Development and Differentiation of Oligodendrocytes

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The central nervous system (CNS) is one of the most complicated organ systems in vertebrates. There are many different kinds of neurons and glial cells, which are believed to be developed from the neuroepithelial cells of the embryonic neural tube (Fig. 1). Glial cells constitute the major component of the CNS. Early studies of gliogenesis in vitro suggested the generation of two main glial classes, astrocytes and oligodendrocytes, during CNS development.

Type-1 astrocytes first appear in the rodent brain several days before birth, whereas oligodendrocytes first appear after birth. Oligodendrocytes form the myelin of the CNS and differentiate from bipotential (O-2A) progenitor cells, which also have the capacity to differentiate into type-2 astrocytes, at least in vitro [1]. However, there is no direct evidence showing its presence in vivo in normal rats. O-2A progenitor cells are known to be highly migratory, as shown by transplantation into the brain of myelin-deficient mutants [2]. Therefore, it is not clear yet from which part of the nervous system the O-2A cells originate.

The developmental processes of the oligodendrocyte in the CNS includes the proliferation, migration, differentiation, and survival processes, all of which are supported by a variety of factors [3]. Various environmental signals are considered to control the proliferation and differentiation of glial precursor cells that generate oligodendrocytes. In this paper, recent progress in identifying the origin of oligodendrocytes and factors affecting oligodendrocyte development are reviewed.

I. Origin of Oligodendrocytes

The differentiation of the O-2A progenitor cells into oligodendrocytes and type-2 astrocytes has been well characterized in vitro and in vivo [4–12]. Furthermore, several intermediate stages can be identified between the O-2A progenitor and the mature oligodendrocyte (galactocerebroside (GalC)-positive cell). The study on the differentiation of O-2A progenitors into oligodendrocytes depends on the application of sequential immunocytochemical markers (Fig. 2). The ordered and partially overlapping expression of surface antigens recognized by monoclonal antibodies, A2B5, O4, and O1, distinguishes three consecutive oligodendrocyte lineages in the rat. The O-2A progenitors are identified by their bipolar morphology and reaction with A2B5 antibody [1, 13], which binds to one or more of the gangliosides. Other antibodies which recognize the O-2A progenitor cell-specific markers have also been obtained, including anti-GD3 ganglioside [14] and anti-NG2 chondroitin-sulfate proteoglycan antibodies [15]. The oligodendroblast cell is recognized by the mouse monoclonal antibody (mAb) O4 [16], but it does not yet express GalC on its surface. The antibody against O4 recognizes two sulfated glycolipids, sulfatide and seminolipid [17, 18]. The GalC-positive cell is a post-mitotic oligodendrocyte that is recognized by the mouse mAb O1 [16].

Fig. 1. Schematic representation of the neuroectoderm lineage. Neurons, astrocytes, and oligodendrocytes are generated from neuroepithelial cell in the CNS.

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O-2A progenitor cell  OL-BLAST  IMMATURE OL

Fig. 2. Schematic representation of three stages of the oligodendrocyte lineage recognized by antibodies against membrane surface components. Studies on the differentiation of O-2A progenitors into oligodendrocytes depend on the application of sequential immunocytochemical markers. The ordered and partially overlapping expression of cell surface antigens recognized by A2B5, O4, and O1 monoclonal antibodies distinguish three consecutive developmental stages of oligodendrocytes.

Furthermore, oligodendrocyte maturation is defined by the expression of myelin-specific proteins, including 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) [19–22], myelin basic protein (MBP) [23, 24] and myelin proteolipid protein (PLP) [6, 25].

It is widely accepted that oligodendrocytes originate from the neuroepithelial cells. Thus, it should be possible to trace its lineage back to the neuroepithelial cells. However, it is not clear which antibodies recognize the precursor of O-2A cells. GD3 seems to be present in the very early precursor cells of oligodendrocytes [9, 11], however, it is also present in other types of brain cells.

Recently, in situ hybridization using probes for the alpha subunit of the receptor for platelet-derived growth factor (PDGFRα) [26, 27], 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) [28, 29] and DM-20 [30], the product of alternative splicing of the myelin proteolipid protein (PLP) gene [31] has yielded evidence that cells of the oligodendrocyte lineage arise from rather restricted regions of the nervous system and then migrate widely into other regions. These observations agree well with the result obtained by Warf et al. [32], who showed that GalC-positive oligodendrocytes developed in cultures of ventral spinal cord cells from E14 rat embryos, but did not develop in cultures of dorsal cells from E14 embryos. In contrast, oligodendrocytes developed in cultures of both ventral and dorsal spinal cord cells from E16 embryos. This suggested that oligodendrocyte precursor cells are present in the ventral half but not the dorsal half of the cord at E14, but spread to the dorsal half by E16. However, there are differences between the localization of PDGFRα mRNA and DM-20 mRNA in the early diencephalon and spinal cord [27–29]. These two populations may be two independent pools of oligodendrocyte precursors, or either one may not be a precursor of oligodendrocytes at an early stage of development. Since evidence is accumulating which indicates that PDGFRα-expressing cells differentiate into oligodendrocytes, we will describe the migration pattern of PDGFRα-positive cells. Most of the results have been obtained by in situ hybridization techniques, but similar results have also been obtained by immunohistochemical analysis using antibody against PDGFRα [33].

