Liver cell-targeted delivery of therapeutic molecules

Jeong-Hun Kang1, Riki Toita2, and Masaharu Murata3,4

1Division of Biopharmaceutics and Pharmacokinetics, Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan, 2Department of Biomaterials, Faculty of Dental Science, 3Department of Advanced Medical Initiatives, Faculty of Medical Sciences, and 4Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan

Abstract
The liver is the largest internal organ in mammals and is involved in metabolism, detoxification, synthesis of proteins and lipids, secretion of cytokines and growth factors and immune/inflammatory responses. Hepatitis, alcoholic or non-alcoholic liver disease, hepatocellular carcinoma, hepatic veno-occlusive disease, and liver fibrosis and cirrhosis are the most common liver diseases. Safe and efficient delivery of therapeutic molecules (drugs, genes or proteins) into the liver is very important to increase the clinical efficacy of these molecules and to reduce their side effects in other organs. Several liver cell-targeted delivery systems have been developed and tested in vivo or ex vivo. In this review, we discuss the literature concerning liver cell-targeted delivery systems, with a particular emphasis on the results of in vivo studies.

Introduction
The liver is the largest internal organ in mammals and consists of parenchymal and non-parenchymal cells. It plays key roles in metabolism, detoxification, synthesis of proteins and lipids, secretion of cytokines and growth factors, and immune/inflammatory responses. However, several liver diseases, such as hepatitis, alcoholic or non-alcoholic liver disease, hepatocellular carcinoma (HCC), hepatic veno-occlusive disease, and liver fibrosis and cirrhosis, can worsen liver function or cause liver damage. Safe and efficient delivery of therapeutic molecules (drugs, genes or proteins) into the liver can increase their efficacy for treating liver diseases and can reduce the risk of side effects in other organs. Several systems based on biological carriers (e.g. viral vectors) and non-viral/synthetic carriers (e.g. polymers and liposomes) have been developed to improve the delivery of therapeutic molecules to liver cells. In this review, we discuss the literature concerning liver cell-targeted delivery systems, with a particular emphasis on the results of in vivo studies.

Liver cells and their physiologic roles
To develop efficient liver cell-targeted delivery systems, it is essential to understand their basic functions and the pathophysiology of liver cell-related diseases. Liver cells can be divided into parenchymal hepatocytes and non-parenchymal cells, which include liver sinusoidal endothelial cells (LSECs), Kupffer cells, stellate cells and intrahepatic lymphocytes (Supplementary Table S1). Non-parenchymal cells form contacts with hepatocytes and regulate the migration, proliferation and differentiation of hepatocytes (Malik et al., 2002; Wisse et al., 1996). Non-parenchymal cells have a variety of roles. In particular, they form a functional network with hepatocytes and play key roles in immune responses, the pathogenesis of liver diseases and the biochemical defense against toxic materials. The physiologic roles of liver cells are described in more detail in the Supplementary Materials.

Strategies for liver cell-targeted delivery
Upon entering the bloodstream, liver cell-targeted delivery systems (nanoparticles) can rapidly bind to plasma proteins, such as albumin, fibrinogen, apolipoproteins (Apo) and immunoglobulins (Igs) (Figure 1). Plasma protein binding is highly dependent on the size, composition, hydrophobicity and surface charge of the delivery system, and influences the biodistribution, uptake by target cells, and therapeutic efficacy of the carried drug (Aggarwal et al., 2009; Saptarshi et al., 2013). Some plasma proteins have direct effects on the uptake of delivery systems by immune cells (e.g. macrophages). For example, delivery systems that bind to opsonins (e.g. complement and Igs) are readily recognized by macrophages and are rapidly cleared by phagocytosis (Lunov et al., 2011; Nagayama et al., 2007a). However, pre-coating the delivery system with dysopsonins (e.g. albumin) can prolong the circulation time and half-life of the delivery
Figure 1. After intravenous injection of a liver cell-targeted delivery system (nanoparticles) containing a therapeutic molecule, the nanoparticle binds to plasma proteins, particularly albumin. Plasma protein binding reduces liver-specific uptake of the nanoparticle delivery system. Nanoparticles can be delivered to hepatocytes through receptor-mediated endocytosis, into Kupffer cells by phagocytosis and receptor-mediated endocytosis, into stellate cells by receptor-mediated endocytosis and into LSECs by pinocytosis and receptor-mediated endocytosis. Of these endocytic routes, receptor-mediated endocytosis is the most commonly used method to deliver nanoparticles into the targeted liver cells. Most of the nanoparticles are taken up and removed by Kupffer cells and LSECs because they express a variety of receptors that limit the number of nanoparticles capable of reaching the hepatocytes and stellate cells.

system (Ogawara et al., 2004; Xia et al., 2013). In addition, covalent attachment of certain polymers [e.g. poly(ethylene-glycol) (PEG)] to the delivery system may reduce or prevent plasma protein binding (Aggarwal et al., 2009).

Receptor-mediated delivery

As described in the Supplementary Materials, liver cells express many different receptors. Therefore, high-affinity ligands that target-specific receptors can be used to develop liver cell-targeted delivery systems (Figure 1). The ligands that can be used for receptor-mediated delivery systems are presented in Table 1. Generally, after receptor-mediated endocytosis of the delivery system, the endosome fuses with the lysosome membrane. This activates vacuolar ATPase pumps in the lysosomal membrane that lower the pH of the endosome to \( \leq 6.0 \) (Bareford & Swaan, 2007; Sun-Wada et al., 2003). The receptors are recycled, but the therapeutic molecules are either degraded in the endosome or escape from the endosome alone or bound to the delivery system. However, systemic administration of a liver cell receptor-mediated delivery system does not guarantee that the delivery system accumulates in the targeted liver cells because some receptors are expressed in multiple cell types (Supplementary Table S2).

Carbohydrate (glycoprotein) receptor-mediated delivery

Hepatocytes express carbohydrate (glycoprotein) receptors such as asialoglycoprotein receptor and mannose receptor (Tolleshaug et al., 1984). Kupffer cells and LSECs also express carbohydrate (glycoprotein) receptors, including the mannose/N-acetylglucosamine receptor (ligand: mannose), the N-acetylglucosamine, galactose particle receptor (galactose and N-acetylglucosamine), the fucose receptor (fucose and N-acetylglucosamine) and the hyaluronan receptor (hyaluronan and chondroitin sulfates) (Bijsbergen et al., 1996; Higuchi et al., 2004; Kogelberg et al., 2007; Smidsrød et al., 1994; Toth & Thomas, 1992). However, several studies have proposed that the fucose receptor expressed on Kupffer cells functions as a galactose receptor (Biessen et al., 1994; Kuiper et al., 1994; Lehrman et al., 1986). The hyaluronan receptor is only expressed in LSECs (Weigel & Weigel, 2003; Weigel et al., 2002). Carbohydrate receptors mediate the uptake of glycoproteins, proteins and cellular particles (e.g. heparin and apoptotic cells) (Dini et al., 1992; Harris et al., 1990).

