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The Simple Life (of Cortical Progenitors)

Asymmetric cell division plays a major role in the generation of cell diversity during development. In this issue of *Neuron*, Sun and colleagues present evidence that the epidermal growth factor receptor is asymmetrically distributed in mitotic cerebral cortical precursors, and the resulting unequal inheritance generates offspring with different responsiveness to growth factor and unique cell fates.

Our ability to acquire and use limited resources plays an important role during our development. Even when we grow up in the same environment, differences in how we exploit our surroundings can shape our futures. Might we have grown taller had we consumed the last glass of milk that was instead seized by our siblings? Some of the factors that regulate how well we obtain or utilize what is available may be intrinsic, such as having a better sense of smell or fondness for dairy products. Much like us, sibling cells in developing animals might not necessarily begin life equally. Recent work by Sun et al. shows that the fate of mammalian neural precursor siblings can be determined by the amount of epidermal growth factor receptor (EGFR) inherited at their births, and their ability to respond to epidermal growth factor depends on how much EGFR they have when they begin life (Sun et al., 2005 [this issue of *Neuron*]).

Variations on two basic themes can be used to establish differences between cells. From the moment of their birth following a cell division, sibling cells can be either identical or different. Initially, identical cells might encounter different environmental cues and consequently develop differently. In contrast, differences between two daughters can be generated through intrinsically asymmetric cell division, or divisions that result immediately in two distinct offspring. Intrinsically asymmetric cell divisions occur during the development of many organisms. The molecular details of asymmetric division are best understood in *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*, where asymmetric division results from an initial establishment of cell polarity, subsequent localization of specific molecules to one pole of the mitotic cell, and asymmetric inheritance of these molecules following an oriented mitotic cleavage (Jan and Jan, 2000).

Although the factors that regulate cell diversity are complex, a wealth of evidence from worms and flies suggests that early neural cell fate is regulated by intrinsic differences between sibling cells that arise from asymmetric divisions. Asymmetric distribution of determinants can regulate whether a precursor generates additional multipotent precursors, more differentiated precursors with more restricted potential, or differentiated progeny. A variety of asymmetrically distributed determinants have been described, including transcriptional activators and repressors, as well as molecules that regulate their asymmetric localization and/or expression (Betschinger and Knoblich, 2004). Asymmetrically distributed molecules regulate other kinds of molecules important for cell fate; for example, asymmetric Numb, Neuralized, and α-adaptin regulate the Notch protein, which serves to regulate a cell's responsiveness to external signals (Betschinger and Knoblich, 2004).

The ability of a cell to respond to its environment can play a major role during differentiation. The timing of differentiation during neuronal development in the *Drosophila* eye appears to be tightly coordinated with extrinsic growth signals (Bateman and McNeill, 2004). EGFR plays multiple roles in cell fate determination during eye development, and its activation is regulated by localized presentation of ligand (Perrimon and Perkins, 1997). In mammals as well, cell fate can be regulated by responsiveness to external signals and growth factors such as EGF. In the developing cortex, the choice between proliferation and differentiation is influenced by EGFR expression in cortical precursors and extracellular ligand concentration (Burrows et al., 1997). Together with the observations in *Drosophila*, these findings suggested that responsiveness to environmental signals can be regulated by limiting expression of receptors such as the EGFR and raised the possibility that combinations of receptor and ligand levels could regulate cell fate decisions in the developing mammalian brain.

Not surprisingly, growing evidence suggests that cell fate decisions in the development of the mammalian cerebral cortex utilize similar mechanisms to those found in invertebrates. Mouse *Numb* and *Numblike* (*Numbl*), the homologs of *Drosophila numb*, appear to regulate neuronal and progenitor number (Castaneda-Castellanos and Kriegstein, 2004), and imaging of dividing mammalian neural precursors in slices of developing cerebral cortex (Chenn and McConnell, 1995) and in utero (Haydar et al., 2003) suggested that mitotic orientation of cortical progenitors could predict cell fate. Targeted mutations in the Lis1 interacting protein Nde1 caused disrupted mitotic spindle orientation and decreased neuronal production, leading to the proposal that the loss of spindle orientation altered neuronal fate decisions (Feng and Walsh, 2004). Whether these mitotic orientation changes resulted in changes in division symmetry remain an intriguing possibility, and how such changes in division symmetry might lead to changes in mammalian precursor cell fate decisions await further study.

