Peer reviewed article

Genetic engineering of embryonic stem cells

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Summary

We describe a novel generation of lentiviral vectors that are particularly well suited for work with embryonic stem cells. The possibility of selecting cell lines with antibiotics and the rapid insertion of any combination of promoters and genes of interest makes them a powerful tool in the generation of transgenic ES cell lines. This vector can also greatly facilitate studies aimed at the improvement of neuronal engineering from ES cells, by making it possible to monitor the emergence and differentiation of neurons.

Key words: embryonic stem cells; lentiviral vectors; genetic engineering

Introduction

Embryonic stem cells have a major potential for studying early steps of development and for use in cell therapy. In many situations, however, it will be necessary to genetically engineer these cells. Such situations include, for example, the use of promoter/reporter constructs to perform live imaging of development and differentiation, or incorporation of security features into cells and thereby increase the safety of transplanted cells. We have developed a novel generation of lentivectors which permit easy genetic engineering of mouse and human embryonic stem cells. The new generation vectors allow rapid insertion of promoters and genes of interest into the lentivector. Also, cells transduced with these lentivectors can be readily selected by antibiotics. This novel generation of lentivectors still has enormous potential for further development and should make for more efficient use of embryonic stem cells for research and ultimately for therapy.

Embryonic stem cells

Embryonic stem cells have received major attention in recent years due to their potential for differentiating into virtually any cell type and thus their potential for use in cell therapy.

Embryonic stem cells (ES cells) are derived from the inner cell mass of the embryo at the blastocyst stage. Adult stem cells are found in specific locations of the developed organism. While adult stem cells usually have a restricted differentiation potential, ES cells are pluripotent, i.e. capable of differentiating into virtually any cell type of our organism. After isolation from blastocysts, ES cells can be expanded in culture indefinitely without losing their pluripotency and thus are particularly attractive candidates for use in cell replacement therapy.

Ethical concerns about the use of these cells have been voiced. In Switzerland the generation and use of human ES cells are authorised only if spare embryos from *in vitro* fertilisation are used to derive them.

At present ES cells are the only readily accessible high quality source of neurons for cell therapy; it is possible to generate large quantities of neurons from ES cells and the outlook for their use in animal models of neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis) is promising [1–3]. However, for future cell therapy applications further studies, both in vitro and in vivo, are necessary for better understanding and control of the neuronal differentiation process. With this aim in view, introduction of transgenes into the genome of stem cells is a major issue in stem cell research.

This work was supported by the NFP46 grant of the Swiss National Science Foundation (NFP 46 Stem Cell Transplants 4046-101109).

Novel vector for genetic engineering of stem cells

Generation of transgenic stem cells has broad applications, ranging from development and cell differentiation studies to redirection of stem cells towards a specific phenotype. Transgenesis of stem cells could also be used to decrease the risk of rejection through down-regulation of immune recognition molecules and decrease the risk of tumour formation through expression of molecules which allow

selective killing of transplanted cells (e.g. herpes simplex thymidine kinase would allow killing of transplanted cells through gancyclovir). So far, however, transgenesis of ES cells has remained a difficult and time-consuming task.

More recently, gene delivery by viral vectors in ES cells has proven increasingly attractive [4–6]. Lentiviral vectors are particularly promising, owing to their capacity to integrate transgenes into the host cell genome. Lentiviral vectors are based on the human immunodeficiency virus (HIV); instead of delivering genetic material of HIV, these vectors are used to introduce transgenes of interest into the host cells. Current generations of lentiviral vectors carry several modifications which render them completely incapable of replicating into the host cell, thus making them compatible with a high biosafety level [7]. They also have important advantages over other viral vectors. Their ability to introduce genetic material into both dividing and non-dividing cells and to mediate long term transgene expression both in vitro and in vivo [8] holds out great promise for lentivectors as tools for gene and cell therapy. In addition, unlike traditional retroviral vectors [9–10], transgene expression is well maintained during propagation and differentiation of embryonic stem cells.