Although Kupffer cells and LSECs play key roles in the endocytosis of carbohydrates, their endocytic kinetics are very different. For example, the carbohydrate receptors of Kupffer cells show greater affinity for mannose/N-acetylglucosamine compared with the carbohydrate receptors expressed on LSECs (Fichter et al., 2013; Sano et al., 1990). However, the endocytic capacity for mannose/N-acetylglucosamine is greater in LSECs than in Kupffer cells (Praanning-van Dalen et al., 1987). This may be because of the 2-fold greater number of LSECs (Praanning-van Dalen et al., 1987) and the greater expression of mannose/N-acetylglucosamine receptors in LSECs (Bijsbergen et al., 1996; Kogelberg et al., 2007; Magnússon & Berg, 1993) than in Kupffer cells. By contrast, endocytosis of galactose/N-acetylglucosamine and fucose/N-acetylglucosamine is greater in Kupffer cells than in LSECs (Higuchi et al., 2004; Magnússon & Berg, 1993). Moreover, Kupffer cells show lower affinity for agalactoorosomucoid than hyaluronidase, while LSECs show similar affinities for both molecules (Sano et al., 1990).

Liver sinusoidal endothelial cells and Kupffer cells contain numerous lysosomes and show greater endocytic and digestive capacities compared with hepatocytes (Smidsrød et al., 1994; Wisse et al., 1996). Generally, the endocytic capacity of Kupffer cells and LSECs is greater in vivo than in vitro (Praanning-van Dalen et al., 1987). The high endocytic capacity of Kupffer and endothelial cells decreases the transfection efficiency of hepatocyte-targeted delivery systems, especially in vivo. Therefore, although several hepatocyte-targeted delivery systems show high transfection
efficiency in vitro, their transfection efficiency is much lower in vivo because of the high endocytic capacity of Kupffer and LSECs.

To facilitate the selective transfer of therapeutic molecules into hepatocytes, the delivery system should have a small diameter to allow the delivery system to pass through the sinusoidal endothelial fenestrae (Rensen et al., 2001), which are ~100 nm in diameter (Wisse et al., 1985). After passing through the fenestrae, the delivery systems can be internalized in hepatocytes.

**Asialoglycoprotein receptor-mediated delivery.** Asialoglycoprotein receptors are among the most important targets for hepatocyte-targeted delivery systems. Glycoproteins (e.g., galactose, lactose, pullulan, N-acetylgalactosamine and asialofetuin) and viruses (e.g., hepatitis B virus (HBV) or hepatitis C virus (HCV)) can be used as selective ligands for the asialoglycoprotein receptor (Hashida et al., 2001; Schwartz, 1984; Stockert, 1995).

Galactose and N-acetylgalactosamine are widely used as hepatocyte- or HCC-specific delivery systems (Hashida et al., 2001; Schwartz, 1984), but N-acetylgalactosamine shows higher affinity for asialoglycoprotein receptors (Iobst & Drickamer, 1996). Over the last two decades, many galactose/N-acetylgalactosamine-modified delivery systems based on galactosylated liposomes or polymers have been developed to specifically target hepatocytes (Hashida et al., 2001; Wang et al., 2013a; Wu et al., 2002; Xiao et al., 2013). Asialoglycoprotein receptor-mediated endocytosis of galactose-modified delivery systems can be influenced by the density of galactose residues (Managit et al., 2005; Staud et al., 1999) and the size of the nanoparticle–molecule complex (Morimoto et al., 2003). Nanoparticles >70 nm in size cannot be endocytosed by asialoglycoprotein receptors (Rensen et al., 2001).

Pullulan is a water-soluble polysaccharide consisting of three α-1,4-linked glucose polymers with different α-1,6-glicosidic linkages. It is frequently used as a ligand to target hepatocytes because of its high affinity for the asialoglycoprotein receptor (Kaneo et al., 2001; Kang et al., 2012).

Asialofetuin is a natural ligand for the asialoglycoprotein receptor. Liposomes containing asialofetuin show significantly greater liver uptake and targeting of hepatocytes compared with conventional liposomes following intravenous injection into mice (Wu et al., 1998).

Regarding HBV surface proteins (large, middle and small proteins), the pre-S1 protein, which is displayed on large proteins, binds to the asialoglycoprotein receptor and specifically recognizes human hepatocytes and HCC (Glebe, 2007; Stockert, 1995). Pre-S1 proteins and pre-S1-derived peptides have been used as delivery systems to target hepatocytes and HCC (Kang et al., 2010a; Yamada et al., 2003).

Several lactosylated delivery systems have been developed to target the asialoglycoprotein receptor (Arima et al., 2010; Hayashi et al., 2012). Antibody-mediated delivery to the

| Liver cell type | Receptor | Ligands | References |
|----------------|----------|---------|------------|
| Hepatocytes    | Carbohydrate (glycoprotein) receptors | Galactose/N-acetylgalactosamine | Iobst & Drickamer (1996), Staud et al. (1999) |
|                | Asialoglycoprotein receptor | Asialofetuin | Wu et al. (1998) |
|                |                     | Pre-S1 protein or pre-S1-derived peptide | Kang et al. (2010a), Yamada et al. (2003) |
|                | Mannose receptor | Pullulan | Arima et al. (2010), Hayashi et al. (2012) |
| Kupffer cells  | Carbohydrate (glycoprotein) receptors | Mannose/N-acetylgalactosamine | Fielding (1992), Jin et al. (2012), Takahashi et al. (2004) |
|                | Mannose receptor | ApoB (or LDL) and ApoE | Wang et al., 2013a; Wu et al., 2002; Xiao et al., 2013 |
| Sinusoidal endothelial cells | Carbohydrate (glycoprotein) receptors | Mannose/N-acetylgalactosamine, gelatin and collagen α-chain | Leitinger & Hohenester (2007), Malovic et al. (2007), Praanning-van Dalen et al. (1987), Sano et al. (1990) |
|                | Mannose receptor | Mannose/N-acetylgalactosamine | Leitinger & Hohenester (2007), Malovic et al. (2007), Praanning-van Dalen et al. (1987), Sano et al. (1990) |
|                | Hyaluronan receptor | Hyaluronan and chondroitin sulfates | Weigel & Weigel (2003) |
|                | Scavenger receptors | LPS and oxidized or acetylated LDL | van Berkel et al., 1991, van Oosten et al. (1998) |
|                | Fc receptors | IgG and IgE | Fridman et al. (1991) |
|                | M6P/IGF-II receptor | Collagen α-chain and M6P | Li et al. (2009), van Beuge et al. (2011), Yang et al. (2009) |
| Stellate cells | Platelet-derived growth factor receptor-β | Polypeptides | Bansal et al. (2011), Li et al. (2012) |

LDL, low-density lipoprotein; Apo, apolipoprotein; Ig, immunoglobulin; IGF, insulin-like growth factor; LPS, lipopolysaccharide; M6P, mannose 6-phosphate.
asialoglycoprotein receptor was also recently described (Coulstock et al., 2013).

