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Human Stem Cells for Modeling Neurological Disorders: Accelerating the Drug Discovery Pipeline

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ABSTRACT

The availability of human stem cells heralds a new era for modeling normal and pathologic tissues and developing therapeutics. For example, the in vitro recapitulation of normal and aberrant neurogenesis holds significant promise as a tool for de novo modeling of neurodevelopmental and neurodegenerative diseases. Translational applications include deciphering brain development, function, pathologies, traditional medications, and drug discovery for novel pharmacotherapeutics. For the latter, human stem cell-based assays represent a physiologically relevant and high-throughput means to assess toxicity and other undesirable effects early in the drug development pipeline, avoiding late-stage attrition whilst expediting proof-of-concept of genuine drug candidates. Here we consider the potential of human embryonic, adult, and induced pluripotent stem cells for studying neurological disorders and preclinical drug development. J. Cell. Biochem. 105: 1361–1366, 2008.

KEY WORDS: STEM CELLS; HUMAN EMBRYONIC; INDUCED PLURIPOTENT; NEUROLOGY; DISEASE MODELS; DRUG DISCOVERY

A major challenge for experimental research of human disease and drug discovery is the use of biologically relevant methods of investigation. The human central nervous system (CNS) is especially difficult to study due to its anatomical and functional complexity, compounded by the limitations and/or cost of live animal models, and the constraints for researching human subjects. The human brain includes neurons, astrocytes, microglia, and oligodendrocytes, which in turn comprises subtypes of cells with specific phenotypes, localizations, and functions. Needless to say, different cell-types contribute to different disease states requiring cell-type specific modeling of disease-specific phenotypes and pharmacologically relevant strategies for drug screening. To this end, animal modeling has been a mainstay of the drug development pipeline, with mice frequently used in pharmaceutical research and development (R&D) as a nonclinical efficacy model. This is despite the fact that drug testing in mice often fails to translate to human studies, with murine results nonpredictive for treating neurodegenerative diseases such as Alzheimer's disease [Schnabel, 2008].

There are many potential causes for the failed translation of neuroactive drug trials from animal models to humans, including species differences in drug penetration of the blood–brain barrier, drug metabolism, and related toxicity, culminating in a variable biological response. In addition, there is the more contentious matter of less than optimal design rigor of testing regimes [Schnabel, 2008].

Although noteworthy, it is not within the scope of this article to consider in detail the merits and shortcomings of in vivo modeling, but rather merely affirm the need for other relevant and complementary methods for laboratory based studies of the human CNS and preclinical identification of bona fide drug candidates. In principle, in vitro human stem cell- and derivative cell-based modeling represents a valuable line of investigation that is practical, relatively cost-effective, and potentially able to accelerate the drug development pipeline; fundamental objectives for the pharmaceutical and biotechnology industries worldwide. Model systems could include disease-specific or transgenic embryonic [Braam et al., 2008], adult [Lovell et al., 2006; Ferrero et al., 2008; Murrell et al., 2008; Zhang et al., 2008], or recently discovered induced pluripotent [Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008a,b] stem cells (ES, AS, or iPS cells, respectively) combined with methods of directed differentiation that mimic the critical stages of CNS development in vivo and endogenous neuronal cell replacement for adult CNS homeostasis. For in vitro assays using lineage-specific cells with limited proliferative potential, stem cells represent a scaleable supply of source cells for bulk derivative cell production. While the efficient generation of neural-subtype cells from stem cells remains a challenge, methods of neural induction are improving [Cho et al., 2008]. Large numbers of high purity and functional neurons [Li and Zhang, 2006; Schwartz et al., 2008] or oligodendrocytes [Nistor et al., 2005; Jessberger et al., 2008] can

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potentially be prepared for pathomolecular analysis and/or high-throughput screening of compound libraries.

