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Diagnostic imaging and therapeutic application of nanoparticles targeting to the liver

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Liver diseases, particularly viral hepatitis, cirrhosis and hepatocellular carcinoma, are common in clinical practice with high morbidity and mortality worldwide. Many substances for diagnostic imaging and therapy of liver diseases may have either severe adverse effects or insufficient effectiveness *in vivo* due to the nonspecific uptake. Therefore, by targeting delivery of drugs into the liver or specific liver cells, the drug efficiency may be largely improved. This review summarizes the up-to-date research progress focusing on nanoparticles targeting to the liver for both diagnostic and therapeutic purposes. Targeting strategies, mechanisms of enhanced effects, and clinical applications of nanoparticles are discussed specifically. We believe that new targeting nanotechnology such as nanoprobes for multi-modality imaging and multifunctional nanoparticles would facilitate significant advancements in this research-active area in the near future.

1 Introduction

Liver is the major site of drug metabolism and excretion. This organ receives blood from the gastrointestinal tract and heart via the portal vein and hepatic arteries, respectively. Blood flows through the sinusoids (terminal vessels between hepatocyte cords and lined with endothelial cells and Kupffer cells), then accumulates in the central veins and leaves the liver from hepatic veins. Structure of liver sinusoids in hepatic lobules is shown in Fig. 1A. Hepatocytes, making up 70-85% of the liver's cell population, are the key functional cells in the liver that play an important role in metabolic, endocrine and secretory functions.[1] Kupffer cells are specialized macrophages in liver, which are located on the wall of the sinusoids.[2] Hepatic stellate cells (HSCs) are another important type of cells in liver. They lie scattered in the space of Disse, a small region between the sinusoid and the hepatocytes. HSCs change into an activated state in response to the liver damage and can lead to collagen scar tissue formation, fibrosis or cirrhosis.[3] Bile is secreted by hepatocytes, drained into biliary ductules, which are lined with epithelial cells, and then exit the liver from bile ducts.[2]

Liver diseases, particularly viral hepatitis, cirrhosis and hepatocellular carcinoma are frequently encountered in clinical practice with high morbidity and mortality worldwide.[4] Although many diagnostic and therapeutic drugs have been developed against these diseases, most drugs are taken up and cleared from the circulation by liver without specific targeting or drug delivery systems due to the first-pass effect.[5] Another important drawback of pharmacotherapy available nowadays is the inability to deliver sufficient amount of drugs to the diseased cells.[6] Many examples can be provided where drugs have beneficial effects in liver or one cell type of liver, yet show adverse effects in the other organs or cells. In the treatment of hepatocellular carcinoma, free doxorubicin showed severe side effects, such as cardiac toxicity, oral mucositis and hair loss.[7] In the treatment of liver fibrosis, anti-inflammatory drugs like cyclooxygenase inhibitors have antifibrotic effects in Kupffer and endothelial cells but could stimulate the fibrotic process after uptake by HSCs.[5, 8] These problems call for persistent efforts through exploring new efficient targeting pharmacological interventions for liver diseases.

Drug carriers have been used in liver targeting delivery, including various types of nanoparticles.[9] Nanoparticles are defined as spherical (or quasispherical) particles with a diameter of less than 100 nm, but often used in the range up to 300-500 nm.[10] Because of their unique size and surface characteristics, nanoparticles have shown great potential in delivering drugs targeting to the liver either passively or actively. This may overcome major clinical and pharmacological problems such as poor solubility, non-specific organ toxicities, short half-life in the blood circulation, and multidrug resistance associated with conventional chemotherapeutic agents.[4]

In liver specific applications, four main types of nanoparticles have been examined: polymers, lipid-based nanoparticles, metal complex and bio-nanocapsules.[4] The applications of nanotechnology in hepatology have been discussed in several review articles. These papers focused on specific nanoparticles such as nano-vectors,[11] bio-nanocapsules,[12] polymers or lipid-based nanoparticles,[4] and just provided a general description of different drugs targeting to the liver [5, 13] or nanotechnology in liver diseases.[6, 14] Although numerous targeting approaches have been proposed, the effect of nanoparticle properties on their disposal by the body at the organ

level and particularly by the liver at the cellular level is largely unclear. The scope of this comprehensive review is to first summarize nanoparticle-based approaches for liver targeting drug delivery systematically. Subsequently, we will discuss and evaluate the efficacy of different nanoparticles in diagnostic imaging and therapeutic application in liver diseases. The information discussed in this review would be useful for the development and assessment for liver targeting drug or gene delivery systems based on nanoparticles, as mentioned in the final section.

2 Approaches for nanoparticles targeting to the liver

To selectively target to the liver, the hepatic-targeted drug delivery system has been designed. In general, nanoparticle delivery strategies could be classically divided into passive and active targeting. The passive targeting approach usually employs the physicochemical properties of the drug carriers such as size, surface properties and routes of administration to selectively increase the nanoparticles accumulation in the liver, and thus reduce the undesirable effects to other organs. Without further identification of the intrahepatic cell types that take up the drug, passive targeting is sometimes described as synonymous for drugs accumulation in this organ.[5] Active targeting, on the other hand, is using specific ligands that can recognize and bind to certain type of cells. Then nanoparticles could be taken up by this specific type of liver cells, minimizing non-specific effects on other types of cells in the liver and other organs. Under such circumstances, selective drug delivery is achieved at the cellular level.[4, 15]

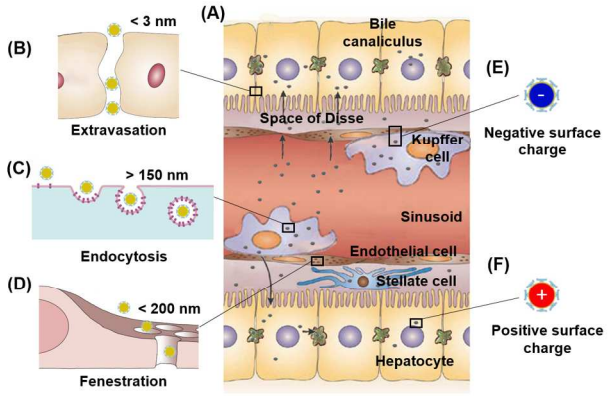

Fig. 1 Mechanisms of nanoparticles passive targeting to the liver. A: Microscopic structure of liver sinusoid in hepatic lobules. B: Nanoparticles smaller than 3 nm in diameter could extravasate different tissues nonspecifically. C: Nanoparticles with large negative surface charge or larger than 150 nm in diameter could be captured by Kupffer cells. D: Nanoparticles less than 200 nm in diameter could pass through sinusoidal fenestrations after intravenous administration. E: Nanoparticles with negative surface charge tend to be taken up by Kupffer cells and endothelial cells. F: Nanoparticles with positive surface charge tend to be taken up by hepatocytes.

2.1 Mechanisms of passive targeting to the liver

Physiological and anatomical characteristics of the liver enable hepatic accumulation of nanoparticles with specific size and surface properties. After entry into the bloodstream, nanoparticles may be subjected to non-specific interactions with serum proteins and rapidly covered by a layer of proteins, *e.g.* the so-called protein corona, leading to uptake by the Kupffer cells on arriving sinusoids and accumulate in the liver.[16] For example, adsorption of immunoglobulin to the surface of liposomes enhances their uptake by Kupffer cells approximately 6-fold.[17] At gap junctions of normal tissues, the intercellular space is roughly 4 nm. Nanoparticles smaller than 3 nm in diameter extravasate different tissues nonspecifically (Fig. 1B).[18] Nanoparticle uptake by the Kupffer cells increases with particle size (Fig. 1C). Thus larger nanoparticles would be more rapidly accumulated in the liver and the blood circulation time would be shorter. With particle size decreasing, the uptake by Kupffer cells is reduced, leading to a prolonged circulation time and increased chance for other liver cells to take up. Usually nanoparticles less than 150 nm in diameter could avoid capture by Kupffer cells, diffuse out of the sinusoids through the fenestrae, and then reach the hepatocytes.[19, 20]

The endothelial cells lining normal liver sinusoids have fenestrae that are holes of 50 - 200 nm in diameter.[21, 22] This means that intravenous administration of nanoparticles less than 200 nm in diameter could pass through these sinusoidal fenestrations (Fig. 1D).[4] Under certain circumstances, some deformable nanoparticles up to 400 nm in diameter could extravasate through the normal sinusoid endothelial fenestrations by a mechanism called forced extrusion.[23] Meanwhile, in some liver diseases, such as hepatocellular carcinoma, the fenestrae of endothelial cells increase to 400-600 nm in diameter, leading to enhanced permeability and extravasation of nanoparticles.[24] It is reported that for cancer treatment, the ideal size requirement for nanoparticles is between 70 and 200 nm.[25] Nanoparticles with a smaller size, usually less than 50 nm in diameter could diffuse deeper in the space of Disse with enhanced localization to the hepatocytes and those on the scale of 10-20 nm often undergo rapid uptake by hepatocytes.[26, 27] In general, nanoparticles larger than 80 nm in diameter would be trapped by the liver.[18]

There are also many factors other than particle size that can affect the cellular uptake of nanoparticles. For instance, Kupffer cells and endothelial cells tend to take up nanoparticles with negative surface charges more efficiently through scavenger receptors (Fig. 1E),[5] while hepatocytes are more likely taking up nanoparticles with positive surface charges (Fig. 1F).[20, 28] Compared to hydrophilic nanoparticles, hydrophobic ones are more rapidly removed from circulation by Kupffer cells.[28] PEGylation, the process of attaching poly(ethylene glycols) (PEG) to the nanoparticle surface, is a widely used mechanism to reduce protein binding, minimize uptake of particles by Kupffer cells and increase uptake by hepatocytes.[29] Unfortunately, it has been reported that PEGylation has a detrimental effect on bioactivity.[30] Zwitterionic coating is an emerging strategy to reduce protein binding on foreign nanoparticles without sacrificing bioactivity.[31, 32] Some liposome formulations, especially the presence of phosphatidylserine, have a modest preference for hepatocytes.[33]

