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Mini-Review

# Liver-directed Gene Therapy

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**Submitted:** 20 November 2013

**Accepted:** 22 November 2013

**Published:** 25 November 2013



Liver is a vital organ in the body responsible for detoxification, protein synthesis and metabolism. Pathologically, the liver is involved in many metabolic and monogenic diseases [1]. The intrinsic anatomic properties of liver make it a preferred target for gene therapy of liver originated or monogenic diseases. Although none of the currently available methods of gene delivery is optimal for liver gene therapy, the concerted effort from researchers has provided a wide range of choices for gene transfer to the liver [2-4]. The objective of this mini-review is to provide a brief summary for various methods developed thus far that are applicable to liver gene therapy **(Table 1)**. Major advantages and disadvantages of each method are also provided for practical consideration

## Virus-based gene-delivery system

Virus-based gene delivery system represents a group of artificially made, replication deficient viruses [5]. The most commonly used ones are adeno-associated viral vectors [6], lentiviral vectors [7], and adenoviral vectors [8]. Viral vectors under the development include foamy viral vectors [9], herpes simplex viral vectors [10], and oncoretroviral vectors [11]. Viral vector-mediated gene delivery to liver can be achieved *via* the hepatic artery [12,13], portal vein [14,15], or bile duct [14] or by direct injection to the liver [14]. Recent progress in a pilot phase-II trial revealed that the hepatic arterial injection of recombinant adenovirus p53 is safe and effective in unresectable hepatocellular carcinoma [15]. Adeno-associated virus 8 prefers hepatocytes [17] and has been used for liver-targeted gene therapy intended for treatment of the citrullinemia [18], hemophilia [19], alpha 1-antitrypsin deficiency [20] and viral hepatitis [21] diseases. Viral vectors are highly effective in gene delivery and have been used in approximately 67% clinical trials [22]. Viral vector based carcinogenesis and immunogenicity represent currently the major hurdle for viral vector-mediated gene therapy.

## Nonviral gene-delivery system

Compared to viral vectors that employ their natural ability to transfer gene into cells, nonviral gene delivery systems use a physical force or cellular function of endocytosis to facilitate gene transfer to target cells. They are divided into two categories including nonviral vector-mediated gene delivery and physical methods.

Nonviral vectors are synthetic or natural compounds that are capable of forming complexes with plasmid DNA or gene coding fragments and facilitating intracellular gene transfer. Materials including lipids [23], polymers [23], proteins [24], and peptides [25] have been shown to be effective for gene delivery. Nonviral vectors have been evaluated for gene therapy of varieties of liver diseases including hepatic fibrosis, viral hepatitis, and liver cancer [26]. Taking advantage of membrane receptors on hepatic stellate cells, liver-targeted gene delivery for hepatic fibrosis has been attempted using mannose 6-phosphate/insulin-like growth factor-II receptor [27], integrins [28], high-affinity membrane receptor for retinol-binding protein [29], and galactosyl receptor [30] as the targets. Target specific gene delivery is a most desirable feature of any gene delivery systems. Clinically, 24% of gene therapy clinical trials have been conducted using nonviral vectors [22]. The major challenge for nonviral vector-mediated gene delivery is its relatively low efficiency.

Physical methods of gene delivery employ a physical force to overcome the membrane barrier of a cell. Compared to viral and nonviral vector-mediated gene delivery, physical approaches do not involve any substances that could be cytotoxic or immunogenic. Physical methods employed for gene delivery include needle injection, gene gun, electroporation, sonoporation, and hydrodynamic gene delivery [2]. Among these methods, hydrodynamic gene delivery has been the most efficient method for gene delivery to the liver, especially in small animals. This method has been used for functional analysis of therapeutic genes and regulatory elements in rodents since its establishment in 1999 [31,32]. Efforts have been made in developing a clinically applicable procedure for hydrodynamic gene delivery to the liver. For instance, Kamimura *et al*. examined a catheter insertion technique to hepatic lobular vein, which is a clinically well-established method, for site-specific, safe, and efficient gene delivery in large animals [33-35]. In combination with computer programming, engineering, and imaging technology, it is highly possible that an effective, simple, and safe hydrodynamic gene delivery to selected site of the liver will be achieved in near future. The remaining challenge for hydrodynamic gene delivery for gene therapy of liver diseases is to conduct safety and efficacy assessment in nonhuman primates to fine-tune different

