# High Efficiency Transduction and Stable Gene Expression of Primary Human Hepatocytes with Lentiviral Vectors

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## ABSTRACT

Lentiviral vectors are retroviral-derived constructs that effectively transduce mammalian cells and integrate stably into the host's genome. We hypothesized that methodologies could be developed enabling the use of lentivectors for the genetic manipulation of primary human hepatocytes in non-dividing cultures. Infection of hepatocytes with green fluorescence protein (GFP)-expressing lentivectors (MOI=5) resulted in >80% GFP positive cells, with GFP expression retained for >2 weeks in primary culture. Realtime RT-PCR analyses demonstrated that lentivectors engineered to express siRNAs successfully knocked down specific nuclear receptor targets. Further, lentiviral infection did not result in detectable alteration of assessed hepatocyte expression markers, including the phenobarbital (PB) induction response – a hallmark feature of the differentiated hepatocyte. Real-time RT-PCR indicated that 500 µM PB treatment produced ~20-fold induction of both CYP2B6 and CYP3A4 mRNA transcripts, independent of viral infection status. In summary, we demonstrate that lentiviral methodology enables highly efficient transduction and stable gene expression in primary human hepatocytes. These approaches establish a powerful platform for conducting toxicological and pharmacological research in a culture model, and offer the potential for clinical cell-based therapeutics to treat hepatic disease.

## INTRODUCTION

The ability to modulate gene expression within the fully differentiated hepatocyte is imperative for the realization of the full potential of hepatic gene therapy, as well as optimal use of the hepatocyte model in research. However, development of an ideal methodology for the genetic manipulation of primary human hepatocyte cultures has proven challenging, as cultures of primary human hepatocytes are refractory to common transfection methodologies and many viral systems have proven ineffective in these quiescent cells.

Lentiviral vectors are unique within the retroviral vector family because they are able to infect both dividing and non-dividing cells and stably integrate into the host genome, thereby facilitating long-term transgene expression.[1-3] Several studies have shown that lentiviral vectors efffectively transduce the widely used hepatic model cell lines, Huh7 and HepG2 as well as cultures of primary hepatocytes.[4-12] While these results are promising, use of these strategies for genetic modulation of primary hepatocyte cultures requires evaluation of the biological impact of the infection event on differentiated hepatic phenotype and function.

## OBJECTIVES

- ❑ Verify infectivity of lentivectors in Huh7s, HepG2s, and primary human hepatocyte cultures.
- ❑ Assess ability of a siRNA expressed from a lentivector to knockdown CAR expression in hepatocytes.
- ❑ Evaluate whether lentiviral infection alters select markers of the differentiated hepatic phenotype.

## MATERIALS & METHODS

❑ **siRNA design and lentiviral siRNA expression vector construction.** siRNA target sequences within the hCAR mRNA were identified using Genscript's "siRNA Target Finder." Template sequences encoding a short hairpin RNA were generated and cloned into pSIH1-H1-copGFP shRNA lentivector (System Biosciences, Mountain View, CA)

❑ **Lentiviral production, titering and target cell infection.** 293T/17 packaging cells were transfected with pPACKH1 packaging plasmid mix and pSIH1 expression vector using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Psuedoviral supernatant was harvested and viral titer was estimated by infecting 293T/17 cells with dilutions of virus and counting the percentage of GFP+ cells in 3-5 random fields.

❑ **Real-time RT-PCR.** Primary human hepatocyte RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA) and converted to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real-time RT-PCR was performed using Assays-on-Demand Gene Expression Products (Applied Biosystems, Foster City, CA). Data were analyzed using the $\Delta\Delta C_T$ Method as previously described.[13]

## RESULTS

**Figure 1**

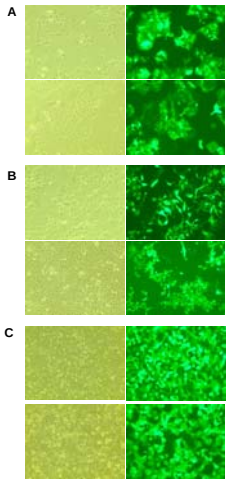


**Figure 1. GFP expression in Huh7s, HepG2s, and primary human hepatocyte cultures infected with lentivirus expressing GFP.** Huh7s, HepG2s, and primary human hepatocytes were infected with lentiviral vectors at a MOI of ~5 TU/cell. Cells were imaged at 100x magnification in both phase-contrast and fluorescence microscopy. (A) Upper panel, Huh7 cells at 5 days post infection; lower panel, Huh7 cells after 5 passages. (B) Upper panel, HepG2 cells at 5 days post-infection; lower panel, HepG2 cells after 5 passages. (C) Primary human hepatocytes at ~2 weeks post-infection.

