Chapter 5

Polylipid Nanoparticle, a Novel Lipid-Based Vector for Liver Gene Transfer

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Additional information is available at the end of the chapter

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1. Introduction

Lipid nanoparticles (LNP) are invaluable carriers for drug and gene delivery, and they are classified as cationic, neutral and anionic depending on the electronic charges existing on the surface of the vesicles [1]. These charges are originated from the charged lipids from which lipid nanoparticles are formulated. Cationic LNP are commonly used for DNA or RNA carriers due to their interaction with negatively-charged nucleotide. Both neutral and negatively-charged LNPs are used for drug delivery [2] and may be formulated as sterically stable LNPs (SSLNPs), which are amendable for cell type-specific or tissue-specific targeting delivery [3]. For liver drug delivery, tremendous efforts have been made to develop cell type-selective lipid-based drug carriers. Effective approaches in targeting hepatocytes, Kupffer cells and hepatic stellate cells have been evaluated in small animals [3, 4], and some of them may be translational to clinical application [5]. These approaches are referable when cationic LNPs are considered for cell type-selective gene delivery. A prerequisite for the success of gene therapy for liver disorders is the development of powerful gene carriers. Non-viral vectors have been very successful for gene transfer in an *in vitro* setting, in terms of efficiency of lipofection, applicability in variety of cell types, and amending ability of cell type-specific delivery (Fig. 1). The clinical application of LNP-mediated gene transfer has been hampered by low efficiency, instability in the bloodstream, short-term transgene expression and toxicity. These shortcomings are the bottle neck hindering the gene transfer employing LNPs as carriers for delivery of function gene(s) to solid organs, and are the challenges in moving from small to large animals of potential gene carriers and approaches, and in the translation to clinical application. However, the polylipid nanoparticles (PLNP) we have developed over the past decade represent one of the few formulations that are applicable for *in vivo*

gene transfer [6], due to a high DNA-packaging capacity, an extremely low binding rate to serum proteins, low toxicity, and amendable synthetic approaches [7, 8]. This chapter intends to introduce the characteristics of this formulation, and to discuss our efforts in moving the non-viral gene transfer platform from small to large animals towards clinical applications.

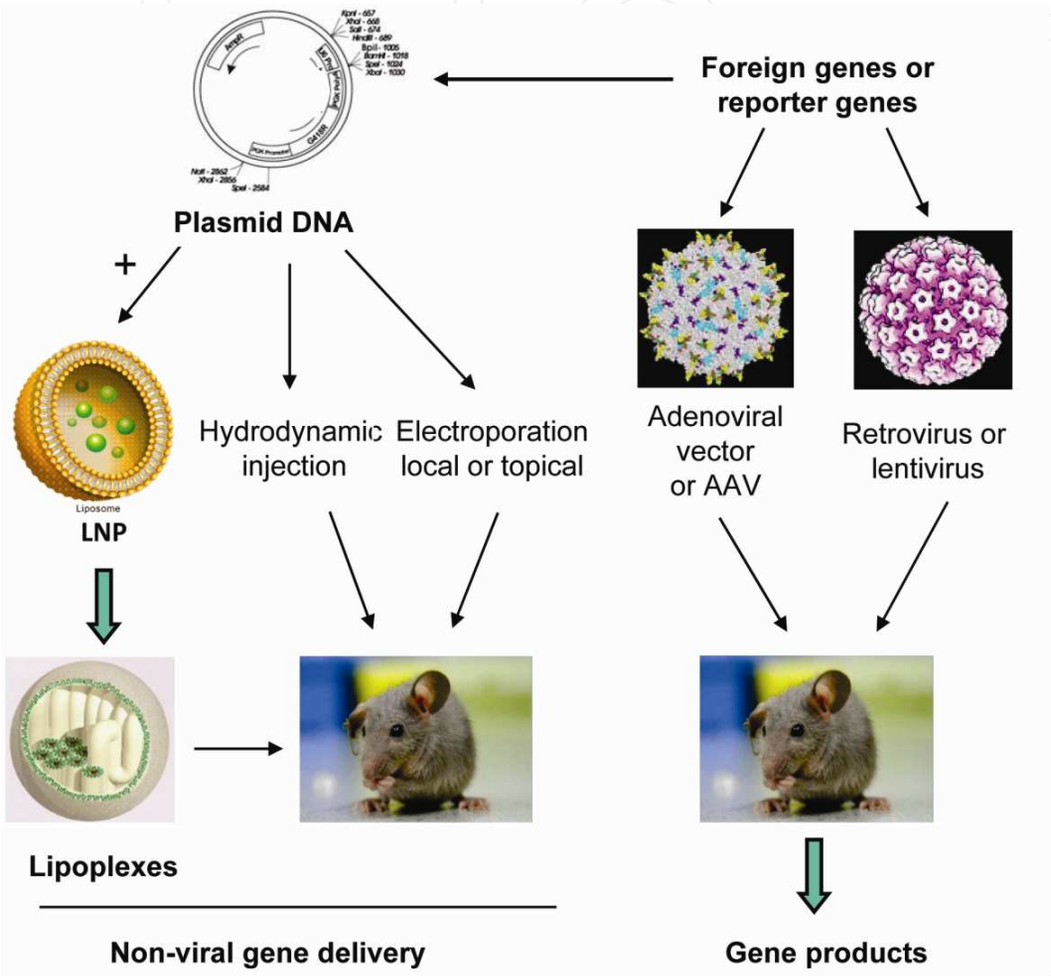

Figure 1. ***In vivo*** **gene transfer mediated by viral or non-viral vectors.** Viral vectors, such as lentiviral or retroviral vectors, which lead to integration of transgene in the host genome, give rise to long-term transgene expression. However, they may cause insertion-induced mutation that is oncogenic. Adenoviral or adeno-associated viral (AAV) vectors often yield a high level of transgene expression in host organs. However, generation of antibodies against viral components is still a concern. Non-viral gene transfer may be achieved by direct plasmid administration with local electroporation or a hydrodynamic approach. The latter is only applicable in mice. Lipid nanoparticle (LNP)-mediated gene transfer becomes a useful approach which is often very successful for *in vitro* gene transfer. Few formulations of cationic LNPs are valuable for *in vivo* gene transfer. There has been a still demand in improving their *in vivo* stability and gene transfer efficacy.