Here we review the rapidly emerging area of stem cell-based modeling of human brain disorders to decipher etiopathologies and advance drug discovery. We propose that the traditional inefficiencies, high attrition rates, protracted R&D cycles, and elevated costs inherent to drug development can be curtailed by using relatively inexpensive and high-throughput in vitro human stem cell-based assays as a first phase of discovery and preclinical development in the pharmaceutical pipeline. In principle, transgenic and disease specific human ES, AS, and iPS cells and their neural derivatives represent important research tools that genuinely emulate the underlying mechanisms of human neurodevelopmental and neurodegenerative diseases and predict in vivo drug response.

HUMAN EMBRYONIC STEM CELLS

Since the first human ES cell lines were derived over a decade ago [Thomson et al., 1998], hundreds have been produced worldwide, including lines suitable for clinical application [Crook et al., 2007]. They are derived from supernumerary blastocysts by established methods of isolation, culture and preservation. Human ES cells can self-renew indefinitely and are pluripotent. Hence they can be expanded to large numbers [Phillips et al., 2008] and be differentiated into most cells of the human body, including the CNS. These properties make them an ideal resource for high-throughput cell-based assayology, including a potentially unlimited supply of neural derivatives [Nat et al., 2007].

When derived from blastocysts identified by preimplantation genetic diagnosis (PGD) as carrying congenital mutations for specific disease states [Verlinsky et al., 2005; Mateizel et al., 2006; Eiges et al., 2007; Ben-Yosef et al., 2008; Peura et al., 2008] or following genetic manipulation [Urbach et al., 2004], human ES cells afford new and relevant perspectives on human disorders. Combined with effective methods of differentiation, they should prove better than conventional mouse stem cell models for recapitulating the human phenotype, and at the very least are complementary to in vivo mouse models. Despite their potential, only a few human ES cell models of CNS diseases have been reported [Urbach et al., 2004; Verlinsky et al., 2005; Mateizel et al., 2006; Eiges et al., 2007]. Moreover, the pharmaceutical industry has been slow to adopt human ES cell-based screening despite a long standing use of in vitro cellular assays and the provision of guidelines for human ES cell research by organizations such as the National Academy of Sciences (http://www.nasonline.org/), the National Institutes of Health (http://www.nih.gov/), and the International Society of Stem Cell Research (http://www.isscr.org/). Interest has been tempered by ethical concerns and in some countries legal restrictions for deriving and using stem cell lines from human blastocysts.

In principle, there are three ways to derive human ES cell lines for in vitro modeling of disease (Fig. 1A): (i) targeted gene disruption of cells by for example RNA interference or homologous recombination; (ii) cell line isolation from congenitally defective preimplantation embryos; or (iii) cell line isolation from blastocysts produced by somatic cell nuclear transfer (SCNT). While there are examples of targeted gene disruption and PGD-derived human ES cell lines, derivation of human ES cells by SCNT is yet to be achieved. Whether or not an SCNT approach is widely adopted once attained will depend on the ethical concerns surrounding oocyte donation and the success of alternative reprogramming methods such as iPS cell generation described below.

Despite the potential of gene targeting in human ES cells, progress has been hindered by poor transfection and cloning efficiencies, with a handful of accessible cell lines seemingly amenable to manipulation [Xia et al., 2007; Braam et al., 2008]. While for the most part achieving efficient gene delivery remains a problem (i.e., >80% transduction efficiency), transient [Braam et al., 2008] stable [Liew et al., 2007] and conditional [Vieyra and Goodell, 2007] expression systems are evolving, together with more compatible methods of human ES cell culture. Adaptation of stem cells to contemporary single cell and feeder-free culture seemingly enhances transfection efficiency by avoiding sequestration of transfection reagents by fibroblasts and maximizing the uptake of reagents by stem cells cultured as monolayers [Braam et al., 2008].

Whereas genetically engineered stem cells typically serve as models of monogenic disorders, ES cell lines derived from defective PGD-embryos can potentially model diseases with complex polygenic traits [Braude et al., 2002]. PGD is used as an adjunct to in vitro fertilization and avoids selective pregnancy termination by identifying genetic defects before embryo implantation. Although traditionally performed by polymerase chain reaction and fluorescence in situ hybridization, DNA chip (microarray) technology offers a new approach to diagnosis or risk prediction of more complex disorders. The use of "gene chips" for preimplantation genetic screening is currently being evaluated by the European Society of Human Reproduction and Embryology (ESHRE) towards developing a code of practice (http://www.eshre.com/emc.asp).

