REVIEW
Development of Oligodendrocyte and Myelination in the Central Nervous System

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Abstract. We demonstrated the cell lineage of oligodendrocytes from the glial precursor cells to mature oligodendrocytes forming myelin sheath around the axon. There are several different stages of oligodendrocyte development in vitro. So far there are no precise data about their morphological changes during oligodendrocyte development, but by the analysis using SEM and immunostaining, the characteristic morphological changes with serial expression of cell markers were observed in each developmental steps of oligodendrocyte. We have also clearly demonstrated how oligodendrocytes wrap around the axon by using video time-lapse movies. These results will be useful for understanding the exact cellular mechanism of myelination in the CNS. (Keio J Med 44 (2): 47-52, June 1995)

Key words: myelination, O2A progenitor, oligodendrocyte, central nervous system, cell culture

Introduction

Myelination is one of characteristic developmental processes carried out by oligodendrocyte in the central nervous system (CNS). The myelin sheaths formed by multilamellar membranes surround individual axons as insulators for fast nerve conduction. In myelination, the plasma membranes of oligodendrocyte extend, envelop and ensheathe each axon. However, little is known about the timing of the differentiation of oligodendrocytes and the way to wrap around each axon making their characteristic shapes.¹² Recently, we succeeded to record the precise time course of myelination by oligodendrocyte in vitro using a combination of cell culture technique and newly developed video time-lapse movies. The continuous observation obtained by the movies lead to new insights into cellular mechanism of myelination in the CNS.³ Here, we review oligodendrocyte development and myelination by the oligodendrocyte plasma membrane.

Oligodendrocyte Development

In the early developmental stage of rat embryonic 14 days, glioblasts congregate in the subependymal zone (also named subventricular zone), which lines the lateral ventricles of the forebrain during development.⁴ The subependymal glioblasts persist throughout life and give rise to all types of glial cells. Most glioblasts migrate into the intermediate or mantle zones, then into fiber tracts and commissures and continue proliferating in the subependymal zones. Generation of oligodendrocytes starts somewhat later than that of astrocyte in the same region. Oligodendrocytes arise from O2A common precursor cells in the subependymal zone during late embryonic and early postnatal periods (Fig 1).⁵ These highly motile cells can be recognized by expression of several marker substances including the platelet derived growth factor α receptor (PDGF-α R), ganglioside GD3 and polysialo-gangliosides recognized by monoclonal A2B5 antibody.⁶-¹¹ Differentiating oligodendrocytes first express sulfatide, galactocerebroside (GC) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP)¹²-¹⁵ and later produce major myelin proteins, such as myelin basic protein (MBP) and proteolipid protein (PLP), taking fully differentiated phenotype of the myelin forming cells.¹⁶,¹⁷ During myelination, both mRNA and proteins of MBP and PLP are markedly increased.

We studied on the proliferation and differentiation of oligodendrocytes in vitro with special reference to their morphology by scanning electron microscopy (SEM).
The scanning electron microphotograph of type-1 oligodendrocytes was obtained from mixed primary cell cultures by the immunopanning method as described previously (Fig 2). As these small, round and glossy cells were vimentin positive (Vim+) and GM1 ganglioside positive (GM1+), but A2B5 negative (A2B5-) by immunological characterization as previously reported, they were classified as pre-O2A progenitor cells. Then type 1 oligodendrocytes (pre-O2A progenitor cells) can evolve into type 2 oligodendrocytes (O2A progenitor cells) which are characterized by additional expression of A2B5 and loss of GM1 ganglioside in their cell surfaces in the culture with only 10% calf serum in MEM. The bipolar or more complexed type-2 astrocytes are able to proliferate in the given condition. In Fig 3a, two type-2 oligodendrocytes in the stage of cell division are shown. By the double labeled immunostaining, more than 90% of type-2 oligodendrocytes are A2B5+ and not GC+/GFAP+, indicating their correspondence with O2A progenitor cells. These cells also exhibit unique proliferation and differentiation responses in culture to growth factors such as PDGF and FGF. Type-2 oligodendrocytes can develop into either mature oligodendrocytes or type-2 astrocytes. The multipolar, functionally distinct oligodendrocytes are identified by reacting both A2B5+ and GC+ in the subcultures supplemented with 10% CS in MEM. Further more these cells differentiate into so-called net complex multipolar process-bearing cell of postmitotic, expressing surface oligodendrocyte marker GC, and ultimately express proteins characteristic of CNS myelin such as PLP, MBP and CNPase. These cells
have been termed type-3 oligodendrocytes and shown in Fig 4. To support the SEM data of oligodendrocyte development shown in Fig 2–4, we have performed further immunocytochemical characterization in the different stages of oligodendrocyte lineage as summarized in Table 1.

Fig 3 Scanning electron microphotograph of type-2 oligodendrocytes. Bipolar type-2 oligodendrocytes of bipolar type at the stage of cell division (a) and the cells with more complexed processes (b). Bar = 4.0 μm (a,b).

Fig 4 Scanning electron microscopy of type-3 oligodendrocytes. Cells contain numerous cell processes of "a spider web". Bar = 65 μm.

