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Reporting on methods to generate and purify rodent and human oligodendrocytes from different sources

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ABSTRACT

Oligodendrocytes are part of the glial cells located in the central nervous system, capable of providing trophic support to neurons and ensheathing their axons. These cells can become dysfunctional under pathologic condition. Rodent and human pluripotent stem cells are inexhaustible sources for producing oligodendrocytes that can be used for disease modeling and cell replacement therapy studies. They also offer many opportunities to model the contribution of oligodendrocytes in non-genetic disorders such as multiple system atrophy. In this method article, we provide robust and reproducible differentiation protocols to obtain oligodendrocyte progenitor cells and purify them using fluorescence activated cell sorting.

Introduction

The inaccessibility to collect oligodendrocytes from patients and the limited access to fetal tissue has until recently hampered studies on neurodegenerative diseases. To date, it is still not possible to obtain oligodendrocytes from patients along disease progression in order to perform mechanistic studies. Assessment of postmortem samples is difficult as adaptive processes are already initiated. In fact, there is a lack of knowledge regarding to the oligodendrocyte biology at the initiation and during the progression of brain diseases. The use of embryonic stem cells (ESCs) and the discovery of induced pluripotent stem cells (iPSCs) (Takahashi et al., 2007) together with the improvement of reprogramming technologies, more recently the direct conversion of both oligodendrocyte progenitor cells (OPC) and oligodendrocytes from fibroblasts (Najm et al., 2013; Yang et al., 2013), now offer an unprecedented opportunity to explore the functional ramifications of a particular genotype in a cell type-specific manner and open doors to large scale drug screening and cell replacement therapy. Nowadays, mouse ESCs (mESC) are used to study in vitro development of cell-therapies and developing drug assays, with the advantage that several pathways of the OPCs commitment lineage are highly conserved between mice and human. In this way, the insights obtained from the mouse developmental biology could be applied to human oligodendrocytes (Murry and Keller, 2008). Yet, the existent protocols for generating OPCs from mESC are not very efficient to produce high yield cultures, and consequently the potential of mESC has not been fully exploited.

During neonatal and post-natal development, oligodendrocytes are abundantly produced in three temporally distinct waves (Kessaris et al., 2006), and differentiated oligodendrocytes express specific markers according to the state of development (Zhang, 2001). In the initial stage of differentiation, several transcription factors are involved in the oligodendroglial lineage restriction, including the downregulation of *Sox2* and upregulation of glial lineage-associated Sox family – *Sox8* and *Sox9* (Wegner and Stolt, 2005). Consequently, expression of transcription factors as *NKX2.2*, *Olig 1* and *Olig 2* and *Sox 10* increase (Kuhlbrodt et al., 1998), and give rise to progenitors that proliferate and differentiate into pre-oligodendrocytes, which express e.g. platelet-derived growth factor receptor-α (PDGFRα) with a bipolar or tripolar morphology. The expression of PDGFRα is the first important step in OPCs differentiation, since this expression establishes the OPC fate acquisition (Hart et al., 1989). During the transition from OPCs to immature oligodendrocytes, a new set of transcription factors is required to promote the OPCs differentiation. These include the transcriptional repression of E2F1 and c-*MYC* and the expression of cell cycle inhibitors such as p27KIP1 (CDKN1B) and *SOX17* (Chew et al., 2011; Larocque et al., 2005). The immature oligodendrocytes exhibit a multipolar morphology, and can be recognized by the O4 antibody. After establishment of the oligodendroglial fate, the immature oligodendrocytes undergo

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extensive cellular growth and mature into myelinating oligodendrocytes expressing myelin basic protein (MBP) and 2′,3′-Cyclic-nucleotide 3′-phosphodiesterase (CNPase) (Seiberlich et al., 2015). This occurs with the transcriptional activation of myelin regulatory factor (MYRF) and the consequent positive-regulation of its targets MBP, CNPase, proteolipid protein 1 (PLP1), myelin-associated glycoprotein (MAG), and myelin oligodendrocyte protein (MOG) (Emery, 2010).

In the past years, several groups, including ours, generated protocols to differentiate OPCs from human pluripotent stem cells (PSCs) (Djelloul et al., 2015; Douvaras et al., 2014; Sim et al., 2011; Stacpoole et al., 2013) to use as models for several diseases involving impaired myelination. Moreover, Vítězslav and colleagues (Vítězslav Bryja, 2006) described a simple protocol for generation of mESC from embryonic day 3.5 blastocysts allowing a starting point for several applications, including the generation of oligodendrocytes. Chen and colleagues have published a protocol for an in vitro isolation and maintenance of enriched rat and mouse OPC, facilitating studies on OPC lineage progression and myelin injury-recovery assays (Chen et al., 2007). Douvaras and colleagues developed a reduced-time protocol to generate oligodendrocytes from human pluripotent stem cells with increased percentage of O4 positive cells isolated (Douvaras and Fossati, 2015). Also, Wang and colleagues were the first to demonstrate the beneficial effect of transplanting hiPSC-derived PDGFRα/CD140a+ cells into hypomyelinated mice (Wang et al., 2013).

