The use of culture systems for the study of oligodendrocyte development and injury:

The erbB2 gene is required for the development of terminally differentiated spinal cord oligodendrocytes

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The nervous system consists of two types of cells, which are neurons and glial cells. Among the glial cells, oligodendrodendrocytes and schwann cells form myelin sheaths in the central nervous system (CNS) and the peripheral nervous system (PNS), respectively. The major function of myelin in vertebrates is to insulate axons and help action potential travel faster. Another important function of myelin is to support axonal function and integrity. In some neuronal diseases, such as multiple sclerosis that is a representative demyelinating disease, myelin sheaths are degenerated. In addition, oligodendrocytes contains several components that inhibit axonal regeneration in the CNS. This seems to be why axonal regeneration is extremely limited in the CNS. Here I will breifly describe some of the current research areas on oligodendricytes and then discuss in detail with a specific study regarding the effect of an axonally-derived signal on the development of oligodendrocytes.

I. The recent trend in the study of oligodendrocyte

1) The genesis of oligodendrocytes
   - *signaling molecules or signal transducers*
     : PDGF-aa, basic FGF, neuregulin, sonic hedgehog, olig-1, olig-2, nkx6.1, pax6

2) The mechanisms of axion-glia interactions
   : cell-cell adhesion molecules, axonally-derived factors

3) The inhibitory effects of oligodendrocytes on axonal regeneration
   : Nogo (A, B, and C), myelin associated glycoprotein (MAG), etc.

4) The injury agents that cause demyelination
: pro-inflammatory cytokines, viruses, auto-antibodies, lipopolysaccharide, glutamate

II. Culture systems to study oligodendrocyte development or injury

1) Pure oligodendrocytes
2) Mixed glial cultures
3) Spinal cord explant culture
4) Brain slice culture
5) Retinal ganglion cell culture
etc.

III. The erbB2 gene required for the development of terminally differerntiated spinal cord oligodendrocytes

1. Abstract

Development of oligodendrocytes and the generation of myelin internodes within the spinal cord depends on regional signals derived from the notochord and axonally derived signals. Neuregulin (NRG)-1, localized in the floor plate as well as in motor and sensory neurons, is necessary for normal oligodendrocyte development. Oligodendrocytes respond to NRGs by activating members of the erbB receptor tyrosine kinase family. Here, we show that erbB2 is not necessary for the early stages of oligodendrocyte precursor development, but is essential for proligodendroblasts to differentiate into galactosylcerebroside-positive (GalC+) oligodendrocytes. In the presence of erbB2, oligodendrocyte development is normal. In the absence of erbB2 (erbB2-/-), however, oligodendrocyte development is halted at the proligodendroblast stage with a >10-fold reduction in the number of GalC+ oligodendrocytes. ErbB2 appears to function in the transition of proligodendroblast to oligodendrocyte by transducing a terminal differentiation signal, since there is no evidence of increased oligodendrocyte death in the absence of erbB2. Furthermore, known survival signals for oligodendrocytes increase oligodendrocyte numbers in the presence of erbB2, but fail to do so in the absence of erbB2. Of the erbB2-/- oligodendrocytes that do differentiate, all fail to ensheath neurites. These data suggest that erbB2 is

required for the terminal differentiation of oligodendrocytes and for development of myelin.

2. Introduction

Development of oligodendrocytes capable of forming myelin internodes requires several distinct environmental cues. These include early, regional, and later axonally derived signals. The initial specification of spinal cord oligodendrocyte precursor cells (OPCs) is dependent on signals from ventral structures, such as the notochord and floor plate. One critical signaling molecule in early OPC specification appears to be Sonic hedgehog derived from floor plate and notochord, which induces the basic helix-loop-helix molecules olg-1 and olg-2 (Lu et al., 2000; Zhou et al., 2000). After their initial appearance in the ventral ventricular zone (Noll and Miller, 1993; Timsit et al., 1995), OPCs migrate widely throughout the neuraxis, mature through antigenically and morphologically distinct stages, and ultimately form myelin internodes. An early stage of OPC is an mAb A2B5 immunoreactive, bipolar, motile cell with a mitogenic response to PGDF-AA and bFGF. These A2B5+ cells mature into proligodendroblasts, less-motile cells (characterized by surface labeling with the O4 mAb, but not antigalactosylcerebroside antibodies), and a fine arbor of processes. Proligodendroblasts proliferate in response to a different spectrum of mitogens from A2B5+/O4- cells. Differentiation of proligodendroblasts is accompanied by exit from the cell cycle and acquisition of galactosylcerebroside expression, identified by mAb O1. Before axonal ensheathment and myelination, oligodendrocytes mature and express myelin genes such as myelin basic protein (MBP). Maturing oligodendrocytes undergo progressive remodeling of their process arbor during a dramatic but poorly understood metamorphosis from premyelinating to myelinating cells. The multiple distinct steps in oligodendrocyte and myelin formation are regulated by distinct signaling systems. Although some of these signaling systems are beginning to be understood, control of the later stages in myelin formation is still poorly understood.

