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SYMPOSIUM: Neural Stem Cells

Postnatal Cerebral Cortical Multipotent Progenitors: Regulatory Mechanisms and Potential Role in the Development of Novel Neural Regenerative Strategies

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In the developing postnatal cerebral cortex, protracted generation of glia and neurons occurs and precise matching of local cell types is needed for the functional organization of regional microdomains characteristic of complex CNS tissues. Recent studies have suggested that multipotent progenitors play an important role in neural lineage elaboration during neurogenesis and gliogenesis after migration from paramedian generative zones. The presence of a separate reservoir of cerebral cortical multipotent cells under strict local environmental regulation would provide an appropriate mechanism for terminal developmental sculpting and for reconstitution of regional cellular pools after injury. We have isolated distinct pools of EGF- and bFGF-responsive multipotent progenitors from the postnatal mammalian cerebral cortex independent of the subventricular zone. These progenitor populations are under tight environmental regulation by specific hierarchies of cytokine subclasses that program the progressive elaboration of intermediate lineage-restricted progenitors and differentiated type I and II astrocytes, myelinating oligodendrocytes and neuronal subtypes that express specific neuromodulatory proteins. Neural lineage development from these cortical multipotent progenitors is a graded developmental process involving sequential induction of specific cytokine receptors, acquisition of factor responsiveness and complex lineage interdependence. The cortical multipotent progenitor pathways program the elaboration of neural lineage species with distinct cellular response properties when compared with analogous species derived from subventricular zone progenitors, indicating that the cortical multipotent cells contribute to the establishment of lineage diversity within the developing cortical cortex. In addition, the cortical multipotent cells generate dynamic intermediate progenitor pools that utilize temporally- coded environmental cues to alter neural fate decisions. These cumulative observations suggest that postnatal cerebral cortical multipotent cells represent a novel set of progenitor pathways necessary for normal mammalian cortical maturation, and may have important implications for our understanding of a wide variety of neuropathological conditions and for the development of more effective regenerative strategies to combat these pervasive neurological disorders.

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Neurobiology of CNS Multipotent Progenitor Cells

There is now considerable evidence that multipotent progenitor cells are present within paramedian generative zones throughout the neuraxis, and may contribute to embryonic neurogenesis, perinatal gliogenesis and even to adult physiological and/or injury responses (24, 42, 78). These *in vitro* and *in vivo* experimental observations were facilitated by the identification of specific CNS cytokines (epidermal growth factor, EGF; basic fibroblast growth factor, bFGF) that regulate multipotent progenitor cell survival, proliferation and their ability to regenerate identical copies of themselves while giving rise to differentiated progeny (self-renewal) (29, 68-70). Although multipotent progenitors represent a small complement of cells within CNS generative zones, including the early postnatal forebrain subventricular zone, their ability to undergo exponential growth over short response intervals *in vitro* and *in vivo* favors the generation of a disproportionally large number of differentiated progeny (70, 78). Within CNS generative zones, multipotent progenitors can exist in a quiescent state or can undergo constitutive proliferation in response to specific cytokines (57). EGF-responsive multipotent progenitors have been shown to migrate