**Figure 2**

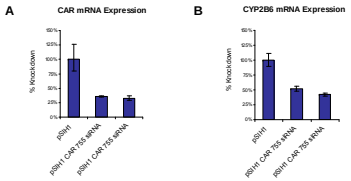


**Figure 2. CAR and CYP2B6 mRNA expression in primary human hepatocyte cultures infected with lentivirus expressing siRNA targeted to CAR.** Cultures of primary human hepatocytes were infected with lentivectors expressing siRNA against CAR (pSIH1 CAR 755 siRNA) or empty lentivector (pSIH1) at a MOI of ~2-4 TU/cell. At 7 days post-infection RNA was extracted, converted to cDNA, subjected to real-time RT-PCR, and the data analyzed using the $\Delta\Delta C_T$ method to determine relative mRNA expression levels. Depicted results are from a single hepatocyte donor, but are representative of data obtained from at least one additional donor. (A) CAR mRNA expression levels. (B) CYP2B6 mRNA expression levels.

## RESULTS (continued)

**Figure 3**

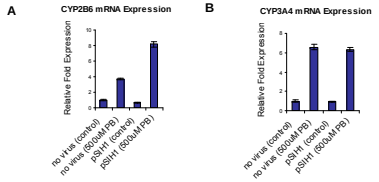


**Figure 3. Effects of lentiviral infection on PB induction responses in primary human hepatocyte cultures. Human** hepatocytes were infected with lentivectors at a MOI of ~5-20 TU/cell. At 3 days post-infection cells were treated with 500µM PB or DMSO control for 24 hours. Cells were harvested at 4 days post-infection, RNA was extracted, converted to cDNA, subjected to real-time RT-PCR, and the data analyzed using the $\Delta\Delta C_t$ method to determine relative mRNA expression levels. Depicted results are from a single hepatocyte donor, but are representative of data obtained from at least one additional donor. (A) CYP2B6 mRNA expression levels (B) CYP3A4 mRNA expression levels.

**Figure 4**

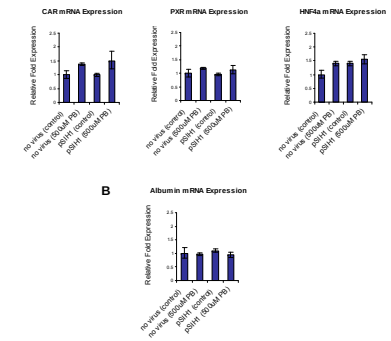


**Figure 4. mRNA expression levels of select hepatic markers in cultures of primary human hepatocytes infected with lentivirus.** Human hepatocytes were infected with lentivectors at a MOI of ~10 TU/cell. At 2 days post-infection cells were treated with 500µM PB or water control for 24 hours. Cells were harvested at 3 days post-infection, RNA was extracted, converted to cDNA, subjected to real-time RT-PCR, and the data analyzed using the $\Delta\Delta C_T$ method to determine relative mRNA expression levels. Depicted results are from a single hepatocyte donor, but are representative of data obtained from at least one additional donor. (A) mRNA levels of the hepatic nuclear receptors CAR, PXR, and HNF4α1. (B) Hepatic albumin mRNA expression levels.

## DISCUSSION

- ❑ Lentiviral vectors efficiently transduce Huh7 and HepG2 cell lines and transgene expression is retained throughout cell division.
- ❑ Cultures of primary human hepatocytes are successfully transduced by lentivectors and expression of the introduced transgene is maintained for the duration of the culture period.
- ❑ siRNA expressed from a lentivector in primary human hepatocytes is an effective strategy to achieve knockdown of CAR mRNA expression to ~35% of endogenous levels, with the functional relevance of this knockdown demonstrated by a corresponding decrease in expression of CYP2B6.
- ❑ Human hepatocytes infected with lentiviral vectors retain the capacity for CYP2B6 and CYP3A4 induction in response to phenobarbital, a sensitive indicator of hepatocyte differentiation status.
- ❑ Lentiviral infection does not alter expression levels of the nuclear receptors ,CAR, PXR, or HNF4a, or expression of the hepatic secretory protein, albumin.

## CONCLUSIONS AND APPLICATION

Lentiviral vectors offer a powerful methodology whereby primary cultures of human hepatocytes may be stably genetically modulated, with little effect on the differentiated hepatic phenotype. These techniques offer promise for pharmacological and toxicological studies in the primary hepatocyte model, as well as ultimately for *ex vivo* gene therapy to ameliorate hepatic disease.

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