Experimental Neurology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review Article

Human stem cell modeling in neurofibromatosis type 1 (NF1)

Michelle L. Wegscheid, Corina Anastasaki, David H. Gutmann *

Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110, United States

ARTICLE INFO

Article history:
Received 28 December 2016
Received in revised form 15 March 2017
Accepted 5 April 2017
Available online xxxx

Keywords:
NF1
Neurodevelopment
Neurofibroma
iPSC
Stem cell
Optic pathway glioma
Retinal ganglion cell
Microglia
Tumor

ABSTRACT

The future of precision medicine is heavily reliant on the use of human tissues to identify the key determinants that account for differences between individuals with the same disorder. This need is exemplified by the neurofibromatosis type 1 (NF1) neurogenetic condition. As such, individuals with NF1 are born with a germline mutation in the *NF1* gene, but may develop numerous distinct neurological problems, ranging from autism and attention deficit to brain and peripheral nerve sheath tumors. Coupled with accurate preclinical mouse models, the availability of NF1 patient-derived induced pluripotent stem cells (iPSCs) provides new opportunities to define the critical factors that underlie NF1-associated nervous system disease pathogenesis and progression. In this review, we discuss the generation and potential applications of iPSC technology to the study of NF1.

Contents

1. Introduction

Neurofibromatosis type 1 (NF1) is a complex multisystem cancer predisposition syndrome, with a birth incidence between 1 in 2500 and 1 in 3000 individuals worldwide (Lammert et al., 2005; Evans et al., 2010). The condition is caused by autosomal dominantly-inherited or *de novo* loss-of-function mutations in the *NF1* gene located on chromosome 17q11.2 (Uusitalo et al., 2014). Affected individuals present with a wide range of clinical manifestations, including pigmentary abnormalities (café-au-lait macules, skinfold freckling, Lisch nodules), peripheral (neurofibromas, malignant peripheral nerve sheath tumors) and central (optic pathway and brainstem gliomas) nervous tumors,

* Corresponding author at: Department of Neurology, Washington University School of Medicine, Box 8111, 660 South Euclid Avenue, St. Louis, MO 63110, United States.
E-mail address: gutmannd@wustl.edu (D.H. Gutmann).

http://dx.doi.org/10.1016/j.expneurol.2017.04.001
0014-4886/

bone abnormalities, vasculopathy and other cancers (Jett and Friedman, 2010). In addition to these medical problems, over 80% of children with NF1 have learning disabilities, social perception deficits (autism spectrum disorder), and attention/or deficits (Hyman et al., 2005).

While there has been enormous progress over the past 25 years since the identification of the *NF1* gene in 1990, there are still a limited number of molecular targets for therapeutic drug design, and few of these molecularly-targeted therapies have been effective when evaluated in human clinical trials. In this regard, oral imatinib mesylate successfully reduced plexiform neurofibroma size and metabolic activity in a preclinical *Nf1* mouse model (Yang et al., 2008), but resulted in variable reductions in tumor volume in a phase 2 study (Robertson et al., 2012). Lovastatin was similarly successful in a preclinical *Nf1* mouse model by normalizing long-term potentiation (LTP) deficits and reversing spatial learning and attention impairments (Li et al., 2005). However, several randomized placebo-controlled lovastatin and simvastatin clinical trials produced no detectable improvements in measures of attention (Bearden et al., 2016; Krab et al., 2008; Payne et al., 2016a; van der Vaart et al., 2013; van der Vaart et al., 2016).

One potential reason for this apparent lack of preclinical translation is the inherent differences between rodents and humans. Although they share substantial genomic homology, there are significant dissimilarities to consider when using animal models to inform about human disorders. Anatomically, rodent brains are unlike human brains in that they are lissencephalic, meaning that their cerebral cortices do not undergo gyrification during development like their human counterparts (Semple et al., 2013). In addition, cerebral progenitor zone complexity and organization differs between rodents and humans (Molnar et al., 2011). Furthermore, specific cell types, like microglia, exhibit striking interspecies differences in proliferation *in vitro*, immune system receptor expression and response to immune stimuli (Smith and Dragunow, 2014).

For these reasons, it would be desirable to complement *Nf1* mouse models with preclinical experiments using actual human biospecimens. One such approach entails the use of patient-derived xenografts (PDX), in which patient tumor tissues are transferred into immunodeficient mice, allowing for preservation of tumor histology, genetic composition, and drug sensitivity. This platform has been highly successful for high-grade brain tumors, such as glioblastoma (Joo et al., 2013), but has been problematic for low-grade gliomas and neurofibromas due to premature senescence and low clonogenic frequencies. Another approach employs pathologic specimens, which maintain intact tissue architecture and gene expression patterns. However, the dynamic changes inherent in these tissues are reduced to a static image, and much of the information in these biospecimens regarding cell-cell interactions, stromal contributions, or the impact of germline genetics on disease development and progression is lost.