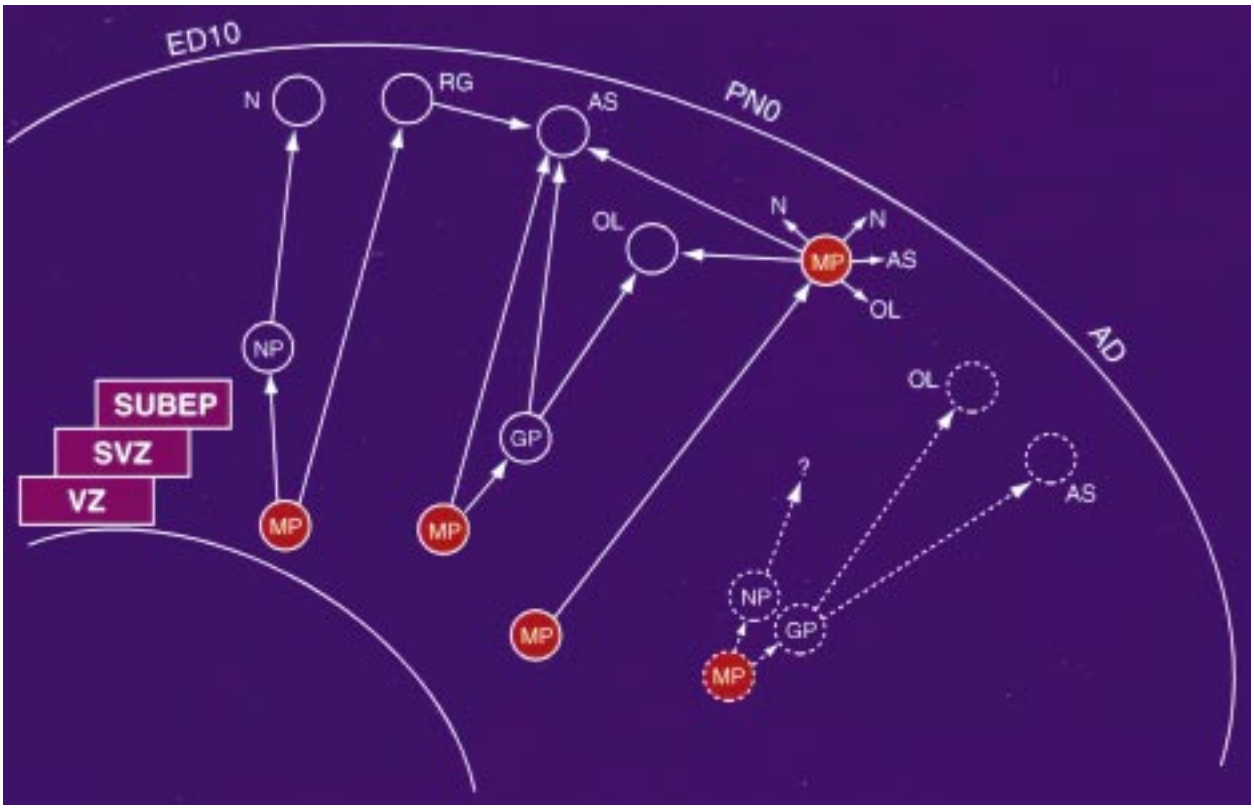

Figure 1. Developmental model of the role of multipotent progenitors in mammalian cerebral cortical maturation. During early embryonic development (ED), multipotent progenitors (MP) that reside within the ventricular zone (VZ) give rise to radial glia (RG), and to neuronal progenitor cells (NP) that migrate to the cortical plate and undergo progressive maturation into differentiated neurons (N) (49). During perinatal (PN) development, there are successive waves of gliogenesis orchestrated, in part, by multipotent progenitors that reside within secondary periventricular generative zones, including the subventricular zone (SVZ). Astrocytes (AS) of the type I variety can arise directly from subventricular zone-derived multipotent progenitors (30) or from radial glial species (47). Oligodendrocytes (OL) arise from glial-restricted progenitors (GP) that originate from the subventricular zone (17, 20, 31, 39, 40, 66). During adult (AD) life, multipotent progenitors continue to reside in the subependyma (SUBEP) of the lateral ventricle, and under certain circumstances can give rise to predominantly oligodendrocytes, astrocytes and may also program the elaboration of neuronal lineage species (13). Multipotent progenitors are also present in the postnatal and adult cerebral cortex independent of periventricular generative zones (25, 26, 45, 46, 51). These cells may be capable of giving rise directly to oligodendrocytes, type I astrocytes and neuronal subtypes.

from the subventricular zone to the cerebral cortex *in vivo* and to undergo cellular differentiation *in situ* in response to environmental cues (13). Numerous *in vitro* studies have begun to define the role of specific cytokines in neural lineage commitment (instructive mechanism), in preferential survival and/or proliferation of intermediate progenitors (selective mechanism) and in cellular differentiation from CNS multipotent progenitors (1, 35, 42, 78). Within phylogenetically-distinct regions of the neuraxis, multipotent progenitors exhibit differential responsiveness to CNS mitogens (77). For example, multipotent progenitors derived from the adult forebrain subependymal zone respond to EGF, whereas multipotent cells derived from IVth ventricular and spinal cord generative zones require a combination of EGF and bFGF for cellular activation. These regional multipotent progenitors also exhibit distinct profiles of cellular proliferation, differentiation and responses to CNS injury. There is now also increasing evidence that EGF-responsive multipotent progenitors play an essential role during perinatal gliogenesis but not during embryonic neurogenesis, whereas bFGF-responsive cells may play a more important role during early embryonic cortical development (9, 65). We have recently identified and isolated EGF-responsive multipotent progenitor cells from the early postnatal cerebral cortex independent of the subventricular zone (Figure 1) (45, 46). These cortical multipotent cells differ from

their subventricular zone counterparts by the uniform expression of the polysialyated form of neural cell adhesion molecule (PSA-NCAM) and by the strict cytokine-mediated regulation of oligodendroglial lineage elaboration from glial-restricted progenitor species. The finding of peak expression of the EGF receptor developmental ligand, transforming growth factor α (TGFα), within the early postnatal cerebral cortex (18, 19, 38) further suggests that these progenitors may be appropriately activated *in vivo*. Our observations lend support to recent observations that CNS progenitor cells with broad lineage potential exist within non-generative "quiescent" regions of the mammalian brain (63). It has been suggested that these progenitor species require more elaborate signals for cellular differentiation (63). The retention of these multipotent progenitors within CNS regions associated with terminal differentiation may be accomplished by continuous migration from generative zones during a discrete developmental period, by long-term self-renewal or by their persistence in a quiescent state (56).