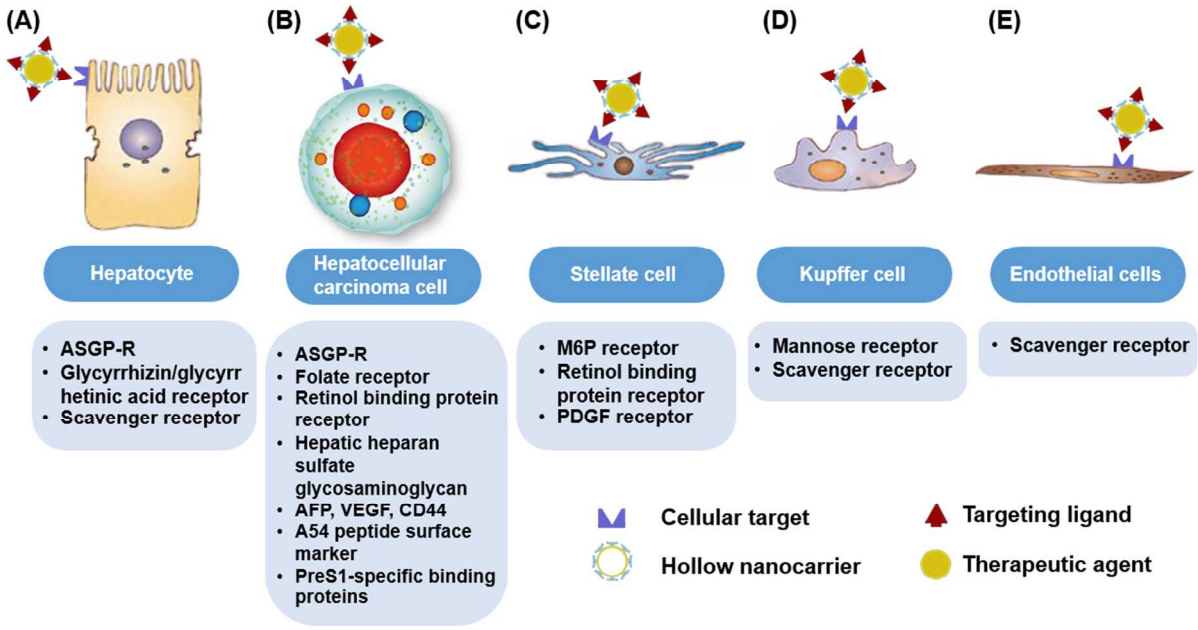

Fig. 2 Receptors or cellular targets on liver cells for nanoparticles active targeting to the liver. After intravenous administration, nanoparticles modified with specific surface ligands could be recognized by their receptors or cellular targets on a specific type of liver cells (A: hepatocyte. B: hepatocellular carcinoma cell. C: Hepatic stellate cells (HSC). D: Kupffer cell. E: endothelial cells.) and then drug/gene was released.

2.2 Strategies for active targeting to the liver

As shown in Fig. 2, several different receptors present on various liver cells have been identified for possible targeting.[13] Generally speaking, specific surface ligands on nanoparticles can be recognized by their receptors or cellular targets on liver cells, and then drug or gene is released into these cells. Until very recently, modified nanoparticles are able to actively target various liver cells, including hepatocytes, HSCs, Kupffer cells, endothelial cells and hepatocellular carcinoma cells. Target of cancer is mostly explored with nine different active targeting strategies. Table 1 lists the cellular targets that have been used for ligand-mediated nanoparticles to enter liver cells for therapeutic and diagnostic purposes.

2.2.1 Targeting to hepatocytes

Targeting strategies to deliver nanoparticles to the hepatocytes (Fig. 2A) and hepatocellular carcinoma cells (Fig. 2B) have focused primarily upon the asialoglycoprotein receptor (ASGP-R). This approach is one of the first and the most widely used options for the cell-specific delivery to liver cells. ASGP-R is expressed in well-differentiated forms of hepatocellular carcinoma cells and the membrane of hepatocytes that face sinusoids. This receptor has binding affinity to a broad range of molecules containing galactose and N-acetyl-galactosamine residues such as lactose, galactoside, galactosamine and lactobionic acid, which could be conjugated to the surface of nanoparticles for active targeting.[4, 13] Nanoparticle that bound to ASGP-R can form a complex and then enter cells by clathrin-mediated endocytosis.[34] After releasing the ligands inside the cell, these receptors recycle back to the cell membrane rapidly. The ASGP-R concentration on liver parenchymal cell surfaces is 100,000–500,000 binding sites per cell,[35] while the density and the activity of the ASGP-R are lower in liver under pathological conditions, because binding inhibitors in serum could significantly reduce the binding capacity of ASGP-R.[36]

Apart from ASGP-R, glycyrrhizin / glycyrrhetinic acid receptor is also employed in active targeting strategies. Glycyrrhizin and glycyrrhetinic acid are the main bioactive compounds of licorice. Nanoparticles modified with glycyrrhizin / glycyrrhetinic acid could be recognized by their receptors on hepatocytes and taken up by hepatocytes via receptor-mediated endocytosis.[37] The number of binding sites for glycyrrhetinic acid is much more than that of glycyrrhizin.[38] Hepatocellular carcinoma cells express more glycyrrhetinic acid receptors on the surface than hepatocytes.[39] Thus, this receptor may have extra value in cancer targeting therapy. It should be noted that physicochemical properties also play important roles in active targeting of nanoparticles to the liver. For example, liposomes that display ASGP-targeting ligands cannot be recognized by the ASGP receptor if the liposome is larger than 70 nm in diameter.[40] More cationic surface charges contribute to the higher anti-tumor efficacy, because nanoparticles more likely bind with the negatively charged phospholipid head groups that are preferentially expressed on tumor cells via electrostatic interactions.[41]

Other ligand / receptor-mediated targeting strategies to hepatocytes include the use of scavenger receptor (Class BI), heparan sulfate proteoglycan, plasma membrane fatty acid transporter, IL-6 receptor and immunoglobulin A binding protein.[4]

2.2.2 Targeting to HSCs

Using the relevant targeting ligands conjugated on the surface of nanoparticles, three groups of receptors over-expressed on HSCs

(Fig. 2C) have been targeted, including mannose 6-phosphate (M6P)/insulin like growth factor-II receptor, retinol binding protein (Type VI collagen and integrin) receptors and platelet-derived growth factor (PDGF) receptor.[4, 6] Human serum albumin modified with mannose 6-phosphate (M6P-HSA) groups can be specifically recognized by the mannose 6-phosphate/insulin like growth factor II receptor. Retinol binding protein receptors can recognize vitamin A and certain cyclic peptide, resulting in a remarkably enhanced uptake of vitamin A and cyclic Arginine-Glycine-Aspartate (cRGD) peptide-coupled nanoparticle by HSCs. The PDGF receptor-β can be up-regulated on activated HSCs during hepatic fibrogenesis and has a special affinity for the cyclic peptide C*SRNLIDC*,[42] which has been employed in the targeting delivery.

2.2.3 Targeting to Kupffer cells and endothelial cells

Uptake by Kupffer cells (Fig. 2D) and sinusoidal endothelial cells (Fig. 2E) can also be mediated by specific receptors. These two types of liver cells share many characteristics relevant for targeting delivery systems. Kupffer cells and endothelial cells specifically recognize oxidized low-density lipoprotein and human serum albumin (HSA) by scavenger receptors.[43] The mannose receptor, one of the sugar receptors, is a transmembrane protein expressed on the surface of macrophages including Kupffer cells and endothelial cells.[44] This feature has been applied to design a cell-specific mannosylated nanoparticles actively targeting Kupffer cells.[45]

Up to date, there are very limited studies focusing on nanoparticles targeting the bile duct epithelial cells. And no drug carrier to this cell type was reported. The αvβ6 receptor[46] and secretin receptor[47] may be used for targeting to bile duct and cholangiocarcinomas cells in the future.

2.3 Combined targeting strategies

It is worth mentioning that passive and active targeting effects could be combined together. Guan *et al.* developed a lactosyl-norcantharidin N-trimethyl chitosan nanoparticles (Lac-NCTD-TMC-NPs)-based novel liver targeting delivery system.[41] The passive tissue targeting of Lac-NCTD-TMC-NPs was based on controlling the particle size at around 120 nm, which warrants their successful penetration through the fenestrated sinusoids. Once inside the tumor site of the liver, the hepatocyte-specific uptake of nanoparticles was achieved by active targeting. NCTD is a small molecule originally secreted by blister beetle. Herein, NCTD modified with a lactobiose-bearing galactose group (Lac-NCTD) was employed to enhance the tumor cells uptake through ASGP-R recognition.

3 Liver targeted nanoparticles for diagnostic imaging

There have been many complementary and supplementary types of techniques for diagnostic imaging of the liver, while detection and characterization of focal hepatic lesions continue to be challenges. In some patients with underlying diffuse liver disease, such as steatosis or cirrhosis, detecting focal liver lesions is a difficult task. Liver targeted nanoparticles can improve the detection of different liver diseases, and play an important role in therapeutic decision-making. Studies on liver targeted nanoparticles for diagnostic imaging are summarized in Table 2 and discussed in detail below.