Galactose/fucose-specific receptor-mediated delivery. Many galactose/N-acetylgalactosamine-modified delivery systems have been developed to target hepatocytes because the predo galactose-specific receptors on hepatocytes are asialoglycoprotein receptors. However, a large proportion of these molecules are taken up by Kupffer cells and LSECs, which express glycoprotein receptors that can remove galactose/N-acetylgalactosamine (Fadden et al., 2003; Lehrman et al., 1986). Galactose/N-acetylgalactosamine-terminated glycoproteins are also high-affinity monosaccharide ligands for galactose particle/fucose receptors expressed on Kupffer cells (Fadden et al., 2003). However, the endocytic capacity of galactose-terminated glycoproteins is much lower in LSECs than in hepatocytes or Kupffer cells (Magnússon & Berg, 1993). Thus, galactose-modified delivery systems are suitable for targeting hepatocytes (Morimoto et al., 2003; Popielarski et al., 2005) and Kupffer cells via galactose particle/fucose receptors (Dong et al., 2008). In fact, several delivery systems have been synthesized to deliver therapeutic molecules into Kupffer cells, including galactosylated low-molecular-weight chitosan (Dong et al., 2009). Shimada et al. (1997) also reported that biodegradable PEG-conjugated galactolipids show greater targeting (>90%) for Kupffer cells when intravenously injected into rats. Thus, several galactose-modified delivery systems show greater transfection efficiency in the liver than in other organs (e.g. lung, kidney, heart and spleen) (Hisayasu et al., 1999; Nishikawa et al., 2000). However, these results do not mean greater transfection efficiency for hepatocytes than other hepatic cells.

The uptake of galactose-modified nanoparticles by hepatocytes and Kupffer cells is influenced by the sizes of these nanoparticles. For example, slightly anionic, galactose-PEGylated nanoparticles of ~50 nm in diameter efficiently target hepatocytes, but nanoparticles of ~140 nm in diameter are more selective for Kupffer cells (Popielarski et al., 2005). Moreover, when galactosylated low-molecular-weight chitosan/oligodeoxynucleotide complexes of ~150 nm in diameter were intravenously injected into mice, Kupffer cells (50.6%) and LSECs (33.2%) endocytosed >83% of the complexed molecules, whereas hepatocytes endocytosed only 16.2% of the complexed molecules (Dong et al., 2008). These studies indicate that hepatocyte- or Kupffer cell-specific delivery can be achieved by controlling the size of the nanoparticles.

Mannose/N-acetylgalactosamine receptor-mediated delivery. In the liver, mannose is mainly removed via mannose receptors expressed on Kupffer cells and LSECs. Therefore, mannose-based delivery systems are more likely to be endocytosed by Kupffer cells or LSECs than by hepatocytes (Chaubey & Mishra, 2014; Kawakami et al., 2000; Nishikawa et al., 1993). As mentioned in the section “Carbohydrate (glyco-protein) receptor-mediated delivery”, the expression and endocytic capacity of the mannose/N-acetylgalactosamine receptor are greater in LSECs than in Kupffer cells (Bijsterbosch et al., 1996; Kogelberg et al., 2007; Magnússon & Berg, 1993). However, the cellular uptake of mannose-mediated delivery systems depends on the amount and type of mannose molecules (Hirata et al., 2010; Jansen et al., 1991). For example, human serum albumin (HSA) terminated with seven para-aminophenyl mannose residues (Man7-HSA) is mainly taken up by Kupffer cells, but Man22-HSA and Man40-HSA are endocytosed by Kupffer cells and LSECs (Jansen et al., 1991).

The mannose receptor, which is mainly expressed on LSECs, is a target for collagen α-chain and gelatin (Leitinger & Hohenester, 2007; Malovic et al., 2007). Recently, Un et al. (2012) reported an efficient method to deliver short interfering RNA (siRNA) into LSECs using ultrasound-responsive, mannose-mediated lipoparticles.

Hyaluronan receptor-mediated delivery. Hyaluronan receptors are expressed in LSECs and can recognize hyaluronan and chondroitin sulfates (Weigel & Weigel, 2003; Weigel et al., 2002). Therefore, hyaluronan-based delivery system may efficiently target LSECs (Kren et al., 2009; Takei et al., 2004).

Scavenger receptor-mediated delivery

The primary function of scavenger receptors is to remove potentially toxic and harmful components, or waste products, as part of the host’s defense system. Scavenger receptors are mainly expressed on Kupffer cells and LSECs, and can endocytose and degrade glycoproteins, lipoproteins [e.g. acetylated low-density lipoprotein (LDL)] (van Berkel et al., 1991) and apoptotic cells (Sambrano & Steinberg, 1995; Terpstra and van Berkel, 2000). Therefore, acetylated or oxidized LDL may be particularly useful to target scavenger receptors (Terpstra et al., 2000; van Berkel et al., 1991). Because viral vectors and liposomes are also removed via scavenger receptors, they may be useful for targeting scavenger receptors (Haisma & Bellu, 2011; Wheeler et al., 2001).

Several delivery systems have been used to demonstrate the uptake of nanoparticles via scavenger receptors (Nagayama et al., 2007b), and scavenger receptor-mediated delivery systems can be used to treat immune and inflammatory diseases in Kupffer cells (Melgert et al., 2003; Wheeler et al., 2001). Furthermore, in an inherited form of hyperlipidemia (i.e. hypercholesterolemia) that shows LDL receptor gene disruption in hepatocytes, a Kupffer cell scavenger receptor-mediated delivery system (e.g. polylactide nanoparticles containing anti-ApoB-100-conjugated LDL) may efficiently decrease LDL levels (Maximov et al., 2010).

Fc or complement receptor-mediated delivery

Fc or complement receptors play key roles in the elimination of circulating pathogens, including soluble immune complexes (Fridman, 1991; Lovdal et al., 2000; Skogh et al., 1985), complement fragments (Helmy et al., 2006) and apoptotic cells (Helmy et al., 2006). Fc receptors recognize the Fc domain of Igs (e.g. IgG and IgE) (Fridman, 1991) while complement receptors target complement fragments and apoptotic cells (Helmy et al., 2006). Hepatic stellate cells express both Fc and complement receptors (Shen et al., 2005; Xu et al., 2013). As LSECs weakly express complement receptors (Schlaf et al., 1999), the uptake of immune...
complexes in these cells is mediated by Fc receptors (Mousavi et al., 2007). Kupffer cells eliminate almost 90% of immune complexes (Johansson et al., 1996). There are two distinct groups of studies examining the uptake of immune complexes by hepatocytes. The first group of studies suggests that immune complexes are not taken up by hepatocytes (Johansson et al., 1996; Kosugi et al., 1992; van der Laan-Klamer et al., 1985). Until recently, there were no reports of Ig-modified delivery systems as asialoglycoprotein receptor ligands for hepatocyte-specific targeting.

Ig- or complement-coated materials may be used to examine the mechanism involved in the removal of pathogenic materials by Fc or complement receptors (Jacquier-Sarlin et al., 1995; Johansson et al., 1996; Kosugi et al., 1992).

**LDL receptor-mediated delivery**

Low-density lipoprotein receptors and very low-density lipoprotein (VLDL) receptors are structurally similar, but have very different distributions and functions. The N-terminal ligand-binding domain of LDL receptors consists of seven cysteine-rich repeats containing negatively charged amino acids, whereas this domain in VLDL receptors consists of eight repeats (Fielding, 1992; Takahashi et al., 2004). LDL receptors are highly expressed in hepatocytes while VLDL receptors are expressed in tissues that metabolize fatty acids (e.g. heart, muscle and fat) and in Kupffer cells, but are weakly expressed in hepatocytes (Multhaupt et al., 1996; Takahashi et al., 1992, 2004). LDL receptors mainly bind to ApoB-100, a component of LDL, whereas VLDL receptors show high affinity for ApoE, a component of VLDL and high-density lipoprotein. Although LDL receptors can also recognize ApoE, their affinity is weaker for ApoE than for ApoB-100 (Fielding, 1992; Takahashi et al., 2004). Thus, ApoB-100 (or LDL-)modified delivery systems may be suitable for targeting hepatocytes via LDL receptors (Jin et al., 2012). To date, however, no reports have described the use of LDL receptor-mediated delivery systems *in vivo*.

