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bFGF, Neurotrophins, and the Control of Cortical Neurogenesis

Minireview

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Early in development, the cerebral cortical epithelium consists of a thin sheet of columnar neuroectoderm cells. This germinal layer, the ventricular zone (VZ), proliferates extensively and generates immature neurons that migrate toward the pial surface, laying down the preplate and then the cortical plate from which the mature cortical layers derive. The earliest born neurons populate deeper cortical layers, with later born neurons populating the more superficial layers. This initial period of embryonic neurogenesis is followed by the largely postnatal production of glial cells, the majority of which derive from a second germinal layer, the subventricular zone, which persists into adulthood in some cortical regions. In part of the cortex, the hippocampus, neurogenesis is extended into the postnatal period. Factors that regulate the proliferation and differentiation of neuroectoderm cells, and the timing of these processes, are critical for normal cortical development. With the establishment of in vitro systems that allow the division and differentiation of neuroectoderm cells in culture, and with the advent of techniques that probe the role of factors during CNS development in vivo, researchers are beginning to examine the characteristics of developing neuroectoderm cells and investigate how their proliferation and differentiation may be regulated. In a recent issue of *Neuron*, two papers converge in their finding that, for the cerebral cortex (Ghosh and Greenberg, 1995) and hippocampus (Vicario-Abejón et al., 1995), basic fibroblast growth factor (bFGF, also known as FGF2) and neurotrophins may interact to regulate division and differentiation of neuroectoderm cells.

How Are Neurons Generated in the Cerebral Cortex?

The CNS neuroectoderm has been described classically as an overtly homogeneous layer of cells. Studies have not distinguished differences that allow prediction of cell fate based on neuroectoderm cell morphology. A number of recent studies indicate, however, that at any one time in development the neuroectoderm cells in a given CNS region may be heterogeneous. In the cerebral cortex, retroviral lineage studies conducted in vivo or in mass cultures in vitro reveal that the majority of clones consist of one cell type, either pyramidal or nonpyramidal neurons, or astrocytes or oligodendrocytes (reviewed in Kilpatrick et al., 1995). These findings suggest that heterogeneous progenitor cells coexist in the cortical VZ, although the possibility that different microenvironments are instructive for cell fate cannot be entirely ruled out.

In clonal analyses of cells developing under standardized culture conditions, where the environment is as near identical for each cell as possible, different cortical neuroectoderm cells still exhibit different fates, providing stronger evidence for cell heterogeneity within the cortical germinal zone (Davis and Temple, 1994). At the start of cortical neurogenesis, embryonic day (E) 12–14 in the murine CNS, the majority of single cells give small clones of neurons, in vivo or in vitro. A small proportion of single neuroectoderm cells appear to be bipotential but limited in their proliferative capacity, generating small clones of neurons and glial cells. In addition, in vitro studies have revealed a rare, multipotential precursor in the cerebral cortex with the properties of a self-renewing stem cell that can generate neurons, astrocytes, and oligodendrocytes (Davis and Temple, 1994; Williams and Price, 1995). Highly proliferative, multipotential cells have also been described in the E10 mouse telencephalon (a structure that generates the cortex), in hippocampus, in cerebral cortex, and in adult subventricular zone (reviewed in Kilpatrick et al., 1995). Although one of the tests for identifying stem cells, the ability to self-renew, has not been carried out in all these systems, a clear picture is emerging: the developing cortex contains a subpopulation of highly proliferative, multipotential cells that can generate both neurons and glial cells, as well as subpopulations of more restricted progenitor cells.

How these neuroectoderm cell types are related to one another is not understood. We have proposed a model for cortical development related to those proposed for production of diverse cell types in blood or neural crest, in which multipotential cortical stem cells generate restricted progenitor cells, perhaps via oligopotent intermediates, and vary the types of progenitor cells that are made as development proceeds (Davis and Temple, 1994). Similar models involving progressive developmental restriction of a highly proliferative, multipotential ancestor cell have been discussed by others in relation to cortical development (Kilpatrick and Bartlett, 1993, 1995; Morshead et al., 1994; Williams and Price, 1995). It is also possible that some restricted neuronal progenitor cells have a different origin from the stem cell population.