3.1 Nanoparticle-based contrast agents for liver MRI

Liver-targeted magnetic nanoparticles have been employed as contrast agents for magnetic resonance imaging (MRI) by reducing relaxation time of protons in absorbing tissues to generate contrast effects under external magnetic fields.[48, 49] There are two types of nanoparticle-based contrast agents for liver MRI to improve the sensitivity and reliability of diagnosis: T_2 contrast agents (superparamagnetic nanoparticles) which can reduce the T_2 relaxation time (transverse relaxation) and T_1 contrast agents (paramagnetic nanoparticles) which work through shortening the T_1 relaxation time (longitudinal relaxation).[50]

3.1.1 Nanoparticle-based T_2 contrast agents for liver MRI

Superparamagnetic iron oxide nanoparticle (SPION) is a typical T_2 contrast agent and is the only nano-sized contrast agent approved clinically. This nanoparticle is a conglomerate of numerous iron oxide crystals coated with dextran, carboxydextran, citrate or PEG.[51] Seven kinds of SPIO particles ranging from 20 to 250 nm were designed and used without specific surface ligand conjugated. SPION accumulation in liver tissues is found to largely depend on the phagocytic Kupffer cells.[52] For example, SPIONs are reported to accumulate in normal liver tissue, benign hepatocellular lesions, and dysplastic nodules, which contain abundant phagocytic Kupffer cells.[53] In non-alcoholic steatohepatitis (NASH)[54] and ischemia–reperfusion,[55] however, the decreased phagocytic function of Kupffer cells leads to much less SPIONs accumulation. It is reported that many carcinomas containing tumor-associated macrophages, which are able to take up SPIONs.[56, 57] While malignant liver tumors, including hepatocellular carcinoma, cholangiocarcinoma and liver metastases from colorectal cancer, are lack of Kupffer cells, and thus do not take up any SPIONs.[58] In addition, the uptake of SPION increases in the fibrosed liver, due to the enhanced permeability and retention.[59]

Two SPIONs are now clinically approved: ferumoxides (Feridex® and Endorem®) with the particle size range of 120 to 180 nm,[60] and ferucarbotran (Resovist®) with the particle size of about 60 nm.[51] At about 8 min following the drip infusion over about 30 min, 80% of ferumoxides are taken up by Kupffer cells.[61] Hypotension, lumbar and leg pain are the most frequent side effects with an incidence ranging from 2 to 10%. Unlike ferumoxides, ferucarbotran can be safely injected rapidly in a bolus fashion, with much less side effects.[62] Besides, Feruglose (Clariscan®) had also been tested clinically for characterization of tumor microvasculature in the liver, but its development in hepatocellular carcinoma has been discontinued due to safety concerns.[62]

Unfortunately, all SPIONs clinically approved exhibit low relaxivity and passively targeting to the liver, which result in relatively low sensitivity in diagnosis. Considering that the efficiency of SPIONs for MRI is strongly dependent on their sizes, magnetic characteristics and surface ligand, strategies for synthesizing SPIONs based on size control, metal doping and surface ligand conjugating have been developed. Recently, Zhao

et al. report a new strategy to achieve high transverse relaxivity (T_2) by controlling the morphology of SPIONs.[49] These octapod iron oxide nanoparticles were fabricated in the presence of chloride anions under thermal decomposition conditions, and exhibited an ultrahigh transverse relaxivity value. These SPIONs were much more effective for *in vivo* liver MRI and hepatocellular carcinoma detection in comparison with conventional spherical SPIONs.

ASGP-R mediated approach has been employed for SPION's active targeting to hepatocytes to increase the sensitivity of diagnosis. Selim *et al*. reported that surface modification of SPIONs using lactobionic acid could improve their intracellular uptake and ability to target hepatocytes.[63]

3.1.2 Nanoparticle-based T_1 contrast agents for liver MRI

Most clinically used MRI contrast agents are gadolinium (Gd) based T_1 contrast agents. Nanoparticle-based T_1 contrast agents (paramagnetic nanoparticles) such as MnO and Gd_2O_3 nanoparticles showed great advantages over clinically approved T_1 contrast agents. Chen *et al*. prepared poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) nanoparticles with gadolinium-diethylenetriamine-pentaacetic acid (Gd–DTPA) absorbed onto the surface. Compared with commercial T_1 contrast agent Magnevist®, this nanoparticle had more biocompatibility, higher plasma stability, better contrast imaging and more specific liver targeting property. At the same dose, this nanoparticle increased the MRI intensity in rat livers five-fold with a longer stagnation time than Magnevist®.[64] Tian *et al*. found that nanoamplifier composed of gadolinium-doped silica nanoparticles and gold nanoparticles (Gd_2O_3@MCM-41@Au) was an effective MRI signal enhancer with sensitive cancer diagnosis potential.[65] The signal enhancement may be explained by the electron transfer between water molecules and Gd_2O_3 doped inside the nanoparticles, which is even more apparent in the presence of the Au nanoclusters. Mn^{2+}-doped SiO_2 nanoparticles[66] and zwitterion-coated gadolinium-embedded iron oxide (GdIO) nanoparticles[50] also showed great a promise as liver-specific MRI contrast agent.

3.1.3 T_1-T_2 dual-modal contrast agents for liver MRI

Some nanoparticle-based MRI agents can provide contrast effects in both T_1 imaging with high tissue resolution and T_2 imaging with high feasibility of hepatic-lesion detection. Im *et al*. integrated nano-sized T_1 contrast agent MnO and T_2 contrast agent Fe_3O_4 into a single hybrid nanocrystal, and found that this dual contrast agent had an obviously higher degree of accuracy than that for any single contrast agent.[67] Zhou *et al*. embedded T_1 contrast agent Gd_2O_3 into T_2 contrast agent SPION, which led to synergistic enhancement of T_1 and T_2 relaxations.[68] The self-confirmation and improved accuracy of liver MRI using T_1-T_2 dual-modal contrast agents promise tremendous potential clinical applications in diagnosing liver diseases, especially for cancers.

3.2 Nanoparticle-based contrast agents for liver CT imaging

In parallel to nanoparticle-based MRI contrast agents, three types of nanoparticles have been employed in computed tomography (CT) imaging of the liver. Compared to conventional iodine-based CT contrast agents, nanoparticle-based contrast agents have shown better imaging efficacy, lower toxicity and longer circulation half-life.

Alkaline earth metal-based nanoparticles (ExiTron nano 6000 and 12000®) have been examined as liver-specific contrast agents for CT in animal.[69] These nanoparticles were stabilized by a polymer coating with an average diameter of 110 nm. Similar to SPIO particles, ExiTron nano 6000 and 12000 were mainly taken up by the Kupffer cells after intravenous injection, and thus specifically accumulated in the normal liver tissue. Primary or metastatic liver cancer did not take up the contrast agent, making it clearly detectable as unenhanced region within the hyperdense normal liver tissue. Particularly, ExiTron nano 12000 could provide X-ray contrast of the liver for up to several weeks after injection (Fig. 3). This allows long-term imaging after a single injection and also reduces additional stress for the animals.[70] Recently, Liu *et al*. designed PEGylated Yb_2O_3:Er nanoparticles which had more significantly enhanced X-ray CT contrast for the liver and longer blood circulation time compared with clinically used contrast agent Iobitridol®.[71] It is worth mentioning that compared with previous reports,[72, 73] the synthesis route of PEGylated Yb2O3:Er nanoparticles (one-pot urea-based homogeneous precipitation for $Yb(OH)CO_3$ nanoparticles and facile in situ calcination technique for Yb_2O_3:Er nanoparticles) was optimized and the content of Yb enhanced, showing great promising of Yb-based nanoparticles as X-ray CT contrast agents.

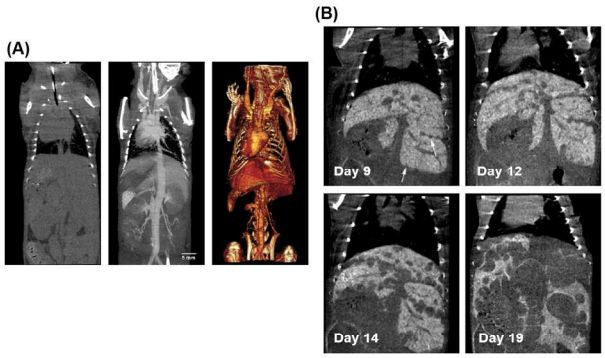

Fig. 3 ExiTron nano 6000 and 12000 provided increasing contrast of liver and spleen up to weeks. A: Coronal micro-CT image of normal mouse liver before intravenous injection of ExiTron nano 12000 (left), during the early intravascular phase (middle) and three-dimensional reconstruction image (right). B: Repeated micro-CT of a mouse with liver metastases after a single injection of ExiTron nano 6000. The smallest liver metastases (300 μm in diameter) was detected 9 days after intrasplenic injection of MC38 colon tumor cells. Reproduced with permission of ref. 69, Copyright 2011, PLoS ONE.

Unexpectedly, no nanoparticle-based CT contrast agent has been reported actively targeting to the liver. Ai *et al*. developed a facile strategy for synthesizing Bi_2S_3 nanodots coated with oleic acid (OA-Bi_2S_3) using commercially available bismuth neodecanoate as the precursor.[74] One of the major advanced features of this nanoparticle-based CT agent is that the surface of

these nanodots can be readily modified with biocompatible agents, which provides possibilities of conjugating specific surface ligand for active targeting to the liver.

Besides MRI and CT, nanoparticles have also been applied as contrast agents for the liver tumor imaging through the near infrared fluorescence (NIRF) optical imaging technique[75] and two-photon imaging technology,[76] indicating their potential as a liver cancer marker.

