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Even Cancers Want Commitment: Lineage Identity and Medulloblastoma Formation

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In this issue of *Cancer Cell*, Yang et al. (2008) and Schüller et al. (2008) show that Hedgehog activation in either multipotent neural stem cells or developmentally restricted progenitors causes only medulloblastomas to form. These data suggest that some stem cell-derived tumors must commit to a specific lineage in order to grow.

The cellular origins of most solid tumors are not well understood, in part because specific markers for the stem, progenitor, and differentiated cell populations from which they might arise are often lacking. Medulloblastomas are central nervous system (CNS) embryonal tumors composed of primitive-appearing cells that can differentiate along multiple lineages (Louis et al., 2007). They arise in the cerebellum, but it is not yet clear whether multipotent stem cells, developmentally restricted progenitors, or other cells give rise to these pediatric malignancies. It is hoped that by defining the cell (or cells) in which they form and the relationship between normal development and oncogenesis, improved therapies can be developed.

The cerebellar ventricular zone (VZ) consists of a band of stem and progenitor cells that line the IVth ventricle of the cerebellum (Figure 1). It gives rise to most cell types in the cerebellum, including Bergmann glia, astrocytes, oligodendrocytes, and Purkinje neurons (Sillitoe and Joyner, 2007). At one edge of this zone lies the rhombic lip, which sends forth a stream of lineage-restricted granule neuron precursors (GNPs) over the cerebellar surface. These proliferate transiently in the external germinal/granular layer (EGL) and then migrate inward and differentiate to form the numerous small neurons of the internal granule cell layer (IGL).

It has become clear that many molecular pathways play similar roles in both cerebellar development and medulloblastoma formation. Hedgehog signaling, for example, drives proliferation of the cerebellar EGL and is also activated in both familial and sporadic medulloblastomas, most commonly by mutations or deletions abrogating function of the inhibitory receptor *Patched* (*Ptc*) (Louis et al., 2007; Pietsch et al., 1997). Transgenic mice lacking one copy of *Ptc* develop medulloblastomas that appear to arise from proliferations of GNP-like cells in the EGL (Kho et al., 2004; Oliver et al., 2005), suggesting that medulloblastomas form in lineage-committed progenitor cells. However, this notion must be reconciled with the fact that medulloblastomas are thought to differentiate into both glia and neurons, occasionally even forming melanocytic or muscle cells (Louis et al., 2007). While such a broad cellular spectrum could result from dedifferentiation of a committed GNP due to the influence of oncogenic molecular events, it is also possible that the tumors truly form in multipotent stem cells with an intrinsic multilineage potential. Indeed, several studies have suggested that some medulloblastomas express markers normally found in VZ stem cells and their descendants, but this is not proof that the tumors came from stem cells (Eberhart, 2007).

In this issue of *Cancer Cell*, Yang et al. (2008) and Schüller et al. (2008) directly address the issue of which CNS cells are capable of being transformed into tumors by Hedgehog activation. They find that while neoplasms can be "initiated" when genetic changes are induced in either multipotent VZ cells or lineage-committed cells, tumor masses appear to first form in the EGL and have a largely GNP-like phenotype.

Yang and colleagues (2008) use conditional *cre*-mediated deletion of *Ptc* in knockout mice ($Ptc^{C/C}$) to activate the Hedgehog pathway in either GNPs or multipotent stem cells. Activation in Math1-expressing GNPs of the rhombic lip and EGL resulted in a marked expansion and inappropriate persistence of this progenitor layer during early postnatal life. Many of these cells were able to exit the cell cycle,

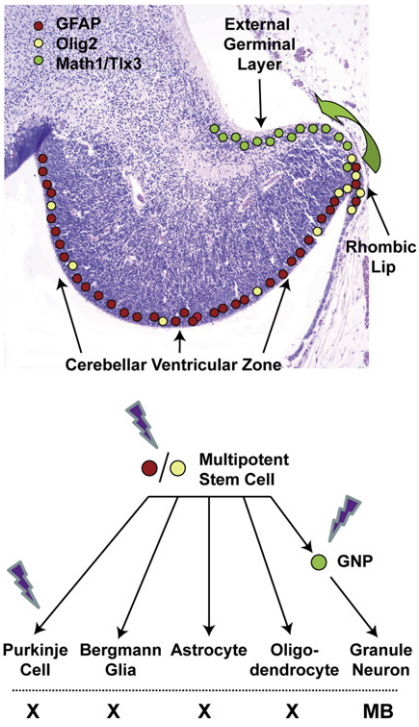

Figure 1. Hedgehog-Induced Medulloblastoma in Multipotent and Lineage-Restricted Cerebellar Cells

