Gene targeting in a HUES line of human embryonic stem cells via electroporation.

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Public Summary:
Genetic engineering of human embryonic stem (ES) cells is important for their application as tools for basic research and therapeutic development. Specifically, gene targeting, the technology to modify an endogenous gene by homologous recombination, has many potential applications in human ES cells. For instance, faulty genes can be repaired using gene targeting before human ES cells or their derivatives are used in transplantation therapies. Fluorescent reporter genes can be introduced by gene targeting into an endogenous gene to monitor gene expression and to trace cell lineages. Mutations can be generated by gene targeting in order to assess the function of an endogenous gene in human ES cells or their derivatives. However, unlike their mouse counterpart, the technology of gene targeting in human ES cells is not well established. Few reports have been published on successful gene targeting in human ES cells. Even among this limited number of reports, the genes targeted were largely limited to genes that can be selected or enriched for when targeted, using strategies that are not adaptable to other genes. In this paper, we described successful gene targeting of the Fezf2 gene in the HUES-9 human ES cell line. Fezf2 is important for the development of corticospinal and related neurons based on work performed in mice. The expression of Fezf2 also marks the corticospinal neurons. By introducing a fluorescent reporter gene into the Fezf2 gene via gene targeting, we will be able to monitor Fezf2 expression in the targeted cell line upon appropriate differentiation. This will allow us to develop a more efficient protocol to differentiate human ES cells towards the corticospinal neurons in future. Because corticospinal neurons (more precisely their connection from the brain to the spinal cord) is often damaged in spinal cord injury, this research may one day lead to the development of cell transplantation therapies that would bring benefits to spinal cord injury patients. Our experience of gene targeting at Fezf2 also provided several valuable lessons for stem cell researchers who plan to apply gene targeting in their work. We found that the HUES-9 human ES cell line has a high tolerance for enzymatic passage and a high clonal expansion capability, which give this cell line a distinct advantage for gene targeting procedures that require single cell dissociation and electroporation. This observation has broad implications as it was previously thought that human ES cells differ from their mouse counterparts due to poor survival after enzymatic dissociation. Our results showed that the choice of cell line used may have a profound effect on the ability of the human ES cells to be genetically manipulated by gene targeting. This is the first report on gene targeting frequency for a gene not expressed in human ES cells using a strategy that is applicable to all human genes. Finally, HUES-9 cells can undergo gene targeting procedures without apparent loss of their characteristic features as human ES cells, which is critical for their widespread utility in basic and clinical research.

Scientific Abstract:
Genetic modification is critical for achieving the full potential of human embryonic stem (ES) cells as a tool for therapeutic development and for basic research. Targeted modifications in human ES cells have met with limited success because of the unique culture conditions for many human ES cell lines. The HUES lines of human ES cells were developed for ease of manipulation and are gaining increased utility in stem cell research. We tested conditions for gene targeting via electroporation in the HUES-9 human ES cell line and demonstrate here successful gene targeting at the gene encoding Fezf2 (also known as Fezl), a transcription factor involved in corticospinal neuron development. With a targeting strategy involving positive and negative selection that is applicable to all genes, we observed a gene targeting frequency of approximately 1.5% for Fezf2, a gene not expressed in human ES cells. We found that conditions developed for gene targeting in mouse ES cells can be readily adapted to HUES cells with few key modifications. HUES-9 cells exhibit an intrinsically high efficiency of clonal expansion and sustain electroporation-based gene targeting procedures without any significant loss of pluripotency marker expression or karyotypic stability. Thus, human ES cell lines adapted for enzymatic passage and efficient clonal expansion can be highly amenable to genetic modifications, which will facilitate their application in basic science and clinical development.

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