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Developmental stage-specific transformation of neural progenitors

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Gliomas represent the most common type of primary brain tumor in both children and adults, showing considerable variability in histologic appearance and clinical outcome. The phenotypic differences between types and grades of gliomas have not been explained solely on the grounds of differing oncogenic stimuli, and current evidence suggests that an interaction between the cell of origin, the tumor microenvironment, and specific cancer-causing genetic changes are all important factors in the evolution of central nervous system tumors.[1,2] Studies performed in neural stem cells (NSC), a possible candidate for the glioma cell of origin, suggest that some of the variability in glioma biology may be, in part, a reflection of regional differences in the NSCs from which they arise.[3,4] However, we don't know whether the developmental stage of the NSC may also influence its response to oncogenic stimuli. A good candidate in which to address this question is the radial glial cell, as it progresses stepwise through distinct developmental stages and is the neonatal origin of adult subventricular zone (SVZ) neural stem cells.[5]

As development progresses, neural progenitors, such as radial glial cells, decrease in number, and their proliferation declines to low levels. The remaining neural stem cells become tightly regulated to ensure they do not hyper-proliferate in adult tissues. These control mechanisms are likely imposed during the cell's progressive restriction in fate potential. Thus, we hypothesized that the susceptibility of these progenitor populations to oncogenic transformation changes as a function of their maturation. To test this, we developed a mouse model that integrates Cre–Lox-mediated, and Tet-regulated expression, to induce expression of activated K-ras into radial glial progenitors at distinct developmental time points. To target radial glial cells, we used the brain lipid binding protein (BLBP) promoter, as it is expressed specifically at the neurogenic and gliogenic stages of radial glial development,[6] allowing us to test the transformation potential of this progenitor population across different stages of their development.

Taking advantage of the lineage-tracing and inducible characteristics of our model, which allowed us to track the progeny that derived from BLBP+ cells and their response to the oncogenic effects of active K-ras, we identified naturally occurring, developmentally dependent variability in the tumorigenic effects of active K-ras.[7] We showed that active K-ras alone was able to induce diffuse malignant gliomas when targeted to embryonic stages, whereas targeting it to late prenatal or postnatal stages did not lead to tumors (**Fig. 1**). The difference in the transforming capacity of active K-ras between prenatal and postnatal stages suggested to us that early progenitors may be less able to engage tumor suppressor pathways than their more mature counterparts. By sorting BLBP+ cells at defined developmental stages we were able to show that, indeed, the level of cell cycle regulators in these cells varies as a function of age, reflecting the changes in cell cycle kinetics that radial glia undergo during development and mirroring the ability of active K-ras to induce transformation. The biggest changes were observed in ARF, which had a robust increase in expression during late prenatal and postnatal time-points, accompanied by the downregulation of cell cycle progression regulators such as CDK4, cdc25A, and cdc25C. The higher expression of the tumor suppressor ARF at late prenatal and postnatal time points inversely correlated with the ability of K-ras mutations alone to initiate radial glial cell transformation. Thus resistance to oncogenic K-ras may reflect a developmental activation threshold for Ink4a/ARF, which might be related to the basal proliferative rate of cells at different stages in their development. This idea is not new, as it has been shown that expression of Ink4a/ARF increases with age in many tissue specific stem cells, including NSCs.[8] However, our analysis was the first lineage-tracing study to show that even though postnatal neural stem cells derive from embryonic radial glial cells, their response to the same oncogenic stimulus is distinct. These distinctions reflect the inherent ability of the cell to engage tumor suppressor pathways in response to an oncogenic stimulus. Interestingly, by deleting p53 in radial glia cells, we were able to overcome the resistance of early postnatal neural progenitors to active K-ras transformation. Thus, it is possible that cancers driven by a single oncogene may in fact derive from earlier precursor populations than their counterparts harboring defects in multiple oncogenic pathways.