3.3 Nanoparticle-based contrast agents for multi-modality liver imaging

Nanoparticles have been individually examined as contrast agents in various noninvasive diagnostic liver imaging techniques, including MRI, CT and fluorescence imaging. However, sometimes information collected from single-modality imaging cannot satisfy the high requirements of the accuracy and efficiency for diagnosis because of the limitation of instruments. For instance, MRI can provide a high spatial resolution of hepatic lesions, but has insufficient sensitivity by other hypointense areas in the liver.[51] CT imaging is usually limited by its poor soft-tissue contrast, while fluorescence imaging is often restricted by the low penetration depth of the excitation and emission light.[77] Thus, a novel nanoparticle-based imaging platform has been proposed recently to combine the merits of each imaging modality.

Three studies employed the physicochemical properties of nanoparticle-based agents without specific surface ligand for multi-modality liver imaging. PEGylated Gd_2O_3:Yb^{3+}, Er^{3+} nanorods,[78] lanthanide-doped Lu_2O_3 nanoparticles[79] and oleic acid-stabilized Gd-doped $NaYbF_4$:Er nanoparticles[73] were successfully developed as long-circulating contrast agents for up-conversion luminescence (UCL), T_1-enhanced MRI and CT trimodal imaging.

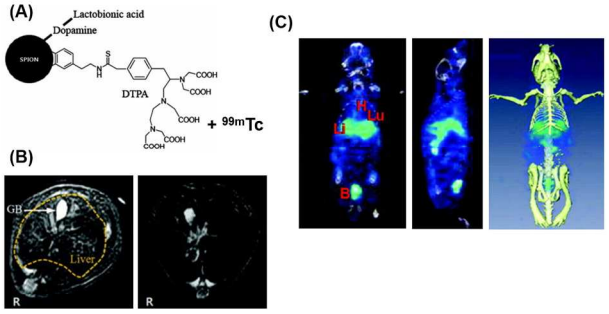

Figure 4. ^{99m}Tc labeled lactobionic acid-SPIONs (LBA-SPION) were used as a hepatocyte-targeted dual imaging probe in mice. A: Structure of LBA-SPION. DTPA (diethylene triamine pentaacetic acid) was conjugated to the nanoparticle for radiolabeling with ^{99m}Tc. B: T2-weighted MRI images of the liver. After LBA-SPION injection (right), the signal of the liver tissue became significantly darker than pre-injection (left). GB, gallbladder. C: SPECT/CT images of mice after LBA-SPION injection in a, coronal (left), saggital (middle) sections and three-dimensional reconstruction view (right). H, heart; Lu, lung; Li, liver; B, bladder. Reproduced with permission of ref. 80, Copyright 2009, Wiley-Liss, Inc.

For actively targeting to the liver, Lee *et al*. synthesized lactobionic acid-SPIONs (LBA-SPION) as hepatocyte-specific contrast agents for MRI and these particles were also labeled with ^{99m}Tc for SPECT (single-photon emission computed tomography)/CT (Fig. 4).[80] The multi-modality imaging using this nanoparticle-based agent provided accurate anatomic and molecular functional information for diagnosis of liver diseases. These multi-functional imaging probes have potential for evaluating hepatocytic functions in certain clinical conditions such as partial liver transplant or hepatitis, and monitoring the disease progress.

4 Therapeutic nanoparticles targeting to hepatocellular carcinoma

Nanoparticles have drawn significant interest not only as diagnostic but also as therapeutic tools. Hepatocellular carcinoma is the most common primary malignancy of the liver in adults. There are two major challenges in the treatment of hepatocellular carcinoma. One is the high recurrence rate even after curative resection (70% in 2 years), and the other is the low response rate to chemotherapy (no survival benefit in the long term).[81] Reasons for chemotherapy failure include the multidrug resistance of hepatocellular carcinoma cells and the insufficient distribution of drugs to the tumor. Thus, the delivery of anti-cancer drugs through nanoparticle targeting to the liver may be beneficial for increasing the drug concentration and prolonging exposure of hepatocellular carcinoma tissue to therapeutic drugs, thereby improving the therapeutic effect and decreasing side-effects.

4.1 Polymeric nanocarriers targeting to hepatocellular carcinoma

Polymers are one of the first types of nanocarriers investigated for liver specific applications. A variety of biodegradable polymers have been used to form nanoparticles to encapsulate therapeutic compounds, such as poly(ε-caprolactone) (PCL), poly(D,L-lactic acid) (PLA), poly(D,L-lactic-co-glycolic acid) (PLGA), and their copolymers diblocked or multiblocked with PEG.[82] Various techniques are employed to prepare polymeric nanocarriers, including solvent evaporation, salting-out, nanoprecipitation, dialysis, supercritical fluid technology, mini-emulsion, micro-emulsion, surfactant-free emulsion, and interfacial polymerization. In spite of these preparation ways, many factors should be taken into consideration for the choice of preparation methods such as the optimum physicochemical properties, biosafety, biodistribution and the field of application, *etc*[83]. For instance, as nanocarriers, polymers should be completely free from additives, reactants or organic solvents. Thus, techniques such as rapid expansion of a supercritical solution can be selected because neither surfactant nor organic solvent is used during preparation. Table 3 lists a summary on up-to-date studies of polymeric carriers for drug/gene targeting delivery to hepatocellular carcinoma.

4.1.1 Passive targeting to hepatocellular carcinoma

In all the published reports, anti-cancer drugs-loaded polymers show more tumor inhibition efficacy or higher concentration in liver or cancer tissue. Six studies employed the physicochemical

properties of polymers without specific surface ligands.[41, 84-88] The size of these polymers was ranged from 55.1 to 219 nm, the while the zeta potential varied from -45 to 37.37 mV. Five anti-cancer drugs (5-fluorouracil, cisplatin, mitoxantrone, doxorubicin and norcantharidin) and one short hairpin RNAs were delivered selectively to liver or cancer tissue. Two polymers passively targeting to the liver are currently under clinical testing. One is doxorubicin transdrug (Livatag®), a doxorubicin-loaded poly-iso-hexyl-cyanoacrylate (PIHCA) nanoparticle which could overcome the multidrug resistance of hepatocellular carcinoma *in vitro* and *in vivo*.[86, 89] Compared to free doxorubicin, Livatag® had reduced the 50% inhibition concentration (IC(50)) in different hepatocellular carcinoma cell lines. A higher anti-tumor efficacy was also observed for Livatag® versus free doxorubicin on hepatocellular carcinoma in transgenic mice overexpressing the mdr1 and mdr3 genes.[89] In a Phase II trial, the 18-month survival rate was 88.9% in Livatag® group, significantly higher than 54.5% in current standard care group (transarterial chemoembolization with a cytotoxic drug). The major side-effects of Livatag® include grade-4 neutropenia, severe hypotension, pseudo-allergic reactions, and acute respiratory distress syndrome.[6] A Phase III study (clinical trial identifier: NCT01655693) is ongoing to determine whether Livatag® is effective in advanced hepatocellular carcinoma after treatment failure or intolerance to Sorafenib.

The other polymer undergoing clinical test is mitoxantrone-loaded polybutyl cyanacrylate (PBCA) nanoparticles. After intravenous injection of ^{3}H-mitoxantrone-PBCA in mice, the radioactivity was observed to be higher in the liver than in other organs, and was even higher in liver tumors than in normal liver tissue.[90] In the phase II clinical trial, patients with unresected hepatocellular carcinoma in the mitoxantrone-loaded PBCA nanoparticles group had 5.46 months median survival periods while survival in the free mitoxantrone group was 3.23 months. Major toxic events were leukopenia and anemia.[87]

4.1.2 Active targeting to hepatocellular carcinoma

Several binding sites on hepatocellular carcinoma cells have been targeted using the relevant targeting ligands conjugated to the surface of polymeric carriers. Five studies examined the delivery of paclitaxel, doxorubicin, 5-fluorouracil, or oridonin through targeting ASGP-R using galactosamine / pullulan conjugated, or galactosylated polymer.[91-95] Among these carriers, galactosamine-targeted poly *N*-(2-hydroxypropyl)methacrylamide (HPMA) doxorubicin is the only nanoparticle actively targeting to the liver that has been tested clinically. In a Phase I trial, the recommended dose was 120 mg/m^2, administered every 3 weeks by intravenous infusion. These nanoparticles were selectively delivered to the liver of cancer patients (15% - 20% of total dose). Severe fatigue, grade 4 neutropenia, and grade 3 mucositis were the major side-effects. In contrast, the control polymer without galactosamine modification had a general body distribution with no significant accumulation in the liver.[92] To reveal the mechanism of the anti-cancer effect of 5-fluorouracil-loaded galactosylated chitosan nanoparticles (GC/5-FU), Cheng *et al*. tested their effect on hepatocellular carcinoma *in vitro* and *in vivo*. They found that the tumor growth inhibition is achieved through activation of the p53 pathway in the orthotopic transplant mouse model.[94]

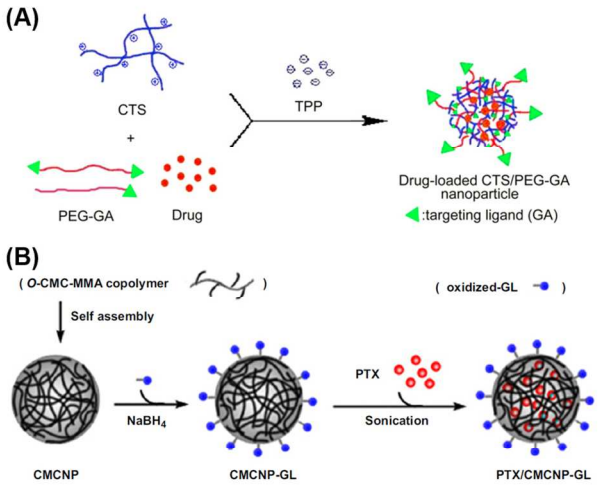

Fig. 5 Scheme of the preparation of the glycyrrhizin / glycyrrhetinic acid-modified nanoparticles. A: Glycyrrhetinic acid-modified chitosan/poly(ethylene glycol) nanoparticles (CTS/PEG-GA). CTS: chitosan; PEG-GA: polyethylene glycols glycyrrhetinic acid; TPP: pentasodium tripolyphosphate. B: Glycyrrhizin-modified O-carboxymethyl chitosan nanoparticles. CMC: carboxymethyl chitosan; GL: glycyrrhizin; PTX: paclitaxel. Reproduced with permission of ref. 96 and 101, Copyright 2010, 2012, Elsevier.

