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Myotube-derived factor promotes oligodendrocyte precursor cell proliferation

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ABSTRACT

Muscle cells secrete numerous molecules that function as endocrine hormones and regulate the functions of distant organs. Myelination in the central nervous system (CNS) is regulated by peripheral hormones. However, the effects of muscle-derived molecules on myelination have not been sufficiently analyzed. In this study, we show that muscle-releasing factors promote proliferation of oligodendrocyte precursor cells (OPCs), which is an element of myelination process. Supernatants of mouse myotube cultures stimulated bromodeoxyuridine (BrdU) incorporation into mouse OPCs. Mouse myotube supernatants did not enhance mouse OPC transmigration and myelin basic protein (MBP) expression. RNA sequencing identified candidate genes with hormonal functions that were expressed in mouse myotubes. These data support the possibility that hormonal molecules secreted by myotubes contribute to OPC proliferation and myelination.

1. Introduction

Myelin covers neuronal fibers in the white matter of the CNS. It increases the speed of nerve conduction and facilitates nervous system functions [1]. Changes of myelin gene expression and alterations in white matter structure are observed in CNS disorders, such as multiple sclerosis [2], Alzheimer's disease [3], stroke [4], and other diseases. Because myelin dysfunction is linked to neurological dysfunction in CNS diseases, myelin repair is recognized as a therapeutic target for the treatment of these diseases [5].

Myelin is formed by oligodendrocytes, which develop from oligodendrocyte precursor cells (OPCs), a tissue stem cell type in the CNS. During development, myelination requires OPC proliferation, their migration to the sites of myelination, and differentiation into the mature oligodendrocyte [6]. OPC development is regulated by extracellular molecules, such as IGF-1 and FGF2, released from CNS cells. These cytokines have been identified as OPC proliferation factors. Intriguingly, recent studies indicate that periphery-derived molecules can also affect OPC proliferation [7–9], suggesting a potential connection between OPC proliferation and peripheral hormones. Among the peripheral hormones implicated, muscle-released factors (called myokines) exert autocrine and/or paracrine effects [10], and muscle-released factors, such as Interleukin (IL)-6 and Brain-derived neurotrophic factor (BDNF), cross the blood–brain barrier [11–13]. In addition, it has been hypothesized that there are interactions between muscle-derived factors and the brain [14]. However, the role of muscle-derived factors in OPC proliferation is still unknown.

Abbreviations: CNS, Central nervous system; OPC, Oligodendrocyte precursor cell; BrdU, 5-bromo-2′-deoxyuridine; MBP, Myelin basic protein; IL, Interleukin; BDNF, Brain-derived neurotrophic factor; PBS, Phosphate buffered saline; FBS, Fetal bovine serum; bFGF, Basic fibroblast growth factor; DMEM, Dulbecco's Modified Eagle Medium; PDGF, Platelet-Derived Growth Factor; PLL, Poly-L-lysine; PFA, Paraformaldehyde; DAPI, 4′,6-diamidino-2-phenylindole; BSA, Bovine serum albumin; IGF, Insulin-like growth factor.

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Here we found that muscle-derived factors promote OPC proliferation *in vitro*. We also observed that muscle-derived factors did not enhance OPC migration and their differentiation into mature oligodendrocytes. We also performed RNA sequence analysis and provide candidate molecules which were secreted from myotubes and stimulate OPC proliferation.

2. Methods

2.1. Mice

All experimental procedures were approved by the Institutional Animal Care Committee of Osaka University. C57BL/6J mice were obtained from Kiwa Laboratory Animals Co., Ltd. Charles River Japan, or Japan SLC. Mice were housed in an air-conditioned room at 23 ± 1 °C with a 12-h light–dark cycle and had free access to water and food.

2.2. Primary cultures of myotubes

Myotubes were obtained from mice at postnatal day 0–1 [15]. Their limb muscles were minced vigorously using razor blades, followed by dissociation into single-cell suspensions using collagenase/$CaCl_2$ solution (phosphate buffered saline [PBS] containing 1.5 U/ml collagenase D and 2.5 mM $CaCl_2$) at 37 °C for 20 min, and filtration through a 40 μm nylon cell strainer. After centrifugation ($350 \times g$, 5 min), cells were suspended in F10-based primary myoblast growth medium (80% Ham's F-10 nutrient mixture [Sigma], 20% fetal bovine serum [FBS], 2.5 ng/ml basic fibroblast growth factor [bFGF; PeproTech]), and were plated in non-coating dishes at 37 °C with 5% CO_2. After 40 min incubation, non-adherent myoblasts were collected and resuspended in myoblast growth medium (40% Ham's F-10 nutrient mixture, 40% Dulbecco's Modified Eagle Medium [DMEM, Sigma], 20% FBS, 2.5 ng/ml bFGF). Cells were plated in collagen type I coated 24-well plates at a density of 2×10^5 cells/well at 37 °C with 5% CO_2. For differentiation of myoblasts to myotubes, cells were cultured in DMEM containing 5% horse serum (Sigma) for 4 days. Myotubes were cultured in DMEM for 3 days and their supernatants were used for assays.

