NeuroToxicology 33 (2012) 518–529

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Review

The potential of induced pluripotent stem cells as a translational model for neurotoxicological risk

Kevin K. Kumar [a,b,c], Asad A. Aboud [a,b], Aaron B. Bowman [a,b,*]

[a] Department of Neurology, Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN, United States
[b] Department of Pediatrics and Vanderbilt Brain Institute, Vanderbilt University, Nashville, TN, United States
[c] Vanderbilt Medical Scientist Training Program, Vanderbilt University, Nashville, TN, United States

ARTICLE INFO

Article history:
Received 1 October 2011
Accepted 3 February 2012
Available online 11 February 2012

Keywords:
Neurotoxicology
Developmental toxicology
Human stem cells
Induced pluripotent stem cells
Environment

ABSTRACT

An important goal of neurotoxicological research is to provide relevant and accurate risk assessment of environmental and pharmacological agents for populations and individuals. Owing to the challenges of human subject research and the real possibility of species specific toxicological responses, neuronal lineages derived from human embryonic stem cells (hESCs) and human neuronal precursors have been offered as a potential solution for validation of neurotoxicological data from model organism systems in humans. More recently, with the advent of induced pluripotent stem cell (iPSC) technology, there is now the possibility of personalized toxicological risk assessment, the ability to predict individual susceptibility to specific environmental agents, by this approach. This critical advance is widely expected to facilitate analysis of cellular physiological pathways in the context of human neurons and the underlying genetic factors that lead to disease. Thus this technology opens the opportunity, for the first time, to characterize the physiological, toxicological, pharmacological and molecular properties of living human neurons with identical genetic determinants as human patients. Furthermore, armed with a complete clinical history of the patients, human iPSC (hiPSC) studies can theoretically compare patients and at risk groups with distinct sensitivities to particular environmental agents, divergent clinical outcomes, differing co-morbidities, and so forth. Thus iPSCs and neuronal lineages derived from them may reflect the unique genetic blueprint of the individuals from which they are generated. Indeed, iPSC technology has the potential to revolutionize scientific approaches to human health. However, before this overarching goal can be reached a number of technical and theoretical challenges must be overcome. This review seeks to provide a realistic assessment of hiPSC technology and its application to risk assessment and mechanistic studies in the area of neurotoxicology. We seek to identify, prioritize, and detail the primary hurdles that need to be overcome if personalized toxicological risk assessment using patient-derived iPSCs is to succeed.

Contents

* Corresponding author at: Department of Neurology, Vanderbilt University Medical Center, 465 21st Ave South, 6140 MRB3, Nashville, TN 37232-8552, USA. Tel.: +1 615 322 2651; fax: +1 615 322 0486.
E-mail address: aaron.bowman@vanderbilt.edu (A.B. Bowman).

0161-813X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2012.02.005

1. Introduction

The field of toxicology has seen rapid innovation in the past two decades by the advent of stem cell technology. Perhaps the first major successful use of stem cells for the study of toxicity was the embryonic stem cell test (EST) developed by Spielmann and colleagues (Heuer et al., 1993; Spielmann et al., 1997). This approach differentiates mouse embryonic stem cells (ESCs) into cardiomyocytes in the presence of potential developmentally toxic agents (Heuer et al., 1993; Seiler and Spielmann, 2011). Although this method utilizes mouse stem cells, and focuses on differentiation into beating cardiomyocytes, the method has been broadly hailed for its ingenuity (Laustriat et al., 2010; Scholz et al., 1999; Wobus and Loser, 2011). However, the method has notable shortcomings in its application to neurotoxicology. For example, although the EST correctly classified the majority of known embryotoxic chemicals tested, it is known that the EST in some cases failed to correctly classify methylmercury as a developmental toxicant (Genschow et al., 2004). There are several potential reasons for these shortcomings of the EST – including species-specific toxicities and tissue-type specific toxicities. Recently, Bremer et al. sought to address both of these issues by adapting the principles of the EST to toxicity testing in human ESCs (hESCs) undergoing neuronal differentiation (Stummann et al., 2009). Their study showed greater sensitivity of early-developing neural precursors over maturing neuronal cells to methylmercury toxicity (*i.e.* greater changes in expression of key early neurodevelopmental markers *versus* more mature neuronal markers) (Stummann et al., 2009). Other groups have also provided proof-of-principle experiments demonstrating the potential of hESCs to evaluate developmental toxicity (Pal et al., 2011). However, ethical and regulatory concerns about the use of cells derived from human embryos have limited adoption of hESC based toxicity testing (Leist et al., 2008; Vojnits and Bremer, 2010).

