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OCT4 TRANSCRIPTION FACTOR IN CONJUNCTION WITH VALPROIC ACID ACCELERATES MYELIN REPAIR IN DEMYELINATED OPTIC CHIASM IN MICE

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Abstract—Multiple sclerosis is a demyelinating disease with severe neurological symptoms due to blockage of signal conduction in affected axons. Spontaneous remyelination via endogenous progenitors is limited and eventually fails. Recent reports showed that forced expression of some transcription factors within the brain converted somatic cells to neural progenitors and neuroblasts. Here, we report the effect of valproic acid (VPA) along with forced expression of Oct4 transcription factor on lysolecithin (LPC)-induced experimental demyelination. Mice were gavaged with VPA for one week, and then inducible Oct4 expressing lentiviral particles were injected into the lateral ventricle. After one-week induction of Oct4, LPC was injected into the optic chiasm. Functional remyelination was assessed by visual-evoked potential (VEP) recording. Myelination level was studied using FluoroMyelin staining and immunohistofluorescent (IHF) against proteolipid protein (PLP). IHF was also performed to detect Oct4 and SSEA1 as pluripotency markers and Olig2, Sox10, CNPase and PDGFRα as oligodendrocyte lineage markers. One week after injection of Oct4 expressing vector, pluripotency markers SSEA1 and Oct4 were detected in the rims of the 3rd ventricle. LPC injection caused extensive demyelination and significantly delayed the latency of VEP wave. Animals pre-treated with VPA + Oct4 expressing vector, showed faster recovery in the VEP latency and enhanced myelination. Immunostaining against oligodendrocyte lineage markers showed an increased number of Sox10+ and myelinating cells. Moreover, transdifferentiation of some Oct4-transfected cells (GFP+ cells) to Olig2+ and CNPase+ cells was confirmed by immunostaining. One-week administration of VPA followed by one-week forced expression of Oct4 enhanced myelination by converting transduced cells to myelinating oligodendrocytes. This finding seems promising for enhancing myelin repair within the adult brains.

Key words: valproic acid, Oct4 transcription factor, myelin repair, visual-evoked potential, multiple sclerosis, trans-differentiation.

INTRODUCTION

Multiple sclerosis (MS) which is characterized by the loss of oligodendrocytes and axon demyelination is a chronic, multifocal and relapsing-remitting disorder in young adults. In MS patients impulse conduction is blocked or slowed in lesion site which results in neurological symptoms (Crowe et al., 1997).

Remyelination is the process in which myelin sheaths are restored around the demyelinated axons, reinstating saltatory conduction and prevents affected axons from secondary damage and degeneration (Franklin and Ffrench-Constant, 2008). Implantation of exogenous remyelinating cells or enhancing endogenous remyelinating cells are of the most interesting approaches to remyelinate naked axons and protect them from further damage (Czepiel et al., 2011). Adult mammalian CNS contains a population of neural stem cells (NSCs) described as immature, undifferentiated and multipotent cells. Subventricular Zone (SVZ) of the ventricles is the major source of endogenous NSCs in the adult brain. Under demyelinating conditions such as MS, NSCs/neural progenitors (NPs) proliferate, migrate, give rise to oligodendrocyte progenitor cells (OPCs) and then differentiate into myelinating oligodendrocytes. Remyelination is often limited or fails in chronic injuries or diseases with extensive myelin loss (Franklin, 2002; Imitola et al., 2003; Aharonowiz et al., 2008). It is proposed that repeated episodes of demyelination in chronic phase of MS may lead to exhaustion of NSCs and progenitors (Franklin, 2002) or pathological conditions of chronic MS lesions may block the differentiation of OPCs to remyelinating cells (Kuhlmann et al., 2008).

Inducing differentiated cells toward pluripotent stem cells opened a new approach for treatment of

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Abbreviations: ANOVA, analysis of variance; IHF, immunohistofluorescent; LPC, lysolecithin; MS, multiple sclerosis; NSCs, neural stem cells; OCT, optimum cutting temperature; OPCs, oligodendrocyte progenitor cells; PBS, phosphate-buffered saline; PLP, proteolipid protein; SVZ, Subventricular Zone; VEP, visual-evoked potential; VPA, valproic acid.

