ELSEVIER

Journal of Hepatology 40 (2004) 337–340

Journal of Hepatology

www.elsevier.com/locate/jhep

Editorial

The promise of lentiviral gene therapy for liver cancer

Antonia Follenzi, Sanjeev Gupta*

Departments of Medicine and Pathology, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Ullmann 625, 1300 Morris Park Avenue, Bronx, NY 10461, USA

See Article, pages 291–297

The liver remains a prominent target for gene therapy, not only because of the importance of liver-directed gene therapy for metabolic and deficiency states, but for liver cancer as well. Hepatocellular carcinoma (HCC) accounts for over 90% of all primary liver cancers and leads to nearly 1 million deaths annually. This will increase further in the future as the incidence of HCC is rising throughout the world in association with large reservoirs of chronic hepatitis B and C. Metastatic disease constitutes another leading cause of liver-related mortalities. Only a very small fraction of patients with HCC or other types of malignant liver disease qualifies for surgical resection. Far fewer patients with liver cancer undergo orthotopic liver transplantation (OLT), although OLT can produce excellent outcomes in early HCC and more patients are receiving OLT following the adoption of new criteria for organ allocation. Nonetheless, the vast majority of liver cancer patients remain untreated, since other therapies are mostly palliative, and novel therapies are desperately needed.

Although gene therapy could be an exciting approach for liver cancer, it has largely been confined to preclinical and experimental settings [1]. Over the past decade, despite extensive work by numerous groups, results of gene therapy in people have been mixed, with few successes and notable complications, e.g. adenoviral and retroviral vector-related toxicities in patients with genetic deficiency disorders [2,3]. These results focused further efforts in the areas of vector development, limiting toxicities of viral vectors, including genomic integrations and activation of deleterious endogenous genes, organ- or cell type-specific gene targeting, and induction of regulatable or sustained gene expression. Fortunately, significant advances in gene therapy have also been made over the past several years, including in the development of effective gene transfer vectors [4].

In particular, lentiviral vectors (LV) are beginning to overcome the challenges of efficient delivery and long-term expression of transgenes without toxicity. Most commonly, replication-defective hybrid LV particles containing core proteins and enzymes of human immunodeficiency virus 1 (HIV-1) are pseudotyped with the G protein of the vesicular stomatitis virus (VSV-G). Pseudotyping refers to the use of an envelope from an unrelated virus to obtain recombinant vector particles. The tropism of vectors can be modulated in this fashion to transduce a broad range of cell types, including hematopoietic cells, neurons and hepatocytes, in the case of VSV-G pseudotyped LV [5]. Moreover, use of robust viral envelopes offers ways to produce LV particles in high titers.

LV are well suited for gene replacement therapy because these vectors efficiently integrate into the genome of non-dividing cells and thus provide stable transgene expression in the long-term. Newer generations of LV can deliver intron-containing cassettes using only exogenous elements to regulate gene expression, which is advantageous [6,7]. Also, biosafety concerns raised by HIV-derived vectors have been alleviated by the findings that LV can be produced by a minimal set of viral genes. As much of the HIV sequences have been eliminated from constructs used to generate late versions of LV, these vectors should be particularly safe. Moreover, generation of self-inactivating (SIN) vectors containing a deletion in the downstream long terminal repeat (LTR) viral sequences, which transcriptionally inactivates upstream LTR following transduction into cells, substantially decrease the risk of vector mobilization and recombination [8].

However, the ability of LV to transduce hepatocytes in vivo can be debated. For instance, an initial report by Kafri et al. documented that LV efficiently transduced the liver in vivo and produced sustained transgene expression [9]. On the other hand, Park et al. reported that intraportal delivery of LV could transduce only a few hepatocytes, whereas

* Corresponding author. Tel.: +1-718-430-2098; fax: +1-718-430-8975.
E-mail address: sanjvgupta@pol.net (S. Gupta).

