventional doxorubicin.[66] One double-blinded dummy-controlled clinical trial assessed the efficacy of RFA with or without post-ablation lyso-thermosensitive liposomal doxorubicin in hepatocellular carcinoma.[67] Subgroup analysis revealed patients with a RFA dwell time for a solitary lesion ≥45 minutes would significantly benefit from the combination of RFA+LTLD (Overall survival: 95% CI: 0.45–0.94; $p < 0.05$). The abovementioned evidence demonstrated the superiority of LTLD over conventional doxorubicin in the locoregional therapy of liver malignancies. To date, only ThermoDox has been approved by FDA for the treatment of liver metastasis.[68] Hopefully, the efficacy of locoregional therapy in

liver metastasis would be greatly improved with the development of next-generation thermosensitive chemotherapeutic or immunotherapeutic liposomes.

Photodynamic Therapy

Photodynamic therapy (PDT) was considered a potential treatment option for liver metastasis.[69] Being activated by light of a certain wavelength, the pre-injected photosensitive drugs were released in irradiated regions, which would generate reactive oxygen species (ROS) to destruct tumor cells locally.[70] The anti-tumor activity of the PDT-induced ROS release process relied on the oxygen concentration in tumor regions.[69] However, the hypoxia microenvironment in the liver would limit the efficacy of PDT.[23] PDT with IR780@O_2-SFNs/iRGD, a novel oxygen-self-sufficient liposomal photosensitizer complex, was found to effectively inhibit the progression of liver metastasis in vivo.[71] IR780@O_2-SFNs/iRGD was composed of four parts, which are IR780, O_2, SFNs, and iRGD. IR780 was a hydrophobic cyanine dye, which acted as a photosensitizer to release ROS under photodynamic irradiations. SFNs were made of pH-sensitive fluorocarbon-functionalized nanoparticles which decomposed in acid microenvironments. iRGD, which could bind to αvβ3 integrin in tumor cell surfaces, helped tumor penetrating of this nano-drug delivery system. When injected intravenously, this complex would use its iRGD and SFNs to selectively target and penetrate tumor regions. When exposed to hypoxic or acidic intratumoral microenvironments, the nano-complex would disassemble and release the oxygen molecules together with IR780 loaded in the core of SFNs. O_2 supplementation would relieve tumor hypoxia and facilitate the generation of tumor-toxic ROS induced by IR780, which further improved the efficacy of PDT. In vivo study showed single IR780@O_2-SFNs/iRGD-mediated PDT would effectively inhibit the progression of liver metastasis in orthotopic breast cancer of nude mice. Another hypoxia-alleviating LNP-based photosensitizer generated by Liang et al[72] also demonstrated decent anti-tumor efficacy in PDT of liver metastasis. The unique arranging mode of porphyrin with perfluorocarbon(PFOB) in this research would increase the O_2 loading content in nanoparticles, improve the generation of singlet oxygen and avoid premature circulatory loss of photosensitizer, which led to elevated PDT efficacy in hypoxic tumors as liver metastasis. In HT-29 colon cancer liver metastasis mouse models, O_2@PFOB@PGL LNPs-mediated PDT was associated with an appealing anti-tumor effect evidenced by fewer metastatic loci compared with conventional photosensitizer-mediated PDT. Coating modification would also be a possible strategy to improve the targeting as well as immunotolerance of LNP-based photosensitizer. Pan et al[73] developed an engineered red blood cell membrane (RBCm)-coating salidroside/indocyanine green nanovesicle (ARISP) as the photosensitizer agent in PDT. ARISP was composed of the following three parts: salidroside, RBCm-coating, and indocyanine as the photosensitizer. Salidroside accounted for down-regulating the expression of hypoxia-inducible factor 1α to attenuate the hypoxia tumor microenvironment. Anti-LDL-r modified RBCm-coatings would not only prolong the circulatory time and help escape the host immune surveillance but also increase the targeting of the whole nano-complex by selectively binding to LDL-r overexpressed hypoxic tumor cells. In vivo study revealed that ARISP plus laser irradiations would significantly suppress the growth of liver metastasis and improve survival in triple-negative breast cancer-bearing mice. Although pre-clinical studies have demonstrated the effectiveness of PDT with LNP-based photosensitizer in liver metastasis, further large randomized clinical trials and real-world studies are needed to validate its performance clinically.

