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Advances in genetic modification of pluripotent stem cells

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ABSTRACT

Genetically engineered stem cells aid in dissecting basic cell function and are valuable tools for drug discovery, in vivo cell tracking, and gene therapy. Gene transfer into pluripotent stem cells has been a challenge due to their intrinsic feature of growing in clusters and hence not amenable to common gene delivery methods. Several advances have been made in the rapid assembly of DNA elements, optimization of culture conditions, and DNA delivery methods. This has lead to the development of viral and non-viral methods for transient or stable modification of cells, albeit with varying efficiencies. Most methods require selection and clonal expansion that demand prolonged culture and are not suited for cells with limited proliferative potential.

Choosing the right platform based on preferred length, strength, and context of transgene expression is a critical step. Random integration of the transgene into the genome can be complicated due to silencing or altered regulation of expression due to genomic effects. An alternative to this are site-specific methods that target transgenes followed by screening to identify the genomic loci that support long-term expression with stem cell proliferation and differentiation. A highly precise and accurate editing of the genome driven by homology can be achieved using traditional methods as well as the newer technologies such as zinc finger nuclease, TAL effector nucleases and CRISPR. In this review, we summarize the different genetic engineering methods that have been successfully used to create modified embryonic and induced pluripotent stem cells.

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1. Introduction

The ability of pluripotent stem cells to indefinitely proliferate in culture and differentiate into multiple cell types under the right cues provides an ideal source for genetic modification for various downstream applications. This enables scientists to dissect basic biology and explore the potential use of pluripotent cells in regenerative medicine and drug discovery. However, a key challenge lies in identifying the ideal platform suited for the intended application. Various viral and non-viral platforms have been utilized for expression of exogenous genes and for targeting endogenous DNA in human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), albeit with varying efficiencies. Methods that have been widely used for murine embryonic stem cells (mESC) have largely been suboptimal for hESC thus necessitating further optimization of culture conditions (Braam et al., 2008).

The fundamental unit for gene delivery into cells is a plasmid DNA or vector carrying the transgenes of choice. The primary architecture of such plasmid DNA comprises a transgene of interest driven by a promoter of choice. Promoter choice is dependent on the type of expression needed. Constitutive promoter is expressed in all cell types, an inducible promoter can be activated or inactivated in the presence of small molecules, and a lineage specific promoter is active in specific cell types. Traditional restriction endonuclease-mediated cloning processes are rapidly being replaced by recombination-mediated cloning methods such as Multisite Gateway® or Lego that enable speedy assembly of multiple DNA fragments. The base plasmid also carries a drug resistance gene which can be utilized for screening cells that harbor the plasmid and a bacterial origin of replication and an antibiotic resistance gene important for propagating the plasmid in bacteria.

An additional factor that is critical for successful gene modification is efficient gene delivery methods to introduce the DNA fragments into pluripotent stem cells. Chemical-based reagents such as Lipofectamine 2000, Fugene HD, Gene Jammer, etc. offer the advantage of direct addition to the culture media but suffer from poor efficiencies. Advances to traditional electroporation devices such as Amaxa Nucleofector and Neon electroporation system have allowed higher efficiency of transfection of hESC (Lakshmipathy et al., 2004; Liu et al., 2009). In cases where chemical and electroporation methods pose a challenge, viral delivery systems have been utilized. A modified *Lentivirus* system has been reported to achieve rapid generation of stable clones in both murine as well as human ESC (Suter et al., 2006). The combination of modular cloning methods, optimal vector design and efficient gene delivery into target cells is critical for modifying hard-to-transfect ESCs (Fig. 1). In addition, choices of culture conditions and media systems are equally important factors. Although ESCs and iPSCs can be cultured under

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feeder-free media systems prior to transfection, recovery and selection of feeders has been the most traditional method used to maintain and clone out the genetically modified stem cells.

Gene modification of cells can be broadly classified into two major categories based on application, (1) gene insertion and (2) gene targeting. Gene insertion methods are most widely used to deliver DNA fragments such as cDNA or shRNA for overexpression or knockdown of specific genes, respectively. The platform of choice is largely dependent on length and strength of expression required. The wide range of gene modification approaches offers unique advantages that can be utilized to robustly express exogenous genes at levels significant enough to alter cellular function. Here, we review the various methods that have been successfully utilized to alter hESCs and iPSCs.