There are a number of critical components for a potential gene therapy product to move from one step to the next in this pipeline. Promisingly, LNP-mediated gene transfection for the treatment of genetic and metabolic disorders or tumors has been moved to clinical trial phases (http://clinicaltrials.gov). A phase I pilot study of gene therapy for cystic fibrosis us-

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ing cationic liposome-mediated gene transfer (NCT00004471) has been completed. A phase I trial of intratumoral epidermal growth factor receptor (EGFR) antisense DNA delivered by DC-Chol liposomes in advanced head and neck cancer, including oral squamous cell carcinoma (NCT00009841) and DOTAP-Chol-Fus1 liposome-mediated gene therapy for non-small cell lung cancer (NCT00059605] [9] were conducted respectively by University of Pittsburg and MD Anderson Cancer Center in collaboration with the National Cancer Institute (NCI). Fus1 is a tumor suppressive gene that has been shown to be effective in suppressing the growth of original or metastatic lesions of non-small lung cancer when it is delivered locally or systemically [10]. Thus, it appears that genetic therapy using LNPs as gene carriers has the potential to be specially tailored for genetic disorders or cancers.

2. Nanoparticle carriers for drug or gene delivery

Lipid-based gene carriers include liposomes (cationic or anionic), polymer and dendrimer nanoparticles. Cationic liposomes are capable of delivering genes to cells or tissues, and achieving maximal therapeutic efficiency with minimal adverse effects [1]. However, the use of cationic LNPs for *in vivo* DNA transfection is hindered by substantial problems; i.e. after intravenous administration, cationic LNPs bind to plasma protein and blood cells due to charge reaction. The resulting aggregates of carriers with proteins or cells block microcirculation or may be cleared rapidly [11, 12]. The common formulations for *in vivo* gene delivery are DOTMA or DOTAP-DOPE or DOTAP-cholesterol (Chol). These formulations are highly serum-reactive [6, 13]. Lungs are the major organ shown to be highly transfected probably due to the accumulation of aggregates of lipoplexes with serum proteins or blood cells when the lipoplexes are administrated intravenously [14]. For this reason, cationic LNPs were once used widely for gene delivery to the lungs; and later for treating lung cancers and metastasis with further optimization [10, 15, 16]. LNP-mediated gene delivery to the liver is more difficult than to lungs. For the development of the gene carriers, cationic LNP formulations, such as DC-Chol, DOTAP-Chol, are available for delivering genes to various tissues [17]. A few LNP formulations targeting hepatocellular carcinoma (HCC) have been developed for improving efficacies of drug therapy [18, 19]. In order to avoid the rapid clearance by the reticuloendothelial system (RES) and to increase the drug delivery through the enhanced permeability and retention (EPR) effect to a tumor site by passive targeting, novel strategies, such as reducing particle size, minimizing rigidity of lipids, generating amphiphilic vesicles and shielding from the recognition by RES system, have been attempted in formulating lipid-based drug/gene carriers [1, 2]. To reduce lysosomal degradation, pH-sensitive LNPs are prepared for drug or gene delivery [20]. These approaches may be instructive in the development of LNPs for gene transfer at different stages of preclinical translation.

Polymeric non-viral vectors have exhibited additional advantages of lower toxicity and immunogenicity [21, 22]. These vectors may offer the possibility of industrial production following good manufacturing practice (GMP). Amphiphilic polyethylene glycol (PEG) has been engineered as a linker, most for coupling peptides to cationic lipids. Other polymers,

such as dendritic poly(L-lysine)-b-poly(L-lactide)-b-dendritic vector [23], poly (ethyleneimine) (PEI) [24], poly (methacrylate) [25] and polyamidoamine dendrimers [26], have been demonstrated to be effective for *in vitro* gene delivery. However, striking issues still exist for cationic polymers regarding whether they are applicable for *in vivo* gene transfer to solid organs such as the liver, without significant adverse effects.

3. Liver-specific gene delivery

Because of our interest in gene therapy of liver disorders, we have focused our efforts on improving liver-based gene delivery. The pathogenesis of liver injury and fibrosis involves complicated interactions among different cell populations in the liver, soluble factors, such as cytokines and reactive oxygen species (ROS), and the extracellular matrix components. In order to improve the efficacy in preventing hepatocellular injury, the use of LNPs that are capable of delivering hepatoprotective agents to the liver, selectively to hepatocytes, will increase local concentration of therapeutic agents, reduce adverse effects, and achieve maximal therapeutic efficiency. The parenchymal cell type in the liver is hepatocytes, which are responsible for an array of metabolic function in the body and are often damaged in a variety of pathological processes. The asialoglycoprotein receptor (ASGP-R) on mammalian hepatocytes provides a unique means for the development of liver-specific drug or gene carriers. The abundant receptors on hepatocytes specifically recognize the natural ligands, lectin and asialofetuin (AF), as well as those with terminal galactose or N-acetylgalactosamine residues, and hepatocytes endocytose these ligands for an intracellular degradation process [27, 28]. The use of its natural or synthetic ligands, such as galactosylated cholesterol, glycolipids or galactosylated polymers to label LNPs has achieved significant targeting efficacy to the liver [4, 28]. AF-labeled LNPs have been used for improving liver-targeting gene transfer in small animals [29], yet there have not been successful reports available in the translation to large animals, such as pigs [30]. Instead, plasmid DNA was directly administrated into the hepatic vein through a catheter with a balloon closure of hepatic vein blood flow [30]. One particular attention has been drawn in terms of the use of AF-labeled drug carriers for HCC targeting. The expression of ASGP-R in HCC cells varies depending on the differentiation status of HCC cells [31]. In general, well-differentiated HCC usually expresses relatively high levels of hepatocyte-specific genes, including ASGP-R; whereas poorly-differentiated HCC expresses minimal or no hepatocyte-specific genes, including ASGP-R [32]. In most cases, there exists the dramatic heterogeneity of liver-specific gene expression in human HCC tissues [33], and decreased expression of ASGP-R was observed in liver cancer tissue [34]. Therefore, using AF or other galactosylated or lactosylated residues to label LNPs for drug or gene delivery may not always be effective for patients with HCC, because HCC develops on a variety of disease backgrounds and there is a striking variation in ASGP-R expression levels in HCC from different patients. Using well-differentiated hepatoma cells, such as HepG2, Hep3B and Huh-7 cells, as an *in vitro* screening tool may not necessarily reflect targeting efficacy to tumor-specific distribution *in vivo* [35].