However, current lentiviral vectors are limited in their cloning flexibility and the possibility of selecting cells expressing the transgene of interest. We have therefore developed a novel generation of lentiviral vectors which allows easy vector construction and rapid generation of mouse and human ES cell lines that homogeneously express a transgene of interest [11]. The novelty of these vectors lies in several features: i) they are based on recombinational cloning technology [12], rendering possible rapid insertion of a promoter and a transgene to express it ubiquitously or in a tissue-specific manner; ii) they carry several elements to optimise transgene expression levels; iii) they carry an antibiotic selection cassette whereby cells carrying the transgene can be selected, thus obtaining pure populations of transduced cells.

The ease of combination of any promoter and gene of interest allows rapid construction of vectors (fig. 1; see also fig. 1 and fig. 2 in ref. [11]) to express proteins at different levels or in a specific cell type. In figure 1 we show expression of a green fluorescent protein in mouse ES cells using three different ubiquitous promoters showing markedly different expression levels. The expression levels of a transgene of interest can thus be fine-tuned by choosing the appropriate promoter.

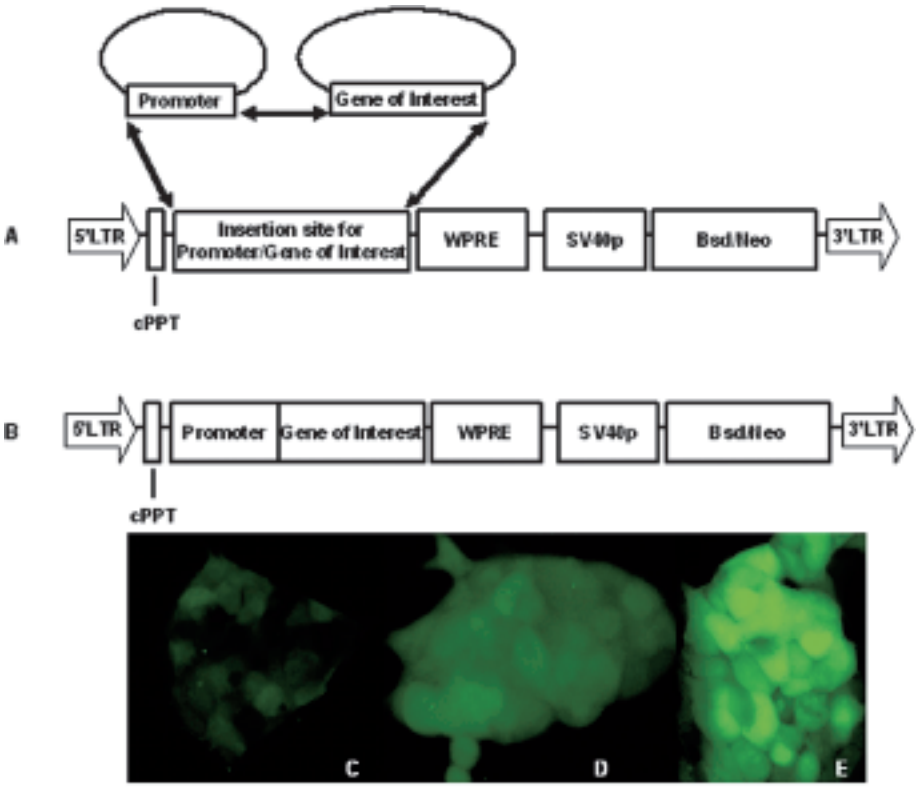


Figure 1

Schematic of the 2K7 lentiviral vector.
A: The backbone of the vector contains a site to insert both a promoter and a gene of interest in a single-step recombination reaction, as shown by the arrows. To increase transgene expression and copy number of the integrated vector in the host cell, Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element (WPRE), and the HIV-1 central polypurine tract (cPPT) were included in the vector backbone. Finally, either blasticidin (bsd) or neomycin (neo) resistance driven by an SV40 promoter allows antibiotic selection of transduced cells.
B: Resulting expression lentiviral construct. Mouse ES cell lines expressing different levels of eGFP were generated using ubiquitous promoters to obtain low (human CMV promoter, C) intermediate, (EF1-α short promoter, D), or high (EF1-α long promoter, E) eGFP expression.