Generation of Glial Lineage Species During Postnatal Cerebral Cortical Development

Early investigations of gliogenesis performed *in vitro* have suggested that oligodendrocytes originate from progenitors that are bipotent (oligodendrocyte – type II astrocytes, O-2As) (66). However, additional studies have shown that during normal cerebral cortical development, these cells are exclusively committed to the oligodendrocyte lineage *in vivo* (17, 20, 31). Thus, oligodendrocytes are now believed to arise from cells that have migrated to the cerebral cortex as committed glial progenitors that may undergo lineage maturation through cell intrinsic mechanisms (39,40). In addition, there is increasing evidence that oligodendrocytes also arise from undifferentiated cells within the subventricular zone, and that these cells are distinct from O-2A progenitors (39,41). Further, *in vitro* and *in vivo* lineage tracer studies suggest that the cellular differentiation of these additional progenitor species is driven by environmental factors present within the post-migratory microenvironment.

The oligodendrocyte lineage is most amenable to the study of neural lineage elaboration from multipotent progenitors because progressive lineage stages are well characterized, and the cytokine regulation of lineage progression has been well defined (54, 64). The earliest identifiable pool of cultured perinatal glial precursors consist of cells that express the polysialyated form of neural cell adhesion molecule (PSA-NCAM) (28). PSA-NCAM-immunoreactive oligodendrocyte precursors (pre-oligodendrocyte progenitors) express the platelet-derived growth factor α receptor (PDGFαR) and later stage-specific cell surface markers (27, 28). Cerebral cortical PSA-NCAM-and PDGFαR-immunoreactive oligodendrocyte precursors are responsive to PDGF for cellular survival and are the foreruners of NG2 proteoglycan – and A2B5-immunoreactive oligodendrocyte progenitors that exhibit optimal responsiveness to PDGF for cellular proliferation (60, 61). NG2 and PDGFαR are colocalized on A2B5- immunoreactive oligodendrocyte progenitors in the neonatal rat cerebrum (60-62). However, NG2 may also be expressed on glial-restricted progenitors that give rise to astrocytes. Additional lineage analysis suggests that PDGFαR-negative progenitors are also capable of migrating from paramedian generative zones and adopting an oligodendrocyte (PDGFαR-immunoreactive) phenotype (10, 33). These studies have shown that oligodendrocytes can be generated from uncommitted neuroepithelial cells throughout the rostrocaudal CNS axis and undergo differentiation in restricted regions (33). These observations suggest that the cellular maturation of oligodendrocyte and astrocyte progenitors derived from uncommitted progenitor species is regulated by local environmental signals (33). PSA-NCAM is downregulated as glial progenitors become post-migratory, proliferative pre-oligodendrocytes (3, 4, 58, 76). Post-mitotic immature oligodendrocytes express an epitope of galactocerebroside (recognized by the monoclonal antibody, O1). Markers of terminal oligodendrocyte differentiation include myelin basic protein (MBP) (64).

Region-specific developmental strategies regulate oligodendrocyte lineage development (54, 71). For example, in some regions like the optic nerve, an intrinsic timing mechanism may control the number of oligodendrocyte progenitor proliferative cycles, resulting in synchronous clonal differentiation (22, 66). By contrast, during spinal cord oligodendrocyte development clonal populations comprised of quiescent oligodendrocyte progenitors and differentiated oligodendrocytes may coexist (asynchronous differentiation), demonstrating that proliferation and cellular differentiation may be uncoupled and regulated by different cellular and environmental cues (81). Early postnatal rat optic nerve and cerebral cortical oligodendrocyte progenitors express trkC and proliferate in response to NT3, whereas embryonic and adult spinal cord oligodendrocyte progenitors do not express trkC or respond to NT3 (12, 15, 71). Transcripts for NT3 are expressed at maximal levels in the neonatal cerebral cortex (16, 21, 44, 75). Neurotrophin-3 is

secreted by type I astrocytes and can exhibit a variety of cellular roles during oligodendrocyte development (11). NT3 mediates the survival of embryonic rat hippocampal oligodendrocyte progenitors in mixed cultures and also of purified optic nerve oligodendrocyte progenitors (5, 8). In combination with CNTF and insulin-associated subclass factors, NT3 promotes the long-term survival of oligodendrocyte lineage species (7). NT3 also regulates the proliferation of oligodendrocyte progenitors *in vivo*, and NT3 antibodies significantly attenuate the proliferation of oligodendrocyte lineage species in mixed glial cultures (6). Concurrent exposure to PDGF and NT3 is required for clonal proliferation of postnatal rat optic nerve oligodendrocyte progenitors (6). For oligodendrocyte progenitors induced to divide by PDGF, NT3 may also partially suppress the generation of differentiated oligodendrocytes (34). Therefore, NT3 may exhibit a number of versatile cellular and region-specific functions during oligodendrocyte progenitor development.