Axonally derived signals appear to be required for several aspects of oligodendrocyte and myelin formation (for review see Barres and Raff, 1999). One potential axonally derived signaling molecule critical for oligodendrocyte and myelin formation is neuregulin (NRG)-1 (for review see Carraway and Burden,

1995; Pinkas-Kramarski et al., 1998; Riese and Stern, 1998; Adlkofer and Lai, 2000). In spinal cord explants bearing a loss-of-function mutation in the NRG-1 gene, O4+ proligodendroblasts completely fail to develop (Vartanian et al., 1999). This early defect in oligodendrocyte development in NRG-1 mutants can be reversed by the addition of recombinant NRG. Consistent with a role for NRGs during early development of the oligodendrocyte lineage, early ventral structures such as the ventral ventricular zone and floor plate of the spinal cord (Vartanian et al., 1999) and the subventricular zone of the forebrain (Vartanian et al., 1994; Corfas et al., 1995) express NRG at the time that OPCs initially arise.

Multiple factors dictate the cellular effects of NRG. These include the specific ligand, levels and repertoire of receptor expression, and the cellular context. The large number of NRG ligands arise from four known genes with multiple splice and promoter variants (for review see Fischbach and Rosen, 1997; Adlkofer and Lai, 2000). Signal transduction through erbB receptors occurs as the consequence of essential ligand-induced receptor dimerizations. For example, erbB2 lacks a binding site for NRG, whereas erbB3 lacks intrinsic tyrosine kinase activity, and neither can transduce NRG signals in isolation. By contrast, erbB4 is both capable of binding ligand and possesses an intact tyrosine kinase domain. Although erbB3 or erbB4 will bind ligand alone, heterodimerization with erbB2 increases the affinity of the receptor complex for its ligand 14-fold. ErbB2 appears to be the preferred receptor partner in most, if not all, erbB heterodimers, and it increases the complexity of signal transduction after NRG stimulation (Pinkas-Kramarski et al., 1997; Riese and Stern, 1998; Yarden and Sliwkowski, 2001). Furthermore, unique and overlapping docking sites for adapter proteins and cytosolic enzymes exist for individual erbBs, making them capable of transducing both common and distinct signals (Plowman et al., 1993; Wang et al., 1998a; Jones et al., 1999).

Here we examine the requirement for erbB2 signaling during development of the spinal cord oligodendrocyte lineage. Through a targeted disruption of the murine erbB2 gene, we demonstrate that, in striking contrast to the absence of NRG-1, the absence of erbB2 has no effect on the formation of the early oligodendrocyte lineage. However, ErbB2 does appear to be essential for normal development of later stages of the oligodendrocyte lineage. In the absence of erbB2, the development of differentiated O1+ oligodendrocytes was dramatically deficient. In addition, the limited O1+ oligodendrocytes that develop in the

absence of erbB2 signaling failed to ensheath neurites and form myelin in long-term cultures.

3. Results

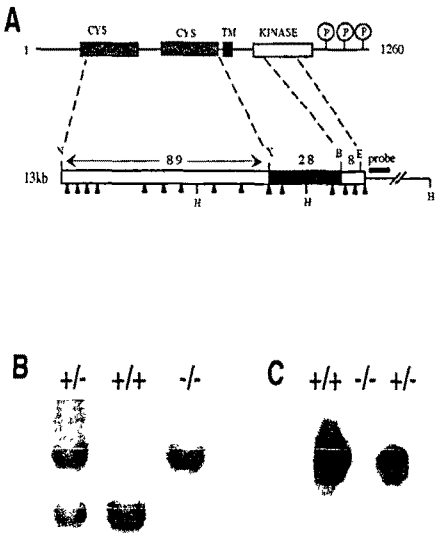


Figure 1. Targeted disruption of the murine erbB2 gene.
(A) Schematic representation of the 1,260 amino acid erbB2/neu protein (top) and the 13-kb genomic fragment (bottom) used to generate the targeting construct.
(B) Southern blot analysis of HindIII-restricted genomic DNA probed with a cDNA fragment found outside the targeting construct.
(C) Western blot analysis of 10.5 dpc embryos homogenized in SDS-PAGE buffer, resolved by 5% SDS-PAGE, and probed for erbB2.

