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# Human embryonic stem cells as a cellular model for human disorders

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**Abstract**

Human embryonic stem cells (HESCs) are pluripotent cell lines derived from the inner cell mass (ICM) of embryos at the blastocyst stage. These cells possess self renewal capacity and differentiation potential to all three embryonic germ layers. These unique characters made HESCs an attractive research tool for studying early human developmental processes as well as a potential therapeutic tool for various human diseases. Here, we focus on HESCs as a cellular model for human disorders. The advantages of such models as well as the various methodologies to achieve HESCs carrying a genetic defect will be discussed.
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*Keywords:* Human embryonic stem cells; Genetic diseases; Human disorders; Nuclear transfer; Preimplantation genetic diagnosis; Genetic manipulation; Gene trap

## 1. Introduction

Human embryonic stem cells (HESCs) are self renewing pluripotent cell lines derived from the inner cell mass (ICM) of blastocyst stage embryos (Thomson et al., 1998). The potency of the cells to differentiate into all three embryonic germ layers was demonstrated in vitro by differentiation into embryoid bodies (EBs) (Itskovitz-Eldor et al., 2000; Schuldiner et al., 2000), as well as in vivo by formation of differentiated tumors (called teratomas) upon injection of HESCs into immunodeficient mice (Thomson et al., 1998; Reubinoff et al., 2000). The self renewal capacity enables growing undifferentiated HESCs in culture for many passages, yet retaining a normal karyotype. The ability to form mesoderm, endoderm and ectoderm tissue components upon EBs formation or injection into immunodeficient mice made HESCs a valuable source for studying early human development. In addition, since these cells have the potential to develop into almost any cell type in vitro, they can be used for cell based therapy as an unlimited source of cells for transplantation. Here, we would like to focus on HESCs as a tool for studying human genetic disorders. HESCs harbor a given genetic defect can be utilized to explore the role of a given gene in human undifferentiated and differentiated cells. Thus, investigation of the molecular and biochemical basis of the disorder can be performed, leading consequently to better understanding of disease progression and to the development of potential therapies for the disorder.

## 2. Human cell models versus animal models

The advantage of studying human disorders using human systems rather than animal models resides in the biochemical, metabolic and genetic differences between human beings and animals. In the past years mice became the mammalian model of choice for human disorders. However, mice varied from human beings in size, growth and anatomy. Likewise, there are various developmental differences (Kaufman, 1997), e.g.: morula formation occurs in human beings two divisions later than in mouse, and there are numerous differences in the formation of tissues such as the liver, pancreas and lung. In addition, 15% of the human genes although have a homologue, lack a true ortholog in mice (Waterston et al., 2002). In some cases, therefore, an animal model for a specific human disorder completely fails to mimic the phenotype of the disease as it displays in human beings. One such a disorder is the Lesch-Nyhan disease, which is caused by a mutation in the HPRT1 gene (Stout and Caskey, 1988). This mutation promotes the overproduction of uric acid, leading to gout-like symptoms and urinary stones, in addition to neurological disorders. In rodent, however, uric acid does not accumulate because of the activity of the urate oxidase (UOX) enzyme that converts uric acid into allantoin (Bedell et al., 1997). For several human genetic diseases the mouse model partially mimic the pathology in humans, but fail to recapitulate some

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major symptoms. Ataxia-telangiectasia is a neurodegenerative disease caused by loss of function mutations in ATM (Savitsky et al., 1995). Though ATM null mutant mice help to elucidate ATM role in cancer, they do not exhibit degeneration of the Purkinje neurons and ataxia as observed in patients (Barlow et al., 1996). Alternative model for such human disorders should be established, therefore, in human cells. Primary cultures from patient's cells can be used to generate such models. This methodology is limited, however, due to the specific range of tissues from which cells can be achieved and the short life span of these primary cultures. In addition, these models based on cultures from patient's cells rely on mutations that occurred naturally in humans in specific genetic background. Another strategy is to induce mutations in already established human cell lines. Thus, gene targeting has been performed on various human cell lines, aiming to explore cellular processes such as senescence (Brown et al., 1997) and cell cycle mechanism (Bunz et al., 1998) as well as for drug discovery applications (Zeh et al., 2003). Nonetheless, the mutations were induced mostly in transformed cells and the phenotype was studied in already differentiated, committed, cell lines.