**Viral vectors**

Although there are some clinical safety concerns of viral vectors, particularly in relation to potential immune/inflammatory responses, they show greater transfection and therapeutic efficiency compared with non-viral vectors, such as synthetic polymers and liposomes (Chowdhury, 2009; Kang et al., 2010c; Parker et al., 2003; Wang et al., 2013b). The most widely used viral vectors in laboratory studies and clinical trials are inactivated retroviruses, adenoviruses, adeno-associated viruses (AAV) and herpes simplex viruses. Viral vectors carrying therapeutic molecules are rapidly taken up and degraded by scavenger receptors expressed on Kupffer cells, which results in low transfection of hepatocytes. The uptake of viral vectors by Kupffer cells may be controlled by mutating the virus domain proteins. For example, mutations in the fiber knob domain of adenovirus (serotype 5) prevent the uptake of adenoviruses by hepatocytes and Kupffer cells, and therefore decrease hepatic toxicity (Shayakhmetov et al., 2005). However, this method may be unsuitable for liver cell-targeted therapy.

Viral vectors, excluding viruses that target the asialoglycoprotein receptor for cell uptake (e.g. HBV), show very low transfection efficiency for hepatocytes. Indeed, one study revealed that just 5% of hepatocytes were stably transfected after infusing $10^{11}$ vector genomes of recombinant AAVs (Xiao et al., 1998). However, of the AAV serotypes, AAV serotype 8 (AAV-8) exhibits high hepatocyte transduction efficiency without activating cytotoxic T lymphocytes (Nakai et al., 2005; Wang et al., 2007). AAV-2 also shows high hepatocyte transduction efficiency, but induces immune responses by activating cytotoxic T lymphocytes (Wang et al., 2007).

The delivery efficiency of viral vectors into hepatocytes can be enhanced by chemically modifying the viral vector (e.g. conjugation with hydrophilic PEG) (Prill et al., 2011), pre-administration of scavenger receptor ligands (e.g. polyminosinic acid) (Haisma et al., 2008) or using hybrid viral vectors (Gallaher et al., 2009). These approaches increase the stability of the circulating viral vectors or reduce their uptake by scavenger receptors expressed on Kupffer cells. Another method is to conjugate the viral vector with a hepatocyte-specific ligand, but there is no direct *in vivo* evidence to support this proposal (Cristiano et al., 1993; Wu et al., 1994). Furthermore, injection of therapeutic viral vectors after pre-injection of clodronate liposomes (Schildner et al., 2003) or high doses of a viral vector (Shashkova et al., 2008) significantly increased hepatocyte transfection efficiency. The former approach leads to short-term depletion of Kupffer cells, while the latter blocks the uptake of the viral vectors by Kupffer cells. However, these methods may have cytotoxic effects against hepatocytes.

**Homing peptides**

Homing peptides are chemically stable molecules that can be conjugated to delivery systems or nanoparticle carriers (Aina et al., 2007; Khandare & Minko, 2006). They have been identified using various library methods, including phage display peptide libraries. The most widely used homing peptide is RGD (cyclic RGD) peptide, which shows strong affinity for integrins αvβ3 and αvβ5 (Aina et al., 2007; Khandare & Minko, 2006).

Homing peptides that specifically target HCC include TTPRDAY (Shimizu et al., 2006), FQHPSPFI (HCBP1) (Zhang et al., 2007a), SFSIIHTPILPL (SP94) (Lo et al., 2008), RGWRPLPKGE (HC1) (Zhang et al., 2011), AGKTPSLETT (A54) (Du et al., 2010), KSLSRHDHHH (HCC79) (Jiang et al., 2006) and AWYLP (Jia et al., 2007). Several HCC-targeted delivery systems using these peptides have been developed (Du et al., 2010; Lo et al., 2008), but further studies are needed to confirm whether they can distinguish between HCC and hepatocytes or other liver cells.

**Hydrodynamics**

Hydrodynamic delivery is based on the principle of intravenous injection of a large volume of solution (8–10% of body
weight) at a high velocity (e.g. 5–7 s for mice; faster rates of injection may cause liver tissue damage and death) (Liu et al., 1999; Zhang et al., 1997). Hydrodynamic delivery is a simple, efficient and liver-specific method for in vivo delivery of genes (DNA and RNA), proteins and polymers (Centelles et al., 2010; Chang et al., 2008; Kobayashi et al., 2004a,b; Zhang et al., 2004). Hydrodynamic injection was also used to establish liver disease models (Kang et al., 2009; Ketzinel-Gilad et al., 2006; Yang et al., 2002). The efficiency of hydrodynamic delivery is influenced by the injection volume (Liu et al., 1999; Zhang et al., 2004), the injection time (Liu et al., 1999), and the size and volume of the injected molecule (Kobayashi et al., 2004a; Liu et al., 1999).

The mechanism of hydrodynamic-based transfer involves changes to the hepatocyte membrane because the increased pressure after hydrodynamic injection opens many pores (mean diameter, 100 nm) in the cell membrane, allowing membrane-permeable substances (genes, proteins and polymers) to freely enter the cytosol of hepatocytes through the pores (Kobayashi et al., 2004b; Zhang et al., 2004). A single hydrodynamic injection was reported to deliver a gene to up to 40% of hepatocytes (Zhang et al., 2004), while avoiding transfection of other organs (e.g. kidney, lung, spleen and heart) (Hattori et al., 2009; Kobayashi et al., 2001; Yang et al., 2001). Hydrodynamic injection may have adverse effects on cardiac function (e.g. irregular cardiac rhythm) and increase venous pressure, but these changes returned to normal within several minutes (Zhang et al., 2004).

Several groups have used hydrodynamic injection for liver-targeted gene delivery in small (e.g. rats and mice) and large animals (e.g. pigs and monkeys), but the transfection efficiency is much lower in large animals than in small animals (Fabre et al., 2008). Furthermore, injection of a large fluid volume into humans (e.g. 5.6–7 L for a 70-kg person) within several seconds may have serious side effects. To overcome these problems, several novel techniques have been reported, including image-guided hydrodynamic injection with catheterization and venous occlusion (Fabre et al., 2008; Kamimura et al., 2009; Yoshino et al., 2006) or the use of a computer-controlled hydroinjector (Suda et al., 2008; Yokoo et al., 2013). These transfer techniques increased transfection efficiency and reduced the risk of serious side effects of hydrodynamic delivery in large animals.

**Use of liver cell-targeted delivery systems for treating hepatic diseases**

**Stellate cell-targeted delivery systems for treating liver fibrosis and cirrhosis**

Stellate cells play major pathologic roles in liver fibrosis and cirrhosis. Therefore, stellate cell-targeted delivery systems may be beneficial for treating these diseases (see Supplementary Materials, Stellate cells). Mannose 6-phosphate (M6P)-modified nanoparticles, such as M6P-HSA (or M6P-HSA-liposome), and N-(2-hydroxypropyl) methacrylamide, which contains M6P and tetrapeptide (GFLG), have been used to target stellate cells (Adrian et al., 2007; Beljaars et al., 2001; Li et al., 2009; van Beuge et al., 2011, 2013; Yang et al., 2009). However, they are also taken up by Kupffer cells and LSECs because M6P-modified delivery systems can accumulate on the scavenger receptors expressed on these cells (Rachmawati et al., 2007). Administration of polyinosinic acid, an inhibitor of scavenger receptors, was found to inhibit the accumulation of M6P-modified nanoparticles in Kupffer cells and LSECs, but not in stellate cells (Adrian et al., 2007), because stellate cells take up M6P-modified nanoparticles through mannose-6-phosphate/insulin-like growth factor II, receptor-mediated endocytosis (Rachmawati et al., 2007). Interestingly, HSA terminated with 28 M6P [M6P(28)-HSA] strongly binds to activated stellate cells, but not to quiescent stellate cells (Beljaars et al., 2001).

