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Neural precursor cells derived from induced pluripotent stem cells exhibit reduced susceptibility to infection with a neurotropic coronavirus

Vrushali Mangale[a], Brett S. Marro[b,1], Warren C. Plaisted[b,2], Craig M. Walsh[b], Thomas E. Lane[a,*]

[a] Department of Pathology, Division of Microbiology & Immunology University of Utah, Salt Lake City, UT 84112, United States
[b] Department of Molecular Biology & Biochemistry and Institute for Immunology, University of California, Irvine 92697, United States

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ABSTRACT

The present study examines the susceptibility of mouse induced pluripotent stem cell-derived neural precursor cells (iPSC-NPCs) to infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV). Similar to NPCs derived from striatum of day 1 postnatal GFP-transgenic mice (GFP-NPCs), iPSC-derived NPCs (iPSC-NPCs) are able to differentiate into terminal neural cell types and express MHC class I and II in response to IFN-γ treatment. However, in contrast to postnatally-derived NPCs, iPSC-NPCs express low levels of carcinoembryonic antigen-cell adhesion molecule 1a (CEACAM1a), the surface receptor for JHMV, and are less susceptible to infection and virus-induced cytopathic effects. The relevance of this in terms of therapeutic application of NPCs resistant to viral infection is discussed.

1. Introduction

Neural precursor cell (NPC) transplantation represents an emerging therapeutic approach to treat several neurological disorders. NPC transplants into rodent models of Alzheimer's disease, Parkinson's disease and spinal cord injury have demonstrated clinical benefits (Blurton-Jones et al., 2009; Cummings et al., 2005; van Gorp et al., 2013). Further, NPCs have been suggested as a potential treatment for the neuroinflammatory autoimmune demyelinating disease multiple sclerosis (MS) as they represent attractive sources for the generation of myelin-competent oligodendrocytes (Ben-Hur et al., 1998; Brustle et al., 1999). NPC-derived glial progenitors have been shown to remyelinate axons following transplantation into regions of acute experimental demyelination (Ben-Hur et al., 1998; Keirstead et al., 1999). In addition, transplantation of neural precursors into a rodent autoimmune model of demyelination resulted in migration of transplanted cells into white matter tracts accompanied by an improvement in clinical outcome (Ben-Hur et al., 2003; Pluchino et al., 2003). In these pre-clinical autoimmune models of MS, NPCs have been suggested to act as modulators of the immune system or directly replace damaged or lost endogenous NPCs that subsequently allows for dampening disease progression, axonal preservation and remyelination (Aharonowiz et al., 2008; Pluchino et al., 2009, 2003).

An important and clinically relevant question is whether transplanted NPCs can alleviate demyelination caused by persistent viral infection. Although the cause of MS has been attributed to multiple factors, viruses have long been considered as a potential triggering agent for MS in genetically susceptible individuals (Gilden, 2005; Olson et al., 2005). Therefore, it is important to study the remyelination potential of NPCs in the context of virally-induced neurologic disease, as this will give important insights into whether cell replacement therapies are effective within the CNS where neurotropic viruses may be persistent. With this in mind, we have previously shown that engraftment of postnatal-derived NPCs into the spinal cords of JHMV-infected mice with established demyelinating disease resulted in the selective colonization of demyelinating white matter tracts by transplanted cells accompanied by remyelination and axonal sparing (Carbajal et al., 2010, 2011; Totoiu et al., 2004; Greenberg et al., 2014). In a clinical setting, NPCs derived from donor-specific iPSCs may be preferable since these cells will retain the genetic background of the donor and potentially bypass the need for immunosuppressive drugs that leave the patient susceptible to opportunistic infections and tumor formation.

There are several known neurotropic viruses that are capable of infecting and replicating in both NPCs and NPC-derived cells (Chucair-Elliott et al., 2014; Huang et al., 2014; Schaumburg et al., 2008). For example, herpes simplex virus type 1 (HSV-1) infects NPCs resulting in

* Corresponding author at: Department of Pathology, Division of Microbiology & Immunology, University of Utah, School of Medicine, Salt Lake City, UT 84112, United States.
E-mail address: tom.lane@path.utah.edu (T.E. Lane).
[1] Present address: Department of Immunology & Microbial Science, The Scripps Research Institute, La Jolla, CA 92037, United States.
[2] Present address: Department of Biotherapeutics & Biotechnology, Genomics Institute of the Novartis Research Foundation, San Diego, CA. 92121, United States.