doi:10.1016/j.jhep.2003.11.023

partial hepatectomy was required before vector administration for more extensive hepatic transduction [10]. In general, it appears that hepatic DNA synthesis induced by partial hepatectomy is necessary for transducing hepatocytes with LV. Also, it was recently established that a DNA sequence, known as the central polypurine tract (cPPT) in the pol gene of HIV-1, functions as a cis-acting determinant for translocation of the vector genome into the nucleus and, consequently, integration into cellular DNA. Presence of the cPPT and an adjacent central termination sequence (CTS) in the virus leads to the formation of a triple-stranded DNA structure during viral reverse transcription, the so-called HIV-1 flap. The transfer constructs used in the generation of early LV were devoid of the cPPT, as well as the CTS, which limited the nuclear translocation of vectors. Reintroduction of this DNA flap sequence in the transfer construct used to build LV significantly enhanced their nuclear translocation, transduction efficiency and expression of transgenes in most tissues examined [11,12]. A series of improved LV have now been developed for targeting various cell types in vivo [13–15]. Promising results have also been obtained in respect of the regulation of gene expression with inducible promoters, which may be turned on or off, depending on the cellular concentrations of activators or inhibitors [16]. Such vectors that are capable of tissue-specific and regulated transgene expression will obviously be very attractive for hepatic gene therapy.

The requirements for cancer gene therapy differ in important ways from gene therapy for metabolic diseases. For instance, elimination of cancer requires removal of all cancer cells, including perhaps those that may be in a precancerous stage, whereas metabolic deficiency states can be corrected by genetic modification of only some cells. This should imply that only extensive gene transfer into cancer cells will be appropriate. Also, the specificity of molecular targets for cancer gene therapy is not well defined at present. This has required testing of many approaches for cancer gene therapy, e.g. strategies using anti-angiogenic factors, cell cycle regulating genes, cytokine genes, cytotoxic prodrug-activating genes, immunomodulatory genes, etc. Alternative strategies have utilized the intrinsic ability of viruses to replicate and eventually cause cell death and such oncolytic viruses have been tested in clinical studies [17], although more work is needed to improve results.

The delivery of vectors constitutes a vexing issue in cancer gene therapy because this bears directly on the extent of gene transfer in cancer cells. For instance, vectors can be injected locally into tumors or delivered systemically by vascular routes. Unfortunately, neither of these mechanisms is entirely satisfactory. Intravenous or intrahepatic arterial injection in animals with liver tumors of adenovirus vectors, which transduce hepatocytes extremely efficiently (90–95%), produces minimal transduction of tumor cells, while exposing normal hepatocytes to toxicity. Similarly, intratumoral injection of vectors generally transduces only a very small fraction of tumor cells, although use of prodrug-activating genes can result in the transmission of intracellular toxins to adjacent tumor cells, the so called ‘bystander effect’, which can significantly amplify cytotoxicity. The prodrug strategies at present have utilized small cytotoxic molecules that can diffuse freely into adjacent cells or tissue, which exposes non-tumor cells to potential toxicities. It will be helpful if ways could be found to either avoid the entry of activated drugs into non-tumor cells or neutralize the bystander toxicity in adjacent normal cells.

Among prodrug activating genes, the thymidine kinase (TK) gene of the herpes simplex virus (HSV) has been widely studied for its potential in cancer gene therapy. The HSV-TK gene phosphorylates several nucleoside analogs, including the antiviral drug, ganciclovir. The triphosphorylated form of ganciclovir inhibits DNA polymerase and induces DNA chain termination, which results in cell death. Thus, HCC cells expressing TK become highly susceptible to ganciclovir. However, the safety margin of this approach requires consideration because although the TK-based ganciclovir activation should be safe for replication quiescent cells, such as mature hepatocytes, hepatic transduction with adenoviral vectors expressing HSV-TK has induced acute liver failure [18,19]. Moreover, the onset of hepatic failure was also described in woodchucks after intratumoral injection of an adenoviral vector in HCC [20]. On the other hand, a recently reported clinical trial from Sung et al. demonstrated the safety of direct intratumoral injection of an adenovirus-encoding TK into metastatic liver tumors treated with ganciclovir [21], presumably reflecting species-specific differences in adenoviral incorporation into liver cells.