As summarized in Figure 1 and Table 1, LNP-based tumor cell-targeting strategies have exhibited promising efficacy in systemic or locoregional treatment of liver metastasis. The advantages of such strategies were featured by the reduction of systemic toxicities and the synergetic effect with local therapy modalities like TACE, HAIC or PDT. However, the lack of internal liver-targeting ability and the incapability to overcome the drug resistance would be the major obstacles in real-world clinical applications. Moreover, most evidence existed at the pre-clinical level, with a paucity of clinical validations. Registered clinical trials evaluating LNP-based strategies in liver metastasis were listed in Table 2.

Liver Tumor Microenvironment Targeted LNP Strategies

As illustrated in Figure 2, the TME in the liver has its unique components like liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), and hepatic satellite cells (HSCs),[23] which played an important role in the progression of liver metastasis.[74] Nonparenchymal components of liver sinusoids, like LSECs and HSCs, would assumptively interact with

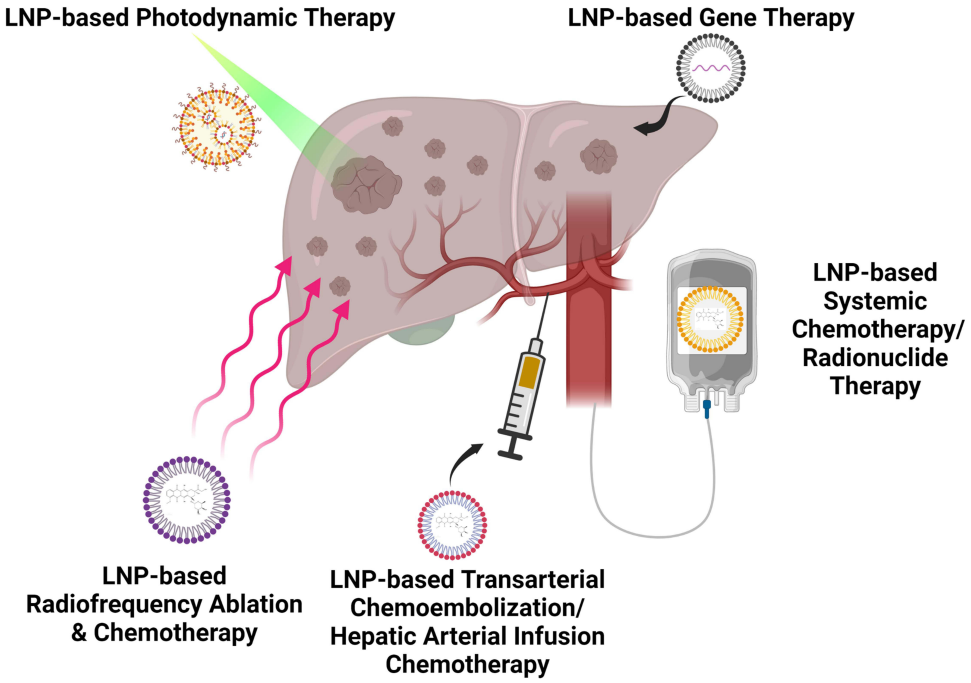

Figure 1 Schematic overview of current LNP-based tumor cell-targeting strategies in liver metastasis management (Created with BioRender.com).
Abbreviation: LNP, lipid nanoparticles.

metastatic tumor cells. Therefore, it would be a feasible approach to target nonparenchymal cells in the liver TME for the treatment of liver metastasis. Systemic drug delivery to hepatocytes has successfully led to the approval of Onpattro by the FDA. However, the delivery to other functional nonparenchymal cells in the liver microenvironment remained challenging. Surprisingly, Paunovska et al[75] found that LNPs would be delivered more potently in stromal cells of the liver microenvironment compared to hepatocytes. These findings suggested the feasibility of targeting the liver microenvironment using drug-loading liposomal nanoparticles. One of the major deficiencies of the liposomal drug was that it would be easily taken by the reticuloendothelial system, which diminished its circulating time and anti-tumor effect. However, as reticuloendothelial systems were important components of liver TME,[76] the increased uptake of TME-targeting LNPs would increase the concentrations of liposomal therapeutics in hepatic nonparenchymal cells, which led to better therapeutic effects.