Although individual cells may have intrinsic differences, evidence suggests that the environment plays a key role in the specification of cortical cells: for example, age-related changes in the cortical environment can direct the generation of neurons to different layers (reviewed in McConnell, 1992). In summary, cortical neuroectoderm cells are heterogeneous; neurons can arise from either highly proliferative, stem-like cells or from more restricted progenitor cells. When considering the action of exogenous factors on the proliferation and differentiation of neuroectoderm cells, it is important to bear in mind the type of cell on which the factor may be acting.

The Influence of bFGF on Cortical Neuroectoderm Cell Division and Differentiation

One of the difficulties encountered in pursuing the development of CNS neuroectoderm cells in vitro is that, when the neuroectoderm is dissociated to single cells, these cells tend to cease division and differentiate. The need for cell–cell contact has been reported for neuroectoderm cells from a number of CNS regions, including cortex. A

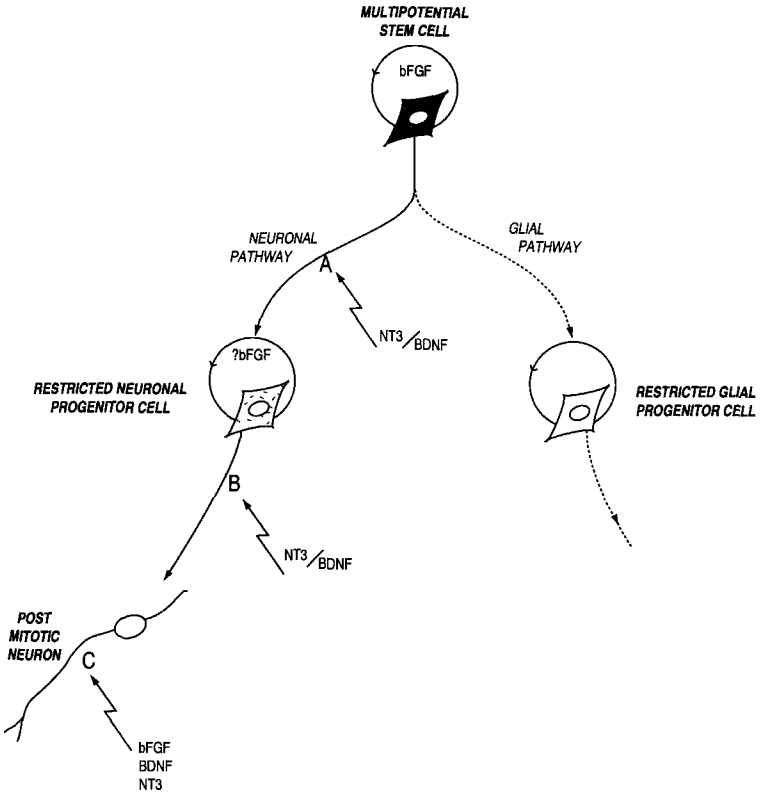

Figure 1. Model of Cortical Neurogenesis and the Influence of bFGF and Neurotrophins

bFGF stimulates division of multipotential stem cells in the cortical VZ. These cells generate restricted neuronal progenitor cells that divide under the influence of mitogens that are as yet uncharacterized. Restricted neuronal progenitor cells become postmitotic and differentiate into cortical neurons. Neurotrophins BDNF and NT-3 may stimulate neuronal differentiation, acting either at the level of the stem cell (A) or at the level of the restricted neuronal progenitor cell (B). bFGF and neurotrophins also act on the postmitotic neuron to stimulate its differentiation and survival (C). Arrowed circle represents cell division.