To date, most of the published work on HCC gene therapy with cytotoxic transgenes used adenoviral vectors. In this issue of the Journal, Gerolami and colleagues [22] describe their unique experience with LV to express HSV-TK in HCC. They used SIN LV, expressing the fusion gene HSV-TK/GFP under control of the CAG constitutive promoter. After in vitro characterization of the vector with human hepatoma cell lines and primary human hepatocytes, the authors studied a rat model of chemically induced HCC, which reproduces the clinical situation better, compared with inoculation of tumor cell lines in animals. Gerolami et al. obtained an antitumoral effect with low liver toxicity in animals treated with the HSV-TK vector and ganciclovir. These studies showed tumor regression after direct intratumoral injection of the HSV-TK LV, whereas intrahepatic arterial injection of the vector resulted in low tumor transduction rates and no tumor regression. The findings are encouraging, although several issues need further analysis. For instance, it was unclear whether cell division was necessary for efficient LV transduction of normal hepatocytes in vivo, because the amount of the vector injected in rats via the hepatic artery might have been insufficient for hepatocellular transduction. While

the authors found superior transduction rates in HCC cell lines compared with normal human hepatocytes, these results cannot be directly extrapolated to the situation in vivo, where additional mechanisms may have regulated cellular access or incorporation of the LV. In studies by others, primary rat hepatocytes were transduced to high frequency with LV in the absence of cell proliferation, both with and without cPPT [14,23,24]. The cPPT sequence was required for efficient replication of HIV-1 by mutation analysis [25,26]. As mentioned above, in early LV vectors, the cPPT sequence was retained only in the packaging construct as part of the *pol* gene, whereas reintroduction of the cPPT sequence in the transfer vector backbone increased transgene integration following increased nuclear translocation of the vector into cells [11,12]. These findings are in agreement with nuclear translocation of LV constituting a rate-limiting step in gene transfer. Therefore, in further studies of liver cancer gene therapy it should be appropriate to address whether use of highly efficient LV would improve tumor transduction rates.

The authors acknowledged the significance of regulating transgene expression in various cell types [27]. LV do offer the potential for modulating transgene expression by substituting constitutive promoters within the vector with tissue- or tumor-specific promoters. This possibility is substantiated by the recent success in developing improved LV for transducing hepatocytes and endothelial cells of tumor vessels in vivo [13,14]. Recently, Chyung et al. proposed an interesting approach for precisely targeting gene expression to diseased tissue [28]. They developed a regulatory system, where a hepatocyte-specific α-1 antitrypsin promoter and an unrelated zinc-induced metallothionein promoter, the latter substituting for a 'disease-specific' promoter, regulated transgene expression in a cell type and promoter-specific fashion. Under these conditions, a transgene will be expressed in a given cell only when both promoters were active. Unlike other transgene-regulatory systems described previously, this system does not require persistent expression of foreign genes that could provoke an immune response or produce toxicity. As an illustration, most melanoma cells express the Fas ligand (FasL), which activates apoptosis pathways, whereas healthy melanocytes do not normally express FasL [29]. By using a melanocyte-specific promoter and the FasL promoter to simultaneously regulate transgene expression, melanoma cells could be exclusively targeted for destruction, while normal melanocytes and non-melanocytes cells expressing FasL would be spared. Such a strategy can potentially be generalized to other tumors and liver diseases, e.g. by utilizing α-fetoprotein and albumin promoter constructs, which should be simultaneously active largely in HCC cells. At present, this regulatory system has been useful only in cultured hepatoma cells, but testing of such dual regulatory constructs in vivo should be of much interest. Use of tissue-specific promoters should also limit potential immunological consequences of cellular transgene expression. Whether vector targeting could be employed to restrict lentiviral transduction to tumor cells, which has been achieved at cell type-specific levels by selecting specific viral serotypes, by pseudotyping vectors with tissue-specific viruses, or by engineering the vector surface to alter tropism [30–32], needs further study. Retroviral display of cell-specific blocking domains offers a paradigm of restricted tropism. For instance, display of epidermal growth factor (EGF) on the amphotropic MLV glycoprotein dramatically inhibits infection of EGF receptor-positive cells, whereas addition of the displayed ligand as a soluble polypeptide can competitively abrogate such sequestration. Thus, EGF-displaying amphotropic vectors can efficiently infect EGF-receptor-negative cells, but do not infect EGF-receptor-positive cells [33]. Intravenous inoculation in mice of non-targeted LV, displaying wild-type glycoproteins, leads to maximal reporter gene activity in the liver and spleen with minimal expression in other organs. In contrast, EGF-displaying vectors inoculated intravenously are expressed mainly in the spleen and very low-level expression is detected in EGF-receptor-enriched liver cells. The capacity of the EGF-displaying vector to transduce liver cells can be restored by pretreating animals with soluble EGF [34]. Such evidence suggests that LV capable of retargeting gene delivery in vivo could be effective.