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High density lipoprotein (HDL) has a high drug carry capacity, and can be recognized by HDL receptors on hepatocytes. Recombinant HDL was utilized to deliver an anti-HBV peptide (nosiheptide) to the liver, and it was shown to achieve a selective distribution in hepatoma cells *in vitro* and a preferential liver distribution in rats [36]. Apolipoprotein E is cleared by hepatocytes, and it has been employed to be carriers for small interfering RNA (siRNA) delivery to hepatocytes [37].

Given the fact that hepatic stellate cells (HSCs) are the major cell type responsible for hepatic fibrosis, a repairing process that causes excess production of extracellular matrix components and deposition of fibrotic scaring in chronically injured liver [38], much attention has been focused on targeting this cell type in the last decade. A couple of cell surface molecules that are overexpressed on activated HSCs during hepatic fibrogenesis, such as insulin growth factor receptor II [39], collagen type VI and platelet-derived growth factor (PDGF) receptor β-subunit [40] are selected as the cell surface targets. Drug carriers labeled with specific peptides recognizing these cell surface molecules, such as cyclic peptide containing arginine-glycine-aspartate (RGD)-labeled sterical lipid nanoparticles [3] or Mannose-6-phosphate human serum albumin (M6P/HAS) [41] exhibited HSC-selective distribution. The RGD cyclic peptide was recently used as a targeting molecule for the recognition of activated HSCs in two animal models for early diagnosis of hepatic fibrosis with a SPECT imaging modality [42]. Using the retinol binding protein (RBP) in activated HSCs seems to be very effective in delivering siRNA against gp46 (rat homolog of human heat shock protein 47), and inhibiting fibrosis in two animal models [43].

Targeting approaches for drug or gene delivery to other non-parenchymal cell types, such as Kupffer cells or sinusoidal endothelial cells, are summarized recently [27]. These approaches are crucial in delivering agents which are anti-inflammatory or anti-oxidants to these cell types due to the fact that Kupffer cells are pivotal in the mediation of inflammatory responses and subsequent fibrogenesis [44].

4. Polylipid nanoparticle-mediated liver gene delivery

Compared to drug delivery, LNP-mediated *in vivo* gene delivery is still in its development stage; and many issues that affect delivery approaches and efficacy remain to be solved. The main issues include: 1) the formation of aggregates between cationic lipids and serum proteins bearing negative charges; 2) the administration routes of LNP-DNA complexes (lipoplexes); 3) intracellular trafficking from the cytoplasm to the nucleus; 4) the proliferative state of cells to be transfected; and 5) transient transgene expression for a short duration [6, 45]. Substantial efforts have been made to address these issues in our previous studies and by others [8, 46, 47]. Particularly, we polymerized an acrylamide lipid to generate a polycationic lipid (PCL), which was able to interact with plasmid DNA effectively and form compacted complexes as demonstrated by Raman microspectral analysis [8]. PCL has a unique molecular configuration and molecular weight distribution as indicated by mass spectrophotometrical analysis [8]. Moreover, this lipid can be synthesized in a multiple gram quan-

tity in a laboratory, and the synthetic approach is amendable for industrial production at a quantity sufficient enough for large animal use [8]. PLNP was formulated with a neutral lipid, cholesterol. The PLNP size was reduced to approximately 100 nm in diameter [7], and the Zeta potential of PLNP was decreased to neutral by neutralizing extra-positive charges with excess plasmid DNA [8]. Not only was this formulation of PLNP non-toxic, but it also displayed transfection efficiency equivalent to other commercially available transfection agents, such as Lipofectamine in hepatoma cell lines [7]. Moreover, high-resolution fluorescent deconvolution microscopy documented that PLNP-mediated gene transfection led to earlier GFP expression in hepatoma cells than Lipofectamine [8]. The unique feature of this formulation is that it is extremely serum-resistant, and exposure to cell culture medium containing 50% fetal bovine serum for 24 hours did not affect its size significantly. PLNP reacted up to 30-fold less with serum proteins or blood cells after intravenous administration in comparison with DOTAP-DOPE or DOTAP-Chol formulations [6]. This feature makes PLNP formulation particularly useful for *in vivo* gene transfer. In the subsequent studies, we have proved that it is very effective in the transfer of reporter genes or function genes to normal mouse livers as demonstrated in Fig. 2 by bioluminescent imaging of firefly luciferase gene expression 24 hours after portal vein injection of PLNP-plasmid DNA complexes (polyplexes) or preclinical models [48, 49].