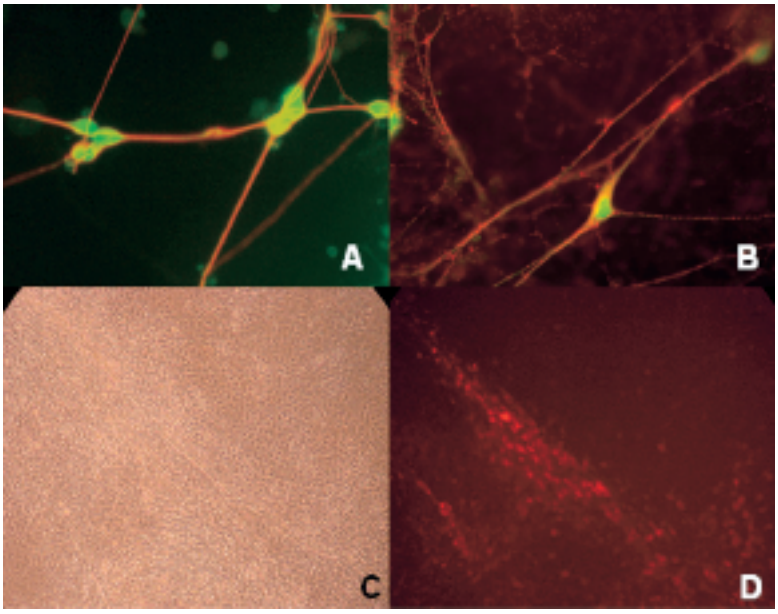
Use of genetic engineering to study neuronal differentiation

Using the novel vector, transgenes can be readily expressed under the control of a tissue-specific promoter. This has a variety of interesting applications for stem cell research. It can be used in studies to monitor neuronal differentiation. The expression of transgenes through tissue-specific promoters also holds major potential for stem cell research and therapy. It may allow selection of a cell type of interest in vitro or in vivo in order to introduce security features (e.g. expression of a suicide gene in unwanted cells).

Using early or late neuron-specific promoters,

Figure 2

A and B: Neurons are identified by a βIII-tubulin staining (in red). The degree of maturity of neurons can be identified using either the Tα1-αtubulin promoter to reveal young neurons (in green in A) or the Synapsin1 promoter to reveal mature neurons (in green in B). This approach can also be used to follow the emergence of neurons in a heterogenous ES-cell derived progeny. For this purpose, mouse ES cells were genetically modified to express a Red Fluorescent Protein (RedFP) driven by the Tα1-αtubulin promoter.
C: Aggregate of ES cells in which newly formed neurons (red spots) can be identified by fluorescence microscopy (D).



the transgene can be used as an indicator of the different stages of neuronal maturation. In figure 2A and 2B (see also fig. 5 in ref. [11]) we show restricted green fluorescent protein expression to ES cells that are already differentiated in neurons (stained in red), using two different neuron-specific promoters. This approach can also be used to follow the emergence of neurons in a heterogeneous cell population. Under certain conditions ES cells aggregate and may differentiate into a variety of cell types. In figures 2C and 2D (see also fig. 6 in ref. [11]) we show an aggregate of this kind containing different cell types derived from ES cells. We can follow the birth of neurons (red spots) thanks to the transgenic expression of a red fluorescent protein controlled by the neuron-specific Tα1-αtubulin promoter.

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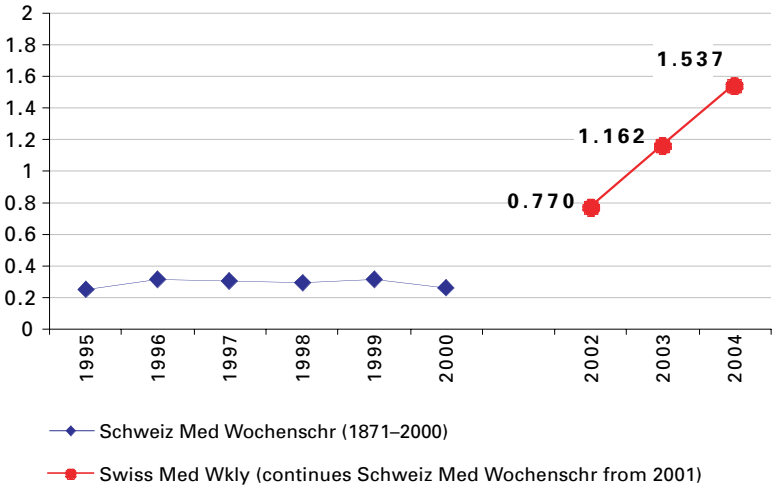

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