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diminished numbers leading to loss of neuroblasts upon differentiation (Chucair-Elliott et al., 2014). NPCs are also susceptible to infection by Enterovirus 71, which causes depletion of their numbers via viral-induced lysis (Huang et al., 2014). In addition, hESC-derived oligodendrocyte progenitor cells (OPCs) are highly susceptible to infection by JC virus, the causative agent of progressive multifocal leukoencephalopathy (PML) (Schaumburg et al., 2008). Coxsackievirus, another virus capable of infecting the CNS, also preferentially replicates in NPCs causing cell death and leading to developmental defects (Ruller et al., 2012). Most recently, Zika virus has been shown in culture to infect human NPCs derived from induced pluripotent stem cells and is linked to fetal abnormalities in pregnant woman exposed to the virus (Tang et al., 2016), while the human coronavirus OC43 was linked to acute disseminated encephalomyelitis within a SCID patient that received a cord-blood transplantation (Morfopoulou et al., 2016). Dampened immune surveillance resulting from treatment with immunosuppressive drugs can cause re-emergence of persistent neurotropic viruses which have the ability to infect and diminish numbers of transplanted cells, thus muting therapeutic benefits. Collectively, these findings suggest that susceptibility of NPCs to infection by neurotropic viruses is clinically important and must be evaluated before considering NPCs as a viable cell replacement therapy for various neurological disorders.

We have previously shown that postnatally-derived NPCs are susceptible to infection by JHMV resulting in virus-induced cell death (Plaisted et al., 2014). Herein we demonstrate that NPCs derived from induced pluripotent stem cells (iPSCs) are functionally similar to cortical NPCs isolated from post-natal transgenic GFP-C57BL/6 mice as they possess tri-potent differentiation potential and can form oligodendrocytes, neurons and astrocytes (Carbajal et al., 2010; Greenberg et al., 2014). However, they differ in their susceptibility to infection by JHMV in that iPSC-derived NPCs (iPSC-NPCs) express low levels of the viral receptor CEACAM1a, making them refractory to infection and virus-induced cell death.

2. Results and discussion

2.1. NPCs generated from mouse induced pluripotent stem cells are functionally similar to postnatal GFP-NPCs

Mouse iPSCs were generated by retroviral transduction of Yamanaka factors (Oct3/4 Sox2, Klf4 and c-Myc) into C57BL/6 fibroblasts using established protocols (Takahashi and Yamanaka, 2006). Mouse iPSCs were grown and differentiated into NPCs according to the schematic outlined in Fig. 1A. Feeder-free iPSCs were generated by supplementing cells with leukemia inhibitory factor (LIF) and stained positive for the stem cell markers- Oct4 and Sox2 (Fig. 1B). iPSCs were differentiated into NPCs by addition of EGF (epidermal growth factor) and bFGF (basic fibroblast growth factor) and were positive for neuronal stem cell markers such as Sox2 (Fig. 1Ci), Nestin (Fig. 1Cii), and Pax6 (Fig. 1Ciii) as assessed by immunofluorescence analysis. iPSC-NPCs were then differentiated for 6 days following withdrawal of EGF and bFGF. Similar to postnatal GFP-NPCs (henceforth referred to as GFP-NPCs) (Carbajal et al., 2010). iPSC-NPCs also terminally differentiated into oligodendrocytes (Fig. 1Di), neurons (Fig. 1Dii) and astrocytes (Fig. 1Diii) (Fig. 1E). These findings illustrate that iPSC-NPCs have similar differentiation properties as compared to postnatal-derived GFP-NPCs.