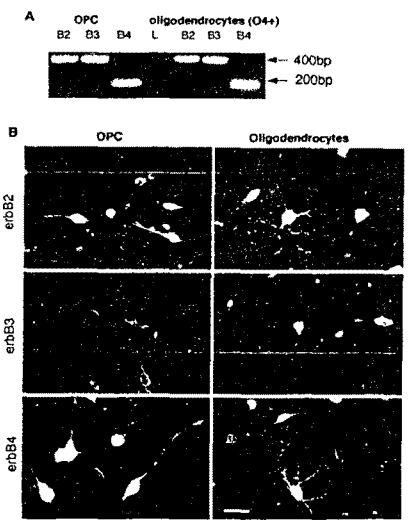
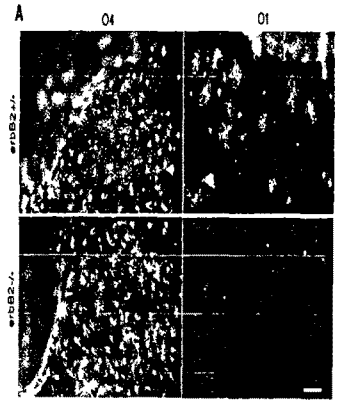
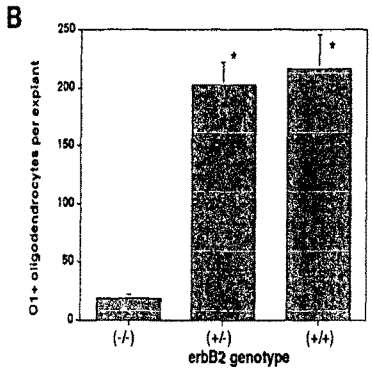


Figure 2. Developing spinal cord oligodendrocytes express the NRG receptors erbB2, erbB3, and erbB4.
(A) RT-PCR of erbB2 (B2), erbB3 (B3), and erbB4 (B4) fragments from immunopanned OPC and oligodendrocyte total RNA.
(B) Immunostaining of OPCs and oligodendrocytes for erbB2, erbB3, and erbB4.

Figure 3. Severe loss of O1+ oligodendrocytes in the absence of erbB2. (A) Development of O4+ oligodendrocytes is normal in the presence of erbB2 (erbB2+/-), as well as in its absence (erbB2-/-). The absence of erbB2 results in a severe loss of O1+ oligodendrocytes. (B) Quantitative analysis of explant data reveals a >10-fold reduction in the number of O1+ oligodendrocytes in the absence of erbB2 compared with erbB2+/- and erbB2+/+ explants. (C) Quantitative analysis of MBP+ oligodendrocytes.





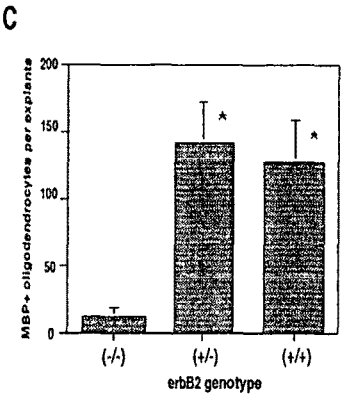
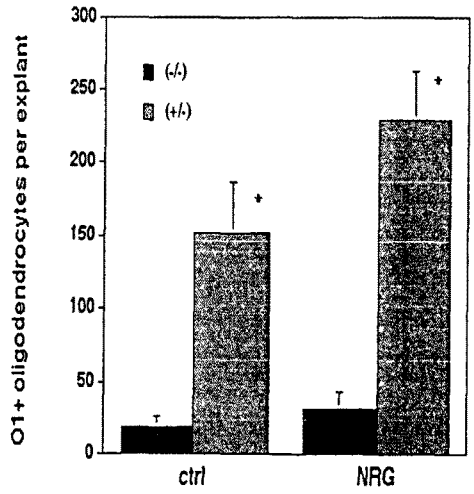


Figure 4. Failed rescue of O1+ oligodendrocytes by addition of recombinant NRG. Spinal cord explants from E9.5 embryos were cultured for 10 d in the persistent presence or absence of 20 nM recombinant NRG-1.