Overall, our results highlight the interplay between genetic alterations and the molecular changes that accompany the temporal development of NSCs, and further emphasize the need to view the

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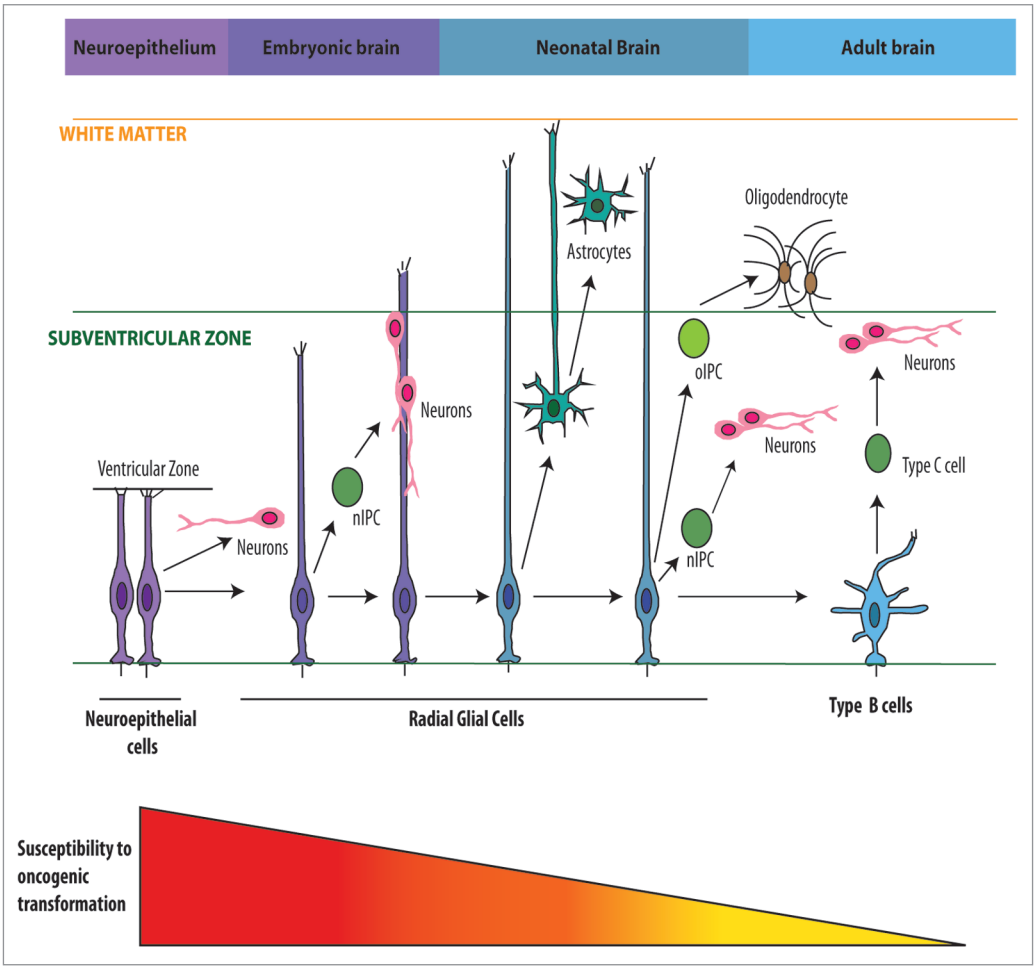

Figure 1. NSCs in the developing forebrain begin as neuroepithelial cells and transform into radial glial cells, which mature into astrocyte-like cells or type B cells. The tumorigenic effects of K-ras^{G12D} in this population, show developmentally dependent variability, reflecting the changes in cell cycle kinetics that radial glia undergo during development. nIPC, neurogenic progenitor cell; oIPC, oligodendrocytic progenitor cell.

tumorigenic process of gliomas in the context of normal brain development. The cell context of oncogene expression may determine the phenotype and biologic aggressiveness of the tumor. Thus, the results of genetic or epigenetic alterations leading to brain tumors may be quite different during the course of CNS development, suggesting that unique treatment strategies may be required.

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