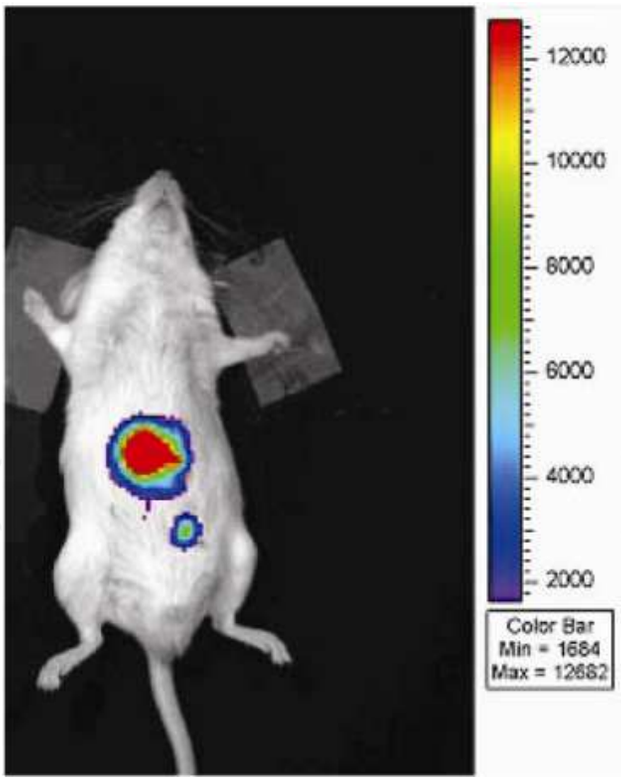

Figure 2. PLNP-mediated gene transfer into mice through portal vein injection. One day after the intravenous injection of polyplexes with pNDLux.2 plasmid encoding the firefly luciferase gene, the animal was imaged by CCD camera. The expression of luciferase was clearly shown in the liver area, demonstrating the effectiveness of this delivery approach and the applicability of a non-invasive imaging modality in the determination of transgene expression in animals.

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We also developed an approach to promote normal hepatocytes to proliferate *in situ* without partial hepatectomy, which favors the transgene expression by lipofection but is not acceptable for clinical application [6]. Furthermore, placing an indwelling catheter in the portal vein allows repeated administration of polyplexes for sustained transgene expression [6]. All these efforts render our formulation of PLNP distinct from other lipid-based nanoparticles. Our animal experiments have clearly demonstrated that PLNP is characterized as extremely stable in the bloodstream, and highly effective in liver-based gene transfer when polyplexes are administrated through the portal vein [6, 17]. In comparison with other commonly used lipid formulations of nanoparticles, our formulation possesses the notable advantages essential for *in vivo* gene delivery as illustrated in Table 1.

Characteristics	PCL	PLNP	Lipofectamine	DOTAP-Chol
Cationic lipid	Yes	LNP	LNP	LNP
Particle size (nm)	Irrelevant	125±54	358 ±85	110±20
Size changes(50%FBS)	Irrelevant	100±20nm	2206 ± 311 nm	1050±100 nm
In vitro transfection efficiency Luciferase activity (in RLU)	Irrelevant	>10E7	>10E7	>10E7
Cytotoxicity (LDH release)	Low or none	Normal	10±3% (>5%)	11±3.5% (>5%)
Binding rate to serum protein	Low	Low	Obvious	20-30-fold higher than PLNP
In vivo stability	Irrelevant	Stable	Not determined	Instability
Usage	Raw material for PLNP	*In vitro* or *in vivo* transfection	*In vitro* transfection	*In vivo* transfection

The content in this table was summarized according to our previous publications [6-8]. FBS = fetal bovine serum. RLU = relative light unit. LDH = lactate dehydrogenase.

Table 1. Comparison of common transfection agents for *in vitro* and *in vivo* application

5. Preclinical trials for proof of the concept

In order to demonstrate that our PLNP formulation is effective in delivering functional genes to the liver, we established a liver injury model in mice caused by the treatment with D-galactosamine (D-Gal) and lipopolysaccharide (LPS). This combination of D-Gal/LPS treatment resulted in a profound acute liver injury characterized by massive liver cell death through apoptosis, elevation of serum alanine aminotransferase (ALT), significant oxidant stress, depletion of the reduced form of glutathione and enhanced lipid peroxidation [50]. In

separate studies we have demonstrated that anti-oxidant enzyme such as extracellular superoxide dismutase (EC-SOD), SOD mimetics (MnTBAP) and catalase are effective in the prevention of hepatic toxicity caused by xenobiotics in primary hepatocytes or hepatoma cells [51-53], and they improved recipient survival and graft function and growth after small-for-size liver transplantation in rats [54]. Therefore, we chose the human EC-SOD gene as a functional gene to prove the feasibility. The EC-SOD gene product was exclusively secreted into the extracellular space and functions as an ROS scavenger. ROS are generated in both intracellular and extracellular spaces, and superoxide anions and hydrogen peroxide (H_2O_2) are able to cross the plasmatic membrane to enter the extracellular space [17]. It was found that two days after portal vein injection of EC-SOD polyplexes, liver EC-SOD gene expression was increased approximately 50-fold compared to the group receiving injection of control plasmid polyplexes, and serum SOD activity was increased accordingly. On the other hand, serum ALT was reduced to nearly one third in mice receiving EC-SOD polyplex injection compared to those with D-Gal/LPS challenge, along with improved liver histology, restored glutathione levels and decreased lipid peroxidation [48]. The findings of this preclinical trial confirmed the effectiveness of PLNP-mediated EC-SOD gene delivery to the liver, and that the delivery protected the mice from oxidant stress-associated liver injury. The results also indicate that this anti-oxidant gene delivery approach could be useful in attenuating xenobiotics or drug metabolite-induced toxicity to the liver.

Ischemia/reperfusion (I/R)-associated donor organ damage is inevitable in all solid organ transplantation, and is caused by enhanced oxidant stress with release of inflammatory cytokines, such as tumor growth factor-α (TNF-α) and interleukin 2 (IL-2). Although the precise molecular mechanism of the I/R-associated liver injury remains to be investigated, enhanced oxidant stress with release of superoxide anions or H_2O_2, depletion of the reduced form of glutathione and increased lipid peroxidation has been the key element in the pathogenesis in orthotopic liver transplantation (OLT) or small size liver graft transplantation (SSLGT) [54-56]. Thus, it is rational to use of antioxidant gene transfer to minimize oxidant stress and improve the donor organ quality and function after the implantation. We delivered either EC-SOD, catalase gene or in combination, using the same approach as described above. Two days after the delivery, the transgene expression was increased for 10-50-fold, with increased SOD or catalase activity in the mouse liver. This delivery led to a marked decrease in superoxide anion levels and H_2O_2 release along with a decrease in serum ALT levels, liver lipid peroxidation and dramatic improvement of liver histology [49]. This study was positively commented by two well-known hepatologists from Europe as an editorial, quoting "beyond a proof of the principle, the study could be the basis for studies with larger animals and may help bridge the gap between the basic understanding of pathophysiologic processes in animal models towards a practical clinical application in liver transplantation" [57]. The findings are especially applicable in living donor liver transplantation, for which small or margin donor livers were used for transplantation. Much more pronounced oxidant stress, a higher rate of graft failure, and retarded graft growth are found in small size liver transplantation than OLT [54, 58]. The margin grafts with small size or steatosis and fibrotic deposition are often used for transplantation in clinics due to severe shortage of donor organs.