The elaboration of alternate neural lineages from postnatal cerebral cortical progenitors may require additional environmental signals. Transcripts for members of specific bone morphogenetic protein (BMP) subgroups are expressed at significant levels within the postnatal cerebral cortex (50). We have previously shown that BMPs promote the elaboration of astrocytes from late embryonic and early postnatal subventricular zone-derived EGF-responsive multipotent progenitors and also from postnatal cortical O-2A progenitors (30, 43). These developmental actions are associated with exit from cell cycle, suppression of alternate lineages and activation of an autonomous response cascade after brief factor exposure. BMP receptor subunits are expressed on EGF-responsive progenitors and also on glial-restricted progenitors (30, 43), suggesting that BMPs can induce the elaboration of distinct astroglial species from multipotent (type IA: GFAF+, A2B5-) and from glial-restricted, including O-2A (type IIA: GFAP+, A2B5+) progenitors.

In Vivo Postnatal Cerebral Cortical Development and Progenitor Cell Responses to Cellular Injury

During postnatal gliogenesis in the rat forebrain, glial differentiation coincides with the establishment of synaptic connections and the elaboration of the regional vascular supply (24). Progenitor migration from the subventricular zone ceases by the second postnatal week, coincident with the collapse of the radial glial scaffold. At postnatal day 4 *in vivo*, there is a wave of production of NG2+/O4- glial precursors that develop into NG2+/O4+ oligodendrocyte progenitors and subsequently into differentiated galactocerebroside-immunoreactive oligodendrocytes (67). A proportion of NG2+/O4+ progenitors remain quiescent or proliferate slowly and are retained throughout adult life without undergoing oligodendrocyte differentiation or the elaboration of alternate lineages (67). The source of NG2+/O4+ progenitors that persist within the cerebral cortex during postnatal and adult life is unclear. PDGFαR-immunoreactive progenitors are initially expressed within the subventricular zone of the late embryonic forebrain, with cellular migration orchestrated by PDGF, secreted by neurons present within the cortical plate (14). PDGFαR-immunoreactive progenitors initially undergo cellular expansion within the subventricular zone and acquire NG2-immunoreactivity at the time of migration (60, 62). Within the perinatal cerebral cortex, NG2 and PDGFαR are co-expressed by glial progenitors throughout gray and white matter tracts (60, 62). Because oligodendrocyte progenitors derived from cortical multipotent cells appear to be under strict environmental regulation, with cellular proliferation and differentiation regulated by separate developmental mechanisms (81), we postulate that oligodendrocyte progenitors that are retained within the postnatal cerebral cortex are derived from cortical multipotent progenitors. These oligodendrocyte progenitors would represent a continuous source of oligodendrocyte lineage species needed to myelinate multiple axonal tracts over extended time frames during normal postnatal development, and could also serve as a reservoir for regional cellular repair following demyelinating injury (24, 54, 59, 79). Recent studies have, in fact, documented that similar progenitor populations may be important in the regeneration of injured CNS cellular populations. Proliferative progenitor cells from the neonatal rat brain can undergo multiple passages *in vitro* and represent a focal reservoir of migrating and myelinating oligodendrocytes following transplantation into neonatal shiverer mice (55). In response to focal demyelinating injury, proliferating cells can differentiate into myelin protein-expressing oligodendrocytes in the adult subcortical white matter (23). EGF-responsive multipotent progenitor cells are highly efficient at remyelination without retention of immature oligodendrocyte progenitor species following transplantation into myelin-deficient rats that exhibit an intrinsic defect in the oligodendrocyte lineage (32). Following bilateral cortical stab wounds in adult rats, pre-oligodendrocytes undergo exuberant cellular proliferation and generate differentiated oligodendrocytes within three days *in vivo* (2). These experimental obser-

vations suggest that myelin repair can be initiated from both multipotent and from oligodendrocyte progenitor species through progenitor cell expansion and oligodendrocyte differentiation following demyelinating and other forms of CNS injury.