Glycyrrhizin / glycyrrhetinic acid receptor is also applied in hepatocellular carcinoma delivery systems. Six kinds of glycyrrhizin / glycyrrhetinic acid receptors-targeted polymers were used to deliver doxorubicin, 5-fluorouracil and paclitaxel to hepatocellular carcinoma cells.[96-101] Fig. 5 shows the illustration of two synthetic routes of the glycyrrhizin / glycyrrhetinic acid-modified nanoparticles as examples.[96, 101] The glycyrrhetinic acid-modified chitosan/poly(ethylene glycol) nanoparticles (CTS/PEG-GA) were prepared in two steps. The first step was to conjugate glycyrrhetinic acid to the PEG molecule, and the second step was the assembly of drug-loaded CTS/PEG-GA nanoparticles, which was achieved by simply adding the pentasodium tripolyphosphate (TPP) solution to the chitosan (CTS) solution containing both PEG-GA molecules and DOX·HCl under vigorous stirring (Fig. 5A).[96] The preparation procedures of paclitaxel/glycyrrhizin-modified *O*-carboxymethyl chitosan nanoparticles are illustrated in Fig. 5B. *O*-carboxymethyl chitosan (CMC) copolymers could spontaneously self-assemble into core-shell CMC nanoparticles (CMCNP) in aqueous solution. The oxidized glycyrrhizin (GL) then reacted with residual amino groups on CMCNP through the formation of Schiff's base. Finally paclitaxel (PTX) was incorporated into the inner core of CMCNP-GL through sonication.[101] To investigate the antitumor efficacy of glycyrrhizin / glycyrrhetinic acid receptors-targeted polymers, Cheng *et al*. compared free 5-fluorouracil and 5-fluorouracil-loaded glycyrrhetinic acid-modified chitosan nanoparticles (GA-CTS/5-FU) in cell lines and orthotropic liver cancer mouse model.[98, 102] The significantly improved anti-cancer effect in GA-CTS/5-FU group was possibly attributed to less immunosuppression from targeted delivery.

Although glycyrrhizin / glycyrrhetinic acid receptor mediated approach is considered one of the most widely efficient options for the cell-specific delivery to hepatocellular carcinoma cells, this has not led to any clinical trials or applications yet.

Vascular endothelial growth factor (VEGF)[103], folate receptor[7], hepatitis B surface antigen[104], RGD peptide receptor[105] and A54 peptide receptor[106] are another five proteins frequently overexpressed on cancer cell surface, and have been utilized in targeting strategies. It should be noted that none of these active targeting approaches has entered any clinic trials or applications. There is still a long way to go to treat patients with hepatocellular carcinoma.

4.2 Non-polymeric nanocarriers targeting to hepatocellular carcinoma

Characteristics of non-polymeric nanocarriers for drug/gene targeting to hepatocellular carcinoma are summarized in Table 4. Liposome is another popular nanocarrier that has been widely used for the drug or gene delivery to liver. They are spherically-enclosed membrane vesicles constructed with lipids.[107] Phosphatidylcholine and phosphatidylethanolamine are the two major structural components of liposome membranes. Other components include cholesterol, hydrophilic polymer conjugated lipids and/or water. There are four main methods to prepare liposomes: hydration of thin lipid film, reverse-phase evaporation, solvent (ether or ethanol) injection, and detergent dialysis,[108] some of which could be combined together in liposome manufacture.

4.2.1 Passive targeting to hepatocellular carcinoma

Two studies employed the physicochemical properties of liposomes to passively deliver doxorubicin or microRNA/siRNA (small interfering RNAs) to liver or cancer tissue.[109, 110] Lyso-thermosensitive liposomal doxorubicin (ThermoDox®), is the only non-polymeric nanoparticle that has been under clinical test.[109] This lyso-thermosensitive liposome consisted of three different synthetic phospholipids: 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine (MSPC), and 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-*N*-methoxypolyethyleneglycol-2000 (DSPE-MPEG-2000), encapsulating doxorubicin to form ThermoDox®. ThermoDox® passively yet rapidly accumulated in the liver and selectively released doxorubicin at above 39.5°C during radiofrequency ablation. Hyperthermia also enhanced the cytotoxicity of doxorubicin by increasing influx of drug into cancer cells. In Phase I trial, the radiofrequency ablation and ThermoDox® combination group showed statistically significant better prognosis. Mild alopecia and neutropenia were the two observed side-effects.[111] Because of the encouraging Phase I results, this therapy has directly entered Phase III testing (clinical trial identifier: NCT00617981). This study is still ongoing and data up to 2014 have revealed that ThermoDox® significantly improved overall survival rate (55%) in patients treated together with radiofrequency ablation for non-resectable hepatocellular carcinoma.

4.2.2 Active targeting to hepatocellular carcinoma

Five ligand-mediated approaches have been used in liposomal treatments of hepatocellular carcinoma.[45, 112] Longmuir *et al*. reported that liposome containing a lipid-anchored 19-amino acid glycosaminoglycan targeting peptide could selectively deliver doxorubicin to hepatocytes.[112] Interestingly, the targeting peptide is buried underneath the dense PEG layer of the liposome. The authors suggested that the linear polysaccharide chains of the heparan sulfate proteoglycans on liver cells can penetrate the PEG layer of the liposome and interact effectively with the targeting peptide ligand, while antibody, receptor, and opson are difficult to penetrate through the PEG layer and interact with the targeting ligand.[112] So this targeting heparan sulfate strategy has resulted in stable and unchanged targeting and biodistribution after repeat liposome administration.[113]

In another study, Opanasopit *et al*. synthesized mannosylated liposome to deliver muramyl dipeptide, an immunomodulator, to Kupffer cells.[45] The mannose receptor is a transmembrane protein expressed on Kupffer cells.[44] Kupffer cells are activated to a tumoricidal state by the administration of muramyl dipeptide, thus reducing the number of metastatic colonies in the liver and increasing the survival of the tumor-bearing mice. ASGP-R[114, 115] and CD44[116] are two other proteins overexpressed on hepatocellular carcinoma cells and also utilized in liposomal targeting treatments.

Apart from liposome, a number of nanoparticles such as solid lipid nanoparticles,[117] nanogel,[118] nanofiber,[119] alginate,[120] protein,[121] and lipoprotein[122] nanoparticles have also been examined to deliver drug/gene targeting to hepatocellular carcinoma. Although most of the drug carriers are organic nanoparticles, some inorganic nanoparticles, such as SPIONs[123] or silica nanorattles,[124] were used to deliver anti-cancer drugs to hepatocellular carcinoma. For example, Li *et al*. reported that hydrophobic antitumor drug docetaxel loaded in PEGylated silica nanorattles showed greater antitumor activity with about 15% enhanced tumor inhibition rate, compared with free docetaxel (Taxotere®) on the subcutaneous H22 cancer bearing mice.[124] These inorganic nanoparticles are promising candidates as drug carriers for cancer therapy in the foreseeable future.

5 Therapeutic nanoparticles targeting to non-tumoral liver diseases

Nanoparticle-drug delivery systems have also shown overwhelming advantages over free therapeutic agents for the treatment of non-tumoral liver diseases such as viral hepatitis, liver fibrosis, liver failure and liver ischemia- reperfusion injury. Nanoparticles for drug/gene delivery targeting to non-tumoral liver diseases are summarized in Table 5. Unfortunately, none of these nanoparticles are currently clinically approved or under clinical testing.

5.1 Nanoparticles targeting to the liver with virus infection

Hepatitis B and hepatitis C are life-threatening infectious diseases of the liver caused by the hepatitis B virus (HBV) and hepatitis C virus (HCV). HBV and HCV replicate in hepatocytes, so the treatment target is mainly hepatocytes. Anti-HBV or HCV drugs can be categorized as interferon (IFN), nucleoside and nucleotide analogues. In practical application, IFN is often associated with

poorly tolerated dose-limiting side-effects such as severe influenza-like symptoms, neurological symptoms, and thrombocytopenia.[125] While for the treatments using nucleoside or nucleotide analogues, relapse in short term therapy and drug resistance in long term therapy are two major challenges.[126] Thus, concentrating drugs in the diseased liver using nanoparticles could contribute to reducing the side-effects and drug resistance.

Liposome is the most commonly utilized nanoparticle that has been reported for the treatment of HBV infection. The cationic liposomes carrying siRNA with 80-100 nm in diameter passively delivered siRNA to hepatocytes.[127, 128] Liposome-mediated siRNA delivery resulted in significant HBV replication knockdown *in vitro* and *in vivo*.