Table 1 Expression of Cell Markers Based on Oligodendrocyte Development

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<th>Cell Types</th>
<th>A2B5</th>
<th>GC</th>
<th>MBP</th>
<th>CNP</th>
<th>Vim</th>
<th>GM1</th>
<th>PLP</th>
<th>MAG</th>
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<td>1) Type-1 Oligodendrocytes</td>
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<td>pre-O2A progenitor cells</td>
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<td>2) Type-2 Oligodendrocytes</td>
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<td>O2A progenitor cells</td>
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<td>3) Type-3 Oligodendrocytes</td>
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<td>A) immature oligodendrocytes</td>
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<td>B) mature oligodendrocytes</td>
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The antibodies used for detection of each cell markers: A2B5, monoclonal anti-ganglioside antibody; GC, antigalactocerebroside antibody; MBP, anti-myelin basic protein antibody; CNP, anti-2',3'-cyclic nucleotide 3'-phosphohydrolase antibody; Vim, anti-vimentin antibody; GM1, anti-ganglioside GM1 antibody; PLP, anti-proteolipid protein antibody; MAG, anti-myelin associated glycoprotein antibody. Note: Expression of MBP, CNP, PLP and MAG are seen only after oligodendrocytes become postmitotic and express GC.
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Fig 5 A sequential time-lapse video micrographs showing the initial process of in vitro myelination on the astrocyte feeder layer. A ruffling lamellipodial membrane extends from the oligodendrocyte process (A; arrows), lamellipodia and filopodial membranes (B; small arrow) and thick filopodia appear. One of the thick filopodia extends and contacts to the axon (B; large arrow). Lamellipodial membrane protrusions extend over the axon (C; arrow) and then underneath the axon (D; arrows). The lamellipodial processes are rearranged (E; arrows) and fasciculate along the oligodendrocyte process. Then, a slender protrusion forms and surrounds the axon (F; arrowheads). The number in the upper right-hand corner of each photograph indicates the time in minutes from the start (A = 43 min) of videotaping. Calibration bar = 7.2 μm (A and B), 9.5 μm (C and D) and 5.6 μm (E and F).

A Cellular Mechanism of Myelination by Oligodendrocytes

The development, differentiation and myelination of oligodendrocytes have been the focus of interests among neurobiologists. We investigated the initiation and wrapping of oligodendrocytes around the axon in vitro using video-enhanced differential interference microscopy analysis. The time-lapse video graphs and schematics of time course of myelination by oligodendrocytes are shown in Fig 5 and 6, respectively. Mature oligodendrocytes form small ruffling lamellipodial membranes
with fine filopodia. The lamellipodial membranes become apposed to the axon, and are retracted subsequently upon its contact to the axon. This retractive behavior is repeated at least three times at 10 min. intervals in real time before the onset of myelination (Data of video time-lapse micrographs are not shown). After that, the ruffling lamellipodia changes its shape as a "veil-like deflated balloon". These events occur in most cases during initial myelination within approximately 40 min. before onset of actual myelin-sheath formation.\(^3\) After initial contact to the axon is finished, the veil-like morphology appears on the tip of the same cell process (Fig 5A). An outgrowth of one filopodium contacts the axon and then the filopodium actively waves from side to side several times near the axon (Fig 5B). Finally a thick filopodium extends straight out towards the axon from the core of the lamellipodium, and one of the arms of the filopodium actively waves from side to side as if to "measure" the length of myelin required to envelop the axon. Thereafter two filopodia come together to form a veil-like process for a moment. At this time, the axon also moves forward (Fig 6c). Within 2–3 min, the position of the large extended arm of the filopodium on the axon shifts from right to left and then the other two filopodial processes also form very thin small lamellipodial structures which create together a large veil-like structure that waves from left to right. Subsequently, a sharp bend occurs suddenly in the lamellipodium and one of the lamellipodial arms becomes twisted. After the bending of an arm, the lamellipodial process near the axon approaches the axon at an acute angle, forming a filopodial footing that anchors to the axon as the lamellipodium folds into double or multiple layers and wraps around the axon (Fig 5C, D and Fig 6f–h). One of the dramatic aspects of the myelination is the wrapping of the axon by the lamellipodium like a transverse wave in a single motion, leading to anchoring of the resting thick filopodial footing. These processes of wrapping around the axon occur within 20–30 min. Lamellipodial protrusions with filopodia are again formed after one turn of the oligodendrocyte lamellipodium around the axon. These filopodia extend and take on a lamellipodial, "bursting" morphology (Fig 5E and Fig 6i–j). We observed the sequential steps of morphological changes in myelination only when the lamellipodial process was folded like a sheet of paper into three or more layers. The lamellipodium consisting of oligodendrocyte plasma membrane elongates and undergoes extensive morphological changes wrapping around the axon again. Then, lamellipodia are rearranged, form filopodia and fasciculate along the oligodendrocyte process. After that, a slender protrusion is formed (Fig 5F and Fig 6l) resulting in fusion of two contacting lamellipodial processes after the lamellipodium wraps around the axon. It usually takes 90–95 min. for one cycle of lamellipodial wrapping around the axon in real time.

References