Pioneering studies have revealed both the feasibility as well as clear advantages for use of stem cell based approaches for neurotoxicological risk assessment. Although the fundamentals of stem cell culture are outside the scope of this review, a number of book chapters and review articles are available on this topic (Neely et al., 2011; Park et al., 2008; Takahashi et al., 2007). Studies using murine stem cells have identified mRNA based expression markers for assessment of neurodevelopmental toxicity (Kuegler et al., 2010; Theunissen et al., 2011). Comparative studies using hESC derived neurons *versus* rodent primary neuronal cultures have revealed important differences in sensitivity, reproducibility, and dynamic ranges by toxicity measures examining neurite outgrowth and cytotoxicity; suggesting further work is needed in developing and interpreting hESC-derived neurotoxicity tests (Harrill et al., 2011). Indeed, toxicogenomic approaches revealed key differences on the influence of a developmental neurotoxicant on expression profiles between *in vivo* models, stem-cell based *in vitro* models and primary tissue/cell culture based models – yet also identified examples of coherent responses from the *in vitro* ESC-based models and *in vivo* measures (Robinson et al., 2011). Furthermore, predictive neurotoxicity testing by hESC-based neuronal differentiation approaches has proven successful in discriminating chemicals and pharmaceuticals with known developmental neurotoxicity (Buzanska et al., 2009). A related approach to hESC-based neurotoxicology has been to start developmentally down-stream of the pluripotent state and utilize multipotent human neuroprogenitors as a starting point for developmental neurotoxicity testing (Breier et al., 2008; Harrill et al., 2010, 2011; Moors et al., 2009; Schreiber et al., 2010; Tofighi et al., 2011a,b). Neuralization of pluripotent stem cells or neuroprogenitors can be accomplished either by adherent culture-based neuronal differentiation or a neurosphere suspension culture, which may be followed by subsequent plating, differentiation and migration. A discussion of the advantages and disadvantages of these two approaches has been recently reviewed by Breier and colleagues (Breier et al., 2010).

In this review, we seek to describe the methods of generating hiPSCs, explore the utility of this technology in the field of neurotoxicology, and discuss technical challenges for these applications. In addition, we will outline the process of generating and maintaining hiPSCs for toxicity testing, characterize multiple exposure paradigms, and attempt to predict the future of the field.

2. The promise of iPSC technology for neurotoxicology

A number of recent reviews have described potential applications of hESC and hiPSC technology to toxicology, pharmacology and the study of human diseases that have environmental contributions to their etiology (Anson et al., 2011; Heng et al., 2009; Marchetto et al., 2011; Saha and Jaenisch, 2009; Vojnits and Bremer, 2010; Winkler et al., 2009; Wobus and Loser, 2011). Here we focus on the promise and roadblocks specifically for neurotoxicological applications. An important advantage of a patient-specific iPSC approach to neurotoxicology is that environmental risk for an individual may be evaluated without *a priori* knowledge of the genetic risk factors. A complex relationship of environmental and genetic risk factors underlies many neurodevelopmental and neurodegenerative diseases – yet identification of causative factors has been severely hampered by the lack of suitable experimental models to account for the combinatorial influence of diverse toxicants and the inherent variation in human susceptibility and exposure. This complexity and variation of genetic and environmental influences between individuals also complicate epidemiological studies to identify contributors. For example, a link between pesticide exposure and Parkinson's disease (PD) became suspected in 1983 with the discovery that exposure to 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), a compound with structural similarity to the pesticide paraquat, causes a selective degeneration of dopaminergic neurons in the substantia nigra (Elbaz and Moisan, 2008; Langston et al., 1983). Despite epidemiological evidence potentially linking pesticide use with risk for PD, discerning the role of specific pesticides in human disease has been difficult (Dick et al., 2007a,b; Frigerio et al., 2009, 2006; Kamel et al., 2007). Likewise, studies have found links between PD and exposure to Mn, Pb, and other metals (Coon et al., 2006; Finkelstein and Jerrett, 2007). Interestingly, a recent study of a Chinese cohort found an association between blood Mn levels and PD, yet no differences in exposure were seen between control and disease groups (Fukushima et al., 2009). This raises the possibility that genetic risk factors may predispose some people to accumulate levels of this environmental toxicant thereby selectively increasing their risk for disease. The advent of hiPSC technology may provide researchers a method to test this and similar hypotheses, by allowing the evaluation of selective sensitivity to neurotoxicants across individual patients.