Hyperactivated, platelet-derived, growth factor receptor (PDGFR) is highly expressed on activated stellate cells during liver injury. PDGFR is implicated in the development of liver fibrosis and cirrhosis by activating both transforming growth factor-β/Smad and extracellular signal-regulated kinase/mitogen-activated protein kinase signaling pathways (Bonner, 2004; Yoshida & Matsuzaki, 2012). Delivery systems containing PDGFR-specific ligands (e.g. cyclic peptides; CSRNLDIC) may be used to target abnormal stellate cells (Bansal et al., 2011; Li et al., 2012). Furthermore, an adenoaviral Cre/loxP system containing the type I collagen promoter efficiently targeted activated stellate cells. Selective overexpression of thymidine kinase under the control of the collagen promoter and the administration of ganciclovir promoted the death of activated stellate cells and reduced hepatic fibrosis (Kinoshita et al., 2007). In addition, a peptide (KTTATDIKGKEV) derived from nerve growth factor receptor-mediated apoptosis of hepatocytes, mainly by upregulation of Fas (CD95) and tumor necrosis factor signaling pathways, plays a key role in virus-induced hepatocyte injury (Herzer, et al., 2007).

**Delivery systems used to treat hepatitis**

Hepatitis (liver inflammation) is mostly caused by long-term exposure to alcohol or toxic agents (e.g. chemicals and drugs), and viral/bacterial infection, especially HBV and HCV infection (Altamirano & Bataller, 2011; Bouchard & Navas-Martin, 2011; Seki & Schnabl, 2012). Viral hepatitis is associated with chronic hepatitis, cirrhosis and HCC. Death receptor-mediated apoptosis of hepatocytes, mainly by upregulation of Fas (CD95) and tumor necrosis factor signaling pathways, plays a key role in virus-induced hepatocyte injury (Herzer, et al., 2007).

Methods aimed at suppressing virus replication and viral protein synthesis have been developed recently to treat hepatitis (Chandra et al., 2012; Deng et al., 2009; Ji et al., 2012; Ryoo et al., 2012; Sakurai et al., 2012; Yang et al., 2010; Zhang et al., 2009), block virus entry into hepatocytes (Liu et al., 2012a; Matsumura et al., 2009; Wong-Staal et al., 2010) and remove virus-infected cells using cytotoxic agents (Shapira et al., 2012).

Oligonucleotides (siRNA, microRNA and antisense nucleotide), interferon and peptide aptamers conjugated to synthetic nanoparticles (Chandra et al., 2012; Ryoo et al., 2012), viral vectors and antibodies that can bind to virus-infected hepatocytes (Deng et al., 2009; Ji et al., 2012;
Sakurai et al., 2012; Shapira et al., 2012; Yang et al., 2010; Zhang et al., 2009) have been used to suppress virus replication and viral protein synthesis. Viral entry can be blocked by inhibiting the docking of viruses to the surface of hepatocytes using peptides or antibodies (Liu et al., 2012a; Matsumura et al., 2009; Wong-Staal et al., 2010). For example, as HCV targets lipoprotein receptors to interact with and enter hepatocytes, ApoE peptides that bind to the LDL receptor efficiently block HCV entry (Liu et al., 2012a).

Hepatitis activates several hepatic cell types, including Kupffer cells, LSECs, stellate cells and intrahepatic lymphocytes. Kupffer cells, the largest population of macrophages in the body, are considered to be an excellent target for treating hepatitis. In fact, several Kupffer cell-targeted delivery systems have been developed to treat hepatitis, and include carbohydrate (e.g. galactose)-conjugated nanoparticles (Dong et al., 2009), β-1,3-glucan schizophyllan (Mochizuki et al., 2013) and viral vectors (Wheeler et al., 2001). As described in the section “Scavenger receptor-mediated delivery”, delivery systems that exploit scavenger receptor-mediated endocytosis can be used to specifically target Kupffer cells.

**HCC-targeted delivery systems**

Hepatocellular carcinoma is highly resistant to conventional chemotherapy and radiotherapy, and has a relatively low 5-year survival rate of <10% (Jemal et al., 2007, 2008). Conventional antitumor drugs, including chemotherapeutic drugs (e.g. doxorubicin) (Cao et al., 2012) and inhibitors that target abnormal molecular signaling pathways (e.g. sorafenib, a multi-targeted tyrosine kinase inhibitor) (Cheng et al., 2012; Llovet et al., 2008) are non-selective for HCC cells, increasing the risk of side effects. Therefore, HCC-targeted delivery systems can increase the therapeutic efficiency of these drugs and decrease the risk of serious side effects.

Hepatocellular carcinoma-specific nanoparticles and oncolytic viruses are the most commonly used HCC-targeted delivery systems. Nanoparticles that target HCC generally contain HCC-specific material (peptides, aptamers or antibodies) (Ashley et al., 2011b; Meng et al., 2012; Toita et al., 2012b; Wang et al., 2012a,b). Unfortunately, most hepatocyte (or liver)-targeted delivery systems cannot distinguish between HCC and normal hepatocytes.

Viral vectors can also be used to deliver therapeutic molecules into HCC. But, as described in the Supplementary Materials, these vectors are mostly taken up by scavenger receptors expressed on Kupffer cells, which decreases the transfection of HCC cells. These vectors can also target hepatocytes, increasing the risk of side effects in hepatocytes. Thus, further research is necessary to improve the safety and therapeutic efficacy of oncolytic viruses.

Several oncolytic viruses (or vectors) have been genetically engineered to improve HCC selectivity, including adeno-viral vector (Liu et al., 2012b), herpes simplex virus (Fu et al., 2012), Newcastle disease virus vector (Altomonte et al., 2010), and measles virus Edmonston strain (Blechacz et al., 2006). HCC-targeted oncolytic viruses contain HCC-specific promoters (e.g. α-fetoprotein promoter) and/or mutated genes (Altomonte et al., 2010; Liu et al., 2012b; Zhang et al., 2007b) or recognize an HCC-specific membrane marker (e.g. CD46) (Blechacz et al., 2006). In a recent study, Fu et al. (2012) designed an oncolytic virus containing a hepatocyte-specific promoter and a hepatocyte-specific microRNA (miR-122). After targeting liver cells with the hepatocyte-specific ApoE-AAT promoter, the miR-122 complementary sequences are degraded in hepatocytes, but not in HCC, promoting HCC-specific replication (Fu et al., 2012). Furthermore, bacteriophage MS2 virus-like particles with a modified peptide (SP94) can selectively bind to HCC, self-assemble in the presence of siRNA and induce the HCC cell apoptosis (Ashley et al., 2011a), although there is no direct *in vivo* evidence supporting this concept.