Applications of our protocols

Here we present, in the same report, procedures to generate and isolate OPCs from different sources: brain and primary cultures from mice, mESC, hPSC (Fig. 1). In addition, we describe a new protocol that allows the generation of cultures highly enriched in mESCs-derived O4+ oligodendrocytes, by use of retinoic acid (RA) and smoothen agonist (SAG). The presented methods will allow a rapid and comprehensive approach to a simple and step-by-step protocol to obtain purified oligodendrocytes.

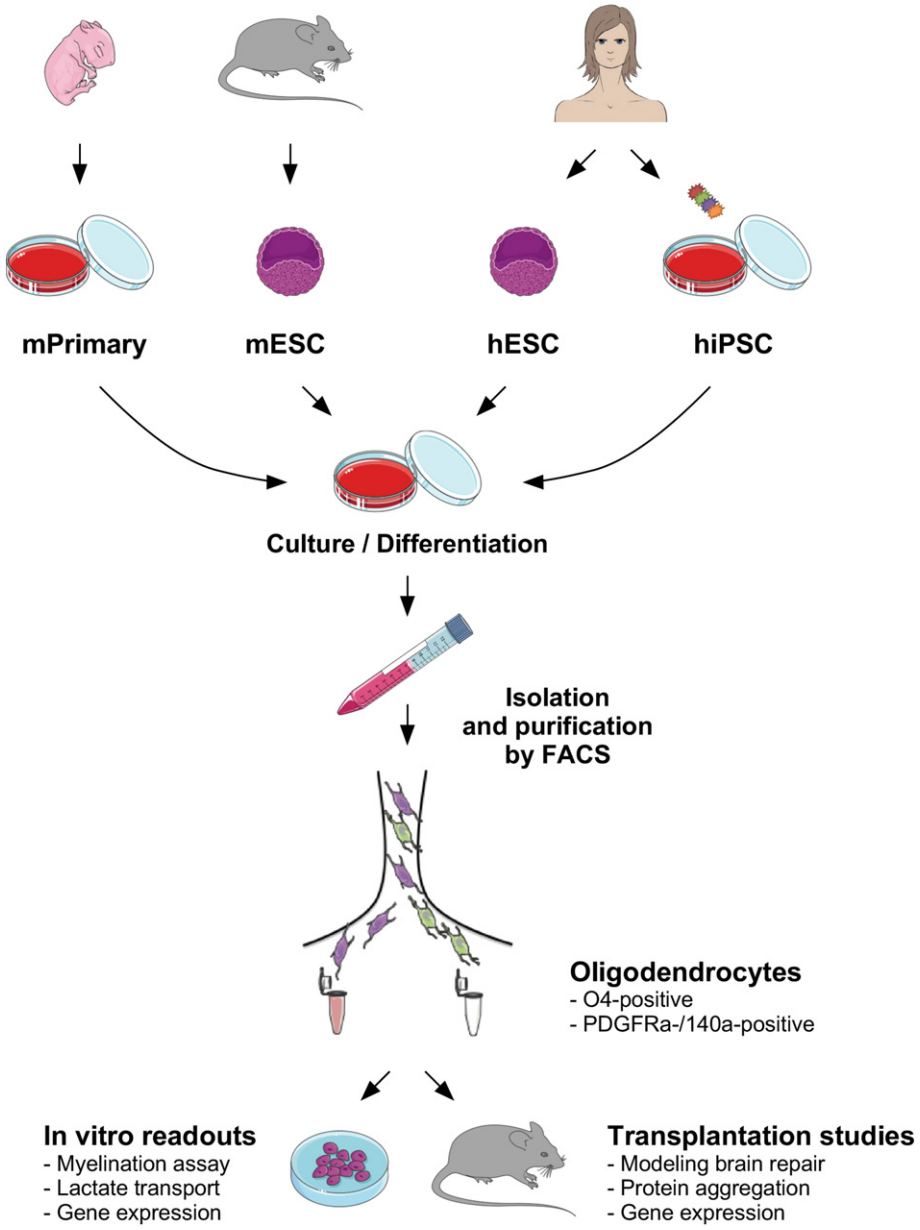

Fig. 1. General overview of the experimental approach. Different sources of OPCs, mouse primary (mPrimary), mouse embryonic stem cells (mESC), human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC). OPCs can be purified by FACS and used for different applications.