While normal PGD-embryos can be used for assisted reproduction, mutant embryos can provide human ES cells exhibiting the same genotype and related defects [Verlinsky et al., 2005; Mateizel et al., 2006; Eiges et al., 2007; Ben-Yosef et al., 2008; Peura et al., 2008]. Once generated, cell lines can be used to decode the pathways through which mutations cause an inherited phenotype. Examples of PGD-derived ES cells representing CNS related disorders include lines for Huntington's disease [Verlinsky et al., 2005] and fragile X syndrome [Eiges et al., 2007]. Once established, these and other PGD-cell lines have the potential to provide a valuable and unlimited source of undifferentiated and differentiating ES cells and derivative neurons, glia, and other somatic cells for investigating the cause, effect, and treatment of aberrant human biology on a cellular level.

HUMAN ADULT STEM CELLS

In contrast to human ES cells, AS cells reside in specialized niches of mature tissues. Nonetheless, they fulfill the basic criteria of stemness, having the capacity to self-renew and give rise to one or more differentiated cell types. The discovery of new AS cell types

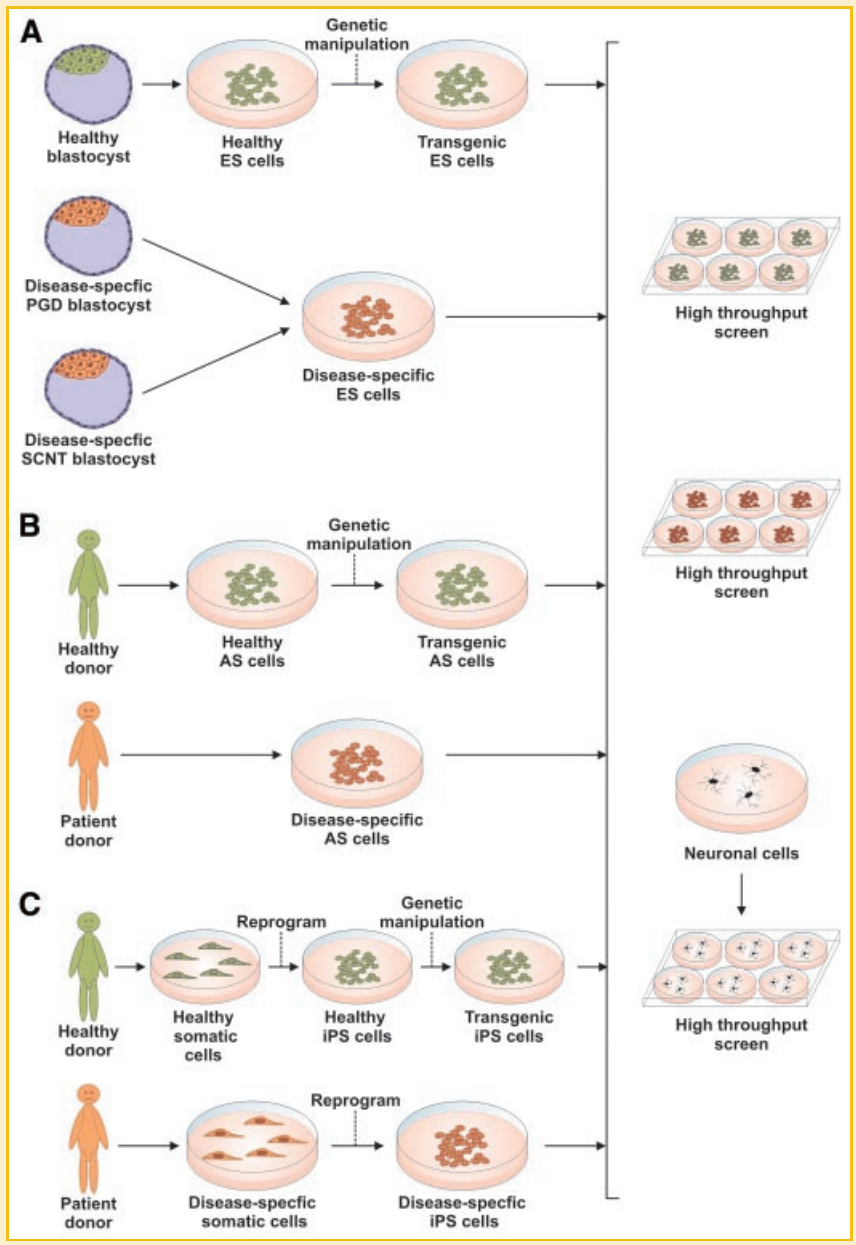

Fig. 1. Schematic illustration of stem cell-based disease modeling and drug discovery. A: Human ES cell disease-models can potentially be produced by: (i) targeted gene disruption of cells derived from healthy blastocysts, (ii) cell line isolation from congenitally defective preimplantation embryos identified by PGD, or (iii) cell line isolation from blastocysts produced by SCNT. B: Human AS cell disease-models can be derived by: (i) targeted gene manipulation of cells from a healthy donor, or (ii) cell line isolation from a patient. C: Human iPS cell disease-models can be produced by reprogramming somatic cells from either a healthy or patient donor, with the former requiring targeted gene disruption to mimic mutations relevant to a disease state. High throughput screening of compound libraries with undifferentiated stem cells and their neural derivatives is subsequently performed to assess the efficacy (including toxicity and other undesirable effects) of potential drug candidates.

in human tissues is ongoing. In addition to expected lineages, many show plasticity seemingly unrelated to their in vivo niche.

Neural stem (NS) cells of the developing CNS are highly proliferative and generate neurons and glia under strict spatial and temporal regulation [Temple, 2001]. Until recently, the adult human brain was thought to be devoid of neurogenic activity. The seminal reports by Altman and Das [1965] of proliferating cells in the mature rat hippocampus, and Reynolds and Weiss [1992] of in vitro isolation, expansion and differentiation of adult mouse striatal cells paved the way for identifying NS cells in the adult human brain. NS cells isolated from human subventricular zone and hippocampal dentate gyrus confirmed their capacity for self-renewal and multipotentiality [Kukekov et al., 1999; Roy et al., 2000; Palmer et al., 2001; Westerlund et al., 2003].

