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Skeletal muscle pericyte subtypes differ in their differentiation potential

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Abstract Neural progenitor cells have been proposed as a therapy for central nervous system disorders, including neurodegenerative diseases and trauma injuries, however their accessibility is a major limitation. We recently isolated Tuj1+ cells from skeletal muscle culture of Nestin–GFP transgenic mice however whether they form functional neurons in the brain is not yet known. Additionally, their isolation from nontransgenic species and identification of their ancestors is unknown. This gap of knowledge precludes us from studying their role as a valuable alternative to neural progenitors. Here, we identified two pericyte subtypes, type-1 and type-2, using a double transgenic Nestin–GFP/NG2–DsRed mouse and demonstrated that Nestin–GFP+/Tuj1+ cells derive from type-2 Nestin–GFP+/NG2–DsRed+/CD146+ pericytes located in the skeletal muscle interstitium. These cells are bipotential as they generate either Tuj1+ cells when cultured with muscle cells or become "classical" α-SMA+ pericytes when cultured alone. In contrast, type-1 Nestin–GFP−/NG2–DsRed+/CD146+ pericytes generate α-SMA+ pericytes but not Tuj1+ cells. Interestingly, type-2 pericyte derived Tuj1+ cells retain some pericytic markers (CD146+/PDGFRβ+/NG2+). Given the potential application of Nestin–GFP+/NG2–DsRed+/Tuj1+ cells for cell therapy, we found a surface marker, the nerve growth factor receptor, which is expressed exclusively in these cells and can be used to identify and isolate them from mixed cell populations in nontransgenic species for clinical purposes.

Introduction

Neural progenitor-based therapies are rapidly emerging as a potential strategy for central nervous system (CNS) regeneration in patients with neurodegenerative diseases or injury. The aim of this therapy is to replace, repair, or enhance the biological functions of damaged tissues.

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However, neural progenitors availability, immune reaction and ethical concerns are limitations for its medical application. The skeletal muscle, as one of the largest organs in the body, represents an alternative source of stem cells, which can be easily obtained in large quantities through a biopsy procedure performed in an outpatient clinic (Wu et al., 2010). Thus, the skeletal muscle may be a convenient and enriched source of neural cells that may circumvent these limitations.

Previously, we isolated Nestin–GFP+ cells in cultured adult skeletal muscle from Nestin–GFP transgenic mice, which exhibit characteristics of neural progenitors such as morphology, specific neural markers profile, replicative capacity, ability to form neurospheres, and functional response to neurotransmitter (Birbrair et al., 2011; Birbrair et al., in press). Our laboratory is working to determine whether these cells form functional neurons in the brain.

However, as their origin remains unclear, here, we aimed to determine their ancestor(s) in the skeletal muscle. The skeletal muscle is highly vascularized and contains a variety of mononucleated cells, including blood, endothelial, fibroblasts, myofiber satellite, immune, and pericytes. Pericytes can be recognized by their position in the microvasculature more than by a precise phenotype (Feng et al., 2011; Sa-Pereira et al., 2012). Their role as stem cells contributing to formation of tissues other than blood vessels has been reported in numerous publications (Alliot-Licht et al., 2001; Caplan, 2007; Crisan et al., 2008; Dellavalle et al., 2007; Dore-Duffy et al., 2011; Feng et al., 2011; Lin et al., 2008; Maier et al., 2010; Nehls and Drenckhahn, 1993; Shi and Gronthos, 2003). Although pericytes may have inherent potential to differentiate into multiple lineages if exposed to appropriate epigenetic signals, this capacity may differ between tissues (Bianco et al., 2008; Sacchetti et al., 2007; Shi and Gronthos, 2003). Here, by using the Nestin–GFP/NG2–DsRed transgenic mouse, we found that skeletal muscle derived Tuj1+ cells differ from classic pericytes but derive from a pericyte subtype that expresses a specific combination of markers, Nestin–GFP+/NG2–DsRed+/CD146+. Interestingly, we also determined that another pericyte subtype, Nestin–GFP−/NG2−DsRed+/CD146+, found in the skeletal muscle, does not have the ability to differentiate into Tuj1+ cells under the same conditions. Moreover, we found that pericyte derived neural Tuj1+ cells are the only cells to express NGF receptor (NGFR, p75) in skeletal muscle cultures, which can be used as a surface marker to distinguish and isolate them from all other cell types obtained from nontransgenic species.

