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学位論文題目	Development of Oligodendrocyte Cell Lineage : The Role of PLP Gene Products in the Early Development of Central Nervous System
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論文内容の要旨

The central nervous system (CNS) is a complex structure containing numerous cell types. Oligodendrocytes are the myelinating cells of the CNS. The myelination of axons by oligodendrocytes is of vital importance to the function of the CNS, since abnormalities in the oligodendrocyte development can lead to the generative neurological diseases. Although the differentiation of the early progenitor cell into oligodendroglia has been well characterized *in vitro*, its relevance to the CNS development is not yet well established. One is limited by the availability of early markers which would specifically recognize oligodendrocyte progenitors among other cell types. In attempt to characterize the oligodendrocyte lineage *in vivo*, they performed *in situ* hybridization using the probes for mRNA encoding PDGF α receptor mRNA, and PLP/DM-20 mRNA encoding PLP and DM-20, two isoforms of the myelin proteolipid protein, structural proteins of the CNS myelin.

PDGFαR mRNA: *In vitro*, oligodendrocyte progenitors were shown to express the alpha-isoform of PDGF receptor, which is down-regulated with development. Immunoselection with an antibody against rat PDGFαR produced the cultures where 95% cells expressed PDGFαR. When these cells were allowed to differentiate, many of them developed into mature oligodendrocytes. *In vivo*, the cells containing PDGFαR transcript appeared in the restricted locations within the ventral portion of the spinal cord. Therefore, PDGFαR has been proposed to be one of the earliest reliable markers for the lineage in the spinal cord and possibly in the brain.

DM-20 mRNA: Products of the PLP/DM-20 gene, proteolipid protein PLP and its isoform DM-20, are the most abundant proteins of the CNS myelin. Besides the structural function, one or both of these proteins seem to be necessary for the early development of oligodendrocytes. It has been demonstrated that a point mutation or a slight overexpression of the PLP/DM-20 gene causes severe effects on myelin formation and survival of oligodendrocytes. The appearance and behavior of the cells which express PLP/DM-20 transcript during early development was relevant of the oligodendrocyte precursors. Thus, mRNA PLP/DM-20 has been proposed as another early marker for the oligodendrocyte development, though the identity of the early PLP/DM-20 expressing cells is still under debate.

Direct comparison of the two markers on the adjacent paraffin sections revealed that cells containing PDGFαR transcript were different from the PLP/DM-20 expressing cells. To reject the possibility, that the PLP/DM-20 expressing cells were not related to the oligodendrocyte lineage, they examined the developmental profile of PLP/DM-20 expression in the brain. Since no drastic changes between the early and the late PLP/DM-20-expressing cells could be found, they assumed that they indeed belong to the same cell lineage. On the next step, they made use of the mRNA encoding UDP-galactose ceramide galactosyltransferase (CGT), the established marker for

premyelinating oligodendrocytes. Obvious regional overlap between PLP/DM-20 and CGT transcripts strongly suggested the oligodendrocyte origin of the PLP/DM-20-expressing cells. Therefore, in the embryonic CNS, the expression of PLP/DM-20 and PDGF α R mRNAs marked different populations of the oligodendrocyte precursors. Similar experiments have been reported by Yu and colleagues (1994), who could trace the cells expressing PDGF α R-mRNA into the oligodendrocyte lineage both *in vitro* and *in vivo*, using another oligodendrocyte marker, 2'-3'-cyclic-nucleotide 3'-phosphodiesterase mRNA (CNP-mRNA).