Isolation and Preliminary Characterization of Cerebral Cortical Multipotent Progenitors

In postnatal day 2 rodent forebrain, our studies indicate that PSA-NCAM is preferentially expressed as a gradient of increasing expression in areas of the cerebral cortex away from the subventricular zone, and that the EGF receptor (EGFR) is expressed on individual cells and cell clusters within these regions (25, 26, 51). Using microdissection techniques and FACS analysis, we have isolated a discrete population (15%) of early neural progenitors from the cerebral cortex independent of the subventricular zone that expresses markers associated with peri-migratory (PSA-NCAM+) cellular species (46). Essentially all of these progenitors expressed markers of undifferentiated (nestin+) cells with negligible expression of neuronal, oligodendroglial and astroglial lineage markers. Completely non-overlapping cellular fractions expressed the PDGFαR (37%) and the EGFR (7%) without expression of the neurotrophin-3 receptor, trkC. The vast majority of progenitors that expressed the PDGFαR also co-expressed NG2 (25, 26). These observations demonstrate that it is possible to isolate undifferentiated progenitor cells from the early postnatal cerebral cortex, and that separate progenitor fractions exhibit a glial progenitor cell phenotype or alternately may be responsive to cytokines that mediate the expansion of a progenitor population of broad lineage potential (EGFR+). Comparison of EGF-responsive proliferative clones derived from the cerebral cortex with those derived from the subventricular zone revealed that virtually all cortical EGF-responsive cells were PSA-NCAM-immunoreactive, while the majority of subventricular zone EGF-responsive cells were not PSA-NCAM-immunoreactive (25, 26, 46). Enzymatic cleavage of the PSA-moiety from primary cortical PSA-NCAM+ cells using endoneuraminidase N (72, 73) attenuated the effects of EGF application on the number and size of progenitor clones generated and on several additional indices of cellular migration (25, 26, 51). Thus, cortical- and subventricular zone-derived EGF-responsive progenitors display distinct cellular properties that may relate to diverse functional roles during postnatal cerebral cortical development, including differences in environmentally-mediated cellular expansion, self-renewal, migration and lineage-specific differentiation (56).

Application of EGF or TGFα to cerebral cortical PSA-NCAM+ cells resulted in the generation of primary proliferative progenitor clones in a proportion (7%) similar to the relative expression of the EGFR in this cellular population (46). These clones were initially comprised of undifferentiated cells, and the large majority of these clonally-derived EGF-responsive progenitors gave rise to all three major CNS lineages, neuronal, oligodendroglial and astroglial lineage species. Dose-response curves for cerebral cortical PSA-NCAM/EGFR-double immunoselected cells propagated at clonal density demonstrated that identical concentrations of EGF (1 ng/ml) promoted the elaboration of the maximum proportion of proliferative progenitor clones, and also the subsequent generation of differentiated neural lineage species (25,26). Cerebral cortical EGF-responsive progenitor species do not initially express the PDGFαR or trkC (as examined by molecular, protein and functional assays). However, application of EGF or TGFα to these cortical multipotent progenitors resulted in progressive expression of both PDGFαR and trkC (15). These findings show that cerebral cortical EGF-responsive progenitors are multipotent, and that exposure to EGF/TGFα results in the expression of two cytokine receptors implicated in glial and neuronal lineage development.

Cortical Multipotent Cells Represent a Distinct Progenitor Pathway for Postnatal Cerebral Cortical Development

To begin to examine whether cerebral cortical EGF-responsive multipotent progenitors represent a distinct progenitor pathway for postnatal cortical development, we chose to compare the cellular properties of glial-restricted progenitors derived from cortical multipotent progenitors with those originally derived from subventricular zone progenitor species. Two cytokines that act on oligodendroglial lineage species were selected to examine the cellular properties of glial progenitors derived from these putative multipotent and glial-restricted progenitor pathways: PDGF has been shown to be important for oligodendrocyte progenitor survival and proliferation, and NT3 is a region-specific cytokine active on this progenitor stage and its receptor, trkC, can be selectively induced in cortical EGF-responsive progenitors following cytokine application (12, 15, 37, 71). When plated and propagated at clonal density in serum-free media, glial progenitors originally derived from the subventricular zone (classical O-2A progenitors) underwent spontaneous oligodendrocyte differentiation, whereas glial progenitors derived from EGF-responsive