Direct evidence indicating PDGFRα-positive cells in the embryonic rat spinal cord are oligodendrocyte precursors came from immunopanning experiments. Cultures of >99% pure PDGFRα-positive cells were established from E17 rat spinal cords by immunoselection with an antibody against the extracellular domain of PDGFRα. When cultured in the presence of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) these cells displayed the characteristic morphology and antigenic phenotype (A2B5+, NG2+) expected for O-2A progenitors. When transferred to defined medium lacking growth factors, they all differentiated into GalC-positive oligodendrocytes [34]. Therefore, PDGFRα-positive cells in the ventral half of the embryonic rat (or mouse) spinal cord are oligodendrocyte precursors; however, it cannot be concluded that all oligodendrocytes originate from PDGFRα-positive cells. It is possible that there are other lineages of oligodendrocytes that differentiate from PDGFRα-negative cells.

PDGFRα-positive cells in the rat spinal cord originate from a very restricted region of the ventricular zone at around E14. These cells are in the ventral half of the spinal cord in two longitudinal columns, one on each side of the central canal. Initially, these columns are only two cells wide but the cells subsequently appear to proliferate and disseminate throughout the spinal cord [27, 28]. This pattern of migration has also been observed in the chick spinal cord. One et al. [35] showed that monoclonal antibody O4 labels all stages of chick oligodendrocyte lineages from the very early stage. In contrast, as mentioned before, O4 only stains much later stages in rodents. Immunolabeling of transverse sections of the E6 chick spinal cord revealed a focus of O4-positive cells in the ventricular zone in the ventral half of the cord that corresponds closely to the PDGFRα-positive cells in the rat. These O4-positive cells migrated radially from the ventricular zone toward the surface, which is also sim-
ilar to what has been observed in the rat.

It is an interesting issue to ask what the signals are
that determine the cells of a particular region of ven-
tricular zone to become oligodendrocytes.

II. Factors Influencing Development of
Oligodendrocytes

O-2A progenitors are stimulated to proliferate by vari-
ous combinations of environmental growth factors. If
these mitogens are absent, O-2A progenitors will stop
dividing and begin to differentiate into oligodendro-
cytes. It has been shown previously that four peptide
growth factors promote DNA synthesis in the O-2A
progenitors in vitro, PDGF, bFGF, neurotrophin-3
(NT-3), and insulin-like growth factor-I (IGF-I), all of
which are synthesized by other type of cells [3,
36–44]. PDGF promotes both the proliferation and the
survival of O-2A progenitors [40–42], whereas IGF-I
or NT-3 alone promote only survival [38]. bFGF alone
does not promote survival or stimulate DNA synthe-
sis, whereas the combination of bFGF and IGF-I stim-
ulates DNA synthesis in the O-2A progenitor cells
[39, 43, 44]. Thus, multiple extracellular signaling
molecules might collaborate to promote proliferation
of O-2A progenitors.

III. PLP Gene Products also Affect
Development of Oligodendrocytes

One approach to identifying environmental signals
that are responsible for neural development is to study
the molecular defect in mutant animals which show
abnormal development in the nervous system. We
have found another humoral factor that affects the
development of oligodendrocytes from the analysis of
a mutant mouse. PLP is one of the major myelin pro-
teins of CNS myelin and was considered to play a role
in stabilization of the myelin membrane (Fig. 3).
However, it has been demonstrated that PLP gene ex-
pression begins long before the myelin structure is
formed (Fig. 4) [45, 46] and mutations within the PLP
gene cause abnormalities in the maturation and sur-
vival of oligodendrocytes [47]. These facts indicate
that the PLP gene products are somehow involved in
oligodendrocyte development. The early PLP gene
product is not PLP, but is DM-20, which is the prod-
uct of alternative splicing of the PLP gene [31].

The jimpy mouse is one of the PLP mutants, which
shows premature cell death of oligodendrocytes and
severe dysmyelination. An A to G conversion at the
conserved “AG” residues of the 3′-splice site of the
fourth intron of the PLP gene was found in jimpy mice
[27, 48], resulting in the deletion of the fifth exon of
jimpy PLP mRNA [49]. Previous studies have re-
ported that jimpy oligodendrocytes in culture survive
and produce more myelin proteins by the addition of
normal astrocyte conditioned medium (Fig. 4) [50,
51], and that partial recovery of jimpy oligodendro-
cyte function was also observed in shiverer brain
transplanted with embryonic jimpy brain fragments
[52]. These results suggest that PLP gene products
could induce the secretion of a humoral factor that
regulates maturation/survival of oligodendrocytes
(oligodendrocyte growth factor, OGF) and this factor
is absent from jimpy brain. It is hard to think that PLP
or DM-20 themselves are secreted into the medium.
These proteins are called proteolipid proteins because

Fig. 3. PLP in the CNS myelin. The major CNS myelin
proteins, myelin basic protein (MBP), and the proteolipid
protein (PLP), represent 70–80% of the total myelin protein
content of the membrane. PLP is thought to promote the
apposition of extracellular surfaces of myelin lamellae.