Liver Sinusoidal Endothelial Cells

Recent research has revealed the crucial role of LSECs in the progression of liver metastasis.[23] As specialized scavenger cells, LSECs were capable of eliminating endogenous macromolecular waste as well as blood-borne pathogens. LSECs

Table 1 Current LNP-Based Strategies in Locoregional Treatment of Liver Metastasis

Procedure	Liposomal Formulation
Transarterial chemoembolization	Liposomal doxorubicin[55] Bletilla striata polysaccharide polymer[56]
Hepatic arterial infusion chemotherapy	PEG-coated 5- fluorouracil[57] Galactosylated doxorubicin liposomes[59]
Radiofrequency ablation	Liposomal doxorubicin[63,64] Lyso-thermosensitive Liposomal doxorubicin[65–67]
Photodynamic therapy	IR780@O_2-SFNs/iRGD[71] ARISP[73] O_2@PFOB@PGL LNPs[72]

Abbreviation: LNP, lipid nanoparticles.

Table 2 Registered Clinical Trials Evaluating Liposomal Formulations in Liver Metastasis

Identifier	Year	Status	Phase	Liposomal Formulations	Conditions
NCT00093444	2004	Completed	1	Lyso-thermosensitive liposomal doxorubicin	Metastatic liver tumors
NCT00441376	2007	Completed	1	ThermoDox™ (Thermally sensitive liposomal doxorubicin)	Metastatic liver tumors
NCT00882180	2009	Completed	1	ALN-VSP02(lipid nanoparticles containing siRNA against VEGF and kinesin spindle protein)	Metastatic liver tumors
NCT01158079	2010	Completed	1	ALN-VSP02	Metastatic liver tumors
NCT01464593	2011	Terminated	2	Lyso-thermosensitive liposomal doxorubicin	Colon cancer liver metastasis
NCT01437007	2011	Completed	1	TKM 080301 (lipid nanoparticles containing siRNA against the PLK1 gene product)	Colorectal cancer with hepatic metastases Pancreas cancer with hepatic metastase Gastric cancer with hepatic metastase Breast cancer with hepatic metastase Ovarian cancer with hepatic metastase
NCT02181075	2014	Completed	1	ThermoDox™ (thermally sensitive liposomal doxorubicin)	Metastatic liver tumors

also helped modulate the anti-tumor immune response in liver.[74] By upregulating the expression of several adhesion molecules like LSECtin, ICAM-1, E-selectin and activating corresponding pro-metastatic signal pathways, LSECs would help circulating tumor cells migrate through the liver sinusoid and further invade the parenchyma.[74,77–79] Collectively, LSECs would be a potential target in treating intra-hepatic metastases. To construct LSEC-targeted RNAi LNPs, Pattipeiluhu et al[80] generated modified Onpattro-like LNPs (srLNPs) which replaced the zwitterionic helper phospholipids in Onpattro with anionic phospholipids. The binding of anionic helper phospholipid on srLNPs to the scavenger receptor stabilin1/2 (stab1/2) on LSECs improved the LSECs-targeting ability. Furthermore, the anionic phospholipid would inhibit the binding of Apo-E on Onpattro to LDL-r on hepatocytes, which redirected the targeting from hepatocytes to LSECs. Cryo-electron microscope showed no major difference in size and ultrastructure between