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6. Challenges in scaling-up and moving towards clinical applications

Our preclinical studies were performed in mice, and there are certainly a number of issues to face when this anti-oxidant gene therapy approach is considered to be evaluated in middle or large size animals such as rabbits, dogs, monkeys or pigs. The first issue is to scale-up, which includes the plasmid DNA generation, synthesis of PCL in a quantity, and formulation of PLNP at a volume sufficient enough for the use in large animals. More challenges exist regarding how to stimulate liver cells to proliferate in large animals and deliver polyplexes locally to the liver. Using a catheter through the femoral vein or jugular vein for retrograde administration into hepatic vein or passing into the portal vein for administration similar to the transjugular intrahepatic portosystemic shunt (TIPS) procedure, which is used to lower portal hypertension in cirrhotic patients, should be feasible in large animals when angiography and the administration are performed by an experienced specialist with the availability of angiographic devices. The latter method was used to administer adenoviral vector in baboons [59]. One trial of plasmid DNA injection into the hepatic vein by blocking the hepatic vein out-flow with an inflated balloon achieved high gene expression levels in selected pig liver lobes [30]. Safety concerns include amount of polyplexes to be administrated locally and the effects of the plasmid DNA, PLNP and polyplexes on the liver as well as systematically. LNP-mediated gene transfer is usually transient; therefore, there will be less concern for long-term effects of the transgene products on the host. However, immune reaction to human gene products in animals may occur if the transgene products are produced at sustained levels for a long period of time. It is preventable by administration of immunosuppressive agents, such as FK506. Moreover, innate immunity to plasmid DNA with bacterial unmethylated CG dinucleotide (CpG) can be eliminated by using CpG-free plasmid [60].

An additional concern is to establish a liver injury model to evaluate the effect of anti-oxidant gene transfer by PLNP in large animals. For pigs, exposure to a loading dose of 0.25 g/kg, maintaining the blood concentration of acetaminophen at 350-450 mg/dl, and adapting enteric maintenance dose of 1,000-3,000 mg/hour resulted in the onset of acute liver failure (prothrombin time value <30%) within 32±4.4 hours, and further mortality in 15.8±2.4 hours [61]. A large dose of acetaminophen intake causes significant oxidant stress and acute liver injury due to its metabolism and generation of an interactive metabolite, n-acetyl-p-benzoquinone imine (NAPQI), which binds to the cytoplasmic membrane, leads to lipid peroxidation, depletion of antioxidants, such as glutathione, and results in hepatic injury. Not only will the delivery of antioxidant genes with PLNP in a pig model of liver injury assess the therapeutic efficacy, but also take advantage of a regenerative response to the injury for high transgene expression. Alternatively, small size graft liver transplantation (SSGLT) at ≤50% graft volume could be performed in rabbits or pigs to mimic living donor liver transplantation in humans. Significant oxidant stress-associated injury and regenerative response in the small size grafts will be the best fit for the high transgene expression and ROS scavenging property of the gene product. Therefore, SSGLT may be considered to be a valuable model for evaluating the feasibility and efficacy of anti-oxidant gene transfer for small-for-size-associated graft failure in a transplant setting.

In summary, moving promising PLNP-mediated antioxidant gene transfer from small animals to large animals may face more challenges than discussed above, and it is even more challenging when further considering for clinical use, in terms of safety concern and administrative approval. Fig. 3 provides a schematic illustration of the roadmap from bench to bedsides of a potential biological therapy. The reality is that with limited funding opportunities from governmental or private agencies, to cope with multi-facet challenges at a large scale, it is less likely to reach the final goal in a short term. Attracting financial investments and taking advantages of cutting-edging technologies and vast resources from biopharmaceutical companies may advance this process in a fast pace. In this context, the net benefits would be the early clinical application of this promising antioxidant gene transfer in patients with critical needs and the financial return from the investment. We would foresee such a movement occurring in the near future.