2. Gene insertion

2.1. Random genomic integration

Randomly integrating technologies enable users to create stable systems leading to lasting expression with or without the use of antibiotic selection. These platforms result in the random insertion of selected DNA fragments into the host genome without the use of DNA homology. Random genomic integration provides a valuable tool for long-term expression in human ES cells despite their rapid and infinite dividing capabilities. However, consequences of random insertion leading to variable copy number per cell are inconsistent integration sites and unpredictable expression patterns. In addition, the locus of insertion can result in partial or complete silencing in human ES cells, which can occur during routine culture and maintenance as well as throughout differentiation. A major risk with these methods is insertional mutagenesis resulting in genome instability and toxicity (Baum et al., 2006).

2.1.1. Naked plasmid

Randomly integrating platforms have progressed with the optimization of transfection system for ES cells. Using solely plasmid DNA provides a relatively simple integrating engineering platform since they require no additional recombination or preparation prior to transfection. As an integrating system, plasmids provide stable expression of complex cassettes with or without selection. The use of plasmid for random integration has the potential to be used in ES cells for a variety of applications including the overexpression of exogenous cassettes (Wobus and Boheler, 2005). In human ES cells, plasmid insertion

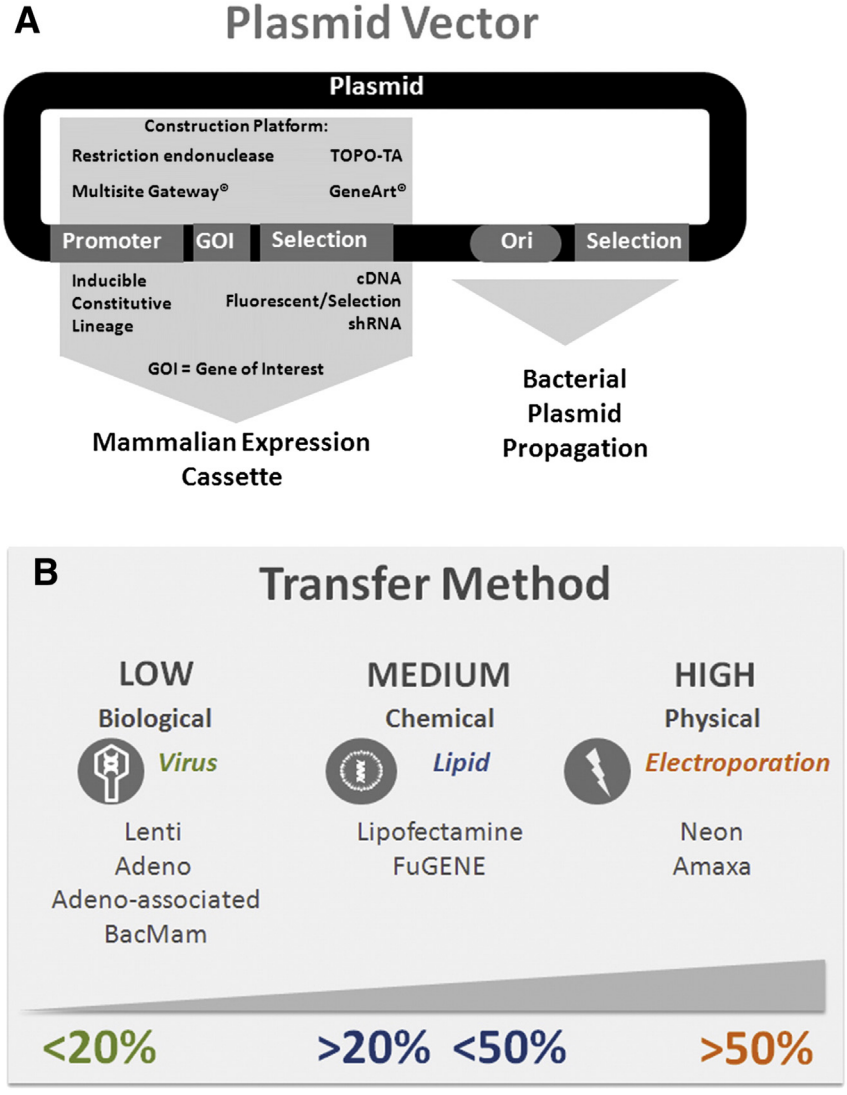

Fig. 1. (A) High throughput efficient cloning systems that can rapidly assemble plasmids of choice and (B) deliver to cells via efficient nontoxic methods, are critical for successful gene modification for gene insertion or targeted gene editing of stem cells.

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