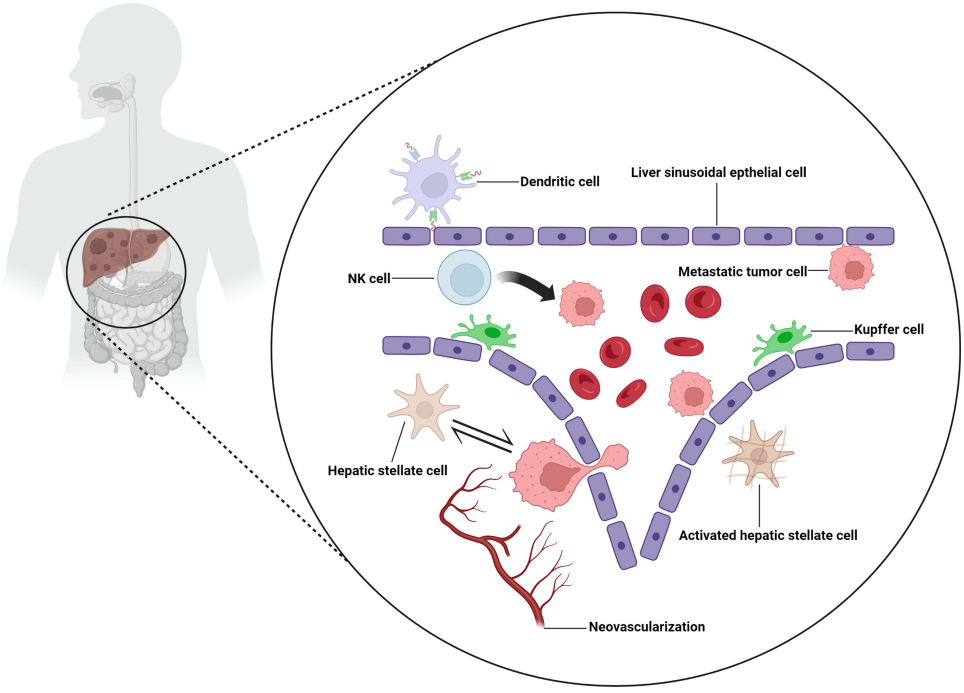

Figure 2 Nonparenchymal components of the tumor microenvironment in liver metastasis (Created with BioRender.com).

Onpattro and srLNPs. In vivo biodistribution assay revealed an increased uptake of srLNPs in LSECs compared with Onpattro analog. In stab1-/-/stab2-/- mutant zebrafish embryos, srLNPs were found to be free-circulatory rather than taken up by LSECs. Furthermore, srLNP-mediated mRNA expression was not observed in LSECs. In older zebrafish embryos with the presence of hepatocytes, srLNP-mediated mRNA expression was still restricted to LSECs. When injected in mice, srLNPs showed increased LSECs tropism with 5-fold targeting enhancement in LSECs compared to hepatocytes. In stab-/- mice, the accumulation of srLNPs was reduced by 80% compared to the wild type. Besides LNP-based RNAi strategies, Campbell et al[81] constructed clodronic acid-encapsulated LNPs to target and destruct LSECs in a stabilin-dependent way. Additionally, the physiochemical property of LNPs, especially the surface charge was found to be involved in the LSECs targeting process. Campbell et al[81] found that cationic LNPs could not be taken up by LSECs in stab-/- zebra fish embryos while LSECs-uptake of positive charged LNPs as EndoTAG-1 and neutral charged LNPs as Myocet was not affected. These abovementioned findings demonstrated the effective LNP-based delivery of novel RNAi or chemotherapeutics to LSECs in both zebrafish and mice models and would support the further clinical exploration of such strategy in treating liver metastasis.

Kupffer Cells

KCs, which are the specialized macrophages in liver TME and sinusoid, were related to the growth and progression of liver metastasis.[74] Despite its pro-metastatic effect, KCs would eliminate the circulatory liposomes, which were thought to be responsible for liposomal chemotherapy resistance.[82] In that, the depletion of KCs in liver microenvironment would be a possible strategy to inhibit the progression of liver metastasis and improve treatment resistance. Clodronate has been found to kill the host macrophages upon phagocytosis.[83] Kruse et al[84] and Shimizu et al[85] have successfully used liposomal clodronate to deplete hepatic KCs, which led to significant repression of liver metastasis growth in colon cancer mouse models. In pancreatic neuroendocrine tumors, Krug et al[86] used liposomal clodronate to treat tumor-bearing RIP1Tag2 mice and found diminished infiltration of F4/80-positive tumor-associated macrophages (TAMs) and density of microvessels, which indicated successful depletion of TAMs in liver metastasis lesions. In vivo study demonstrated that the use of liposomal clodronate would significantly reduce the cumulative tumor burden compared to the controlled group ($6.9 \times 106\ \mu m^2$ versus $2.6 \times 106\ \mu m^2$; $p = 0.036$). Since clodronate could also be ingested by circulatory and non-hepatic macrophages, this clodronate-induced macrophage depletion would cause potentially systemic side effects.[83] Thus, novel liposomal formulations with specific liver-targeting were urgently needed for KCs targeting strategy in liver metastasis treatment. Another possible Kupffer cell-targeting strategy was the use of liposomal oxaliplatin. Ukawa et al[82] found that the injection of liposomal oxaliplatin would decrease the number of KCs without injury to normal hepatocytes. Regarding liposomal RNAi therapy, Pattipeiluhu et al[80] found that RNAi-containing anionic LNPs would specifically target KCs evidenced by threefold targeting enhancement in murine KCs compared to murine hepatocytes. In summary, the deficiencies in current LNP-based-Kupffer-cell-targeting strategies like the off-target toxicity and nonspecific targeting ability warranted the discovery of novel targeting ligands on KCs.