2.2. Expression of MHC class I and II following exposure to JHMV

It has been previously shown that, under normal physiologic conditions, MHC class I and II are undetectable on NPCs and IFN-γ treatment can induce expression of MHC on these cells (Chen et al., 2011; Plaisted et al., 2014; Weinger et al., 2012). We found there was constitutive expression of MHC class I on iPSC-NPCs and exposure to either JHMV or recombinant mouse IFN-γ (100 U/mL) did not modulate expression levels (Fig. 2A, C). In addition, MHC class II expression was undetectable on iPSC-NPCs exposed to either medium or JHMV, yet treatment with IFN-γ dramatically increased expression (Fig. 2B, D)

2.3. NPCs derived from mouse iPSCs express low levels of JMHV receptor CEACAM1a

The primary receptor for JHMV is murine carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (Hirai et al., 2010; Williams et al., 1991). There are two alleles for murine CEACAM1: m*Ceacam1a* and m*Ceacam1b* and inbred strains of mice such as C57BL/6 and Balb/c are homozygous for m*Ceacam1a* and are highly susceptible to infection by JHMV (Williams et al., 1991). CEACAM1a is widely expressed in a variety of mouse tissue and can be detected on the surface of epithelial cells lining the respiratory tract, endothelial cells and also on hematopoietic cells (Hemmila et al., 2004). We have recently shown that cultured NPCs derived from 1-day old C57BL/6 mice express CEACAM1a as determined by CEACAM1 mRNA transcripts as well as surface protein expression (Plaisted et al., 2014). In order to determine whether NPCs derived from iPSCs express CEACAM1a similar to GFP-NPCs, cell surface expression of the receptor was evaluated by staining NPCs with an anti-CEACAM1 antibody. GFP-NPCs and mixed splenocytes from C57BL/6 were used as controls. Flow cytometry analysis revealed that iPSC-NPCs expressed low levels of surface expression of CEACAM1 compared to GFP-NPCs (Fig. 3A, B). Furthermore, qPCR analysis of mRNA isolated from cultured iPSC-NPCs revealed a significant ($p < 0.0001$) reduction in CEACAM1a transcripts compared to mRNA isolated from GFP-NPCs or splenocyte controls (Fig. 3C). Collectively, we interpret these findings to indicate iPSC-NPCs express proportionally less CEACAM1a compared to GFP-NPCs.

2.4. NPCs derived from iPSCs are less susceptible to JHMV infection and virus induced cell death

We next evaluated the susceptibility of NPCs to JHMV infection. In order to do this, cultured iPSC-NPCs were infected with JHMV for 18 h and fixed 72 h post-infection (p.i.). Cells were then stained with either an anti-Sox2 antibody (to confirm NPC status) or an antibody specific for the carboxyl terminus of JHMV nucleocapsid protein and imaged by fluorescence microscopy (Plaisted et al., 2014). Compared to GFP-NPCs, iPSC-NPCs express low levels of JHMV nucleocapsid protein, suggesting these cells are less susceptible to JHMV infection (Fig. 4A, B). To determine if JHMV-infection of NPC cultures induced cell death, lactate dehydrogenase (LDH) was measured in the supernatants of GFP-NPC and iPSC-NPC cultures at 24, 48 and 72 h p.i. GFP-NPCs cultures infected with JHMV revealed a significant ($p < 0.001$) increase in cell death compared to JHMV-infected iPSC-NPC cultures (Fig. 4C). Congruent with reduced cell death, we detected lower viral titers within the supernatants of JHMV-infected iPSC-NPCs at 24 ($p < 0.05$), 48 ($p < 0.001$), and 72 ($p < 0.05$) hours p.i. compared to infected GFP-NPCs (Fig. 4D).

This study provides a comparative analysis of *ex vivo* expanded neural precursor cell populations with regard to their susceptibility to infection by a neurotropic coronavirus. These studies demonstrates that although iPSC-NPCs are functionally similar to postnatally-derived NPCs in their ability to differentiate into oligodendrocytes, astrocytes and neurons, they are unique with respect to susceptibility to viral infection and expression of MHC class I on the cell surface. iPSC-NPCs express low levels of the JHMV receptor CEACAM1a, providing reduced susceptibility and limited replication. Furthermore, due to the impaired ability of virus to enter these cells and replicate, the cells are resistant to virus-induced cell death. Nonetheless, we demonstrate that after 72 h following infection of iPSC-NPCs there is an increase in cell