The role of the PDGF α R expression in the oligodendrocyte progenitors has been characterized, while the significance of the early PLP/DM-20 expression is not yet clear. PDGF causes mitogenic response in the early progenitors which carry receptors to this growth factor. As these cells mature, they louse the receptors and do not respond to the PDGF any more. PLP/DM-20 gene is expressed and abnormality in the PLP mutants found before the myelinating period, suggesting premyelinating function of the PLP/DM-20 gene product. Previously they showed that the PLP gene expression is directly associated with secretion of a factor, which increases the number of oligodendrocytes. Here they present the data suggesting that this activity is mediated by a fragment containing C-terminal portion of PLP or DM-20, secreted into the medium. Furthermore, a synthetic peptide corresponding to 215-232 residues of PLP/DM-20 also exhibited a similar activity. Purified PLP or its C-terminal fragment could affect the development of oligodendrocytes at extremely low concentrations (0.3pM). The dose-response curve of PLP/DM-20 or PLP-peptide showed reduced activity at higher concentration, and accordingly they did not affect cultured oligodendrocytes after major PLP gene expression occurred within these cells. Thus, PLP gene product can be secreted into the medium and exerts biological activities within a narrow window in development before the major induction of the PLP gene expression occurs.

A systematic investigation of the role of the PLP/DM-20 gene products in the developing murine CNS has been accessed both *in vivo* and *in vitro*. PLP/DM-20 mRNA is expressed in the embryonic hindbrain by the subset of the oligodendrocyte progenitors. The PLP C-terminal fragment can be secreted and is capable to promote the differential/survival of oligodendrocytes *in vitro*. The combined evidence suggests that PLP/DM-20 gene products can regulate the development of distinct oligodendrocyte lineage in the developing CNS.

論文の審査結果の要旨

オリゴデンドロサイトは中枢神経系ミエリン形成細胞であり、神経上皮細胞由来である。しかし、脳内のどの領域から発生し、移動・分化・成熟するのか、未だ明らかにされていない。本研究においては、オリゴデンドロサイトの前駆細胞を発生段階の早期から検出することが示された２種類のプローブ [Platelet Derived Growth Factor α Recepter (PDGFαR) cDNA および Myelin Proteolipid Protein (PLP)/ DM20 cDNA をテンプレートとしてリボプローブを作製]を用いて、マウス脳内オリゴデンドロサイトの神経系譜を in situハイブリダイゼーション法で解析した。

胎仔期マウス脳内において、PDGFαR mRNAとPLP/ DM20 mRNAを産生している細胞は、異なった細胞であることが示された。さらに、延髄領域では PLP/DM20 mRNA産生細胞が、また視神経においては PDGFαR mRNA産生細胞がオリゴデンドロサイトに分化することが示され、脳内の領域によってオリゴデンドロサイトの系譜が異なることが明らかとなった。

オリゴデンドロサイト前駆細胞は、PDGFによってその分化・増殖が制御されていることが知られているが、今回新たにオリゴデンドロサイト前駆細胞であることが示された延髄における PLP/DM20 mRNA産生細胞は、PDGF受容体を発現しておらず PDGFによる制御を受けられない。そこで、新たな分化・増殖因子の検索を、グリア培養系を用いて行った。PLPはミエリン膜に大量に存在し、ミエリン形成期に産生されるようになるが、その遺伝子の alternative splicing産物である DM20はミエリン形成期のはるか以前から産生されている。本研究により DM20が細胞に産生されると、その C-末端約６０アミノ酸残基を含んだ領域は細胞外に分泌され、オリゴデンドロサイトの分化・増殖を制御し得ることが示された。

以上の結果より、脳内においてオリゴデンドロサイトには少なくとも２種類の異なった細胞系譜が存在することが明らかとなり、そのうち一つは PDGFにより細胞数が制御され、他方は DM20の断片により制御されていることが示唆された。

申請者の論文は、オリゴデンドロサイトの細胞系譜に関する重要な新しい知見を提供するものであり、学位論文として十分ふさわしい内容であるものと審査委員会で一致して判定した。

また審査委員会において、提出論文の発表をさせた後に、その内容のみならず学問的背景や関連分野や研究動向についての口頭試問を行ったが、いずれに対する応答も的確であった。本論文は英語で書かれており、審査も英語で行われたが、英語力にも何ら問題はないものと判断された。

以上、総合的に判断し、学位を取得するに足る水準に十分達しているものと判断した。