key component of the cell contact effect appears to be membrane associated (Temple and Davis, 1994). Although the identity of this activity is not known, bFGF is a strong candidate. In addition to its known association with extracellular matrix and cell membranes, bFGF is present in the telencephalon as early as E9.5, and in the cerebral cortex throughout neurogenesis and into adulthood (reviewed in Baird, 1994; Kilpatrick et al., 1995). Addition of bFGF has been shown to stimulate proliferation of cortical neuroectoderm cells in vitro, leading to an increase in neuronal number (Gensburger et al., 1987). More recently, the issue of which type of cortical cell is being stimulated by bFGF has been addressed. In vitro, bFGF has been shown to stimulate cells with characteristics of multipotential stem cells from embryonic telencephalon, E17 cortex, adult hippocampus, and subventricular zone (Gage et al., 1994, Soc. Neurosci., abstract; Kilpatrick et al., 1995). It is not known whether bFGF influences the division of more restricted cortical neuronal progenitor cells, although it does stimulate the division of committed neuronal progenitor cells derived from embryonic striatum (Vescovi et al., 1993) and olfactory epithelium (DeHamer et al., 1994).

Is bFGF sufficient to stimulate cortical neuroectoderm cell division by itself? In cases where bFGF has been tested in cultures containing more than one cell, or where cells have been exposed to serum, an interaction between bFGF and other growth factors is possible. For multipotential, highly proliferative cells from embryonic telencephalon, E17 cortex, and adult subventricular zone, the addition of fetal calf serum was required along with bFGF to stimulate division (Kilpatrick and Bartlett, 1993, 1995), suggesting the need for interaction with other factors. In addition, whether bFGF is acting directly as a mitogen in these settings, or whether it is in some way permissive for the mitogenic action of other factors, is not clear. Hence, although we may conclude that there is substantial evidence that bFGF can stimulate division of at least a subset of cortical neuroectoderm cells, the mechanism by which it acts and the role of putative interacting factors remain to be elucidated.

Besides its involvement in neuroectoderm cell proliferation, bFGF has been implicated in neuronal differentiation and survival in the cortex and hippocampus (reviewed in Baird, 1994). Vicario-Abejón et al. (1995) clearly show that in cultures of E16 hippocampus there is a population of cells that proliferates on administration of bFGF and a population that is stimulated to differentiate. This dual function of bFGF on early phases of division and later phases of differentiation is consistent with the distribution of bFGF and its receptors in both the cortical VZ and the cortical plate (Weise et al., 1993; Baird, 1994).

Neurotrophins Stimulate bFGF-Treated Neuroectodermal Cells to Differentiate into Neurons

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) are well established as survival and differentiation factors in the PNS. In addition, there is growing evidence for their action as survival factors in the adult CNS. However, their role in CNS development is not clear at this point. BDNF and NT-3, and their receptors TrkB and TrkC, are abundant in the developing cortex from an early age (reviewed in Klein, 1994). There is evidence that these

two factors may play a role in the differentiation of neocortical neurons. Recently, BDNF was shown to be involved in activity-dependent survival of embryonic cortical neurons, suggesting a role in the pruning of cortical neurons during development (Ghosh et al., 1994). NT-3 has been shown to stimulate the differentiation of calbindin-positive neurons in the embryonic hippocampus in vitro (Collazo et al., 1992).

Now Ghosh and Greenberg (1995) and Vicario-Abejón et al. (1995) have studied the effect of neurotrophins on bFGF-expanded neuroectoderm cells. For both cortex and hippocampus, anti-NT-3 antibodies significantly reduce the numbers of neurons developing in these cultures, while addition of recombinant neurotrophin significantly increases neuronal differentiation. These findings suggest that these neurotrophins can stimulate differentiation of neuroectodermal cells toward neuronal phenotypes. It is still unclear whether the neurotrophins act directly on the multipotential stem cell to direct cell fate toward a neuronal phenotype, or on a restricted progenitor cell, which may be a product of the stem cell, to promote its differentiation (Figure 1). However, the fact that the effect of added NT-3 on cortical cells was found to be transient, present at 4 days but not at 7 days, suggests that the neurotrophin acts to accelerate the differentiation of committed neuronal progenitor cells rather than to stimulate the production of added neuronal progenitors from a more primitive cell (Ghosh and Greenberg, 1995). In the hippocampus, calbindin-positive neurons appear to be preferentially produced by neurotrophin treatment (Vicario-Abejón et al., 1995). Perhaps neurotrophin activity can direct the differentiation of specific subpopulations of neurons. There is some evidence for this activity from studies in other systems: for example, NT-3 stimulates motor neuron differentiation from avian neural tube progenitor cells (Averbuch-Heller et al., 1994), and in the PNS, BDNF stimulates pluripotent crest cells to adopt a sensory neuron fate (Sieber-Blum, 1991).