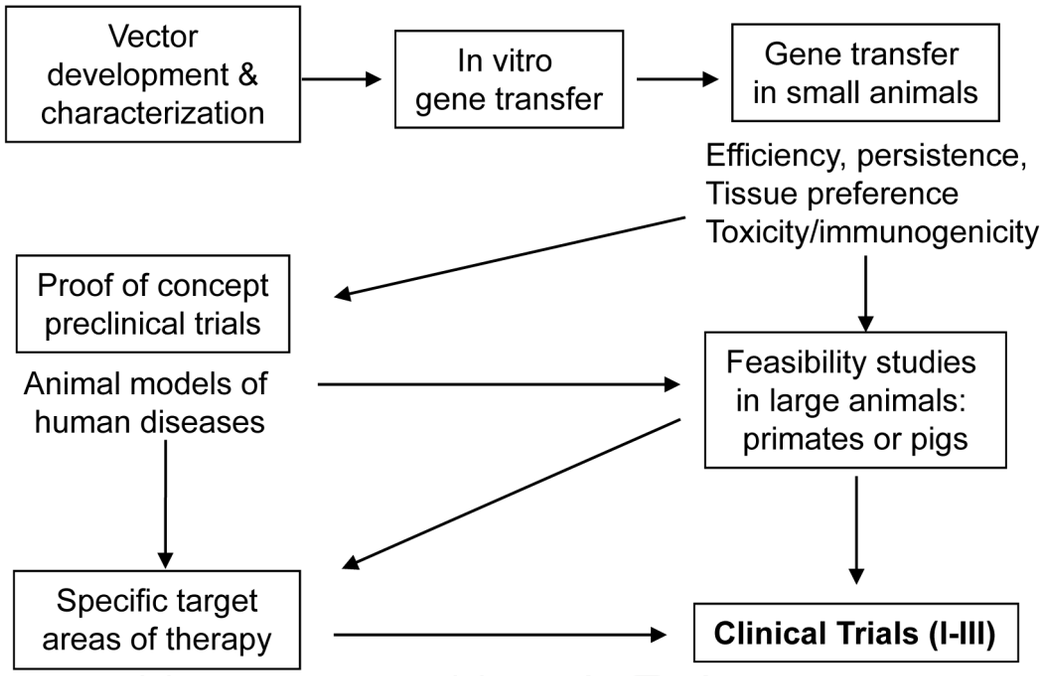

Figure 3. Translation roadmap of a potential gene therapy platform from bench to bedside. This illustration summarizes the major steps in moving a potential gene transfer approach from laboratory research to clinical trials. The actual actions could be more complicated than described. However, for the guarantee of patient safety, each new therapeutic agent must be well characterized, and evaluated in preclinical settings, and then move to large animals for feasibility assessment. The balance between therapeutic benefits and potential risks of an innovative therapy platform always leans on the patient safety as the first priority.

7. Conclusion and prospectives

Non-viral vector-mediated gene transfer has less concern in terms of integration-associated long-term transgene expression and insertion-induced mutation. In general, non-viral vector elicits minimal immune responses in contrast to adenoviral vectors [17]. However, non-viral

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vectors, such as lipid nanoparticles (LNPs) possess their own drawbacks when they are considered for *in vivo* use. One prominent issue is the interaction of cationic LNPs with serum protein and blood cells, and this causes a series of issues, such as instability of the lipoplexes or polyplexes and adverse effects to the host, including non-preferential distribution, embolism of the aggregates of lipoplex-protein or blood cells, and inflammatory responses. For these reasons, many gene transfer agents are very effective in cell culture; whereas they have less applicability *in vivo*. Up to date, only a few formulations of cationic LNPs have proved to be effective and safe in animals and have reached the stage of clinical trials, such as DOTAP-Chol and DC-Chol. Our PLNP formulation has a superior stability profile, and displayed much less reactivity to serum proteins and blood cells when compared to other commercially available formulations. At the same time, it has proved to be the most effective liver-based gene transfer agent [6]. Two preclinical trials with different models of oxidant-stress-associated liver injury have demonstrated the effectiveness of the anti-oxidant gene delivery in the liver, and the efficacy of the gene delivery in minimizing oxidant-stress, attenuating liver cell death, and improving liver histology [48, 49]. Further efforts have been made to move this promising PLNP-mediated anti-oxidant gene transfer technology from bench to bedside. The strategies in pushing this movement towards clinical trials include: 1) Scaling-up of the polycationic lipid production and generation of PLNPs; 2) Generation of specific antioxidant gene plasmids in a GMP facility at the standard for clinical use; 3) Establishing large animal models for safety and efficacy assessment; and 4) Preparation for obtaining administrative approval of clinical application. Although the clinical translation of this potential technology will need tremendous efforts, we anticipate that this technology will eventually reach to patients with critical needs as a novel therapy. Potential indications which may benefit from this therapy range from alcohol or drug toxicity to living donor liver transplantation with a margin graft. This technology is also applicable in oxidant stress-associated disorders in other systems, such as ischemic cardiac, pulmonary, brain or renal damage, etc. [17]. With the combination of our extensive expertise in drug and gene delivery, advanced knowledge and skills in liver injury, fibrosis, transplant and cancer research and practice, in addition to the engine of financial investment from various sources, such as venture capital and governmental support in entrepreneurship, we are optimistic to foresee the benefits of this technology in indicated patients in a near future. Nevertheless, the road to reach this goal will not be smooth, and various challenges demand powerful solutions.

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Abbreviations used in the chapter

ASGP-R = asialoglycoprotein receptor; DOTAP = (dioleoyloxy)-3-(trimethylamonio) propane; DOPE = L-a dioleoyl phosphatidylethanolamine; EC-SOD = extracellular superoxide dismutase; HCC = hepatocellular carcinoma; LDLT = Living donor liver transplantation; LNP = lipid nanoparticles; OLT = orthotopic liver transplantation; PEG = polyethylene glycol; PLNP = polylipid nanoparticles; polyplex = PLNP-plasmid DNA complex; RES = reticuloendothelial system.

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References

[1] Wu J, Zern MA. Modification of liposomes for liver targeting. J Hepatol 1996;24:757-763.

[2] Wu J, Wu GY, Zern MA. The prospects of hepatic drug delivery and gene therapy. Expert Opin Investig Drugs 1998;7:1795-1817.

[3] Du SL, Pan H, Lu WY, Wang J, Wu J, Wang JY. Cyclic Arg-Gly-Asp peptide-labeled liposomes for targeting drug therapy of hepatic fibrosis in rats. J Pharmacol Exp Ther 2007;322:560-568.

[4] Wu J, Liu P, Zhu JL, Maddukuri S, Zern MA. Increased liver uptake of liposomes and improved targeting efficacy by labeling with asialofetuin in rodents. Hepatology 1998;27:772-778.

[5] Abegunewardene N, Schmidt KH, Vosseler M, Kreitner KF, Schreiber LM, Lehr HA, Gori T, et al. Gene therapy with iNOS enhances regional contractility and reduces delayed contrast enhancement in a model of postischemic congestive heart failure. Clin Hemorheol Microcirc 2011;49:271-278.

[6] Liu L, Zern MA, Lizarzaburu ME, Nantz MH, Wu J. Poly(cationic lipid)-mediated in vivo gene delivery to mouse liver. Gene Ther 2003;10:180-187.

[7] Wu J, Lizarzaburu ME, Kurth MJ, Liu L, Wege H, Zern MA, Nantz MH. Cationic lipid polymerization as a novel approach for constructing new DNA delivery agents. Bioconjug Chem 2001;12:251-257.

[8] Nyunt MT, Dicus CW, Cui YY, Yappert MC, Huser TR, Nantz MH, Wu J. Physicochemical characterization of polylipid nanoparticles for gene delivery to the liver. Bioconjug Chem 2009;20:2047-2054.