Finally, it should be stressed that notwithstanding the expectations generated by extensive public interest, the field of gene therapy is still in its infancy and much more remains to be done. We feel encouraged by the potential for lentiviral gene therapy in the treatment of liver cancer and look forward to continuing progress in this important area.

Acknowledgements

Supported in part by NIH grants P01 DK-0529562 and R01 DK-46952.

References

[1] Sangro B, Herraiz M, Prieto J. Gene therapy of neoplastic liver diseases. Int J Biochem Cell Biol 2003;35:135–148.
[2] Raper SE, Yudkoff M, Chirmule N, Gao GP, Nunes F, Haskal ZJ, Furth EE, et al. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum Gene Ther 2002;13:163–175.
[3] Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302:415–419.
[4] Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 2001;7:33–40.
[5] Follenzi A, Naldini L. Generation of HIV-1 derived lentiviral vectors. Methods Enzymol 2002;346:454–465.
[6] Zufferey R, Donello JE, Trono D, Hope TJ. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of

transgenes delivered by retroviral vectors. J Virol 1999;73: 2886–2892.
[7] Miyoshi H, Smith KA, Mosier DE, Verma IM, Torbett BE. Transduction of human CD34 + cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. Science 1999;283: 682–686.
[8] Vigna E, Naldini L. Lentiviral vectors: excellent tools for experimental gene transfer and promising candidates for gene therapy. J Gene Med 2000;2:308–316.
[9] Kafri T, Blomer U, Peterson DA, Gage FH, Verma IM. Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors. Nat Genet 1997;17:314–317.
[10] Park F, Ohashi K, Chiu W, Naldini L, Kay MA. Efficient lentiviral transduction of liver requires cell cycling in vivo. Nat Genet 2000;24: 49–52.
[11] Follenzi A, Ailles LE, Bakovic S, Geuna M, Naldini L. Gene transfer by lentiviral vectors is limited by nuclear translocation and rescued by HIV-1 pol sequences. Nat Genet 2000;25:217–222.
[12] Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P. HIV-1 genome nuclear import is mediated by a central DNA flap. Cell 2000;101:173–185.
[13] De Palma M, Venneri MA, Naldini L. In vivo targeting of tumor endothelial cells by systemic delivery of lentiviral vectors. Hum Gene Ther 2003;14:1193–1206.
[14] Follenzi A, Sabatino G, Lombardo A, Boccaccio C, Naldini L. Efficient gene delivery and targeted expression to hepatocytes in vivo by improved lentiviral vectors. Hum Gene Ther 2002;13:243–260.
[15] Lotti F, Menguzzato E, Rossi C, Naldini L, Ailles L, Mavilio F, Ferrari G. Transcriptional targeting of lentiviral vectors by long terminal repeat enhancer replacement. J Virol 2002;76:3996–4007.
[16] Vigna E, Cavalieri S, Ailles L, Geuna M, Loew R, Bujard H, Naldini L. Robust and efficient regulation of transgene expression in vivo by improved tetracycline-dependent lentiviral vectors. Mol Ther 2002;5: 252–261.
[17] 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.
[18] Qian C, Idoate M, Bilbao R, Sangro B, Bruna O, Vazquez J, Prieto J. Gene transfer and therapy with adenoviral vector in rats with diethylnitrosamine-induced hepatocellular carcinoma. Hum Gene Ther 1997;8:349–358.