This current study by Sun and colleagues provides one possible mechanism by which asymmetric divisions might regulate cell fate decisions in developing cortical precursors. Examining sections through the developing forebrain, they found that the EGFR was asymmetrically distributed in approximately one-fifth of mitotic progenitor cells that expressed EGFR. Elegant in vitro studies of single cortical progenitor cells indicated that even in progenitor cells isolated from the

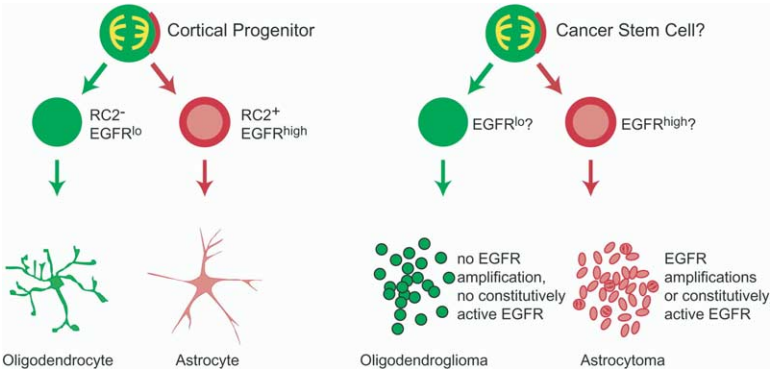

Figure 1. Potential Similarities between Normal Cortical Development and Brain Tumor Development

(Left) Asymmetric distribution of EGFR (red crescent) in dividing cortical precursor gives rise to one daughter expressing high levels of EGFR and one daughter expressing low levels. The EGFR^{high} daughter also expressed the radial glial marker RC2 and gives rise to astrocytes, while the EGFR^{low} daughter is RC2 negative and generates oligodendrocytes. (Right) The proposed cancer "stem cell" could give rise to cells that differentiate toward astrocyte or oligodendrocyte lineages, depending on the level of EGFR activation. Although the asymmetric division depicted is hypothetical, small-cell astrocytomas are characterized by EGFR amplifications or expression of a constitutively activated form of the EGFR. In contrast, the morphologically similar high-grade oligodendroglioma does not have EGFR amplification or activated receptors.

cortex and cultured away from normal environmental tissue cues, asymmetric EGFR could be observed in dividing progenitors. Furthermore, the asymmetric distribution of EGFR gave rise to daughter cells that had different EGFR levels.

To examine the functional consequences of differential inheritance of EGFR, the authors then assessed proliferation and migration in the resulting daughters. When grown in culture media without fibroblast growth factor (FGF), daughters that inherited EGFR were more likely to incorporate BrdU. Interestingly, in the presence of FGF, this functional asymmetry disappeared, with both daughters equally likely to incorporate BrdU. Imaging studies revealed further asymmetry between the daughters that inherited different quantities of EGFR, with daughters that inherited more EGFR consistently migrating further. These findings suggested that the asymmetrically distributed EGFR provided responsiveness to environmental EGF.

What did these cells become following these molecularly asymmetric divisions? The expression of several cellular markers was highly correlated with high EGFR expression in the daughter pairs. High EGFR expressing daughters were more likely to express the radial glial markers RC2, GLAST, and CD-15/Lewis X. Comparing the daughters of E16 progenitors suggested that the daughter that inherited more EGFR resembled radial glial progenitors (RC2^{+}, GLAST^{+}, nestin^{+}), while the EGFR^{low} daughter appeared to resemble oligodendrocyte precursors (RC2^{-}, GLAST^{-}, β-tubulinIII^{-}, nestin^{+}).

These expression characteristics suggest that EGFR^{high} daughters had a different developmental potential than EGFR^{low} daughters. In addition to radial glial markers in EGFR^{high} daughters, it was found that EGFR is colocalized with RC2 in radial glial cells in vivo. In contrast, EGFR^{low} daughters only expressed Olig1 and Olig2, bHLH transcription factors involved in neuronal and oligodendrocyte cell differentiation. Further studies of clonal lineage in culture suggested that, indeed, asymmetric distribution of EGFR in these late cortical progenitors marked distinct lineages, so that high EGFR expression correlated with RC2 expression and future astrocyte differentiation while low EGFR resulted in the generation of oligodendrocytes (Figure 1).