[9] Lu C, Stewart DJ, Lee JJ, Ji L, Ramesh R, Jayachandran G, Nunez MI, et al. Phase I clinical trial of systemically administered TUSC2(FUS1)-nanoparticles mediating functional gene transfer in humans. PLoS One 2012;7:e34833.

[10] Ito I, Ji L, Tanaka F, Saito Y, Gopalan B, Branch CD, Xu K, et al. Liposomal vector mediated delivery of the 3p FUS1 gene demonstrates potent antitumor activity against human lung cancer in vivo. Cancer Gene Ther 2004;11:733-739.

[11] Sakurai F, Nishioka T, Saito H, Baba T, Okuda A, Matsumoto O, Taga T, et al. Interaction between DNA-cationic liposome complexes and erythrocytes is an important factor in systemic gene transfer via the intravenous route in mice: the role of the neutral helper lipid. Gene Ther 2001;8:677-686.

[12] Fumoto S, Kawakami S, Shigeta K, Higuchi Y, Yamashita F, Hashida M. Interaction with blood components plays a crucial role in asialoglycoprotein receptor-mediated in vivo gene transfer by galactosylated lipoplex. J Pharmacol Exp Ther 2005;315:484-493.

[13] Fumoto S, Kawakami S, Ito Y, Shigeta K, Yamashita F, Hashida M. Enhanced hepatocyte-selective in vivo gene expression by stabilized galactosylated liposome/plasmid DNA complex using sodium chloride for complex formation. Mol Ther 2004;10:719-729.

[14] Liu Y, Mounkes LC, Liggitt HD, Brown CS, Solodin I, Heath TD, Debs RJ. Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. Nat Biotechnol 1997;15:167-173.

[15] Schleh C, Rothen-Rutishauser B, Kreyling WG. The influence of pulmonary surfactant on nanoparticulate drug delivery systems. Eur J Pharm Biopharm 2011;77:350-352.

[16] Ramesh R, Ito I, Saito Y, Wu Z, Mhashikar AM, Wilson DR, Branch CD, et al. Local and systemic inhibition of lung tumor growth after nanoparticle-mediated mda-7/IL-24 gene delivery. DNA Cell Biol 2004;23:850-857.

[17] Wu J, Hecker JG, Chiamvimonvat N. Antioxidant enzyme gene transfer for ischemic diseases. Adv Drug Deliv Rev 2009;61:351-363.

[18] Wei M, Xu Y, Zou Q, Tu L, Tang C, Xu T, Deng L, et al. Hepatocellular carcinoma targeting effect of PEGylated liposomes modified with lactoferrin. Eur J Pharm Sci 2012;46:131-141.

[19] Zheng S, Chang S, Lu J, Chen Z, Xie L, Nie Y, He B, et al. Characterization of 9-nitrocamptothecin liposomes: anticancer properties and mechanisms on hepatocellular carcinoma in vitro and in vivo. PLoS One 2011;6:e21064.

[20] Khalil IA, Hayashi Y, Mizuno R, Harashima H. Octaarginine- and pH sensitive fusogenic peptide-modified nanoparticles for liver gene delivery. J Control Release 2011;156:374-380.

[21] Karmali PP, Chaudhuri A. Cationic liposomes as non-viral carriers of gene medicines: resolved issues, open questions, and future promises. Med Res Rev 2007;27:696-722.

[22] Al-Jamal WT, Kostarelos K. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine. Acc Chem Res 2011;44:1094-1104.

[23] Li Y, Zhu Y, Xia K, Sheng R, Jia L, Hou X, Xu Y, et al. Dendritic poly(L-lysine)-b-Poly(L-lactide)-b-dendritic poly(L-lysine) amphiphilic gene delivery vectors: roles of PLL dendritic generation and enhanced transgene efficacies via termini modification. Biomacromolecules 2009;10:2284-2293.

[24] Byeon JH, Kim HK, Roberts JT. Monodisperse Poly(lactide-co-glycolic acid)-Based Nanocarriers for Gene Transfection. Macromol Rapid Commun 2012; 23:1821-1825.

[25] Nogueira N, Conde O, Minones M, Trillo JM, Minones J, Jr. Characterization of poly(2-hydroxyethyl methacrylate) (PHEMA) contact lens using the Langmuir monolayer technique. J Colloid Interface Sci 2012;385:202-210.

[26] So H, Lee J, Han SY, Oh HB. MALDI In-Source Decay Mass Spectrometry of Polyamidoamine Dendrimers. J Am Soc Mass Spectrometry 2012:DOI: 10.1007/s13361-13012-10445-13364.

[27] Poelstra K, Prakash J, Beljaars L. Drug targeting to the diseased liver. J Control Release 2012;161:188-197.

[28] Wu J, Nantz MH, Zern MA. Targeting hepatocytes for drug and gene delivery: emerging novel approaches and applications. Front Biosci 2002;7:d717-725.

[29] Alino SF, Benet M, Dasi F, Crespo J. Asialofetuin liposomes for receptor-mediated gene transfer into hepatic cells. Methods Enzymol 2003;373:399-421.

[30] Alino SF, Herrero MJ, Noguera I, Dasi F, Sanchez M. Pig liver gene therapy by noninvasive interventionist catheterism. Gene Ther 2007;14:334-343.

[31] Li Y, Huang G, Diakur J, Wiebe LI. Targeted delivery of macromolecular drugs: asialoglycoprotein receptor (ASGPR) expression by selected hepatoma cell lines used in antiviral drug development. Curr Drug Deliv 2008;5:299-302.

[32] Chen X, Lingala S, Khoobyari S, Nolta J, Zern MA, Wu J. Epithelial mesenchymal transition and hedgehog signaling activation are associated with chemoresistance and invasion of hepatoma subpopulations. J Hepatol 2011;55:838-845.

[33] Hyodo I, Mizuno M, Yamada G, Tsuji T. Distribution of asialoglycoprotein receptor in human hepatocellular carcinoma. Liver 1993;13:80-85.

[34] Sawamura T, Nakada H, Hazama H, Shiozaki Y, Sameshima Y, Tashiro Y. Hyperasialoglycoproteinemia in patients with chronic liver diseases and/or liver cell carcinoma. Asialoglycoprotein receptor in cirrhosis and liver cell carcinoma. Gastroenterology 1984;87:1217-1221.