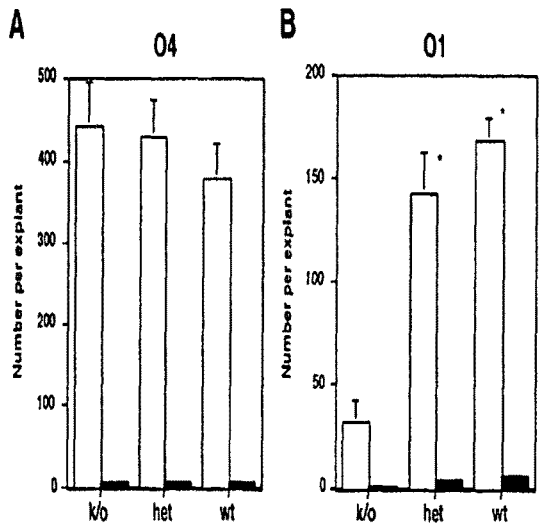


Figure 5. Lack of erbB2 signaling does not result in increased cell death of OPCs or oligodendrocytes in spinal cord explants. Spinal cord explants from erbB2-/-, erbB2+/-, and erbB2+/+ E9.5 mouse embryos were cultured for 9 days and double-labeled with propidium iodide, and the stage-specific markers O4 (A) or O1 (B) and the number of apoptotic cells identified by nuclear pyknosis.

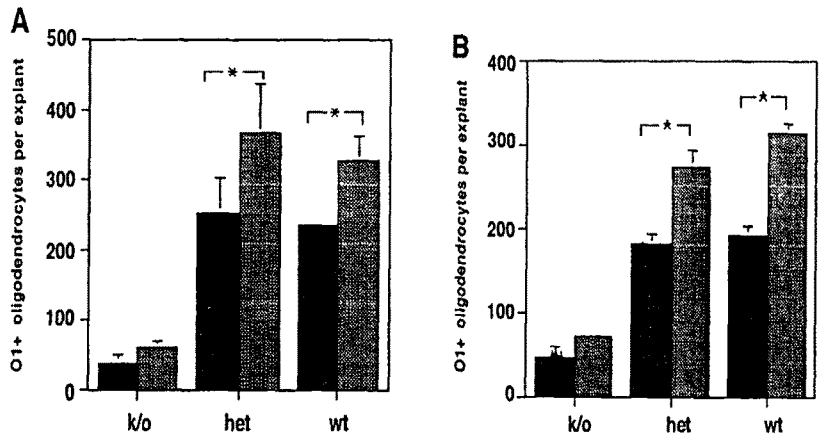


Figure 6. Ligands that transduce survival signals to oligodendrocytes through distinct transmembrane receptors do not rescue the erbB2-/- phenotype. (A) PDGF-AA does not rescue GalC+ oligodendrocytes in erbB2 loss-of-function mutants. (B) LIF does not rescue the GalC+ oligodendrocytes in the erbB2 loss-of-function mutants.

Figure 7. Low-density cultures from erbB2-/- explants have significantly decreased numbers of O1+ oligodendrocytes compared with cells expressing erbB2. There is a significant reduction in the number of O1+ oligodendrocytes in the absence of erbB2.

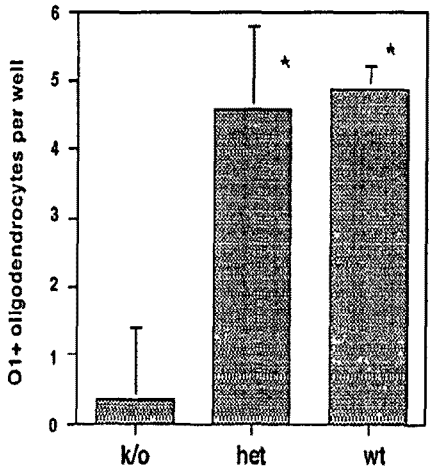
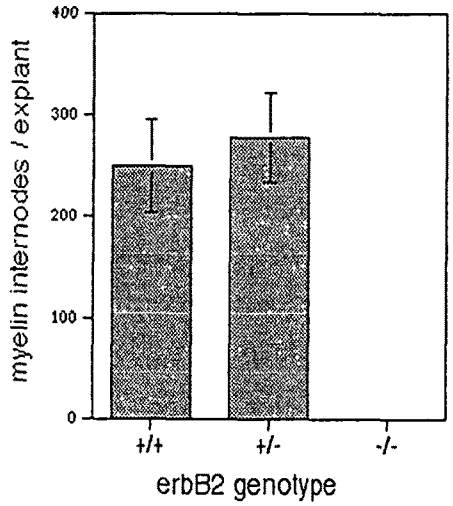


Figure 8. Deficient axonal ensheathment in erbB2 loss-of-function mutants. Quantitation of the number of myelin internodes per explant from erbB2+/+, erbB2+/-, and erbB2-/- mice. In the presence of erbB2, numerous myelin internodes are identified, whereas in the absence of erbB2, none were observed.



4. Conclusions

- NRG-mediated signals transduced through diverse erbB receptors are required for distinct stages in oligodendrocyte development.
- Axonally derived signals are necessary for oligodendrocyte development and myelination.
- NRG is a candidate axonal signal for oligodendrocyte formation and

myelination

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