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Advanced Drug Delivery Reviews 57 (2005) 1904–1917

Advanced DRUG DELIVERY Reviews

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ES cell technology: An introduction to genetic manipulation, differentiation and therapeutic cloning☆

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Received 30 January 2005; accepted 4 September 2005
Available online 25 October 2005

Abstract

ES cells are extraordinary cells, capable of proliferating in a pluripotent state indefinitely and of differentiating spontaneously into all cell types in vivo and many in vitro. However, the manipulation and modification of ES cells by processes such as directed differentiation and genetic modification have placed ES cells at the forefront of many biological studies and could lead to their application in biopharmaceutical areas such as cellular therapy and drug screening. Here we describe some of the ES cell based technologies that have lead to this realisation of ES cell potential.

Keywords: Lineage selection; Gene targeting; Differentiation; Drug discovery; Cell therapy

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Embryonic Stem Cell Science and the Therapeutic Interface", Vol. 57/13, 2005.

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doi:10.1016/j.addr.2005.07.018

1. Genetic manipulation of ES cells

1.1. Gene targeting: (homologous recombination)

Gene targeting by homologous recombination in ES cells has revolutionised the study of many biological systems [1,2]. Since ES cells contribute to all tissues upon injection into a recipient blastocyst, including the germ line [3,4] modification in an ES cell genome can be transmitted, by the breeding of ES cell/wild-type chimaeras, to generate mice containing the desired mutations in all cells. In this way mice with a variety of modifications such as null and point mutations, chromosomal rearrangements and large deletions have been generated. In addition, it is possible to target reporter genes under the control of specific promoters to study gene expression patterns.

In order to target a region of the genome by homologous recombination, a targeting vector is constructed which contains two 'arms' one of which is homologous with a region 5′ and one a region 3′ of the locus to be altered (Fig. 1). Commonly genes conferring drug resistance and or a reporter gene are cloned between these two homology arms to allow for the selection of transfected cells and subsequent analysis of targeted clones. Some of the ways in which gene targeting has been used in ES cells are described below.

1.1.1. Gene function

The most common use of gene targeting in ES cells is to study gene function by generating mice deficient in a gene of interest—i.e. 'knockout' mice. To generate a gene knockout, typically part or all of the coding region of the gene is replaced by a selection and or reporter gene. If the reporter gene is placed under the control of the targeted gene's promoter, either directly or through an IRES element (see Section 2.2) then this is termed a knock-in and gives further information on the function of the gene by the ability to study gene expression and to trace targeted cells. More recently gene knockout technology has progressed to allow for the generation of tissue specific and conditional knockout animals [5,6]. These are particularly useful when the ubiquitous knockout of a gene either causes early lethality or a severe phenotype, making it impossible to study the function of the gene in a tissue of choice. Both tissue and temporal specific knockouts

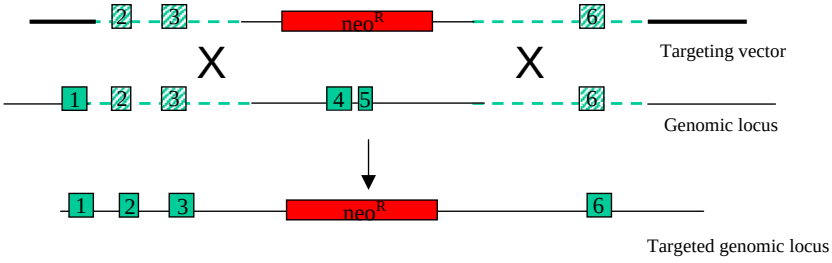

Fig. 1. Homologous recombination: the targeting vector contains two regions of homology with the endogenous genomic locus to be targeted. Homologous regions are indicated by hatching and dashed lines. Exons 4 and 5 of the endogenous locus are replaced by the neomycin resistance gene in the targeting vector. Homologous recombination between the incoming targeting vector and the endogenous locus results in the formation of a targeted locus in which the region between the homology arms is replaced with exogenous vector sequences, in this case a neomycin resistance gene. Exons are depicted as filled in boxes.