For liposomes to active deliver to the liver, scavenger receptor and ASGP-R are two binding sites on hepatocytes that have been targeted.[129, 130] Kim *et al*. designed liposomes with apo A-I linked onto the surface to deliver siRNA to the liver to reduce viral protein expression.[129] These liposomes were predominantly taken up by the liver via scavenger receptor-mediated endocytosis. Apo A-I is a protein component of high-density lipoprotein that guides the transport of cholesterol from cells of the arterial wall to the liver and steroidogenic organs. Since this protein is an endogenous product, there will be no concerns on its immunological side effects in future clinical applications.[129] In another study, Qi *et al*. established a highly efficient ASGP-R-mediated gene transfer to hepatocytes using cationic liposomes modified with soybean sterylglucoside and PEG.[130]

In the treatment of HBV infection, other types of nanoparticles such as lipoprotein,[131] gold nanoparticles[132] and galactosylated polymers,[133] have been used for the target delivery of acyclovir and IFN-α to the liver or hepatocytes. All therapeutic nanoparticles in the treatment of viral hepatitis have achieved success in the experimental stage with great potential in clinical application in the foreseeable future.

5.2 Nanoparticles targeting to the fibrotic liver

Liver fibrosis originates from chronic liver injury that occurs in most types of liver diseases, including viral hepatitis, alcoholic hepatitis or genetic abnormalities. Advanced liver fibrosis results in cirrhosis, and may lead to hepatocellular carcinoma. Since the activation of the HSCs is the most important event in fibrogenesis, HSCs are the major target for drug delivery in the treatment of liver fibrosis. However, the conventional anti-fibrotic treatments are of little clinical success and no standard treatment of liver fibrosis is available at the moment.[6]

Liposome is the only type of nanoparticles applied in the targeted therapy for liver fibrosis. Three types of receptors expressed on HSCs (retinol binding protein receptors, mannose-6-phosphate receptor and PDGF receptor-β) have been actively targeted by liposomes. Retinol binding protein receptors are able to recognize vitamin A and certain cyclic peptide such as cRGD peptide. Sato *et al*. used vitamin A–coupled liposomes to deliver siRNA against gp46 to HSCs in rat models of both acute and chronic liver fibrosis.[134] The protein gp46 is the rat homolog of human heat shock protein 47. In this study, liver fibrosis was completely resolved and survival in rats was prolonged after the treatment. Du *et al*. developed cRGD peptide-labeled liposomes to improve IFN-α1b delivery to HSCs in a rat model of liver fibrosis induced by bile duct ligation.[135] The extent of liver fibrosis was significantly reduced after administration of these liposomes. Mannose 6-phosphate/insulin like growth factor II receptor on activated HSCs can also be targeted by M6P-HSA groups coupled nanoparticles. Dilinoleoylphosphatidylcholine (DLPC) or rosiglitazone in M6P-HSA conjugated liposomes was observed to be selectively delivered to HSCs and significantly reduced fibrosis grade in rat.[136-138]

Besides viral hepatitis and liver fibrosis, nanoparticles have also been examined in the targeted therapy for inflammation,[139, 140] fulminant hepatitis,[141] liver failure,[142] malarial[143] and liver ischemia- reperfusion injury.[144] Fichter *et al*. took the advantage of the natural capture of polymers by Kupffer cells to deliver dexamethasone in order to suppress the inflammatory response of liver macrophages.[139] These polymers without surface modifications had negative surface charges with the average size between 175 and 240 nm. In addition, ASGP-R on hepatocytes[140, 141, 143-145] and mannose receptor on Kupffer cells[142] have been targeted using the relevant targeting ligands conjugated to polymers, liposomes, dendrimers and solid lipid nanoparticles.

6 Multifunctional nanoparticles targeting to the liver

In order to achieve better diagnosis and therapy outcomes, new multifunctional nanoparticles with both diagnostic and therapeutic capabilities have emerged as promising protocols in the treatment of liver diseases (Table 6). SPIONs are usually used for liver diagnostic imaging as discussed previously. These nanoparticles could also deliver minicircle DNA into normal liver via intrabiliary infusion.[146] Mouli *et al*. designed an innovative image-guided local delivery strategy using doxorubicin-functionalized SPIONs, and resulting in significantly increased intratumoral drug uptake with limited off-target.[123] Doxorubicin was used as an anti-cancer drug and SPIO-platform as the MRI contrast agents to image therapeutic delivery. In this work, they also employed nanoablation and nanoembolization strategies (Fig. 6). Nanoablation is the image-guided placement of electrodes into the tumor tissue to electroporate tumor cells, which could result in the formation of temporary channels in the membrane of tumor cells, permitting nanoparticles with SPIONs to enter rapidly. The influx of nanoparticles is independent on the stage of cell cycle or cellular uptake machinery. Nanoembolization is the image-guided delivery of embolic agents and nanoparticles into the blood supply of tumors directly. This strategy could minimize the off-target delivery and maximize the uptake by tumor cells.

Three types of multifunctional nanoparticles have been developed to actively target to hepatocellular carcinoma cells and HSCs. As shown in Fig.7, Huang *et al*. designed a MRI-visible smart polymeric drug delivery system. In this study, a new pH-sensitive polymer poly(aspartate)-graft-poly(ethylene glycol)-dodecylamine-dodecylaminehydrazone-(adriamycin-levulinic acid) (PASP-g-PEG-DDA-Hyd DDAHyd-(ADR-LEV)) was modified with the antibody against VEGF to deliver the anticancer drug doxorubicin (trade name Adriamycin) to hepatocellular carcinoma cells in mice. SPIONs were also encapsulated into the polymers for the MRI purpose.[103] Maeng *et*

al. designed a polymer (YCC-DOX) composed of poly (ethylene oxide)-trimellitic anhydride chloride-folate (PEO-TMA-FA) carrying both doxorubicin and SPIONs, using folate as the targeting moiety.[7] This nanoparticle could specifically target to folate receptor expressed on tumor cells. YCC-DOX was superior to MRI contrast agent SPIONs (Resovist®) in diagnostic imaging and had more obvious anticancer effect than free doxorubicin (FD) or commercial liposomal doxorubicin (DOXIL®). To treat liver fibrosis, Li *et al*. reported that pPB-SSL-IFN-γ-DIR (DIR labeled cyclic peptide C*SRNLIDC* conjugated liposomes) could target HSCs specifically (Fig.8) via PDGF receptor-β, presenting enhanced anti-fibrotic effect.[42, 147] Since all these multifunctional nanoparticles remain in the experimental stage, more efforts to initiate clinical testing of these delivery systems are required to assess their potential for use in patients with liver disease.

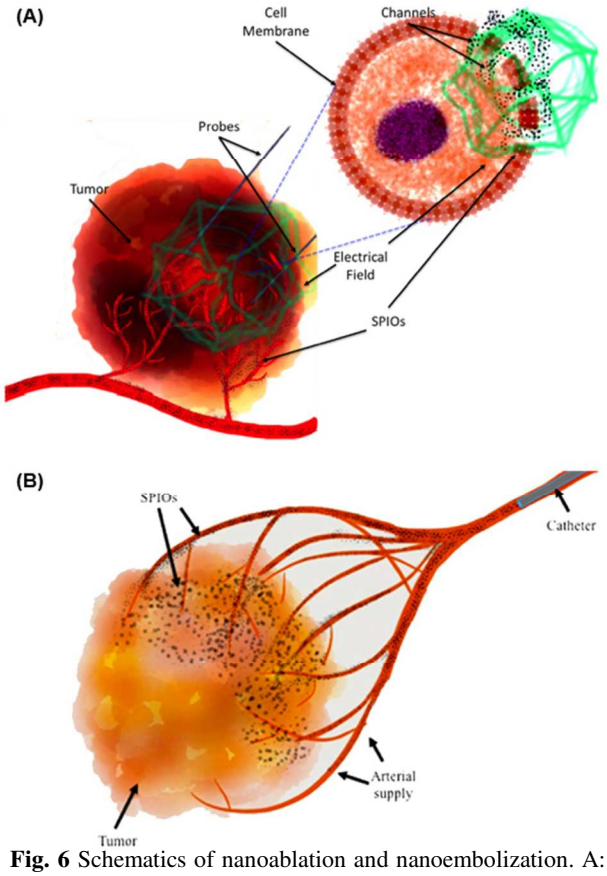

Fig. 6 Schematics of nanoablation and nanoembolization. A: In nanoablation, ablation probes are inserted into tumor tissue (left) and generate an electrical field (green) around tumor cells (right). B: In nanoembolization, nanoparticles and embolic agents are delivered directly to a tumor's arterial supply using a microcatheter. Reproduced with permission of ref. 123, Copyright 2013, American Chemical Society.