**Table 1:** Features of Liver-directed Gene Delivery Systems.

| Method | Functional Component | Advantages | Disadvantage |
|---|---|---|---|
| **Viral Vectors** | | | |
| **Oncoretrovirus** | RNA | High efficiency | Random integration, low titer |
| **Lentivirus** | RNA | High efficiency, sustained gene expression | Random integration, low titer |
| **Foamy virus** | RNA | High efficiency, sustained gene expression | Random integration, low titer |
| **Adenovirus** | Double stranded DNA | High efficiency, sustained gene expression, infect non-dividing cells | Host innate immune response |
| **Adeno-associated virus** | Single stranded DNA | No pathogenic, sustained gene expression, infect to non-dividing cells | Integration may occur, small capacity of transgene, low titer |
| **Herpes simplex virus** | Double stranded DNA | No integration, sustained gene expression | Low transduction efficiency |
| **Nonviral Vectors** | | | |
| **Lipids** | Cationic lipids | High efficiency *in vitro*, ease to prepare | Low efficiency *in vivo*, acute immune response |
| **Polymers** | Cationic polymers | Highly effective *in vitro*, ease to prepare | Toxic to cells, acute immune response |
| **Proteins** | Natural or chemically modified proteins in cationic nature | Highly effective *in vitro*, less toxic, can be target specific | Low activity *in vivo* |
| **Peptides** | Lysine or arginine residues in peptides | Highly effective *in vitro*, less toxic, can be target specific | Low activity *in vivo* |
| **Physical Methods** | | | |
| **Needle injection** | Mechanic force | Simple | Low efficiency, expression limited to needle track |
| **Gene gun** | Pressure | Good efficiency | Limited to target area, need surgical procedure for internal organ |
| **Electroporation** | Electric pulse | High efficiency | Tissue damage, limited target area, need surgical procedure for internal organ |
| **Sonoporation** | Ultrasound | Site specific | Low efficiency, tissue damage |
| **Hydrodynamic delivery** | Hydrodynamic pressure | Simple, high efficiency, site specific | Need catheter insertion technique in large animals |

parameters in order to ensure clinical success in gene therapy for various liver diseases.

## PERSPECTIVES

Despite the progress made in developing various methods for effective gene delivery, gene therapy for treatment of liver diseases remains in its infancy. This is primarily due to the fact that many of the liver diseases progress into a fibrotic stage with significant change of liver parenchyma, vasculature, and sinusoids. Consequently, efficient gene delivery by various highly effective methods established using health liver in animals cannot be achieved, resulting in insufficient production of gene product and failure to achieve a successful cure. Evidently, future studies need to take into the consideration of disease status when optimizing a method of gene delivery. There is no doubt, however, gene therapy will become one of the most effective treatments for liver diseases that are not curable with currently available modalities.

## ACKNOWLEDGMENT

The authors would like to thank Enago for the English language review. The research in the authors' laboratory has been supported in part by grant support from the Japanese Society for the Promotion of Sciences (22890064 and 23790595), Takara Bio Research Grant from JSGT, and Adaptable and Seamless Technology Transfer Program through target-driven R&D, JST to Kenya Kamimura, and from the National Institute of Health (EB002946 and HL075542) to Dexi Liu.

## REFERENCES

1. Dooley S, Lok A, Burroughs A, Heathcote J. Sherlock's diseases of the liver and biliary system, 12th Edition, Wiley-Blackwell, 2011.
2. Kamimura K, Suda T, Zhang G, Liu D. Advances in Gene Delivery Systems. Pharmaceut Med. 2011; 25: 293-306.
3. Kamimura K, Liu D. Physical approaches for nucleic acid delivery to liver. AAPS J. 2008; 10: 589-595.
4. Suda T, Kamimura K, Kubota T, Tamura Y, Igarashi M, Kawai H, et al. Progress toward liver-based gene therapy. Hepatol Res. 2009; 39: 325-340.
5. Thomas CE, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. Nat Rev Genet. 2003; 4: 346-358.
6. van der Laan LJ, Wang Y, Tilanus HW, Janssen HL, Pan Q. AAV-mediated gene therapy for liver diseases: the prime candidate for clinical application? Expert Opin Biol Ther. 2011; 11: 315-327.
7. Dismuke D, Samulski RJ. Hepatic gene therapy using lentiviral vectors: has safety been established? Hepatology. 2013; 58: 13-14.
8. Vetrini F, Ng P. Liver-directed gene therapy with helper-dependent adenoviral vectors: current state of the art and future challenges. Curr Pharm Des. 2011; 17: 2488-2499.
9. Lindemann D, Rethwilm A. Foamy virus biology and its application for vector development. Viruses. 2011; 3: 561-585.
10. Manservigi R, Argnani R, Marconi P. HSV Recombinant Vectors for Gene Therapy. Open Virol J. 2010; 4: 123-156.
11. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. New Microbiol. 2013; 36: 1-22.