Hepatic Satellite Cells

As key stromal components of liver TME, HSCs were also involved in the progression of liver metastasis. Accounting for around 15% of nonparenchymal cells in the liver, HSCs located within the lumen of Disse would switch to myofibroblast-like phenotype and excrete various cytokines and extracellular matrix (ECM) when activated, thus would be an important modulator in liver metastasis pathogenesis.[23] It has demonstrated that activated HSCs would construct a pro-metastatic microenvironment in liver by modulating the ECM or inducing immunotolerance to exotic tumor cells.[87,88] In CRLM and intrahepatic metastasis of hepatocellular carcinoma, HSCs would promote the growth of liver metastasis by the action of the SDF-1/CXCR4 axis and PI3K-AKT-ERK pathway, respectively.[89,90] Therefore, HSC was a pivotal modulator in the pathogenesis of liver metastasis. Using LNPs targeting HSCs has long been investigated in the management of liver fibrosis and cirrhosis,[91] while the explorations of HSCs-targeting LNPs in liver metastasis were flourishing. Li et al[92] constructed a novel LNP named CAP/GA-sHA-DOX to target HSCs in treating liver malignancy. This LNP was designed as follows: (1) capsaicin (CAP) would inhibit the proliferation of HSCs by blocking the substance-P-induced HSCs activation. (2) GA (glycyrrhetinic acid) was designed to bind the highly-

expressed GA receptor on the surface of tumor cells. (3) HA (hyaluronic acid) was designed to specifically bind to the over-expressed CD44 on HSCs, which would improve the endocytic uptake of LNPs in HSCs. (4) doxorubicin (DOX) was the cytotoxic chemotherapeutics that would kill the tumor cells in the liver. Accordingly, this LNP aimed to disrupt the crosstalk between HSCs and tumor cells while also destroying tumor cells using co-loaded cytotoxic agents. In vitro studies showed CAP/GA-sHA-DOX LNPs were stable under physiologic PH conditions and would effectively release CAP and DOX under the acidic PH environment comparable to lysosomes. The use of CAP/GA-sHA-DOX in the HSCs-tumor cells co-culture system exhibited enhanced cytotoxicity compared to doxorubicin monotherapy, which revealed the synergic effect of CAP plus DOX in destroying cancer cells. Furthermore, the use of CAP/GA-sHA-DOX alleviated the drug resistance and tumor migration induced by the HSC-tumor cell crosstalk. As designated, CAP/GA-sHA-DOX was taken up by both HSCs and tumor cells simultaneously. In vivo biodistribution assay showed increased intra-tumor CAP/GA-sHA-DOX accumulation observed in tumor-bearing mice, which confirmed the superior active targeting ability of this novel LNP. In accordance with in-vitro results, CAP/GA-sHA-DOX exhibited superior anti-tumor efficacy by inhibiting tumor growth and blocking the activation of HSCs. Immunochemistry staining showed CAP/GA-sHA-DOX-treated tumor tissue had less angiogenesis and reversed epithelial to mesenchymal transition phenotype compared to tumor tissue treated with single liposomal chemotherapeutics. Moreover, CAP/GA-sHA-DOX would effectively inhibit the metastasis of tumor cells in orthotopic mice models. Besides CAP/GA-sHA-DOX, some other active-targeting LNPs have also been fabricated to inhibit the crosstalk between HSCs and tumor cells. One dual-targeting strategy generated by Li et al[93] and Qi et al[94] used aprepitant to block the activation of HSCs while using co-delivered curcumin to induce tumor cell apoptosis. Both in vitro and in vivo experiments demonstrated that these dual-targeting LNPs would successfully inhibit the growth of tumor cells and reverse the chemotherapy resistance in the liver by blocking the HSC-tumor cell interaction. Another LNP-targeting strategy from Guo et al[95] used oxymatrine to inhibit the activation of HSCs while using cysteine-end FH peptide (CFH) as the HSCs-targeting component. This novel LNP incorporating oxymatrine with CFH exhibited enhanced intra-tumor drug delivery as well as TME modulating ability both in vivo and in vitro. Collectively, using liposomal HSCs-targeting strategy successfully inhibited the progression of liver malignancy both in vitro and in vivo therefore would be a promising modality in future liver metastasis management.

