Transduction of Human Cells with Polymer-complexed Ecotropic Lentivirus for Enhanced Biosafety.

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Public Summary:
Stem and tumor cell biology studies often require viral transduction of human cells with known or suspected oncogenes, raising major safety issues for laboratory personnel. Pantropic lentiviruses, such as the commonly used VSV-G pseudotype, are a valuable tool for studying gene function because they can transduce many cell types, including non-dividing cells. However, researchers may wish to avoid production and centrifugation of pantropic viruses encoding oncogenes due to higher biosafety level handling requirements and safety issues. Several potent oncogenes, including c-Myc and SV40 large T antigen, are known to enhance production of induced pluripotent stem cells (iPSC). All other known iPSC-inducing genetic changes (OCT4, SOX2, KLF4, NANOG, LIN28, and p53 loss of function) also have links to cancer, making them of relatively high safety concern as well. While these cancer-related viruses are useful in studying cellular reprogramming and pluripotency, they must be used safely. To address these biosafety issues, we demonstrate a method for transduction of human cells with ecotropic lentivirus, with additional emphasis on reduced cost and convenient handling. We have produced ecotropic lentivirus with sufficiently high titer to transduce greater than 90% of receptor-expressing human cells exposed to the virus, validating the efficacy of this approach. Lentivirus is often concentrated by ultracentrifugation; however, this process takes several hours and can produce aerosols infectious to human biomedical researchers. As an alternative, viral particles can be more safely sedimented onto cells by complexation with chondroitin sulfate and polybrene (CS/PB). This technique increases the functional viral titer up to 3-fold in cells stably expressing murine retrovirus receptor, with negligible added time and cost. Transduction of human dermal fibroblasts (HDFs) is maximally enhanced using CS/PB concentrations approximately 4-fold lower than the optimal value previously reported for cancer cell lines, suggesting that polymer concentration should be titrated for the target cell type of interest. We therefore describe the use of methylthiazolyldiphenyl-tetrazolium bromide (MTT) to assay for polymer toxicity in a new cell type. We observe equivalent viability of HDFs after viral transduction using either polymer complexation or the standard dose of polybrene (PB, 6 mug/ml), indicating minimal acute toxicity. In this protocol, we describe the use of ecotropic lentivirus for overexpression of oncogenes in human cells, reducing biosafety risks and increasing the transduction rate. We also demonstrate the use of polymer complexation to enhance transduction while avoiding aerosol-forming centrifugation of viral particles.

Scientific Abstract:
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