Efficient Transduction of Zebrafish Melanoma Cell Lines and Embryos Using Lentiviral Vectors

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Abstract

The establishment of *in vitro* cultures of zebrafish cancer cells has expanded the potential of zebrafish as a disease model. However, the lack of effective methods for gene delivery and genetic manipulation has limited the experimental applications of these cultures. To overcome this barrier, we tested and optimized vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped lentiviral and retroviral vector transduction protocols. We show that lentivirus successfully and efficiently transduced zebrafish melanoma cell lines *in vitro*, allowing antibiotic selection, fluorescence-based sorting, and *in vivo* allotransplantation. In addition, injection of concentrated lentiviral particles into embryos and tumors established the feasibility of *in vivo* gene delivery.

Keywords: viral vectors, retrovirus, gene delivery, zebrafish cell lines, lentivirus, lentiviral

ZEBRAFISH CANCER CELL lines are useful tools to unravel various aspects of cancer biology. For instance, their transplantation into optically clear *casper* zebrafish allows the study of cancer cell invasion and interaction with the microenvironment.^{1–4} The possibility of expanding their applications by fluorescent labeling, barcoding, or genome editing has been limited by the lack of efficient gene delivery methods. Although viral vectors have been shown to transduce some zebrafish cell lines (e.g., PC2,⁵ Sertoli⁶), although often with low efficiency, their successful use as delivery vectors in zebrafish cancer cell lines has not been reported.

We investigated whether vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped retroviral or lentiviral vectors could efficiently transduce zebrafish melanoma cell lines. The QC:GFP retroviral⁷ and the pLV-mCherry lentiviral vectors containing a cytomegalovirus (CMV) promoter driving the expression of GFP or mCherry fluorescent reporters, respectively, were tested on a panel of melanoma cell lines

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FIG. 1. (A) Representative flow cytometry plot showing transduction of the zf-MEL-A melanoma cell line with retroviral vector QC-GFP (*green*) or lentiviral vector pLV-mCherry (*red*). The plot shows gated live single cells (SYTOX[®] *Blue* negative) analyzed 48 h post-transduction. *Y*-axis: fluorescence intensity, *X*-axis: forward scatter. (B) Quantification of transduction efficiency of four zebrafish melanoma cell lines, measured as percentage of fluorescent positive cells. (C) Confocal microscopy (60×) illustrating *in vitro* transduced ZMEL1 (*mitfa:GFP*) with pLV-mCherry showing transgene expression >20 passages after sorting for GFP⁺mCherry⁺ cells. (D) Epifluorescent microscopy illustrating tumor engraftment and transgene expression of GFP⁺mCherry⁺ ZMEL1 cells upon allotransplantation into irradiated *casper* recipients. (E) Confocal imaging (20×) of intracranial microinjection aiming with a ventral approach to the posterior forebrain of pLV-mCherry PEG concentrated viral particles captured at 20–24 hpi (68–72 hpf). hpf, hours postfertilization; hpi, hours postinjection.

established in the our laboratory from tumors obtained from the *Tg(mitfa:B-RAF^{V600E}); mitfa^{-/-}; p53^{-/-}* MiniCoopR model.⁸ A representative transduction experiment with $\sim 2.3 \times 10^9$ TU (transduction units; pLV-mCherry) or $\sim 0.76 \times 10^9$ TU (QC-GFP) of four different zebrafish melanoma cell lines illustrates a robust transduction efficiency with both retroviral (0.9%–41%) and lentiviral (98%–99%) vectors (Fig. 1A, B). The lentiviral vectors showed more consistent results, with a much higher transduction efficiency than retroviral vectors. This difference could be partially because of the higher viral titer and the ability of lentiviral vectors to transduce nondividing cells. Indeed, the latter might explain some of the variability in retroviral transduction efficiency (Fig. 1B), given the differences in proliferation rate among the zebrafish cancer cell lines used (not shown). Although it is well known that transduction efficiency is highly cell line dependent in mammalian systems, we performed a direct comparison of titration of concentrated pLV-mCherry virus in human 293T and zebrafish zf-MEL-A cells to assess differential sensitivity to viral transduction. Compared with 293T cells ($\sim 10.16 \times 10^9$ TU/mL), zf-MEL-A cells had a lower sensitivity to viral transduction ($\sim 0.36 \times 10^9$ TU/mL), highlighting the importance of using high titer concentrated viral particles to efficiently transduce zebrafish cancer cells.

In an independent experiment, the previously established GFP⁺ ZMEL1 melanoma cell line² was transduced *in vitro* with pLV-mCherry (Fig. 1C). Transgene expression was retained *in vitro* for >20 passages (Fig. 1C), but a reduction of fluorescence intensity was observed over time, similar to known counter-selection effects in mammalian cells (not shown). Sorted GFP⁺mCherry⁺ ZMEL1 cells were allo-transplanted subcutaneously into the dorsum of irradiated *casper* recipients.² The transduction did not affect engraftment and the transplanted cells showed long-term retention of transgene expression *in vivo* (Fig. 1D). In addition, the direct injection of $\sim 2 \times 10^7$ TU of concentrated pLV-mCherry viral particles into primary GFP⁺ *BRAF^{V600E}; p53^{-/-}* zebrafish melanomas showed evidence of mCherry transgene expression 24 hours postinjection (hpi), offering proof of principle of the feasibility of intratumor *in vivo* gene delivery with lentiviruses (Supplementary Fig. S1A; Supplementary Data are available online at www.liebertpub.com/zeb).

We tested several vectors with different promoters *in vitro* to assess which available viral backbones could be used in zebrafish. The SV40 promoter was found to be effective, whereas mammalian PGK and EF1 α promoters were not, similar to previous reports.^{5,6} The transduction of the ZMEL1 cell line with pLenti-puro⁹ or pLenti-CMV-Blast-DEST¹⁰ allowed for fast antibiotic selection within 1 week with 0.5 μ g/mL puromycin or 2 μ g/mL blasticin (Gibco), respectively (not shown). Interestingly, an attempt to pseudotype the QC-GFP or pLV-mCherry vectors with the envelope protein of a virus known to infect zebrafish tissues (IHNV-G)¹¹ did not result in successful transduction (not shown).

Finally, although the use of viral vectors in one-cell stage embryos or adult brain has been previously reported,^{5,12} we tested the use of lentiviral vectors for *in vivo* delivery in developing embryos. We microinjected $2\text{--}10 \times 10^3$ TU of pLV-mCherry concentrated viral particles (1–5 nL) either into the forebrain (anterior to the mid-brain–hindbrain boundary) or into the heart of developing embryos at 48 hours postfertilization. Confocal imaging at 20–24 hpi showed robust and specific *in vivo* mCherry expression (Fig. 1E and Supplementary Fig. S1B). Interestingly, the embryos were kept at 28°C throughout the experiment and efficient transduction did not require incubation at 37°C for 24 h after injection, as previously reported for adult brain.⁵

In conclusion, our work shows that VSV-G pseudotyped lentiviral vectors allow efficient transduction *in vitro* of zebrafish melanoma cell lines, and could also be utilized as an *in vivo* gene-delivery tool in zebrafish.

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Disclosure Statement

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