Hyperactivated intracellular signals in HCC are also useful targets for developing HCC-targeted delivery systems (Kang et al., 2008, 2010a; Toita et al., 2012a). For example, combining the human hepatocyte-specific pre-S1 protein from HBV with a tumor cell-specific gene regulating nanoparticle, which targets hyperactivated protein kinase C-α in HCC, increases transfection efficiency and achieves HCC-specific gene delivery (Kang et al., 2010a).

**Clinical trials of liver-targeted delivery systems**

Although numerous viral and non-viral liver-targeted delivery systems have been developed, very few clinical trials of these systems have been performed. In a clinical study using AAV-8 vectors, which show high hepatocyte transduction efficiency (see “Viral vectors” section), peripheral vein infusion of AAV-8 vectors expressing a codon-optimized human factor IX transgene improved the bleeding phenotype in patients with severe hemophilia B, with low immune responses (Nathwani et al., 2011). However, in a clinical study of AAV-2 hemophilia B gene therapy, Manno et al. (2006) reported that the delivery system achieved therapeutically relevant levels of factor IX but was associated with unexpected liver toxicity caused by cytotoxic T lymphocyte-mediated immune responses to AAV-2-transduced hepatocytes.

HAb18G/CD147, a member of CD147 family, is over-expressed in HCC cells and plays an important role in HCC invasion and metastasis (Xu et al., 2007). Recent clinical trials of Licartin, an immunoradioconjugate containing the radioisotope $^{131}$I and metuximab (HAb18G/CD147-specific antibody fragment), in combination with chemoembolization, significantly extended the survival time in patients with HCC (He et al., 2013; Wu et al., 2010). Furthermore, intravenous administration of a doxorubicin-galactosamine-modified polymer conjugate was reported to target normal hepatocyte and HCC cells, and had unsatisfactory results, with two partial responses and one minor response in a study of 31 patients (Seymour et al., 2002).

**Summary**

Several liver cell-targeted delivery systems have been developed for safe and efficient delivery of therapeutic molecules into the liver. These systems are based on biological carriers (e.g. viral vectors) or non-viral/synthetic carriers (e.g. polymers and liposomes). Biological carriers show relatively high therapeutic efficacy, but are associated
with safety concerns, including activation of the host’s immune responses. Conversely, non-viral carriers show low pathogenicity, but their therapeutic efficacy is low, a limitation that needs to be resolved.

Hepatocytes are an important target for treating HCC, hepatitis, and alcoholic or non-alcoholic liver disease. It is essential to reduce the uptake of nanoparticles by non-parenchymal cells to achieve high therapeutic efficacy in hepatocytes. Receptor-mediated delivery systems are the most widely used methods for delivering therapeutic molecules into hepatocytes, but non-parenchymal cells also express receptors with similar functions. For example, galactose/N-acetylgalactosamine-modified delivery systems that target hepatocytes are also taken up by Kupffer cells and LSECs (Dong et al., 2008; Shimada et al., 1997). The lack of specific ligands for each receptor and differences in the activity/expression levels of receptors between patients may restrict the development and efficacy of receptor-mediated delivery systems.

It is essential that more specific delivery systems capable of differentiating between normal cells and diseased/damaged cells in the liver are developed. Such systems should display increased therapeutic efficacy for diseased/damaged cells and low risk of side effects in normal cells. Abnormal stellate cell-specific delivery systems, such as M6P-modified nanoparticles and PDGFR-targeted delivery systems, may satisfy these requirements. Although numerous delivery systems have been developed to treat hepatic diseases, including HCC, hepatitis, liver fibrosis and cirrhosis, further development of these delivery systems is essential.

**Declaration of interest**

This work was supported financially by a Health Labour Sciences Research Grant (Research on Publicly Essential Drugs and Medical Devices) from the Ministry of Health, Labour and Welfare of Japan and a grant-in-aid for Scientific Research (B) (KAKENHI Grant Number 23310085) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. The authors report no declaration of interest.

**References**

Adrian JE, Kamps JA, Scherphof GL, et al. (2007). A novel lipid-based drug carrier targeted to the non-parenchymal cells, including hepatic stellate cells, in the fibrotic livers of bile duct ligated rats. Biochim Biophys Acta, 1768, 1430–9.

Aggarwal P, Hall JB, McLeland CB, et al. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv Drug Deliv Rev, 61, 428–37.

Aina OH, Liu R, Sutcliffe JL, et al. (2007). From combinatorial chemistry to cancer-targeting peptides. Mol Pharmaceut, 4, 631–51.

Altamirano J, Bataller R. (2011). Alcoholic liver disease: pathogenesis and new targets for therapy. Nat Rev Gastroenterol Hepatol, 8, 344–9.

Altmontone J, Marozin S, Schmid RM, Ebert O. (2010). Engineered newcastle disease virus as an improved oncolytic agent against hepatocellular carcinoma. Mol Ther, 18, 275–84.

Arima H, Yamashita S, Mori Y, et al. (2010). In vitro and in vivo gene delivery mediated by Lactosylated dendrimer/alpha-cyclodextrin conjugates (G2) into hepatocytes. J Control Release, 146, 106–17.

Ashley CE, Carnes EC, Phillips GK, et al. (2011a). Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles. ACS Nano, 5, 5729–45.

Ashley CE, Carnes EC, Phillips GK, et al. (2011b). The targeted delivery of multicomponent cargos to cancer cells by nanoporous particle-supported lipid bilayers. Nat Mater, 10, 389–97.

Bansal R, Prakash J, Ruijter M, et al. (2011). Peptide-modified albumin carrier explored as a novel strategy for a cell-specific delivery of interferon gamma to treat liver fibrosis. Mol Pharm, 8, 1899–909.

Bareford LM, Swan PW. (2007). Endocytic mechanisms for targeted drug delivery. Adv Drug Deliv Rev, 59, 748–58.

Beljaars L, Olinga P, Molema G, et al. (2001). Characteristics of the hepatic stellate cell-selective carrier mannose-6-phosphate modified albumin (M6P(28)-HSA). Liver, 21, 320–8.

Biessen EA, Olinga P, Molema G, et al. (1994). Ligand size is a major determinant of high-affinity binding of fucos- and galactose-exposing (lipo)proteins by the hepatic fucose receptor. Biochem J, 299, 291–6.

Björnberg MK, Donker W, van de Bilt H, et al. (1996). Quantitative analysis of the targeting of mannos-termanal glucocerebrosides. Predominant uptake by liver endothelial cells. Eur J Biochem, 237, 344–9.

Blechacz B, Splinter PL, Greiner S, et al. (2006). Engineered measles virus as a novel oncolytic viral therapy system for hepatocellular carcinoma. Hepatology, 44, 1465–77.

Bonner JC. (2004). Regulation of PDGF and its receptors in fibrotic disease. Cytokine Growth Factor Rev, 15, 255–73.

Bouchard MJ, Navas-Martin S. (2011). Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. Cancer Lett, 305, 123–43.

Cao H, Phan H, Yang LK. (2012). Improved chemotherapy for hepatocellular carcinoma. Anticancer Res, 32, 1379–86.

Centelles MN, Isasi JR, Qian C, et al. (2010). Influence of the chitosan nature on the transfection efficacy of DNA-loaded nanoparticles after hydrodynamic administration in mice. J Microencapsul, 27, 460–9.