The actions of BDNF and NT-3 on bFGF-treated cortical and hippocampal cells contrast with those of NGF on bFGF-treated striatal progenitor cells from a similar developmental age. Application of NGF had little effect on embryonic murine striatal neuroectoderm cells alone, but significantly augmented bFGF-induced proliferation of these cells (Cattaneo and McKay, 1990). Whether this was a direct effect on cell division or an indirect effect via increased cell survival is not clear. The difference in neurotrophin action is heightened by the observation that removal of NGF and bFGF then induced neuronal differentiation in these striatal cultures. These differences may be attributed to the type of neurotrophin used. However, given the high levels of NGF added to the striatal cultures, it is possible that the action of NGF was not via TrkA receptors. Alternatively, the differences may reflect regional variations in the roles of neurotrophins on neuroectoderm cells.

The proportion of neurons that develop in bFGF-treated cortical and hippocampal cultures (defined by microtubule-associated protein 2 staining) is small: around 10% when stimulated by exogenous neurotrophin. In contrast, around 90% of cells in cortical cultures differentiate into neurons in the absence of bFGF (Ghosh and Greenberg, 1995). Ghosh and Greenberg discuss the possibility that bFGF partially inhibits the differentiation of neurons from these cultures, similar to its inhibition of oligodendrocyte differentiation from oligodendrocyte progenitor cells (McKinnon et al., 1990). For clonal telencephalic cells expanded in bFGF, it was necessary to remove bFGF before adding Ast-1-conditioned medium in order to get neuronal differentiation (Kilpatrick and Bartlett, 1993).

In considering the interactions between bFGF and neurotrophins, modulation of bFGF activity may be influenced by binding to heparan sulfate proteoglycans, low affinity receptors for FGFs that can protect FGFs from degradation and regulate the way they are presented to active receptors. These proteoglycans may themselves be modulated in the cortex as development progresses, providing an additional level of control on FGF activity (reviewed in Kilpatrick et al., 1995). In this regard, it will be interesting to assess the role of both low affinity and truncated neurotrophin receptors as possible regulators of neurotrophin activity on the differentiation of neuroectoderm cells (Klein, 1994; Biffo et al., 1995). An alternative explanation for the minor proportion of neurons that develop in these cultures is that the majority of neuroectoderm cells expanded in bFGF may be neuronal progenitors that require the action of other growth factors for their differentiation, or progenitor cells that are restricted to give nonneuronal cell types.

These data provide evidence for the involvement of neurotrophins in directing neurogenesis. Individual knockouts of TrkB, TrkC, BDNF, and NT-3 do not have a major impact on cortical development (reviewed in Klein, 1994), suggesting that neurotrophins are probably not essential on an individual basis. It is quite likely that redundancy within the neurotrophin system, or between neurotrophins and other growth factors, can compensate for these losses. Certainly the similarity in BDNF and NT-3 action shown in hippocampus (Vicario-Abejón et al., 1995) demonstrates that parallel pathways may exist to regulate neuronal differentiation in cortex.

In summary, the factors that control the proliferation of different classes of cortical neuroectoderm cells and their timely differentiation into different cell types are still mainly unknown. The search for these factors, including exogenous factors and transcription factors, will be facilitated by the application of model culture systems and by the analysis of naturally occurring and genetically constructed murine mutants that influence proliferation and differentiation of cortical cell types. The identification of growth factors as instigators and modulators of proliferation and differentiation programs will be an important step toward understanding how cortical neurogenesis is environmentally regulated; the actions of bFGF and neurotrophins described here represent a fruitful beginning.

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