To examine whether EGFR played a causal role in this lineage distinction, Sun and colleagues infected cultured E14/15 cortical precursors with retrovirus to overexpress EGFR. They observed that EGFR overexpression caused a reduction in clone size that was dependent on EGF in the growth media. Furthermore, they observed that EGF in the media reduced the frequency of oligodendrocytes generated, and overexpression of EGFR reduced this frequency yet further. This reduction was again dependent on the presence of EGF in the media. These studies suggested that asymmetrically inherited EGFR leads to intrinsic differences in sibling cells to respond to environmental EGF, and these differences can result in changes in cell fate decisions.

Of course, cell fate decisions in the developing cortex are influenced by many players. In addition to Olig1 and Olig2, a host of other transcription factors with bHLH motifs also play key roles in cortical development, and different combinations of bHLH activities promote the formation of neurons versus astrocytes versus oligodendrocytes (Ross et al., 2003). The findings of the current study raise the intriguing possibility that EGFR signaling might influence the activity of bHLH signaling networks, or even the possibility that specific bHLH factors or activity might be asymmetrically inherited.

How is EGFR asymmetrically localized in mammalian neural progenitors? Although immunofluorescence studies indicated that EGFR colocalized with Numb in asymmetric divisions, EGFR could still be asymmetrically localized in cells from Numb and Numblike double-knockout mice. Latrunculin A treatment of dividing precursors disrupted the asymmetric localization of EGFR, suggesting that like Numb localization in *Drosophila*, EGFR asymmetric distribution of EGFR in mouse neural precursors is actin dependent.

These studies raise further questions about how EGFR segregation is regulated. Many asymmetrically inherited molecules appear to exploit a cell's intrinsic polarity (Jan and Jan, 2000), yet EGFR can be asymmetrically localized in cultured precursors, seemingly removed from their normal tissue context and polarity signals. Furthermore, although the bulk of EGFR appears to be localized apically with Numb in vivo, asymmetric EGFR can seemingly ignore apical-basal cues

when favoring one daughter. Moreover, asymmmetric EGFR localization occurs both in the ventricular zone where progenitors have clear apical basal polarity, as well as in the subventricular zone, where polarity is less well defined. Although it remains unexplored whether disruptions in EGFR asymmetry lead to subsequent changes in glial fate determination, the findings of Sun et al. suggest that asymmetric distribution of surface receptors during mitosis can predict distinct cell fates in the glial lineage.

The importance of EGFR in regulating cell fate and differentiation has been suggested by studies of human cancers with amplifications of EGFR. The current studies of Sun and colleagues provide evidence that asymmetric EGFR inheritance following mitosis may be one point of lineage divergence in the production of astrocytes and oligodendrocytes, with $EGFR^{high}$ precursors giving rise to astrocyte lineages and $EGFR^{low}$ precursors generating oligodendrocytes. An intriguing consequence of this observation may be directly relevant to some particularly troublesome human brain cancers. The small cell variant of glioblastoma (also called small-cell astrocytoma) is often confused for high-grade oligodendrogliomas. While small-cell astrocytomas are resistant to chemotherapy and follow an aggressive clinical course, in contrast, high-grade oligodendrogliomas are more responsive to chemotherapy and carry a more favorable prognosis. Recent studies have shown that EGFR amplification is common in small-cell astrocytoma, and a mutated constitutively activated form of the EGFR (EGFR-vIII) is often found specifically in these astrocytomas, but not in high-grade oligodendrogliomas (Perry et al., 2004). Recent evidence suggesting that brain tumors resemble stem cells (Oliver and Wechsler-Reya, 2004) make the findings of Sun et al. demonstrating the role of EGFR in astrocyte/oligodendrocyte lineage choices of neural precursors potentially illuminating. Could differences in EGFR signaling in multipotent cancer cells underlie the distinctions between small-cell astrocytomas and high-grade oligodendrogliomas? Together, these studies raise the tantalizing possibility that the factors that regulate normal cell lineages from neural precursors may serve similar function in the development of brain cancers from stem-like cancer cells (Figure 1). Further understanding of the diversity of inherited factors that function in generating cell diversity during development may lead to insights into how cancer cells determine their fates.

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