7 Summary

Nanoparticles targeting to the liver have shown high potential to offer a wide variety of diagnostic imaging and therapeutic applications in liver diseases. As summarized in this review, drug or gene can be specifically delivered to hepatocytes, HSCs,

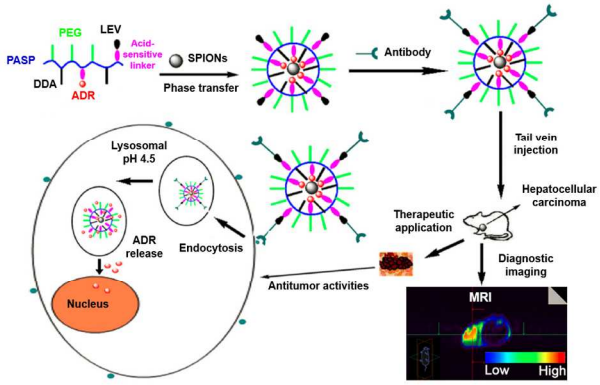

Fig. 7 Schematic illustration of the formation of novel multifunctional nanoparticles and their dual-functional process. The ADR is stable in polymer at neutral pH of the physiological environment and release rapidly in acidic endosomal/lysosomal compartments of tumor cells after the breaking of acid-sensitive linker. The hydrophobic SPIONs are encapsulated inside the core of the polymer for MR imaging. PASP: polyaspartate; PEG: polyethylene glycols; LEV: levulinic acid; DDA: dodecylamine; ADR: Adriamycin. Reproduced with permission of ref. 103, Copyright 2013, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

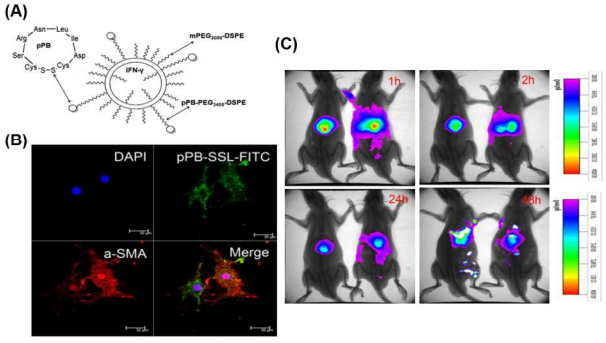

Fig. 8 Cyclic peptide C*SRNLIDC* (pPB) conjugated liposome delivered IFN-γ delivered to HSCs. A: The scheme of pPB conjugated liposome. B: The pPB conjugated liposome was detected in the cytoplasm of HSCs by confocal microscope. The blue fluorescent signal indicates nuclei via the staining of DAPI. The red fluorescent signal indicates positive staining for α-SMA in activated HSCs. The red fluorescent signal indicates the location of pPB conjugated liposomes. C: The living-body images of rat indicated that the fluorescence was more predominant in the liver region injected with liposomes with pPB conjugating (right) than without (left). Reproduced with permission of ref. 147, Copyright 2012, Elsevier.

Kupffer cells, endothelial cells and hepatocellular carcinoma cells using different targeting nanoparticles. Polymer and liposome are two most widely used nanocarriers, while some inorganic nanoparticles, such as SPIONs or silica nanorattles, are tested to deliver anti-cancer drugs to hepatocellular carcinoma. Unexpectedly, no nanoparticle has been reported passively or actively targeting to epithelial cells in liver.

To date, the most important application of nanotechnology in hepatology is to treat hepatocellular carcinoma. Although most of the aforementioned researches are based on animal studies,

several nanoparticles such as Feridex®, Endorem®, Resovist®, Livatag®, and ThermoDox®, are currently approved clinically or under clinical tests. All commercialized nanoparticles for liver diagnostic imaging are designed passively targeting to the liver except one type of clinically tested nanocarriers, mitoxantrone-loaded PBCA, which is actively targeting to the liver. Some nanocarriers are able to deliver both drugs and one or several imaging probes or several imaging probes simultaneously. It is evident from this review that the development of these multifunctional nanoparticles has opened up possibilities for image-guided drug delivery systems and multi-modality imaging systems.

8 Challenges and perspective

Many foreign compounds including nanoparticles are mostly taken up by the liver even without the use of any targeting strategies because of the first pass-effect and the prominent role of liver in metabolism. This specific physiological function enables nanoparticles to accumulate more rapidly in liver than in any other organs. While a major challenge is that these nanoparticles may not accumulate in the proper type of liver cells. Eighty percent of macrophages in human body is located within the liver (Kupffer cells), and xenobiotics, especially particles larger than 150 nm, are often captured by this type of cells. Hepatocytes express many endocytic receptors and transporters on the cell membrane, and also take up a large portion of xenobiotics. It is now essential to continue developing new types of nanoparticles and new targeting ligands for liver cell-specific delivery. For example, in the near future, more efforts might focus on inorganic nanoparticles such as clay and calcium phosphate, and organic-inorganic hybrid nanoparticles, which have unique combined material- and size-dependent physicochemical properties, but have not been examined in the diagnosis and treatment of liver diseases yet.

It is still unclear why many nanoparticles actively targeting to the liver do not reach the clinic in spite of their demonstrated positive outcomes in animals. One possible reason is that many specific ligands used for active targeting are exogenous products, so they are suspected to trigger immunological side effects in clinical applications. Endogenous products such as apo A-I and small molecules such as vitamin A or mannose could be ideal surface ligands for active targeting and should not be detrimental to human immunity in clinical applications. Another possible reason is that the ligands homing to target receptors in animal may not be able to bind to human receptors specifically and effectively. Thus, a proper experimental animal model should be chosen and maybe more than one animal model should be used to test the targeting efficacy. Although there is still a long way for nanoparticles towards clinical application, it is our opinion that forthcoming research efforts would focus on the development of nanoparticles with biosafety and efficient active targeting to the liver to treat not only hepatocellular carcinoma but also other liver diseases.

As for newly designed nanoprobes for multi-modality imaging and multifunctional nanoparticles, future long-term studies would also focus on further validating their safety and effectiveness. In particular, image-guided drug delivery system is more helpful in screening for early effects of drugs and selecting the patients eligible for an experimental therapy in clinical trials. These multifunctional nanoparticles also light up the trend of "personalized treatment" that only patients who demonstrate high drug uptake by diseased liver cells would continue to receive the treatment. These multifunctional nanoparticles as well as novel techniques such as nanoablation and nanoembolization will provide opportunities for more effective liver therapies in the future.

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Table of contents

This review summarizes research progress focusing on nanoparticles targeting to the liver for both diagnostic and therapeutic purposes.

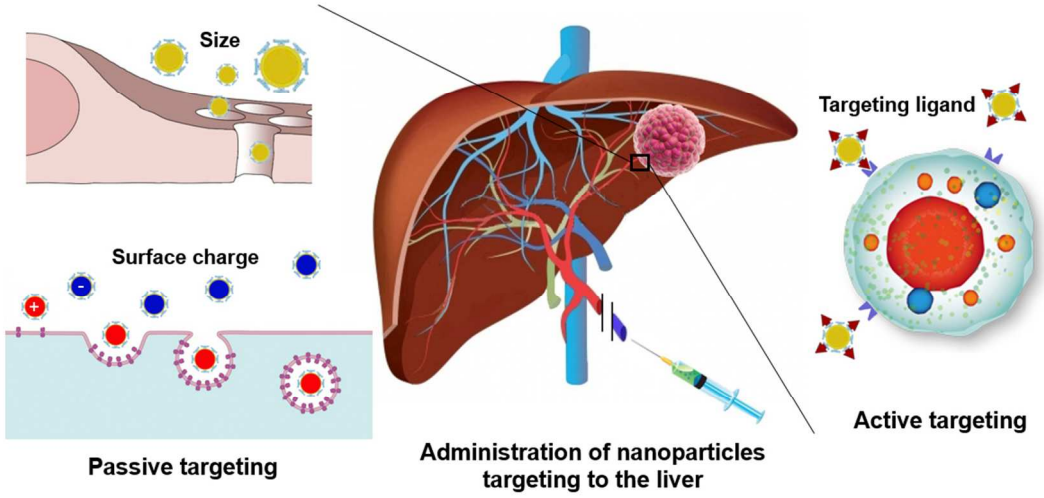

Table 1 Strategies used in nanoparticles active targeting to the liver

Cellular target	Cell type	Targeting ligand	Reference
Asialoglycoprotein (ASGP) receptor	Hepatocytes, hepatocellular carcinoma cells	Galactosamine, galactoside, lactose, lactobionic acid, pullulan, soybean sterylglucoside	63, 80, 91-95, 114, 115, 117, 118, 130, 133, 140, 141, 143-145
Glycyrrhizin / glycyrrhetinic acid receptor	Hepatocytes	Glycyrrhizin, glycyrrhetinic acid	96-101, 120
Scavenger receptor	Hepatocytes, Kupffer cells, and endothelial cells	Apolipoprotein A-I, human serum albumin (HSA)	43, 129
Mannose receptor	Kupffer cells	Mannose	45, 142
Mannose-6-phosphate (M6P) receptor	Hepatic stellate cells	Mannose-6-phosphate	136, 138
Retinol binding protein (Type VI collagen and integrin) receptor	Hepatic stellate cells, hepatocellular carcinoma cells	Vitamin A, RGD peptide	105, 119, 134, 135
Platelet-derived growth factor (PDGF) receptor-β	Hepatic stellate cells	Cyclic peptide C*SRNLIDC*	42, 147
Alpha-fetoprotein (AFP)	Hepatocellular carcinoma cells	AFP antibody	76
Vascular endothelial growth factor (VEGF)	Hepatocellular carcinoma cells	Anti-vascular endothelial growth factor antibody	103
Hepatic heparan sulfate glycosaminoglycan	Hepatocellular carcinoma cells	Glycosaminoglycan targeting peptide	112
CD44	Hepatocellular carcinoma cells	Anti-CD44 antibody	116
Specific cell surface marker	Hepatocellular carcinoma cells	A54 peptide	106
Specific receptors (with PreS1-specific binding proteins)	Hepatocellular carcinoma cells	PreS1 domain of hepatitis B surface antigen	104
Folate receptor	Hepatocellular carcinoma cells	Folate	7