[35] Dorasamy S, Narainpersad N, Singh M, Ariatti M. Novel targeted liposomes deliver siRNA to hepatocellular carcinoma cells in vitro. Chem Biol Drug Des 2012:10.1111/j.1747-0285.2012.01446.x.

[36] Feng M, Cai Q, Shi X, Huang H, Zhou P, Guo X. Recombinant high-density lipoprotein complex as a targeting system of nosiheptide to liver cells. J Drug Targeting 2008;16:502-508.

[37] Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, Jayaraman M, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol Ther 2010;18:1357-1364.

[38] Wu J, Zern MA. Hepatic stellate cells: a target for the treatment of liver fibrosis. J Gastroenterol 2000;35:665-672.

[39] Beljaars L, Molema G, Weert B, Bonnema H, Olinga P, Groothuis GM, Meijer DK, et al. Albumin modified with mannose 6-phosphate: A potential carrier for selective delivery of antifibrotic drugs to rat and human hepatic stellate cells. Hepatology 1999;29:1486-1493.

[40] Li F, Li QH, Wang JY, Zhan CY, Xie C, Lu WY. Effects of interferon-gamma liposomes targeted to platelet-derived growth factor receptor-beta on hepatic fibrosis in rats. J Control Release 2012;159:261-270.

[41] Beljaars L, Olinga P, Molema G, de Bleser P, Geerts A, Groothuis GM, Meijer DK, et al. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P(28)-HSA). Liver 2001;21:320-328.

[42] Li F, Song Z, Li Q, Wu J, Wang J, Xie C, Tu C, et al. Molecular imaging of hepatic stellate cell activity by visualization of hepatic integrin alphavbeta3 expression with SPECT in rat. Hepatology 2011;54:1020-1030.

[43] Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, Takimoto R, et al. Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol 2008;26:431-442.

[44] Yen RD, Zern MA, Wu J. Molecular therapy for hepatic fibrosis. Hauppauge, NY.: Nova Science Publishers, 2006: 1-23.

[45] Nishikawa M, Huang L. Nonviral vectors in the new millennium: delivery barriers in gene transfer. Hum Gene Ther 2001;12:861-870.

[46] Diez S, Navarro G, de ICT. In vivo targeted gene delivery by cationic nanoparticles for treatment of hepatocellular carcinoma. J Gene Med 2009;11:38-45.

[47] Li S, Rizzo MA, Bhattacharya S, Huang L. Characterization of cationic lipid-protamine-DNA (LPD) complexes for intravenous gene delivery. Gene Ther 1998;5:930-937.

[48] Wu J, Liu L, Yen RD, Catana A, Nantz MH, Zern MA. Liposome-mediated extracellular superoxide dismutase gene delivery protects against acute liver injury in mice. Hepatology 2004;40:195-204.

[49] He SQ, Zhang YH, Venugopal SK, Dicus CW, Perez RV, Ramsamooj R, Nantz MH, et al. Delivery of antioxidative enzyme genes protects against ischemia/reperfusion-induced liver injury in mice. Liver Transpl 2006;12:1869-1879.

[50] Wu J, Danielsson A, Zern MA. Toxicity of hepatotoxins: new insights into mechanisms and therapy. Expert Opin Investig Drugs 1999;8:585-607.

[51] Wu J, Karlsson K, Danielsson A. Effects of vitamins E, C and catalase on bromobenzene- and hydrogen peroxide-induced intracellular oxidation and DNA single-strand breakage in Hep G2 cells. J Hepatol 1997;26:669-677.

[52] Wu J, Karlsson K, Danielsson A. Protective effects of trolox C, vitamin C, and catalase on bromobenzene-induced damage to rat hepatocytes. Scand J Gastroenterol 1996;31:797-803.

[53] Wu J, Soderbergh H, Karlsson K, Danielsson A. Protective effect of S-adenosyl-L-methionine on bromobenzene- and D-galactosamine-induced toxicity to isolated rat hepatocytes. Hepatology 1996;23:359-365.

[54] Cui Y-Y, Qian J-M, Yao A-H, Ma Z-Y, Qian X-F, Zha X-M, Zhao Y, et al. SOD mimetic improves the function, growth, and survival of small-size liver grafts after transplantation in rats. Transplantation 2012; 94:687-694.

[55] Qian JM, Zhang H, Wu XF, Li GQ, Chen XP, Wu J. Improvement of recipient survival after small size graft liver transplantation in rats with preischemic manipulation or administering antisense against nuclear factor-kappaB. Transplant Int 2007;20:784-789.

[56] Liu PG, He SQ, Zhang YH, Wu J. Protective effects of apocynin and allopurinol on ischemia/reperfusion-induced liver injury in mice. World J Gastroenterol 2008;14:2832-2837.

[57] Luedde T, Trautwein C. The role of oxidative stress and antioxidant treatment in liver surgery and transplantation. Liver Transpl 2006;12:1733-1735.

[58] Cerullo V, Seiler MP, Mane V, Brunetti-Pierri N, Clarke C, Bertin TK, Rodgers JR, et al. Toll-like receptor 9 triggers an innate immune response to helper-dependent adenoviral vectors. Mol Ther 2007;15:378-385.

[59] Brunetti-Pierri N, Stapleton GE, Palmer DJ, Zuo Y, Mane VP, Finegold MJ, Beaudet AL, et al. Pseudo-hydrodynamic delivery of helper-dependent adenoviral vectors into non-human primates for liver-directed gene therapy. Mol Ther 2007;15:732-740.

[60] Hyde SC, Pringle IA, Abdullah S, Lawton AE, Davies LA, Varathalingam A, Nunez-Alonso G, et al. CpG-free plasmids confer reduced inflammation and sustained pulmonary gene expression. Nat Biotechnol 2008;26:549-551.

[61] Thiel C, Thiel K, Etspueler A, Morgalla MH, Rubitschek S, Schmid S, Steurer W, et al. A reproducible porcine model of acute liver failure induced by intrajejunal acetaminophen administration. Eur Surg Res 2011;46:118-126.

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