12. Bell P, Gao G, Haskins ME, Wang L, Sleeper M, Wang H, et al. Evaluation of adeno-associated viral vectors for liver-directed gene transfer in dogs. Hum Gene Ther. 2011; 22: 985-997.

13. Brunetti-Pierri N, Liou A, Patel P, Palmer D, Grove N, Finegold M, et al. Balloon catheter delivery of helper-dependent adenoviral vector results in sustained, therapeutic hFIX expression in rhesus macaques. Mol Ther. 2012; 20: 1863-70.

14. Sobrevals L, Enguita M, Rodriguez C, Gonzalez-Rojas J, Alzaguren P, Razquin N, et al. AAV vectors transduce hepatocytes in vivo as efficiently in cirrhotic as in healthy rat livers. Gene Ther. 2012; 19: 411-417.

15. Sabatino DE, Lange AM, Altynova ES, Sarkar R, Zhou S, Merricks EP, et al. Efficacy and safety of long-term prophylaxis in severe hemophilia A dogs following liver gene therapy using AAV vectors. Mol Ther. 2011; 19: 442-449.

16. Tian G, Liu J, Zhou JS, Chen W. Multiple hepatic arterial injections of recombinant adenovirus p53 and 5-fluorouracil after transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: a pilot phase II trial. Anticancer Drugs. 2009; 20: 389-95.

17. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. Clin Microbiol Rev. 2008; 21: 583-593.

18. Chandler RJ, Tarasenko TN, Cusmano-Ozog K, Sun Q, Sutton VR, Venditti CP, et al. Liver-directed adeno-associated virus serotype 8 gene transfer rescues a lethal murine model of citrullinemia type 1. Gene Ther. 2013; .

19. Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. N Engl J Med. 2011; 365: 2357-2365.

20. Li H, Lu Y, Witek RP, Chang LJ, Campbell-Thompson M, Jorgensen M, et al. Ex vivo transduction and transplantation of bone marrow cells for liver gene delivery of alpha1-antitrypsin. Mol Ther. 2010; 18: 1553-1558.

21. Suhy DA, Kao SC, Mao T, Whiteley L, Denise H, Souberbielle B, et al. Safe, long-term hepatic expression of anti-HCV shRNA in a nonhuman primate model. Mol Ther. 2012; 20: 1737-1749.

22. Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. Gene therapy clinical trials worldwide to 2012 - an update. J Gene Med. 2013; 15: 65-77.

23. Jones CH, Chen CK, Ravikrishnan A, Rane S, Pfeifer BA. Overcoming Nonviral Gene Delivery Barriers: Perspective and Future. Mol Pharm. 2013; .

24. Lam AP, Dean DA. Progress and prospects: nuclear import of nonviral vectors. Gene Ther. 2010; 17: 439-447.

25. Margus H, Padari K, Pooga M. Cell-penetrating peptides as versatile vehicles for oligonucleotide delivery. Mol Ther. 2012; 20: 525-533.

26. Reddy LH, Couvreur P. Nanotechnology for therapy and imaging of liver diseases. J Hepatol. 2011; 55: 1461-1466.

27. Adrian JE, Poelstra K, Scherphof GL, Meijer DK, van Loenen-Weemaes AM, Reker-Smit C, et al. Effects of a new bioactive lipid-based drug carrier on cultured hepatic stellate cells and liver fibrosis in bile duct-ligated rats. J Pharmacol Exp Ther. 2007; 321: 536-543.

28. 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.

29. Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, 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.

30. Mandal AK, Das S, Basu MK, Chakrabarti RN, Das N. Hepatoprotective activity of liposomal flavonoid against arsenite-induced liver fibrosis. J Pharmacol Exp Ther. 2007; 320: 994-1001.

31. Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther. 1999; 6: 1258-1266.

32. Zhang G, Budker V, Wolff JA. High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. Hum Gene Ther. 1999; 10: 1735-1737.

33. Kamimura K, Suda T, Xu W, Zhang G, Liu D. Image-guided, lobe-specific hydrodynamic gene delivery to swine liver. Mol Ther. 2009; 17: 491-499.

34. Kamimura K, Zhang G, Liu D. Image-guided, intravascular hydrodynamic gene delivery to skeletal muscle in pigs. Mol Ther. 2010; 18: 93-100.

35. Kamimura K, Suda T, Zhang G, Aoyagi Y, Liu D. Parameters Affecting Image-guided, Hydrodynamic Gene Delivery to Swine Liver. Mol Ther Nucleic Acids. 2013; 2: e128.

**Cite this article**

*Kamimura K, Abe H, Suda T, Aoyagi Y, Liu D (2013) Liver-directed Gene Therapy. JSM Gastroenterol Hepatol 1(1): 1005.*