Table 2 Liver targeted nanoparticles for diagnostic imaging

Category / surface ligand	Size (nm)	Imaging technique	Target	Reference
SPION[a]	20 – 250	MRI	Liver lesions of human	51, 60, 62
SPION[b]	10-100		Ischemia–reperfusion liver of Wistar rats	55
	86		Liver of Kunming mice	53
	50		Fibrosed liver of Wistar rats	59
	14-20		Liver of SD rats with NASH	54
Octapod SPION	30		Liver and cancer of orthotopic HepG2 bearing mice	49
SPION with lactobionic acid modified	10		Liver of SD rats / rabbit	63
Gd-DTPA	187.9		Liver of Wistar rat	64
Gd-doped silica and gold	~30		Cancer of BALB/c mice with colon cancer liver metastasis	65
Mn-SiO_2	25		Cancer of HepG2 orthotopic tumor mice	66
GdIO	4.8		Liver of BALB/c mice	50
MnO/Fe_3O_4	11		Liver and cancer of HepG2 orthotopic tumor mice	67
Gd_2O_3/SPION	14		Liver of BALB/c mice and cancer of HepG2 orthotopic tumor of nude mice	68
ExiTron nano	110	CT	Liver and metastasis of mice with colon cancer liver metastasis	69, 70
PEGylated Yb_2O_3:Er	170		Liver of rats	71
Oleic acid coated Bi_2S_3 nanodots	2-3		Liver of rats	74
Polymer[c]	30–40	NIRF	Cancer of HepG2 /EF-Luc orthotopic transplant tumor mice	75
Quantum dots linking to AFP antibody	4	Two-photon imaging technology	Cancer of nude mice	76
Gd_2O_3:Yb^{3+}, Er^{3+} nanorods	110-180 in length, 15-30 in diameter	UCL/MRI/CT	Liver of C57BL/6 mice and rats	78
Lanthanide-doped Lu_2O_3	115	UCL/MRI/CT	Liver of C57BL/6 mice and rats	79
Oleic acid-stabilized Gd-doped NaYbF4:Er	20 - 52	UCL/MRI/CT	Liver of rats	73
SPION with lactobionic acid modified	12	MRI/SPECT/CT	Liver of BALB/c mice	80

a: Commercial SPIO: Feridex, Endorem, Resovist and Clariscan; b: a product family of several SPIO particles, commercial name: FeraSpin; c: delivering ICG (indocyanine green)
SPION: superparamagnetic iron oxide nanoparticle; NASH: Non-alcoholic steatohepatitis; DTPA: diethylenetriamine-pentaacetic acid; AFP: alpha-fetoprotein; NIRF: near infrared fluorescence optical imaging technique; UCL: up-conversion luminescence; SPECT: single-photon emission computed tomography

Table 3 Polymeric carriers for drug gene targeting to hepatocellular carcinoma

Specific surface ligand	Size (nm)	Zeta potential (mV)	Drug/gene delivered	Target	Reference
-	139	-	Doxorubicin	Hepatocellular carcinoma of human	86
	55.11	-2.42	Mitoxantrone	Unresected hepatocellular carcinoma of human	87
	114.9	-45	5-Fluorouracil	Liver of mice	84
	71.3	-5.4	Cisplatin	Cancer of subcutaneous H22 bearing mice	85
	120.6	37.37	Lactosyl- norcantharidin	Cancer of subcutaneous H22 bearing mice	41
	219	-	short hairpin RNAs	Liver and cancer of subcutaneous Hepa 1–6 bearing mice	88
Galactosamine conjugated	-	-	Doxorubicin	Liver and tumor of human with primary or metastatic liver cancer	92
Galactosamine conjugated	127.5	-10.6	Paclitaxel	Cancer of subcutaneous HepG2 bearing mice	91
Pullulan conjugated	102.8	-22.1	Doxorubicin	Liver of rats	93
Galactosylated	35.19	10.34	5-Fluorouracil	Cancer of subcutaneous /orthotropic liver cancer mice	94
Galactosylated	282.2	-12.88	Oridonin	Liver of rats and mice	95
Glycyrrhizin/glycyrrhetinic acid-modified	195.6	18.42	Doxorubicin	Cancer of subcutaneous H22 tumor-bearing mice	96
	214.2*	-	Doxorubicin	Liver of rats	97
	193.7/ 217.2	27.4 / 30.6	5-Fluorouracil	H22 orthotopic transplant tumor in mice	98, 102
	274.2	-45.6	Doxorubicin	H22 orthotopic transplant tumor in mice	99
	164.5*	-26.9	Doxorubicin	Liver of mice	100
	100-205	-30	Paclitaxel	Cancer of subcutaneous H22 bearing mice	101
Attaching cyclic RGD peptide	15 - 30	-	Doxorubicin	Liver of mice	105
Hepatitis B surface antigen conjugated	~50	-	Paclitaxel	Cancer of subcutaneous HepG2 or A431 bearing mice	104
A54 peptide functionalized	77.85	33.3~36.2	Doxorubicin	Cancer of subcutaneous BEL-7402 bearing mice	106

Cyclic RGD peptide: cyclo(–Arg–Gly–Asp–d–Phe–Cys–)

Table 4 Other nanocarriers for drug/gene targeting to hepatocellular carcinoma

Category	Specific surface ligand	Size (nm)	Zeta potential (mV)	Drug/gene delivered	Target	Reference
Liposome	-	-	-	Doxorubicin	Liver of patients with liver cancer underwent radiofrequency ablation	109
	-	102.2	- 3.94	MicroRNA/siRNA	Liver and cancer of subcutaneous Sk-Hep-1 cells bearing mice	110
	Containing glycosaminoglycan targeting peptide	123.6	8.8	Doxorubicin	Hepatocytes of mice	112
	Mannosylated	95	-	Muramyl dipeptide	Liver non- parenchymal cells (Kupffer cells) of colon carcinoma liver metastasis mice	45
	Galactosylated	79	-	Doxorubicin	Liver parenchymal cells of mice	114
	Lactobionic acid conjugated	124	- 17.1	Doxorubicin	Cancer of subcutaneous HepG2 cells bearing mice	115
	Anti-CD44 antibody- mediated	100	-	Doxorubicin	Cancer of orthotopic HepG2 cells bearing mic	116
Solid lipid nanoparticles	Galactosylated	120.4	-12.4	Docetaxel	Cancer and liver of mice bearing subcutaneous tumor	117
Nanogel	Galactosylated	130	-18.4	Doxorubicin	Cancer of diethylnitrosamine-induced hepatocellular carcinoma rats	118
Nanofiber	RGD peptide	10–20	-	Curcumin	Cancer of mice bearing orthotopic transplant tumor	119
Alginate	Glycyrrhetinic acid-modified	241.2	-43.1	Doxorubicin	Liver and cancer of subcutaneous H22 cells bearing mice	120
Protein nanoparticles	-	68-80	-	Doxorubicin	Cancer of diethylnitrosamine-induced hepatocellular carcinoma rats	121
Lipoprotein	-	90	-4.2	Cholesterol-conjugated siRNA	Cancer and liver of subcutaneous HepG2 bearing mice	122
Silica nanorattle	-	125	-	Docetaxel	Cancer of subcutaneous H22 bearing mice	124

Table 5 Nanocarriers for drug/gene targeting to non-tumoral liver disease

Disease/ treatment	Category	Specific surface ligand	Size (nm)	Zeta potential (mV)	Drug/gene delivered	Target	Reference
HBV/ HCV infection	Liposome	-	80-100	-	siRNA	Hepatocytes of mice	127
	Liposome	-	~100	13.6	siRNA	Hepatocytes of mice	128
	Liposome	Apolipoprotein A-I mediated	177	39.1	siRNA	Liver of acute HBV-infected mice	129
	Liposome	Soybean sterylglucoside modified	183	-	Antisense oligonucleotides	Hepatocytes of mice	130
	Lipoprotein	-	33.5	-	Acyclovir	Liver of rats	131
	Gold nanoparticles	-	29.25	-	IFN α	Liver of mice	132
	Polymer	Galactosylated	198.1	-8.5	Acyclovir	Liver of mice	133
Liver fibrosis	Liposome	Vitamin A–coupled	153.9	-	siRNA	Hepatic stellate cells of dimethylnitrosamine, CCl4 or bile duct ligation induced liver cirrhosis rats	134
		Cyclic RGD labeled	101	-	IFN-α1b	Hepatic stellate cells of bile duct ligation induced liver fibrosis rats	135
		M6P-HSA modified	81	-	DLPC	Hepatic stellate cells of bile duct ligation induced liver fibrosis rats	136
		M6P-HSA modified	92.37	- 30.5	Rosiglitazone	Liver of CCl4 induced liver fibrosis rats	138
Inflammation	Polymer	-	215	-28	Dexamethasone	Kupffer cells of mice	139
Inflammation	Polymer*	Lactose-modified	26.4	17.16	Diammonium glycyrrhizinate	Liver of rats	140
Fulminant hepatitis	Liposome	Galactose- conjugated	115.9	14.17	siRNA	Liver of Concanavalin A- induced hepatitis mice	141
Liver failure	Liposome	Mannosylated	61.6	54.7	NFκB decoy	Liver of mice	142
Malarial	Dendrimer	Galactose coating	2.4	-	Primaquine phosphate	Hepatocytes of rats	143
Hepato- protective	Solid lipid NPs	Galactosylated	135	-31.6	Cucurbitacin B	Liver of rats	145
Liver ischemia-reperfusion injury	Liposome	Galactose- conjugated	130	50	siRNA	Hepatocytes of liver ischemia-reperfusion mice	144

IFN: interferon; DLPC: dilinoleoylphosphatidylcholine

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Table 6 Multifunctional nanoparticles targeting to the liver

Category	Specific surface ligand	Size (nm)	Drug/gene delivered	Imaging technique	Target	Reference
SPION	-	15	Doxorubicin	MRI	Liver cancer of VX2 rabbit bearing orthotopic N1S1 tumor	[123]
Polymer	Anti-VEGF modified	70	Adriamycin and SPION	MRI	Cancer of subcutaneous H22 cells bearing mice	[103]
Polymer	Containing folate	84.7	Doxorubicin/SPION	MRI	Liver cancer of rabbits bearing VX2 rabbit tumor	[7]
Liposome	Cyclic peptide C*SRNLIDC*	83.5 -86.9	IFN-γ	*in vivo* fluorescence image system	Hepatic stellate cells of thioacetamide rats with liver fibrosis	[42, 147]

Anti-VEGF: anti-vascular endothelial growth factor