This work demonstrates the heterogeneity of the pericyte population in the skeletal muscle and its distinct differentiation potential. Based on this analysis, we envision the possibility of using pericyte derived Nestin–GFP+/NGF receptor+/Tuj1+ cells to treat diverse pathologies, including neurodegenerative diseases, neoplasias, and CNS trauma lesions.

Materials and methods

Animals

Nestin–GFP transgenic mice were maintained homozygous for the transgene on the C57BL/6 genetic background (Mignone et al., 2004). C57BL/6 wild-type mice were used as controls. NG2–DsRed transgenic mice expressing DsRed-T1 under the control of the NG2 promoter (Zhu et al., 2008) were purchased from the Jackson Laboratory. Nestin–GFP mice were crossbred with NG2–DsRed mice to generate Nestin–GFP/NG2–DsRed double-transgenic mice. All mouse colonies were housed at Wake Forest School of Medicine (WFSM) in a pathogen-free facility of the Animal Research Program under 12:12-h light/dark cycle and fed *ad libitum*. Both male and female homozygous mice were used, and their ages ranged from 3 to 5 months. Animal handling and procedures were approved by the WFSM Animal Care and Use Committee.

Flexor digitorum brevis (FDB) muscle culture preparation

FDB muscle from Nestin–GFP transgenic, NG2–DsRed transgenic, Nestin–GFP/NG2–DsRed transgenic, and C57BL/6 wild-type mice were used for most experiments in this work. FDB muscle was preferred over more traditional muscles for most experiments because it is small and flat, allowing more complete dissociation by trituration in a single step, shortening the experiment significantly (Zhang et al., 2011). Methods for FDB culture preparation have been described (Birbrair et al., 2011). Briefly, muscles were carefully dissected away from the surrounding connective tissue and minced, then digested by gentle agitation in 0.2% (w/v) Worthington's type-2 collagenase in Krebs solution at 37 °C for 2 hours. They were resuspended in growth medium and dissociated by gentle trituration. The growth medium used to plate cell cultures consisted of DMEM-high glucose (Invitrogen, Carlsbad, CA, USA), supplemented with 2% L-glutamine, 50 U/ml penicillin and 50 mg/ml streptomycin, 10% (v/v) horse serum (Invitrogen) and 0.5% (v/v) CEE (Gemini Bio-products, West Sacramento, CA, USA). It supported both proliferation and differentiation of myogenic cells (Zammit et al., 2004).

Immunocytochemistry

Cultured cells were fixed with 4% PFA for 30 min, then permeabilized in 0.5% Triton X-100 (Sigma, St. Louis, MO, USA), and blocked to saturate nonspecific antigen sites using 5% (v/v) goat serum/PBS (Jackson Immunoresearch Labs, West Grove, PA, USA) overnight at 4 °C. The next day, the cells were incubated with primary antibodies at room temperature for 4 h and visualized using appropriate species-specific secondary antibodies conjugated with Alexa Fluor 488, 568, 647 or 680 at 1:1000 dilution (Invitrogen). They were counterstained with Hoechst 33342 reagent at 1:2000 dilution (Invitrogen) to label the DNA and mounted on slides for fluorescent microscopy with Fluorescent Mounting Medium (DakoCytomation, Carpinteria, CA, USA).

Primary antibodies

Table 1 shows antibodies, their dilution, and source.

Skeletal muscle processing

To detect DsRed and GFP fluorescence, nondissociated extensor digitorum longus (EDL) muscles from 3-month-old Nestin–GFP/NG2–DsRed mice were dissected, fixed in 4%