Comparison of gene-transfer efficiency in human embryonic stem cells.

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Public Summary:

Scientific Abstract:
Technologies designed to allow manipulation and modification of human embryonic stem (hES) cells are numerous and vary in the complexity of their methods, efficiency, reliability, and safety. The most commonly studied and practiced of these methods include electroporation, lipofection, nucleofection, and lentiviral transduction. However, at present, it is unclear which protocol offers the most efficient and reliable method of gene transfer to hES cells. In this study, a bi-fusion construct with ubiquitin promoter driving enhanced green fluorescent protein reporter and the firefly luciferase (pUb-eGFP-Fluc) along with neomycin selection marker was used for in vitro and in vivo studies. In vitro studies examined the transfection efficiency and viability of each technique using two hES cell lines (male H1 and female H9 cells). Lentiviral transduction demonstrated the highest efficiency (H1: 25.3 +/- 4.8%; H9: 22.4 +/- 6.5%) with >95% cell viability. Nucleofection demonstrated transfection efficiency of 16.1 +/- 3.6% (H1) and 5.8 +/- 3.2% (H9). However, minimal transfection efficiency was observed with electroporation (2.1 +/- 0.4% (H1) and 1.9 +/- 0.3% (H9)) and lipofection (1.5 +/- 0.5% (H1) and 1.3 +/- 0.2% (H9); $P < 0.05$ vs. lentiviral transduction). Electroporation also demonstrated the highest cell death (62 +/- 11% (H1) and 42 +/- 10% (H9)) followed by nucleofection (25 +/- 9% (H1) and 30 +/- 15 (H9)). Importantly, lentiviral transduction generated a greater number of hES cell lines stably expressing the double-fusion reporter gene (hES-DF) compared to other transfection techniques. Finally, following subcutaneous transplantation into immunodeficient nude mice, the hES-eGFP-Fluc cells showed robust proliferation as determined by longitudinal bioluminescence imaging. In summary, this study demonstrates that lentiviral transduction and nucleofection are efficient, simple, and safe techniques for reliable gene transfer in hES cells. The double-fusion construct provides an attractive approach for generating stable hES cell lines and monitoring engraftment and proliferation in vitro and in vivo.