Immune Cells

Immune cells like NK cells and dendritic cells were also involved in the progression of liver metastasis. NK cells activated upon recognizing cancer antigens would directly kill metastatic cancer cells or secret cytokines and growth factors to modulate TME.[23] OK-432, a modified streptococcus pyogenes formulation, was found to enhance the activity of multiple immune cells like macrophage and NK cells when systemically used.[96] Uehara et al[97] assessed the immunomodulatory effect of liposomal OK-432 on liver NK cells in tumor-bearing mice. The increased proportion of NK cells and Interferon-γ in the liver was observed in tumor-bearing mice treated with liposomal OK-432. Corresponding to the augmented immune infiltrations in the liposomal OK-432-treated group, the survival in this group was also superior compared to either the conventional OK-432 group or the controlled group. This study suggested a possible liver NK cell-targeted strategy in treating liver malignancies, which would be a potential approach in liver metastasis management.

Neovascularization

Neovascularization mediated by vascular endothelial growth factor (VEGF) in liver TME was correlated with the progression and metastasis of various cancer types.[23] In that, the VEGF-directed strategy was considered an effective approach to treating metastatic cancer and has been confirmed in large randomized clinical trials.[74] Moreover, co-inhibition of VEGF would synergize immunotherapy efficacy based on the results of the IMbrave150 study.[98] Chen et al[99] constructed an LNP named HLBBRT which was composed of Cu^{2+} ion-based intracellular bio-nanoreactor, the PD-1 inhibitor, VEGF-targeted RNAi and hypoxia-responsive liposomal shell. When this liposomal complex encountered the hypoxic liver TME, the hypoxia-sensitive liposomal shell degraded and released the inner bio-nanoreactor. After cellular uptake of the bio-nanoreactor, the reduction of Cu^{2+} to Cu^{+} intracellularly was coupled with the generation of •OH, which led to ROS-mediated cell death. Furthermore, VEGF-targeting RNAi would silence the expression of VEGF, thus alleviating the hypoxia-induced neovascularization and

Table 3 Current Nonparenchymal-Cell-Targeting LNPs Strategies in Liver Metastasis

Targeted Cell	Ligand	Co-Loaded Cell-Specific Cytotoxic Agent	Reference
Liver sinusoidal endothelial cells	Scavenger receptor	N/A	[80]
	Scavenger receptor	Clodronate	[81]
Kupffer cells	N/A	Clodronate	[84–86]
	N/A	Oxaliplatin	[82]
	Scavenger receptor	N/A	[80]
Hepatic satellite cells	CD44	Capsaicin	[92]
	CD44	Aprepitant	[93]
	Cysteine-end FH peptide	Oxymatrine	[95]
NK cells	N/A	OK-432	[97]

Abbreviation: N/A, not applicable.

potentiating the co-encapsulated PD-1 inhibitor. Besides direct ROS-mediated cytotoxicity, in vitro studies also showed that HLBBRT-treated cancer cells would promote dendritic cell maturation and enhance the immunogenic cancer death effect. The assumed TME-modulating effect of HLBBRT has been validated in vivo, which found that HLBBRT would increase the infiltration of CD8+ T cells while reducing the immunosuppressive components such as T-regular cells, TAMs and inhibitory cytokines including TNF-α and IFN-γ. Additionally, the survival of mice with CRLM was significantly prolonged in the HLBBRT-treated group compared with controlled groups. Based on these findings, VEGF-targeting LNP would be a potential strategy in modulating TME of liver metastasis.

In summary, as listed in Table 3, LNP-based liver TME-targeting strategies were promising in treating liver metastasis with enhanced liver-targeting ability and possible synergetic efficacy with TME-modulatory systemic therapies like the checkpoint inhibitors or the VEGF inhibitors. Existed deficiencies such as the lack of real-world validations in clinical practice and potential disturbance to liver functions warranted further explorations.