The main impetus for NS cell research has been to provide transplantable cells for neurotrauma and neurodegenerative

diseases, with less interest directed to disease-modeling and drug discovery. However, the specific relevance of neurogenesis to emerging theory of neurodevelopmental disorders such as schizophrenia and autism is bolstering interest in NS cells as research tools. For example, antidepressant drugs used to treat schizophrenia and autism increase cell proliferation in adult hippocampus, suggesting a role for neurogenic activity in the etiology and treatment of these disorders [Santarelli et al., 2003; Encinas et al., 2006]. Disease-specific or genetically engineered NS cell lines represent cellular systems for discerning disease etiology and the actions of existing and novel drug candidates (Fig. 1B).

While human NS cell cultures are a gold standard for neurological disease modeling, limited accessibility to source tissues impedes their use. For example, surgical or postmortem removal of brain tissues is insufficient for the requirements of large-scale cell-based drug screening. Tissue biopsies from olfactory epithelium represent more practical sources [Roisen et al., 2001; Winstead et al., 2005]. Importantly, new evidence supports the differentiation of AS cells across lineages so that easily accessible stem cells could be used in the therapy of degenerative diseases of the CNS [Ross and Verfaillie, 2008]. For example, neurons and glia can be produced from skin derived precursor cells isolated from human scalp and foreskin [Toma et al., 2005], marrow stromal stem cells [Cho et al., 2005; Togel and Westenfelder, 2007], umbilical cord stem cells [Sanchez-Ramos et al., 2001; Low et al., 2008], and adipose tissue-derived stromal cells [Safford et al., 2002; Schaffler and Buchler, 2007]. Given their accessibility, abundance, ethical justification, and plasticity, these non-NS cells represent viable alternatives for discerning etiopathologies and pharmaco-screening. Examples include disease specific AS cells from Parkinson's disease and amyotrophic lateral sclerosis (ALS) patients [Ferrero et al., 2008; Murrell et al., 2008; Zhang et al., 2008]. Neurological disease-specific AS cells potentially offer a superior model system than conventional immortalized human neural cell lines often used by the pharmaceutical industry.