## 3. Human embryonic stem cells as a cellular model for human disorders

As mentioned above, HESCs can be maintained in culture for long periods without losing their basic characteristics. Moreover, HESCs recapitulate in vitro, as well as in vivo, early developmental processes, differentiating to all three embryonic germ layers (reviewed in Dvash and Benvenisty, 2004). Thus, using embryonic stem cells as a cellular model for human disorders enables to exam the effect of a given genetic defect on early embryo development and organogenesis, both in vitro and in vivo, implying the necessity of specific genes to these processes. Furthermore, as HESCs can be grown in culture for many passages they may serve as a convenient tool for investigating the molecular and biochemical basis of medical disorders. Generation of HESC lines harbor mutations that confer a predisposition to a specific disease could give insight into the process by which the disease arises and potentially lead to the development of new therapies.

Several methodologies can be utilized in order to achieve HESCs with various genetics defects, which could be used as a cellular model for the respective human disorders. The various methodologies can be divided into two groups. (1) Methods that manipulate wild type HESCs to harbor an artificial genetic mutation; (2) methods for achieving HESCs from blastocysts, which already harbor a genetic mutation.

## 4. Manipulating human embryonic stem cells to harbor a genetic defect

The ability to introduce foreign DNA into HESCs, either by transfection or infection methods, has been widely used (Eiges et al., 2001; Pfeifer et al., 2002; Ma et al., 2003; Zwaka and Thomson, 2003; for review see Meyshar, 2004). Human genetic disorders are caused by gain of function or loss of function mutations.

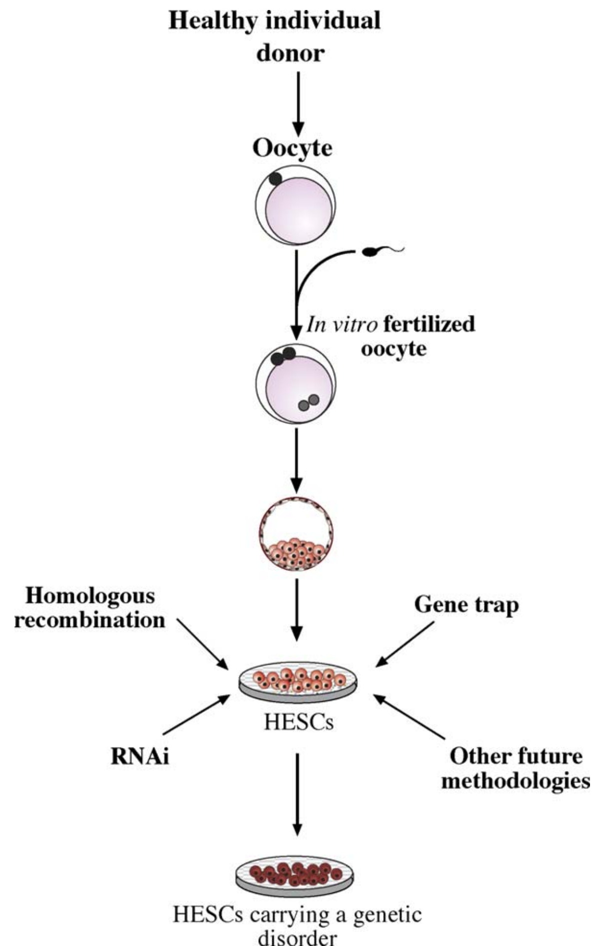


Fig. 1. Creating a cellular model of human disorders by manipulating HESCs. Insertion of a mutation by various techniques into HESCs, derived from blastocyst stage embryos of a healthy individual, can lead to establishment of a HESC line that carries a genetic disorder. Homologous recombination, RNAi and gene trap methods were shown to be useful in HESCs. Other methodologies, such as chemical mutagenesis, need to be tested.

Gain of function mutations lead to an aberrant function of a protein that causes the disease. Such dominant disorders may be modeled simply by introducing a gene harboring a mutation. However, most inherited genetic disorders involve loss of function mutations. Various genetic mutations have to be inserted into HESCs in order to generate a model for the respective human disorders. Genetic mutations could be randomly inserted or could be targeted to silence a specific gene. Here, we discuss the gene specific targeting methods, homologous recombination and RNA interference (RNAi), as well as the gene trap random insertion mutagenesis method. These methodologies were already applied to HESCs and could potentially be used to generate a cellular model for human disorders (Fig. 1). The feasibility of other methodologies, such as chemical mutagenesis and deletion induction, have to be examined in HESCs.

### 4.1. Homologous recombination

Since it was first shown that homologous recombination can be utilized to disrupt gene function in HESCs (Zwaka and

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