Chandra PK, Kundu AK, Hazari S, et al. (2012). Inhibition of hepatitis C virus replication by intracelular delivery of multiple siRNAs by nanosomes. Mol Ther, 20, 1724–36.

Chang ML, Chen JC, Yeh CT, et al. (2008). Gene gun bombardment with DNA-coated gold particles is a potential alternative to hydrodynamics-based transfection for delivering genes into superficial hepatocytes. Hum Gene Ther, 19, 391–5.

Chaubey P, Mishra B. (2014). Mannose-conjugated chitosan nanoparticles loaded with rifampicin for the treatment of visceral leishmaniasis. Carbohydr Polym, 101, 1101–8.

Cheng AL, Guan Z, Chen Z, et al. (2012). Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma according to baseline status: subset analyses of the phase III Sorafenib Asia- Pacific trial. Eur J Cancer, 48, 1452–65.

Chowdhury EH. (2009). Nuclear targeting of viral and non-viral DNA. Expert Opin Drug Deliv, 6, 697–703.

Coulstock E, Sosabowski J, Ovečka M, et al. (2013). Liver-targeting of interferon-alpha with tissue-specific domain antibodies. PLoS One, 8, e57263.

Cristiano RJ, Smith LC, Kay MA, et al. (1993). Hepatic gene therapy: efficient gene delivery and expression in primary hepatocytes utilizing a conjugated adenovirus-DNA complex. Proc Natl Acad Sci USA, 90, 11548–52.

Danieels CK, Schmucker DL, Jones AL. (1989). Hepatic asialoglycoprotein receptor-mediated binding of human polymeric immunoglobulin A. Hepatology, 9, 229–34.

Deng L, Li G, Xi L, et al. (2009). Hepatitis B virus inhibition in mice by lentiviral vector mediated short hairpin RNA. BMC Gastroenterol, 9, 73.

Dini L, Autuori F, Lentini A, et al. (1992). The clearance of apoptotic cells in the fibrotic livers of bile duct ligated rates. Biochim Biophys Acta, 1768, 1430–9.

Dong L, Zuo L, Xia S, et al. (2009). Reduction of liver tumor necrosis factor-α expression by targeting delivery of antisense oligonucleotides into Kupffer cells protects rats from fulminant hepatitis. J Gene Med, 11, 229–39.
Du B, Han H, Wang Z, et al. (2010). Targeted drug delivery to hepatocarcinoma in vivo by phage-displayed specific binding peptide. Mol Cancer Res, 8, 135–44.

Fabre JW, Grehan A, Whitehorne M, et al. (2008). Hydrodynamic gene delivery to the pig liver via an isolated segment of the inferior vena cava. Gene Ther, 15, 452–62.

Fadden AJ, Holt OJ, Drickamer K. (2003). Molecular characterization of the rat Kupffer cell glycoprotein receptor. Glycobiology, 13, 529–37.

Fichter M, Baier G, Dediers M, et al. (2013). Nanocapsules generated out of a polymeric dexamethasone shell suppress the inflammatory response of liver macrophages. Nanomedicine, 9, 1223–34.

Fielding CJ. (1992). Lipoprotein receptors, plasma cholesterol metabolism, and the regulation of cellular free cholesterol concentration. FASEB J, 6, 3162–8.

Finbloom DS, Magilavy DB, Harford JB, et al. (1981). Influence of antigen on immune complex behavior in mice. J Clin Invest, 68, 214–24.

Fridman WH. (1991). Fc receptors and immunoglobulin binding factors. FASEB J, 5, 2684–90.

Fu X, Rivera A, Tao L, et al. (2012). Construction of an oncolytic herpes simplex virus that precisely targets hepatocellular carcinoma cells. Mol Ther, 20, 339–46.

Gallaher SD, Gil JS, Dorigo O, Berk AJ. (2009). Robust in vivo hepatic gene expression mechanisms following intravenous administration of a hydrodynamics-based procedure. Vet J, 181, 336–9.

Gallagher SJ, Gil JS, Dorris O, Berk AJ. (2009). Robust in vivo transduction of a genetically stable Epstein-Barr virus episome to hepatocytes in mice by a hybrid viral vector. J Virol, 83, 3249–57.

Glebe D. (2007). Viral and cellular determinants involved in hepadnaviral entry. World J Gastroenterol, 13, 22–38.

Iobst ST, Drickamer K. (1996). Selective sugar binding to the mannose receptor in the human liver. Hepatology, 14, 1070–5.

Kang JH, Tachibana Y, Obika S, et al. (2012). Efficient reduction of serum cholesterol by combining a liver-targeted gene delivery system with chemically modified apolipoprotein B siRNA. J Control Release, 163, 119–24.

Herzer K, Sprienzl MF, Galie PR. (2007). Hepatitis viruses: live and let die. Liver Int, 27, 293–301.

Hirata K, Maruyama T, Watanabe H, et al. (2010). Genetically engineered mannosylated-human serum albumin as a versatile carrier for liver-selective therapeutics. J Control Release, 145, 9–16.

Hirata K, Maruyama T, Watanabe H, et al. (2010). Genetically engineered mannosylated-human serum albumin as a versatile carrier for liver-selective therapeutics. J Control Release, 145, 9–16.

Jemal A, Siegel R, Ward E, et al. (2007). Cancer statistics, 2007. CA Cancer J Clin, 57, 43–66.

Kang JH, Tachibana Y, Obika S, et al. (2012). Efficient reduction of serum cholesterol by combining a liver-targeted gene delivery system with chemically modified apolipoprotein B siRNA. J Control Release, 163, 119–24.

Kawakami S, Sato A, Nishikawa M, et al. (2000). Mannose receptor-mediated gene transfer into macrophages using novel mannosylated cationic liposomes. Gene Ther, 7, 292–9.

Kettenl-Gilad M, Zauberman A, Nussbaum O, et al. (2006). The use of the hydrodynamic HBV model to study HBV biology and anti-viral therapy. Hepatol Res, 34, 228–37.

Khandare JJ, Minko T. (2006). Antibodies and peptides in cancer therapy. Bioconjug Chem, 17, 583–9.

Khandare JJ, Minko T. (2006). Antibodies and peptides in cancer therapy. Bioconjug Chem, 17, 583–9.
Kren BT, Unger GM, Sjeklocha L, et al. (2009). Nanoparticle-delivered Sleeping Beauty mediates therapeutic Factor VIII expression in liver sinusoidal endothelial cells of hemophilia A mice. J Clin Invest, 119, 2086–99.

Kuiper J, Bakkeren HF, Biessen EA, Van Berkel TJ. (1994). Characterization of the interaction of galactose-exposing particles with rat Kupffer cells. Biochem J, 299, 285–90.

Lehrman MA, Haltiwanger RS, Hill RL. (1986). The binding of fucose-containing glycoproteins by hepatic lectins. The binding specificity of the rat liver fucose lectin. J Biol Chem, 261, 7426–32.

Leitinger B, Hohenester E. (2007). Mammalian collagen receptors. Matrix Biol, 26, 146–55.

Li F, Li QH, Wang JY, et al. (2012). Effects of interferon-gamma liposomes targeted to platelet-derived growth factor receptor-beta on hepatic fibrosis in rats. J Control Release, 159, 261–70.

Li FQ, Su H, Chen X, et al. (2009). Mannose 6-phosphate-modified bovine serum albumin nanoparticles for controlled and targeted delivery of sodium ferulate for treatment of hepatic fibrosis. J Pharm Pharmacol, 61, 1155–61.