multipotent progenitors failed to undergo spontaneous oligodendrocyte differentiation despite prolonged propagation *in vitro* (45). Similar results were obtained when NG2-immunoselection (60, 61) was used to generate corresponding glial-restricted cellular populations from primary subventricular zone-derived cells and from EGF-generated cortical progenitors, respectively (25, 26). These observations demonstrate that oligodendrocyte maturation from subventricular zone glial-restricted progenitors is driven by cell autonomous mechanisms, while oligodendrocyte differentiation of glial progenitors derived from cortical multipotent cells may require additional environmentally-mediated cues. To examine cytokine responsiveness, the two cortical and subventricular zone-derived glial progenitor populations were plated at moderate density and propagated in the presence of PDGF or NT3. When applied to subventricular zone-derived glial progenitors, PDGF but not NT3 significantly increased the proportion of O4-immunoreactive pre-oligodendrocytes, stimulated DNA synthesis and promoted the elaboration of differentiated oligodendrocytes (46). By contrast, when applied to glial progenitors derived from cortical multipotent cells both PDGF and NT3 enhanced these three indices of progressive oligodendrocyte maturation. In addition, propagation of glial progenitors derived from primary subventricular zone-derived cells for extended periods *in vitro* failed to promote the expression of transcripts and proteins for trkC. These cumulative experimental findings demonstrate that glial-restricted progenitors derived from primary subventricular zone cells and from cortical multipotent cells exhibit significant differences in their potential to undergo spontaneous oligodendrocyte differentiation and in their responsiveness to NT3. Thus, these observations lend support to the proposal that cortical EGF-responsive cells represent a novel progenitor pathway for postnatal cerebral cortical development.

Cortical Multipotent Progenitors Require Specific Classes of Environmental Signals for the Elaboration of Myelinating Oligodendrocytes, Type I and II Astrocytes and Neuronal Subtypes

We next sought to define the environmental signals required for oligodendrocyte differentiation from cortical EGF-responsive multipotent progenitors. When propagated in the absence of added cytokines at clonal density in serum-free media, cortical EGF-responsive progenitors exhibited excellent long-term survival, and were capable of generating large complements of oligodendroglial (O4), astroglial and neuronal lineage species, as well as a significant complement of glial-restricted (NG2+) progenitors (25, 26, 45). Application of cytokines with known actions on oligodendrocyte lineage species (bFGF, PDGF, NT3) differentially increased the proportion of pre-oligodendrocytes (O4+) (45). However, only cytokines that activate gp130/leukemia inhibitory factor β receptors (LIFβRs), such as ciliary neurotrophic factor (CNTF) and LIF, promoted the dose-dependent elaboration of differentiated oligodendrocytes. In these studies, CNTF did not alter indices of proliferation or survival, indicating that the cytokine does not act through a selective lineage mechanism (56). In addition, CNTF did not alter the relative proportions of neuronal and glial lineage species, indicating that CNTF does not support oligodendrocyte development at the expense of alternate neural lineages (instructive mechanism) (56). These findings suggest that CNTF (and LIF) act on cells already committed to the oligodendrocyte lineage. We then wanted to quantify the proportion of progenitor species derived from cortical multipotent cells that respond to CNTF and to establish their lineage identities. Prior studies have shown that activation of gp130/LIFβRs results in nuclear translocation of the latent transcription factor, STAT3 (74, 82). Using a STAT3 translocation assay, we have shown that a proportion of these cells (17%) respond to saturating doses of CNTF (and LIF) with nuclear translocation of STAT3 (16). In addition, virtually all pre-oligodendrocytes and a smaller proportion of cortical glial-restricted (NG2+) progenitors exhibited STAT3 nuclear translocation, indicating a direct effect on these cellular species (25, 26, 45). These clonal density effects could be reproduced using NG2-immunoselected glial progenitors derived from cortical multipotent cells (25, 26). The CNTF-mediated enhancement of oligodendrocyte differentiation could be attenuated by application of both CNTF-neutralizing and LIFβR-blocking antibodies. Further, application of NT3 to the NG2-immunoselected cells promoted the time-dependent expression of the CNTFαR protein on pre-oligodendrocytes. By contrast, when cortical multipotent cells were plated and propagated in moderate and high density cultures, a significant complement of differentiated oligodendrocytes were generated (45). Application of CNTF resulted in a significant dose-dependent enhancement of the number of advanced differentiated (myelin basic protein-expressing) oligodendrocytes elaborated. In clonal density cultures of cortical multipotent progenitors, LIFβR-blocking antibodies significantly inhibited the CNTF-mediated elaboration of differentiated oligodendrocytes, whereas gp130-blocking anti-

bodies had no demonstrable effect. Further, moderate density cultures of cortical multipotent progenitors propagated with LIFβR and/or gp130-blocking antibodies failed to exhibit significant differences between control and experimental conditions in the proportions of differentiated oligodendrocytes generated. Finally, conditioned media from moderate density cultures of cortical multipotent progenitors when applied to identical cellular populations in clonal density cultures could not reproduce the effects of exogenous application of CNTF (or LIF) on the elaboration of differentiated oligodendrocytes. These overall observations suggest that environmental cues, including membrane-associated signals and activation of gp130/LIFβRs, are required for oligodendrocyte differentiation of glial progenitors derived from cerebral cortical EGF-responsive multipotent progenitors.