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degenerative diseases (Takahashi and Yamanaka, 2006; Takahashi et al., 2007a,b; Nakagawa et al., 2008; Yamanaka, 2008). Recently, direct reprograming of somatic cells to desired cells became an interesting research priority in the field of stem cells (Ambasudhan et al., 2011; Kim et al., 2011; Ring et al., 2012). Within the nervous system, transcription factors were used to trans-differentiate somatic cells. Rouaux and Arlotta showed that post-mitotic callosal projection neurons of layer II/III can be reprogramed into layer-V/VI corticofugal projection neurons following the forced expression of transcription factor Fezf2. Reprogramed neurons acquired the properties of corticofugal projection neurons and reset their axonal connectivity (Rouaux and Arlotta, 2013). Niu and colleagues showed that administration of a single transcription factor Oct4 or Sox2 was able to induce astrocytes to neuroblasts, while Sox2 was more efficient. Induced neuroblasts could be generated even in aged brains and in the presence of BDNF and noggin or under treatment of mice with valproic acid (VPA), the induced neuroblasts differentiated to electrophysiologically mature neurons and integrated into the local neural network (Niu et al., 2013). In Another report, transplanted human fibroblasts and human astrocytes engineered to express inducible forms of neural reprograming genes Ascl1, Brn2, and Myt1, were converted to neurons by chemical induction (Torper et al., 2013). Later Su and colleagues showed that within the injured adult spinal cord, astrocytes could be converted to neuroblasts by forced expression of transcription factor Sox2. Interestingly, these induced neuroblasts could differentiate into the synapse-forming neurons, *in vivo* (Su et al., 2014).

The efficiency of reprograming and transdifferentiation could be enhanced by defining the best chemical cocktail of small molecules. Small molecules modulate specific targets in signaling and/or epigenetic mechanisms and promote reprograming efficiency and ultimately replace reprograming factors (Yuan and Arias-Carrion, 2008; Feng et al., 2009; Li and Ding, 2010; Masip et al., 2010). VPA is a chromatin modifier frequently used for enhancing reprograming efficiency. We previously showed that *in vivo* forced expression of Oct4 following VPA administration converted somatic cells to neural progenitors or reprogramed them back to a less differentiated state (Dehghan et al., 2014). This finding was promising for overcoming the limitations in the repair potential of adult brains and for the compensation of cell loss in neurological diseases. In the present study, we examined if the neural progenitors or partially reprogramed cell induced by VPA and Oct4 expression participate in myelin repair in an animal model of demyelination. We show that VPA pretreatment followed by *in vivo* overexpression of Oct4, accelerates myelin repair following lysolecithin (LPC)-induced local demyelination in the mouse optic chiasm.

EXPERIMENTAL PROCEDURE

Animals

Adult male C57BL/6 mice were obtained from the Pasteur Institute, Karaj, Iran and housed in groups of 3–4 mice/cage. All animals were housed under a 12-h light/12-h dark cycle with *ad libitum* access to food and water. All experimental practices were conducted in compliance with NIH guidelines for the care and use of laboratory animals in research with the approval of the Tarbiat Modares University Committee on Ethics in Research. Efforts were made to minimize the animals suffering and to reduce the number of animals used.

Preparation of viral particles

DOX-inducible Fuw-based lentiviral vectors inserted with mouse Oct4 cDNA were transfected by a Virapower Lentiviral Packaging Mix (Invitrogen, K4975-00) into 293T cells using the Lipofectamine 2000 (Invitrogen) transfection reagent. At 48 h post-infection, the supernatant including viral vectors was collected, filtered, concentrated and then re-suspended. A same vector expressing GFP instead of Oct4 was prepared in parallel to check the efficacy of producing viral particle for transfection both *in vitro* and *in vivo*. For tracing the fate of transfected cells, a lentiviral vector with an Oct4-IRES-GFP expressing sequence was used. The final count of functional viral particles was approximately 700,000/μl. Three microliter of medium (2,100,000 viral particle) was applied to each animal.

Interventions

For intracerebroventricular (i.c.v.) injections, a cannula was stereotaxically implanted just over the right cerebral ventricle of animals (A: 3.6 and L: 1.1 from the lambda, V: 2.2 from the dura). Prior to surgery all animals were anesthetized with ketamine (70 mg/kg, i.p., Alfasan, Holland) and xylazine (10 mg/kg, i.p., Alfasan, Holland).

VPA was used in the form of sodium valproate (Abidi Co., Tehran, Iran). Animals received 150 mg/kg of sodium valproate twice a day via oral gavages for 7 days, then Oct4 expressing viral particles (3 μl) were injected i.c.v. and the induction was done by 2 μl of doxycycline solution (6 ng/mouse, i.c.v.) from the consequent day and continued for seven days. All i.c.v. injections were administered into the right ventricle of animal over a period of 10 min.

Induction of demyelination

Surgery was performed on anesthetized mice as described above. Optic chiasm demyelination was done as mentioned in our previous report (Dehghan et al., 2012). Briefly, one microliter of 1% LPC (Sigma, St. Louis, USA) was injected using a Hamilton syringe into optic chiasm. The stereotaxic coordinates of injection site were; anterior: +0.5 mm to the Bregma, lateral: 0, and ventral: 4.9 mm from the dura. The syringe was kept at the place for 5 min before gentle removal.

Visual-evoked potential (VEP) recording procedure

VEP measure the electrical activity of the optical pathway in response to stimulation of photoreceptors. Optic pathway is frequently affected in patients with MS and VEP is routinely used to assess the involvement of optic pathway; moreover, it is sensitive to experimentally