Multipotent cells expressing GFAP or Olig2 populate the fetal cerebellar ventricular zone and give rise to Purkinje cells, Bergmann glia, astrocytes, and oligodendrocytes. They also generate granule neuron precursors (GNP) in the rhombic lip that migrate over the cerebellar surface to form an external germinal layer of progenitors committed to differentiate into granule neurons. Activating Hedgehog signaling in either multipotent stem cells or GNPs results in the formation of medulloblastomas (MB) that express markers of granule neuron differentiation, but not tumors resembling other glial or neuronal cell types. Activation of Hedgehog in Purkinje cells fails to generate tumors.

migrate appropriately into the IGL, and differentiate. Some did not, however, and by 10–11 weeks of age, medulloblastomas had formed in all animals. Further temporal control of *Ptc* deletion, achieved using a *Math1-CreER* mouse, indicated that Math1-positive cells giving rise to deep cerebellar nuclei and brain stem neurons could not generate medulloblastomas, while those fated to populate the EGL could, further implicating GNPs as the true cell of origin. Indeed, activation of Hedgehog signaling as late as postnatal day 10 was able to initiate tumor formation in GNPs.

Yang et al. (2008) also activated Hedgehog in GFAP-expressing neural stem cells of the VZ, which give rise to multiple types of neurons and glia. Increased proliferation of neural stem cells was observed following *Ptc* deletion, but glia and non-granule neurons derived from these stem cells appeared normal. In contrast, marked changes were present when *Ptc*-deficient cells migrated to the rhombic lip and committed to the granule cell lineage. Both the rhombic lip and EGL were massively expanded, and cerebellar tumors formed rapidly in all animals. The tumors themselves resembled those generated when *Ptc* was deleted in Math1-expressing cells, with a similar proliferation index, capacity for differentiation, and transplantability. Thus, earlier genetic tumor initiation did not seem to generate an intrinsically more aggressive neoplasm.

Schüller and colleagues (2008) activated Hedgehog signaling using a mutated *Smoothened* receptor (*SmoM2*). They also found that aberrant Hedgehog activation in committed GNPs defined by expression of Math1 resulted in efficient generation of medulloblastomas. Crosses resulting in SmoM2 expression in GNPs defined by Tlx3 expression yielded similar results, despite the fact that in *Tlx3-cre:SmoM2* animals, cells activate Hedgehog signaling after they leave the rhombic lip. Indeed, the authors were able to induce tumors in GNPs defined by Gli1 expression in the EGL up to postnatal day 10 in *Gli1-CreERT2:SmoM2* mice.

Schüller et al. (2008) also generated medulloblastomas by activating Hedgehog signaling in multipotent stem/progenitor cells expressing GFAP or Olig2. The authors demonstrate for the first time that this latter protein, previously shown to be expressed in multilineage progenitor populations in the spinal cord and forebrain, also marks a subset of multipotent cerebellar VZ cells destined to give rise to oligodendrocytes, Purkinje cells, and interneurons, as well as granule cells concentrated in the posterior cerebellar lobes.

One significant finding in both studies was the inability of Hedgehog signaling to induce astrocytomas, oligodendrogliomas, or neuronal tumors, despite the fact that the pathway was activated in multipotent stem cells in several brain regions. Indeed, when Schüller and colleagues (2008) expressed SmoM2 specifically in Purkinje neurons, no tumors formed. It has recently been shown that Hedgehog activity is required for growth of stem-like malignant glioma cells (Bar et al., 2007; Clement et al., 2007), but activation of the pathway, at least by itself, seems insufficient to generate glial tumors.

A second intriguing finding was the requirement that cells commit to a granule cell lineage prior to generating fully transformed tumor masses. This appears to result from an intrinsic property of GNPs rather than local factors in the cerebellum, as once murine medulloblastomas formed, they could be transplanted with equal efficiency into both cerebellar and forebrain locations. It has been suggested that some molecular signals important in normal cell lineage development function as lineage dependency/lineage addiction factors in cancer (Garraway and Sellers, 2006), and it may be that Hedgehog plays a similar role in at least a fraction of medulloblastomas.