Despite the demonstrated potential of AS cells, the derivation of functional neural cells remains a challenge. The majority of studies claiming generation of specific neural cells from AS cells do not include definitive characterization, with many only showing pan-neuronal marker expression (e.g., beta-III-tubulin, MAP-2, Neu-N). Few studies profile neurotransmitter subtypes and electrophysiological properties of differentiated neurons [Wislet-Gendebien et al., 2005; Fernandes et al., 2006]. Nevertheless, similar to ES cells, AS cells can have an immediate impact as models for human CNS diseases and research tools for drug discovery. Compound screening will benefit from assays of undifferentiated stem cells as well as their lineage specific progenies (i.e., neuronal and nonneuronal/non-target). Analyses of stem cells are important due to their role in the physiological homeostasis of the CNS and other tissues/organs relevant to systemic drug effects.

INDUCED PLURIPOTENT STEM CELLS

The recent creation of iPS cells by somatic cell reprogramming to an embryonic stem cell-like state offers another tool to study human disease, including developmental and degenerative disorders of the CNS, and model-based drug development. Similar to human ES and AS cells, iPS cells can either be modified by targeted gene disruption or created from patients as disease-specific cell lines (Fig. 1C) [Dimos et al., 2008; Park et al., 2008a]. Currently, oncogenes and viral vectors are used to produce iPS cells, which are unsuitable for cell replacement therapy. While the method of reprogramming is likely to be less of an issue for disease modeling and drug discovery, it remains to be shown that viral integration and transduction of foreign genes does not confound endogenous cellular processes. In addition, it is unclear whether iPS cells are functionally equivalent to ES cells.

The first example of a disease-specific iPS cell line was generated from a patient with ALS [Dimos et al., 2008]. The ALS lines provide proof-of-principle of modeling neurological disorders by reprogramming fibroblasts and differentiation to nerve cells for characterization. Interestingly, mouse models of ALS rely on mutations of the superoxide dismutase-1 gene, which only partially emulate the various forms of the disease in humans. By contrast, iPS cell lines have been derived from patients with mild and severe forms of ALS and are expected to more faithfully recapitulate genetic patterning and variant pathophysiology.

A more recent report of disease-specific iPS cells describes the generation of lines from patients with a variety of diseases with either Mendelian or complex inheritance, including Down syndrome, Gaucher disease type III, Parkinson disease, and Huntington disease [Park et al., 2008a]. Again, by carrying specific genetic lesions these cell lines and the wave of other cell lines likely to follow will hopefully provide new and accurate insights to diseases previously deemed difficult or nigh on impossible to investigate.

CONCLUDING REMARKS

Overall, less than 10% of compounds that enter clinical phase testing are approved for market, at an estimated cost of US$1.2–1.7 billion per drug [Kaitin, 2008; Sollano et al., 2008]. Historically, CNS class compounds that are new chemical entities have a slightly higher success rate of ~14% [Dimasi, 2001]. The high failure rate is reflected by the number of new drugs approved for use in the category of neurology by the US Food and Drug Administration (FDA) in 2006, 2007, and 2008 with one, four, and one drugs approved respectively (http://www.centerwatch.com/patient/drugs/druglist.html). There is clearly a need to increase productivity and decrease the cost of drug development using strategies that concomitantly bolster innovation and facilitate R&D for early assurance of drug safety and efficacy. To this end, human stem cell based models of CNS development, function, and disease represent a useful research tool to complement in vivo experimentation.

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