Fig. 4. Cell differentiation and PLP gene expression.
A: The differentiation of glial cells has been well character-
ized in vitro and in vivo. The reverse transcription-PCR am-
plicated product from DM-20 mRNA was detected throughout
the development stages investigated. B: DM-20 mRNA is
formed by an alternative splicing from the PLP gene. PLP
mRNA becomes detectable in P2 brain.
they can be extracted by chloroform-methanol, and so are very hydrophobic. OGF may be a processed fragment of PLP/DM-20 or it may be a totally unrelated factor induced by the presence of PLP/DM-20. Identification and characterization of this OGF are important in understanding the development of oligodendrocytes.

Previous studies have reported that conditioned medium from B104 neuroblastoma cell line promotes the growth of O-2A progenitor of neonatal and mature rat brain, and increase the number of oligodendrocytes [53–55]. B104 cells produce mRNA for DM-20 [45]. Moreover, it has also been shown that the relative amounts of the factors secreted is B104>C6>RN22 [52], which correlates well with the amount of DM-20 mRNA found in these cells [44]. These findings prompted us to speculate that the factor(s) secreted by B104 cells is the gliogenic factor(s) (OGF) missing in the jinpy brain.

Culture supernatants from DM-20-producing cell lines (G26, B104, B16) or non-producing cells (NIH3T3) were added to primary glial cell cultures from E17 mouse brain. After 4 d, the number of oligodendrocytes present in cultures with supernatants from DM-20-producing cells was significantly higher than that of control cultures, but not with the NIH3T3-supernatant [56].

To investigate whether PLP gene expression is directly involved in this process, NIH3T3 cells were forced to produce PLP or DM-20. By the addition of the supernatants from the PLP/DM-20 transformants, the number of oligodendrocytes increased [56] (Fig. 5). This activity present in the PLP/DM-20 supernatant was completely inhibited by anti-PLP antibody (AA3), which recognizes the C-terminus of PLP. Thus, PLP/DM-20 themselves (although this possibility is low as mentioned above) or their fragments (containing C-terminal portion) were released into culture medium and increased the number of oligodendrocytes (Yamada et al., manuscript in preparation).

To investigate more directly that the PLP gene products themselves contain the growth activity, purified PLP was added to the same assay system. Purified PLP clearly increased the number of oligodendrocytes in culture, which was inhibited by anti-PLP antibody. Furthermore, the OGF activity present in the conditioned medium from DM-20-producing cell lines, including B104, was also inhibited by anti-PLP antibody. Since the supernatant from B104 is known to promote the growth of O-2A progenitors, PLP/DM-20 themselves or their fragments are suggested to cause the increase in the number of O-2A progenitors (Yamada et al., manuscript in preparation).

The PLP gene products acting extracellularly increased the number of oligodendrocytes. This function may be modulated by the activity of other growth factor(s). In the assay system of the primary mouse glial cell culture, chemically defined medium contains insulin at the concentration of 10 μg/ml. This concentration of insulin is high enough to activate IGF-I receptors. Thus, it is highly possible that the combination of PLP and IGF-I promotes the proliferation of O-2A progenitor. This possibility is now under investigation.

Previous results from studies on dysmyelinating PLP mutants suggested that a product of the PLP gene may be involved in cell survival and differentiation. This study clearly demonstrated that PLP gene products containing the C-terminal region of PLP can be secreted as a humoral factor that promotes the growth of oligodendrocytes.

IV. Possible Generation of Oligodendrocyte Cell Lineage by Sonic Hedgehog

An interesting and important issue to ask in understanding the development of oligodendrocytes is: what determines a specific region (only one or two cell
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widths) of E14 rat spinal cord ventricular zone to express the PDGFRA gene and thus to become oligodendrocyte-lineage. An important factor in determining the dorso-ventral pattern in the spinal cord is the Sonic hedgehog (Shh). Shh is expressed by both the notochord and the floor plate. When ectopic notochord was grafted into a dorsal-lateral position relative to the endogenous notochord of a chick embryo, a second floor plate was induced, as expected. It also resulted in the appearance of a new focus of PDGFRA-positive oligodendrocyte precursors in the ventricular zone, the same distance from the induced floor plate as was the original focus from the endogenous floor plate [34]. Thus, Shh seems to play a major role in determining neuroepithelial cell fate to oligodendrocyte, as well as to motor neurons [57].

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