2.3. SW10 culture

Mouse Schwann cells (SW10) were purchased from the American Type Culture Collection (ATCC, catalog number CRL-2766). Cells were maintained in DMEM containing 10% FBS. To collect supernatants of SW10 cells, cells were plated on non-coated 24-well plates in DMEM at a density of 2×10^5 cells/well for 3 days.

2.4. Proliferation assay

OPCs were isolated from the brains of postnatal day 1 mice as described previously [9]. Cerebral cortices were dissected in PBS and dissociated into single-cell suspensions using the 0.25% Trypsin in PBS for 20 min at 37 °C. Platelet-derived growth factor (PDGFR)α-positive cells were isolated using PDGFRα-specific antibody-coated magnetic beads (Miltenyi Biotec). Cells were plated in poly-L-lysine (PLL, Sigma) precoated 96-well plates at a density of 1×10^4 cells/well. Cells were maintained in the following OPC medium: DMEM containing 4 mM L-glutamine, 1 mM sodium pyruvate, 0.1% bovine serum albumin (BSA; minimum 98% electrophoresis grade), 50 μg/ml apo-transferrin, 5 μg/ml insulin, 30 nM sodium selenite, 10 nM biotin, 10 nM hydrocortisone (all Sigma), 10 ng/ml PDGF-AA (PeproTech), and 10 ng/ml bFGF.

To evaluate the effect of myotube supernatants on OPC proliferation, OPCs were treated with myotube supernatants for 2 days. Cell proliferation was evaluated with a BrdU incorporation assay (Cell Proliferation ELISA, BrdU[colorimetric], Sigma). BrdU was added into wells 2 h before the end of the culture period.

2.5. Migration assay

PDGFRα-positive OPCs were plated on PLL-precoated transwell cell-culture inserts (6.5 mm diameter, 8 μm pore size, Corning Costar) at a density of 3×10^4 cells/well as previously described [16]. After 2 h incubation, OPCs were treated with myotube supernatants for 16 h. OPCs were fixed with 4% paraformaldehyde (PFA) in PBS for 30 min, and nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI, 1 μg/ml) in PBS for 20 min at room temperature. Cells that migrated to the lower membrane of the transwell insert were counted. Images of the membranes were acquired by fluorescence imaging, using an upright microscope equipped with a 4× objective (Olympus).

2.6. Differentiation assay

OPCs and immature oligodendrocytes were obtained from the brains of postnatal day 1 mice as previously described [9]. Cerebral cortices were dissected in PBS and dissociated into single-cell suspensions using 0.25% Trypsin-PBS incubation at 37 °C for 15 min. After neutralization, cells were centrifuged at $300 \times g$ for 5 min, suspended in 10% FBS-DMEM, and filtered through a 70 μm nylon cell strainer. Cells were plated at a density of 3×10^6 cells/dish in PLL-precoated 10 cm dishes (Greiner Bio-One) and maintained at 37 °C with 7% CO_2 in 10% FBS-DMEM. Ten days after culturing, dishes were rotated 20 times, and detached cells were removed. The adhesive cells were incubated in 0.05% trypsin in PBS at 37 °C for 5 min. The detached cells were filtered through a 70 μm nylon cell strainer and plated in non-coated dishes. After 30 min incubation at 37 °C, non-adherent cells were collected and plated at a density of 1.5×10^4 cells/well into PLL-coated 96-well plates in OPC medium.

To evaluate the effect of myotube supernatants on the differentiation of oligodendrocyte-lineage cells, cells were treated with myotube supernatants for 2 days. After fixation with 4% PFA in PBS for 30 min, cells were permeabilized with a blocking solution (PBS containing 0.1% Triton X-100 and 5% bovine serum albumin [BSA]) for 30 min at room temperature. The cells were then incubated with antibodies against rat anti-MBP (1:500; Abcam) and goat anti-Olig2 antibody (1:300; R&D Systems) overnight at 4 °C. Antibodies were diluted in blocking solution. The next day, cells were incubated with Alexa Fluor 488-conjugated donkey antibody to rat IgG (1:500) and Alexa Fluor 647-conjugated donkey antibody to goat IgG (1:500, both Life Technologies) for 1 h at room temperature. Images of stained cells were acquired using an IN Cell Analyzer 6000 (GE Healthcare), an automated microscope. These images were analyzed by the IN Cell Investigator image analysis software (IN Cell Developer) that quantified MBP-positive areas in Olig2-positive cells.

2.7. RNA sequence

Total RNA was extracted from myotubes and SW10 cells with the RNeasy Mini Kit (Qiagen). Strand-specific RNA-seq libraries were prepared from the depleted RNA using the TruSeq Stranded mRNA Sample prep kit v2 (Illumina) and sequenced on an Illumina HiSeq 2500 device at Microgen Corp, Japan (HiSeq 2500 System User Guide Part #15011190 Rev. V HCS 2.2.70 protocol. 2.9.). The paired-end insert size was approximately 180 base pairs. We quantified the expression level of each gene/transcript by counting the number of aligned sequence reads with Bowtie 2 [17] and eXpress [18]