We have previously shown that BMPs promote the generation of astrocytes from subventricular zone-derived EGF-responsive multipotent progenitors and from early postnatal cortical O-2A progenitors, with concurrent suppression of alternate lineages (30, 43). Further, transcripts for BMP2 and BMP7 are expressed within the early postnatal cerebral cortex (50). These next experiments were undertaken to examine whether similar BMP-mediated astroglial-inductive actions occur following BMP application to cortical multipotent cells or to their glial-restricted progeny. When cortical multipotent progenitors were plated and propagated at clonal density in the presence of BMP2 or BMP7, both factors displayed a dose-dependent enhancement in the elaboration of type I (GFAP+/A2B5-) astrocytes with concurrent suppression of oligodendroglial and neuronal lineage species (25, 26, 45). In addition, when NG2-immunoselected glial progenitors derived from the cortical multipotent cells were examined using identical paradigms, both BMPs enhanced the generation of type II astrocytes (GFAP+/A2B5-) with suppression of the elaboration of pre-oligodendrocytes (25, 26). Further, these BMP-mediated effects were associated with nuclear translocation of the BMP-selective latent transcription factor, SMAD1. To examine whether BMP responsiveness of EGF-responsive cortical multipotent cells changes when these cellular species begin to express PDGFαR and trkC, delayed BMP addition paradigms were employed. Delayed BMP application resulted in a progressive reduction in the elaboration of astrocytes and the *de novo* elaboration of oligodendroglial lineage species (46). This cytokine effect was associated with the concurrent appearance of receptor proteins for PDGF and NT3. We have previously shown that BMPs can upregulate the expression of trkC on developing superior cervical ganglia cells and can induce responsiveness to NT3 (80). In a parallel fashion, application of the BMPs to NG2-immunoselected cells derived from cortical multipotent progenitors enhanced the expression of trkC (25, 26). Similar inductive effects were seen for NG2-negative cells when BMPs were applied directly to cortical EGF-responsive multipotent progenitors. These cumulative findings demonstrate that BMPs can promote the elaboration of the astroglial lineage from both cortical multipotent cells and from their glial-restricted progeny. In addition, the BMP-mediated induction of trkC on intermediate progenitor pools suggests a potentially significant degree of lineage interdependence: a negative feedback loop for the astroglial lineage (NG2+ cells) with the concurrent NT3-mediated potentiation of the oligodendrocyte lineage, and parallel promotion of NT3-mediated elaboration of the neuronal lineage from EGF-responsive cortical progenitor cells of broader lineage potential (NG2- cells).

Our experimental findings have shown that sequential or concurrent exposure to bFGF and NT3 can selectively potentiate the elaboration of neuronal lineage species from cortical EGF-responsive multipotent progenitors (25, 26). The evolving neuronal lineage species expressed a wide variety of neuromodulatory proteins and their biosynthetic precursors, including GABA, glutamic acid decarboxylase, somatostatin, choline acetyltransferase, substance P, tyrosine hydroxylase and serotonin; neuronal subpopulations derived from EGF- and bFGF-responsive multipotent progenitors expressed distinct profiles of these neuromodulatory molecules (25,26) (see below). These observations first suggested that the cortical multipotent progenitors display an unusual degree of developmental plasticity *in vitro*. There is not, as yet, sufficient evidence that neuronal lineage species are generated from these cells *in vivo*. This observation may reflect the presence of a spectrum of inhibitory signals or the lack of appropriate environmental cues for neuronal differentiation. An alternate explanation for the EGF-responsive cells may be that upregulation of the EGFR by cortical progenitors during postnatal life may selectively promote glial lineage elaboration (65). Finally, we have previously shown that a subset of hematopoietic cytokines, including interleukin 7, are expressed in the developing postnatal cerebral cortex and are capable of promoting neuronal lineage commitment and cellular differentiation from multipotent progenitors (48, 52, 53).

Characterization of Distinct Cerebral Cortical EGF- and bFGF- Responsive Multipotent Progenitor Populations