Challenges for LNP-Based Therapy in Liver Metastasis Management

One major challenge is how to translate the in vitro performance of LNP formulations into in-vivo efficacy. The efficacy disparity between in vitro and in-vivo experiments was considered to be related to the formation of serum protein corona and anti-PEG antibodies on LNPs in vivo, which activated the host immune systems.[100,101] The pH discordance between the lab and in-vivo environment also contributed to the efficacy disparity.[102] Moreover, the heterogeneity of cancer cells and the TME in liver metastasis would limit the targeting ability of active targeting LNPs in vivo.[103]

Another challenge remaining for active-targeting LNPs was the choice of targeting ligands. For instance, GA has been widely used as a ligand for liver-targeting LNPs.[92] However, it has been reported that GA would interfere with the drug metabolism process by modulating the enzymic activity of several CYP450 isoforms, which may lead to drug safety concerns.[104] Excessive GA uptake was also found to correlate with pseudo-hyperaldosteronism, which was characterized by severe hypertension and hypokalemia.[105] The example of GA reminded the importance of exploiting the physiological function of common targeting ligands in LNPs.

Finally, the efficacy of LNP-based in clinical trials might be confounded by the anti-tumor effect of drug-free liposomes. In that, future clinical trials should incorporate an extra controlled group with drug-free liposomes to better evaluate the exact effectiveness of LNP-based formulations.

Prospective

There were some opportunities for researchers to improve LNP-based strategies in the future. First, the development of biocompatible LNPs with controlled delivery of multiple drugs acting on different targets was urgently needed. Second, the development of novel coating materials would optimize the controlled-release of active-targeting LNPs in treating liver metastasis. Recently research showed nanoparticles equipped with a novel urea-based periodic mesoporous organosilica (Ur-

PMO) material would promote the controlled-release process and exhibit an improved biological effect, which showed the potential in tumor-targeted LNP-based strategy.[106] Another potential opportunity existed in stimuli-responsive LNPs, which would be syngeneic with the use of locoregional therapy modalities for liver metastasis. Third, identifications of novel ligands on metastatic cancer cells such as cell senescent markers would promote the development of metastasis-targeting LNPs. Additionally, as most of current LNPs targeting cytoplasmic or membrane markers, the targeting of mitochondria markers was a promising strategy in liver metastasis.[107] LNPs containing natural-extracted polyphenol such as GA would be promising in treating metastasis by enhancing the ROS-eliminating process of mitochondria in metastatic tumor cells.[107] Moreover, optimizing the pharmaceutical property of existing LNPs would help improve the therapeutic index and reduce systemic toxicity. Finally, as current data remained as pre-clinical and no comparative studies were reported, data from large randomized clinical studies was needed to evaluate the comparative efficacy of tumor cell-directed strategy, liver TME-directed strategy and the joint treatment strategy in real-world clinical settings.

In conclusion, the LNP-based strategy showed great promise for individualized precise management of liver metastasis, while future studies were imperatively needed to enhance the efficacy and safety profile. This review highlighted the current status of two LNP-based strategies, ie, metastatic tumor cell-directed and TME-directed strategy in the management of liver metastasis, which showed great potential in future real-world clinical oncology practice.

Summary

1. Conventional treatments in liver metastasis were lack of efficacy and targeting ability.
2. Lipid nanoparticles-based therapy appeared to be a promising strategy to optimize current management modalities in liver metastasis.

Abbreviations

TME, tumor microenvironment; LNPs, liposomal nanoparticles; AUC, area under curve; siRNA, small interfering RNAs; LDL-r, low-density lipoprotein receptor; Apo-E, apolipoprotein E; CI, confidence interval; CAPEOX, oxaliplatin + capecitabine; CRLM, colorectal cancer liver metastasis; RNAi, RNA interference; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9; MTH1, mutT homolog 1; PS, protamine sulfate; TR, transferrin receptor; TACE, transarterial chemoembolization; R+PGLD, raltitrexed plus liposomal doxorubicin; T+P, tegafur plus pirarubicin; BSP, bletilla striata polysaccharide; HAIC, hepatic arterial infusion chemotherapy; 5-FU, 5-fluorouracil; PEG, polyethylene glycol; DSM, degradable starch microspheres; RFA, radiofrequency ablation; LTLD, lyso-thermosensitive liposomal doxorubicin; PDT, photodynamic therapy; ROS, reactive oxygen species; PFOB, perfluorocarbon; RBCm, red blood cell membrane; LSECs, liver sinusoidal endothelial cells; KCs, Kupffer cells; HSCs, hepatic satellite cells; stab1/2, stabilin1/2; CAP, capsaicin; GA, glycyrrhetinic acid; HA, hyaluronic acid; DOX, doxorubicin; VEGF, vascular endothelial growth factor.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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