[19] van der Eb MM, Cramer SJ, Vergouwe Y, Schagen FH, van Krieken JH, van der Eb AJ, et al. Severe hepatic dysfunction after adenovirus-mediated transfer of the herpes simplex virus thymidine kinase gene and ganciclovir administration. Gene Ther 1998;5:451–458.
[20] Bilbao R, Gerolami R, Bralet MP, Qian C, Tran PL, Tennant B, et al. Transduction efficacy, antitumoral effect, and toxicity of adenovirus-mediated herpes simplex virus thymidine kinase/ganciclovir therapy of hepatocellular carcinoma: the woodchuck animal model. Cancer Gene Ther 2000;7:657–662.
[21] Sung MW, Yeh HC, Thung SN, Schwartz ME, Mandeli JP, Chen SH, Woo SL. Intratumoral adenovirus-mediated suicide gene transfer for hepatic metastases from colorectal adenocarcinoma: results of a phase I clinical trial. Mol Ther 2001;4:182–191.
[22] Gerolami R, Uch R, Faivre F, Garcia S, Hardwigsen J, Cardoso J, et al. Herpes simplex virus thymidine kinase–mediated suicide gene therapy for hepatocellular carcinoma using HIV-1-derived lentiviral vectors. J Hepatol 2004;40:291–297.
[23] Seppen J, Rijnberg M, Cooreman MP, Oude Elferink RP. Lentiviral vectors for efficient transduction of isolated primary quiescent hepatocytes. J Hepatol 2002;36:459–465.
[24] Oertel M, Rosencrantz R, Chen YQ, Thota PN, Sandhu JS, Dabeva MD, et al. Repopulation of rat liver by fetal hepatoblasts and adult hepatocytes transduced ex vivo with lentiviral vectors. Hepatology 2003;37:994–1005.
[25] Charneau P, Alizon M, Clavel F. A second origin of DNA plus-strand synthesis is required for optimal human immunodeficiency virus replication. J Virol 1992;66:2814–2820.
[26] Charneau P, Mirambeau G, Roux P, Paulous S, Buc H, Clavel F. HIV-1 reverse transcription. A termination step at the center of the genome. J Mol Biol 1994;241:651–662.
[27] Uch R, Gerolami R, Faivre J, Hardwigsen J, Mathieu S, Mannoni P, Bagnis C. Hepatoma cell-specific ganciclovir-mediated toxicity of a lentivirally transduced HSV-TkEGFP fusion protein gene placed under the control of rat alpha-fetoprotein gene regulatory sequences. Cancer Gene Ther 2003;10:689–695.
[28] Chyung YH, Peng PD, Kay MA. System for simultaneous tissue-specific and disease-specific regulation of therapeutic gene expression. Hum Gene Ther 2003;14:1255–1264.
[29] Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, et al. Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape. Science 1996;274: 1363–1366.
[30] Auricchio A, Kobinger G, Anand V, Hildinger M, O'Connor E, Maguire AM, et al. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. Hum Mol Genet 2001;10:3075–3081.
[31] Lavillette D, Russell SJ, Cosset FL. Retargeting gene delivery using surface-engineered retroviral vector particles. Curr Opin Biotechnol 2001;12:461–466.
[32] Reynolds PN, Nicklin SA, Kaliberova L, Boatman BG, Grizzle WE, Balyasnikova IV, et al. Combined transductional and transcriptional targeting improves the specificity of transgene expression in vivo. Nat Biotechnol 2001;19:838–842.
[33] Fielding AK, Maurice M, Morling FJ, Cosset FL, Russell SJ. Inverse targeting of retroviral vectors: selective gene transfer in a mixed population of hematopoietic and nonhematopoietic cells. Blood 1998; 91:1802–1809.
[34] Peng KW, Pham L, Ye H, Zufferey R, Trono D, Cosset FL, Russell SJ. Organ distribution of gene expression after intravenous infusion of targeted and untargeted lentiviral vectors. Gene Ther 2001;8: 1456–1463.