During mammalian cerebral cortical development and in the adult forebrain, bFGF is also known to promote the survival and expansion of multipotent progenitor cells (29, 36). We therefore utilized differential EGF and FGF receptor immunoselection to determine whether separate populations of EGF- and FGF-responsive multipotent progenitor populations are present in the postnatal cerebral cortex independent of the subventricular zone. Starting with PSA-NCAM- expressing cortical progenitor cells, we utilized sequential immunoselection techniques with antibodies to the extracellular domains of the EGFR and FGFRs1-4 to generate three separate progenitor populations: FGFR+/EGFR-, EGFR+/FGFR- and EGFR+/FGFR+ (25, 26). Clonal density analysis demonstrated that cytokine-responsive multipotent progenitors expressing these three distinct cellular phenotypes were present in the early postnatal cerebral cortex in a ratio of 4:2:1, respectively. Similar populations of multipotent progenitors were also present in the adult cerebral cortex, albeit at slightly lower relative proportions (Figure 1). Analysis of cellular response profiles for these separate progenitor populations using immunocytochemical assays of the activation (phosphorylation) of the cAMP-response element binding protein (pCREB) demonstrated distinct signaling properties among these three multipotent progenitor populations. FGFR+/EGFR- progenitors displayed delayed acquisition of EGF-responsiveness and negative synergy between EGF and bFGF, EGFR+/FGFR- progenitors did not subsequently acquire bFGF responsiveness, while EGFR+/FGFR+ progenitors exhibited earlier signal activation following application of either cytokine and positive growth factor synergy. Detailed lineage analysis demonstrated that cortical bFGF-responsive multipotent progenitors displayed a distinctive profile of responsiveness to cytokines that program neural lineage elaboration when compared to EGF-responsive multipotent progenitors: for example, changing neural fate decisions from one lineage to another (astrocytes to oligodendrocytes: bFGF/LIF) or accelerating cellular maturation within a single neural lineage (oligodendrocytes:bFGF/NT3) (26).

Intermediate Progenitor Pools Derived From Cortical Multipotent Cells Exhibit a Novel Form of Cellular Memory That Imparts Developmental Plasticity to Evolving Neural Fate Decisions

The exquisite degree of environmental regulation exhibited by the cortical multipotent progenitors suggested that intermediate progenitor populations derived from these cells may represent dynamic progenitor pools ("transit amplifying cells") (78) capable of utilizing the information encoded in sequential environmental cues to modify neural fate decisions, a novel form of developmental memory. To examine this exciting issue, cortical intermediate progenitor populations were isolated by immunoselection and exposed to sequential growth factor paradigms using specific cytokines that promote initial cellular expansion (priming step) and selective elaboration of oligodendroglial, astroglial and neuronal lineage species (differentiating step) (26). Additional factors were used in the priming step to determine whether they could differentially alter parameters of cellular maturation without affecting the initial phenotype of the expanded intermediate progenitor pools. Application of each of four distinct growth factor permutations (bFGF, alone or in combination with PDGF, LIF or BMP2) promoted the clonal expansion of intermediate progenitor species without altering their cellular properties or promoting developmental maturation. However, these initial factor manipulations significantly and differentially altered the responsiveness of these progenitors to subsequent growth factor applications that normally program the selective maturation of a specific complement of oligodendrocytes (glial growth factor, PDGF), astrocytes (BMP2, LIF) and neurons (NT3). Our experimental findings demonstrate that the specific molecular properties of the priming stimulus dramatically modulates the relative proportions and the state of cellular maturation of each neural lineage in response to the secondary (differentiating) cytokine stimulus. Thus, intermediate progenitor species in the developing cerebral cortex may represent dynamic cellular pools that are capable of utilizing temporally-coded environmental signals in novel ways. These observations suggest a greater degree of developmental plasticity and context-dependent signaling than previously recognized.

Conclusions

The presence of an intrinsic reservoir of multipotent cells within the early postnatal cerebral cortex would provide a general mechanism for the elaboration of specific cell types to precisely match local needs and to reconstitute regional cellular pools following injury (Figure 1). Thus, the phylogenetic rationale for the presence of postnatal cerebral cortical multipotent cells independent of periventricular generative zones might

encompass: *1.* elaboration of specific cell types to precisely match local needs, *2.* generation of diverse cellular progeny with unique phenotypic properties and environmental responsiveness, *3.* terminal sculpting of neural connections within complex regional microenvironments, *4.* terminal differentiation of specific lineage species separated in time and space:oligodendrocyte myelination, *5.* correction of random morphogenetic errors, and *6.* reconstitution of regional cellular pools following injury in ways that recapitulate normal development and would therefore promote the reestablishment of integrated neural networks with preservation of long-term memory traces originally encoded through epigenetically-driven mechanisms. The different cortical multipotent cellular pools may be derived from a variety of developmental sources that could endow them with alternate cellular response properties and forms of lineage plasticity: concurrent migration from the subventricular zone during postnatal cerebral cortical development, prior migration to the cerebral cortex during earlier embryonic stages and persistence as a self-renewing or as a relatively quiescent population or, alternatively, their presence as an intrinsic constituent of the postnatal cerebral cortex. These experimental observations will further our understanding of early progenitor cell regulatory events in neural lineage development during normal mammalian cerebral cortical maturation. These findings may also have important implications for elucidating pathological mechanisms underlying cellular responses to a broad range of genetic and environmental insults, including traumatic and ischemic brain injury, dysmyelinating and inflammatory demyelinating conditions, cortical migration disorders and neuropsychiatric diseases such as schizophrenia, and for the development of effective regenerative stategies to combat these and other pervasive neurodevelopmental, neurodegenerative and neuropsychiatric conditions.

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