It is important to remember that the results presented in these two papers only reflect oncogenesis driven by Hedgehog signaling and that the capacity of other molecular events to cause either multipotent or more restricted cells to form medulloblastomas could be quite different. Nevertheless, it is now clear that even tumors made up largely of a single cellular lineage may well have been initiated in a multipotent stem or progenitor cell and that expression studies of the bulk of tumor cells may not always indicate the cell of origin.

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Drug-Resistant Phosphatidylinositol 3-Kinase: Guidance for the Preemptive Strike

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In this issue of *Cancer Cell*, Zunder et al. (2008) describe surprising findings from investigating inhibitor-resistant mutations in the affinity pocket of p110α of phosphatidylinositol 3-kinase (PI3K). Information on these critical residues provides a road map for generating novel PI3K inhibitors that can overcome the anticipated resistance mutations.

Small-molecule inhibitors targeting the tyrosine kinases Abl and EGFR have been spectacularly successful as cancer drugs (Druker, 2004). However, these successes have been curtailed by the appearance of kinase mutants that are resistant to the inhibitors (Gorre et al., 2001; Pao et al., 2005). The inhibitors typically bind to a conserved structural motif, referred to as an affinity pocket, that is located in the immediate vicinity of the ATP binding site. They compete with ATP for binding to the kinases. Most of the resistance mutations block inhibitor binding. A particularly effective and commonly encountered mutation occurs at a position referred to as the ''gatekeeper'' that controls access of inhibitors to the affinity pocket. The resistance mutations have now led to the development of second-generation inhibitors that are effective against many of the mutant kinases (Burgess and Sawyers, 2006; Druker, 2006; Kwak et al., 2005). However, targeting gatekeeper mutant kinases remains a significant challenge. This situation illustrates the general problem of mutation-induced drug resistance that needs to be anticipated in all therapeutic strategies.

In recent years, phosphatidylinositol 3-kinase (PI3K) has emerged as an exceedingly attractive and promising drug target. The PI3K signaling pathway is upregulated in most cancers as a result of various genetic and epigenetic changes. *PIK3CA*, the gene encoding the catalytic subunit p110α of PI3K, is frequently mutated in cancers of the breast, colon, endometrium, and prostate (Samuels et al., 2004). About 80% of these mutations map to one of three hot spots in the gene. They induce a gain of function in enzymatic and signaling activity. The mutant p110α is also oncogenic in cell culture and in animal model systems, strongly suggesting that it contributes to the oncogenic cellular phenotype in human cancers. Academic and industrial laboratories have responded to this development by generating PI3K inhibitors, some of which are entering clinical trials (Marone et al., 2008). Mutations resulting in inhibitor resistance will surely arise. Can we apply the lessons learned from the protein kinases to the lipid kinase PI3K? This is the question asked in a study published in the current issue of *Cancer Cell* (Zunder et al., 2008).

The recently determined structure of p110α shows some broad similarities to that of protein kinases, notably a hydrophobic cavity that corresponds to the affinity pocket of protein kinases (Huang et al., 2007). Several PI3K inhibitors target this structural motif and therefore could be affected by resistance mutations similar to those found in protein kinases. These basic similarities guided Zunder and colleagues (2008) in their study of drug resistance in PI3K. The researchers mutagenized selected residues lining the p110α affinity pocket, including the homolog of the gatekeeper, I848. The mutants were tested against several PI3K inhibitors using an ingenious yeast lethality test. The assay is based on the fact that *Saccharomyces cerevisiae* does not contain class I PI3Ks and that expression of p110α depletes its essential stores of phosphatidylinositol 4,5-bisphosphate, thus interfering with cell replication. Inhibition of p110α activity restores growth and viability of p110α-expressing *S. cerevisiae* (Figure 1). The simplicity and rapidity of the yeast screen allowed coverage of a sizable number of mutant/inhibitor combinations, including saturation mutagenesis and diverse inhibitor chemotypes.

The results of these mutagenesis studies are both unexpected and instructive. In contrast to the affinity pockets of protein kinases, which can accommodate diverse mutations, the pocket of p110α is relatively intolerant to change. Most mutations led to a loss of enzymatic activity. Gatekeeper mutations were another surprise: not only did they fail to induce resistance, most of them were also catalytically inactive or retained only minimal enzymatic activity. A single residue in the affinity pocket gave rise to resistant