Liu F, Song Y, Liu D. (1999). Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther, 6, 1258–66.

Liu S, McCormick KD, Zhao W, et al. (2012a). Human apolipoprotein E peptides inhibit hepatitis C virus entry by blocking virus binding. Hepatology, 50, 484–91.

Liu X, Cao X, Wei R, et al. (2012b). Gene-into-therapy targeting liver cancer by a dual-regulated oncolytic adenoviral vector harboring IL-24 and TRAIL. Cancer Gene Ther, 19, 49–57.

Llovet JM, Ricci S, Mazzaferro V, et al. (2008). Sorafenib in advanced hepatocellular carcinoma. N Engl J Med, 359, 378–90.

Liu F, Cao X, Wei R, et al. (2012). Gene-into-therapy targeting liver cancer by a dual-regulated oncolytic adenoviral vector harboring IL-24 and TRAIL. Cancer Gene Ther, 19, 49–57.

Kuiper J, Bakkeren HF, Biessen EA, Van Berkel TJ. (1994). Endocytosis of ricin by rat liver cells in vivo and in vitro. J Biol Chem, 269, 3789–90.

Lo A, Lin CT, Wu HC. (2008). Hepatocellular carcinoma cell-specific peptide ligand for targeted drug delivery. Mol Cancer Ther, 7, 579–89.

Lolvad T, Andersen E, Brech A, Berg T. (2000). Fc receptor mediated endocytosis of small soluble immunoglobulin G immune complexes in Kupffer and endothelial cells from rat liver. J Cell Sci, 113, 3255–66.

Lunov O, Syrovots T, Loos C, et al. (2011). Differential uptake of functionalized polysynery nanoparticles by human macrophages and a monocytic cell line. ACS Nano, 5, 1657–69.

Magnusson S, Berg T. (1993). Endocytosis of ricin by rat liver cells in vivo and in vitro is mainly mediated by mannose receptors on sinusoidal endothelial cells. Biochem J, 291, 749–55.

Malik R, Selden C, Hodgson H. (2002). The role non-parenchymal cells express the mannose receptor on murine liver sinusoidal endothelial cells is the main receptor on murine liver sinusoidal endothelial cells. A monocytic cell line. ACS Nano, 5, 1657–69.

Managat C, Kawakami S, Yamashita F, Hashida M. (2005). Effect of galactose density on asialoglycoprotein receptor-mediated uptake of galactosylated liposomes. J Pharm Sci, 94, 7266–75.

Mannu CS, Pierce GF, Arruda VR, et al. (2006). Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med, 12, 342–7.

Matsumura T, Hu Z, Kato T, et al. (2009). Amphipathic DNA polymers inhibit hepatitis C virus infection by blocking viral entry. Gastroenterology, 137, 673–81.

Maximov VD, Reuko VV, Barry JN, et al. (2010). Protein-nanoparticle conjugates as potential therapeutic agents for the treatment of hyperlipidemia. Nanotechnology, 21, 265103.

Miyagi S, Weert BN, Schellekens H, et al. (2003). The pharmacokinetic and biological activity profile of dexamethasone targeted to sinusoidal endothelial and Kupffer cells. J Drug Target, 11, 1–10.

Meng L, Yang L, Zhao X, et al. (2012). Targeted delivery of chemotherapy agents using a liver cancer-specific aptamer. PLoS ONE, 7, e33434.

Mochizuki S, Morishita H, Sakurai K. (2013). Macrophage specific delivery of TNF-α siRNA complexed with β-1,3-glucan inhibits LPS-induced cytokine production in a murine acute hepatitis model. Bioorg Med Chem, 21, 2535–42.

Morigoto K, Nishikawa M, Kawakami S, et al. (2003). Molecular weight-dependent gene transfection activity of unmodified and galactosylated polyethyleneimine on hepatoma cells and mouse liver. Mol Ther, 7, 254–61.

Mousavi SA, Sparstol M, Fladeby C, et al. (2007). Receptor-mediated endocytosis of immune complexes in rat liver sinusoidal endothelial cells is mediated by FcγRIIb. Hepatology, 46, 871–84.
demonstrated with a novel monoclonal antibody and by C5a anaphylatoxin-induced Ca\textsuperscript{2+} release. Lab Invest, 79, 1287–97.

Schwartz AL. (1984). The hepatic asialoglycoprotein receptor. CRC Crit Rev Biochem, 16, 207–33.

Seki E, Schnabl B. (2012). Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut. J Physiol, 590, 447–58.

Seymour LW, Ferry DR, Anderson D, et al. (2002). Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin. J Clin Oncol, 20, 1668–76.

Shapira A, Shapira S, Gal-Tanamy M, et al. (2012). Removal of hepatitis C virus-infected cells by a zymogenized bacterial toxin. PLoS ONE, 7, e32320.

Shashkova EV, Doronin K, Senac JS, Barry MA. (2008). Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. Cancer Res, 68, 5896–904.

Shimizu A, Maruta F, Akita N, et al. (2006). Identification of an oligopeptide binding to hepatocellular carcinoma. Oncology, 71, 135–45.

Shmaklova EV, Bordon K, Senac JS, Barry MA. (2008). Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. Cancer Res, 68, 5896–904.

Shmaklova EV, Bordon K, Senac JS, Barry MA. (2008). Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. Cancer Res, 68, 5896–904.

Shmaklova EV, Bordon K, Senac JS, Barry MA. (2008). Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. Cancer Res, 68, 5896–904.

Shmaklova EV, Bordon K, Senac JS, Barry MA. (2008). Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. Cancer Res, 68, 5896–904.
Xu J, Xu HY, Zhang Q, et al. (2007). HAb18G/CD147 functions in invasion and metastasis of hepatocellular carcinoma. Mol Cancer Res, 5, 605–14.

Xu R, Lin F, He J, et al. (2013). Complement 5a stimulates hepatic stellate cells in vitro, and is increased in the plasma of patients with chronic hepatitis B. Immunology, 138, 228–34.

Yamada T, Iwasaki Y, Tada H, et al. (2003). Nanoparticles for the delivery of genes and drugs to human hepatocytes. Nat Biotechnol, 21, 885–90.

Yang J, Chen S, Huang L, et al. (2001). Sustained expression of naked plasmid DNA encoding hepatocyte growth factor in mice promotes liver and overall body growth. Hepatology, 33, 848–59.

Yang N, Ye Z, Li F, Mahato RI. (2009). HPMA polymer-based site-specific delivery of oligonucleotides to hepatic stellate cells. Bioconjug Chem, 20, 213–21.

Yang PL, Althage A, Chung J, Chisari FV. (2002). Hydrodynamic injection of viral DNA: a mouse model of acute hepatitis B virus infection. Proc Natl Acad Sci USA, 99, 13825–30.

Yang X, Haurigot V, Zhou S, et al. (2010). Inhibition of hepatitis C virus replication using adeno-associated virus vector delivery of an exogenous anti-hepatitis C virus microRNA cluster. Hepatology, 52, 1877–87.

Yokoo T, Kamimura K, Suda T, et al. (2013). Novel electric power-driven hydrodynamic injection system for gene delivery: safety and efficacy of human factor IX delivery in rats. Gene Ther, 20, 816–23.

Supplementary material available online

Supplementary Tables 1–2