These limitations support the pressing need for an *in vitro* human system amenable to genetic engineering, as well as dynamic molecular and functional analyses. The discovery of somatic cell reprogramming to a pluripotent state by Shinya Takahashi and colleagues in 2006 (Takahashi and Yamanaka, 2006) ushered in an era of *in vitro* human disease modeling. The work that Dr. Yamanaka received the Nobel Prize for in 2012 involved retroviral delivery of transcription factors *Oct3/4*, *Sox2*, *c-Myc* and *Klf4* into mouse embryonic fibroblasts, generating induced pluripotent stem cells (iPSCs) with the capacity to differentiate into any cell type in the body (Fig. 1) (Takahashi and Yamanaka, 2006). Within the last ten years, refinements in reprogramming and differentiation techniques have resulted in the generation and application of human-derived iPSCs (Takahashi et al., 2007) to model complex genetic disorders, such as Rett syndrome (Marchetto et al., 2010), Fragile X syndrome (Mor-Shaked and Eiges, 2016), schizophrenia (Brennand et al., 2011), and bipolar disorder (Chen et al., 2014a). In this review, we discuss the current capabilities of somatic cell reprogramming, iPSC differentiation and the potential of iPSC technology to provide multidimensional models of neurodevelopment and tumorigenesis in NF1. In addition, we will highlight potential applications of iPSC technology to therapeutic delivery and screening, as well as discuss the inherent limitations of this approach.

2. iPSC sources and reprogramming

Induced pluripotent stem cells, like most stem cells, are capable of generating more iPSCs (self-renewal), but also can give rise to cell types from any of the three germinal layers formed during embryogenesis (ectoderm, mesoderm, and endoderm). In this regard, they are similar to human embryonic stem cells (hESCs), but do not carry the ethical concerns associated with the use of embryos for hESC isolation. Importantly, iPSCs do not derive from embryonic tissues, and are instead generated by genetic reprogramming of non-germ cells (somatic cells).

There are multiple somatic cell types that can be reprogrammed to generate iPSCs (Fig. 2), each with unique advantages and disadvantages. Dermal fibroblasts from skin punch biopsies were the first source of human-derived iPSCs (Takahashi et al., 2007; Yu et al., 2007), and are the most frequently used cell type for reprogramming. iPSC sources have since been expanded to include stem cells from adult peripheral blood and umbilical cord blood collected after birth (Loh et al., 2009). Exfoliated renal tubular epithelial cells isolated from urine (Zhou et al., 2011) and keratinocytes from hair (Aasen et al., 2008) are also viable reprogramming sources for the generation of iPSCs. There is currently no consensus regarding the ideal tissue from which to harvest cells for reprogramming, but the cell type of origin has been shown to affect programming efficiency (Kim et al., 2011; Maherali et al., 2008).

For example, reprogramming of primary human keratinocytes using conventional retroviral transduction with *OCT4*, *SOX2*, *KLF4* and *MYC*

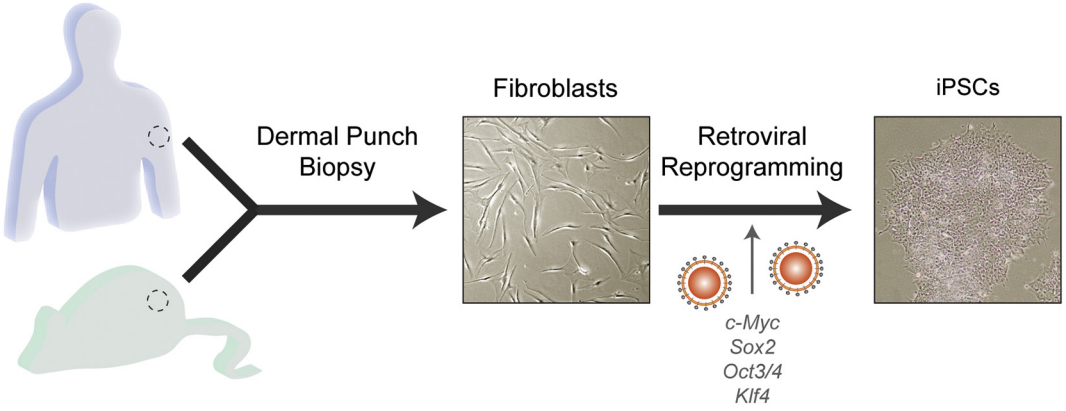

Fig. 1. iPSCs can be generated from somatic cells by transcription factor-mediated reprogramming. Mouse fibroblasts and human dermal fibroblasts were originally reprogrammed by Shinya Takahashi and colleagues *via* retrovirus-mediated transfection of transcription factors *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*.

Please cite this article as: Wegscheid, M.L., et al., Human stem cell modeling in neurofibromatosis type 1 (NF1), Exp. Neurol. (2017), http://dx.doi.org/10.1016/j.expneurol.2017.04.001