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Commentary

Medulloblastoma in Mice Lacking p53 and PARP

All Roads Lead To Gli

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Medulloblastomas, embryonal neoplasms arising in the cerebellum, are the most common malignant brain tumors in children. They are composed of primitive cells with the potential to differentiate along neuronal and glial lines. Similar lesions, known as primitive neuroectodermal tumors (PNETs), arise outside the posterior fossa, albeit rarely. Several molecular pathways important in cerebellar development have been implicated in medulloblastoma pathogenesis (reviewed in[1–3]). A better understanding of the cells and signaling events involved in medulloblastoma tumorigenesis will be critical to the development of targeted therapies for these aggressive neoplasms, as evidenced by the inhibition of medulloblastoma growth by drugs blocking Hedgehog pathway activity.[4] In this issue of *The American Journal of Pathology*, Tong and colleagues[5] describe a new medulloblastoma model in which tumors arise from the cerebellar external granule cell layer (EGL) of mice lacking *p53* and *PARP*. The up-regulation of the Hedgehog pathway effector Gli in all of their tumors suggests a more general role for Hedgehog signaling than was previously appreciated. In medulloblastomas, some cellular pathways may be more equal than others.

Cerebellar Development and Medulloblastoma

The analysis of cerebellar development has shed considerable light on medulloblastoma pathogenesis, as several genetic pathways seem to be critical for the development of both normal cerebellar structures and central nervous system (CNS) embryonal tumors. Unlike cerebral cortex, which derives from a single subventricular matrix, the cerebellum develops from two germinal matrix regions (reviewed in[6]). In cerebellum, subventricular matrix cells give rise to neurons of the deep nuclei, Purkinje cells, Golgi neurons, and glial cells. A second matrix region, the EGL, is formed by neuroblasts from the rhombic lip that migrate over the cerebellar surface. These neuroblasts first proliferate in the outer EGL, then exit the cell cycle and move to the inner EGL (Figure 1). Immature granule neurons migrate inwards from the EGL along Bergmann glia, transiting through the molecular layer and past the Purkinje cells to take up residence in the internal granule cell layer (IGL).

The Hedgehog pathway is the best-characterized regulator of EGL proliferation and cerebellar size. The ligand Sonic Hedgehog is secreted by Purkinje cells, and promotes proliferation of granule cell precursors in the EGL by binding to its receptor PTCH.[7–9] Several markers can be used to track the exit of EGL neuroblasts from the cell cycle and their progressive differentiation. Proliferating neuroblasts in the outer EGL express Math-1, p53, and NeuN.[10–12] As cells move to the inner portion of the EGL, they down-regulate proliferation markers and begin expressing promoters of neuronal differentiation such as the cell cycle-dependent kinase inhibitor p27Kip1 and the bHLH transcription factors NeuroD and NeuroD2.[13–16] Markers of neuronal differentiation such as class III β-tubulin, MAP-2, synaptophysin, and nestin are also expressed in post-mitotic inner EGL cells or in differentiated neurons of the IGL.[17–20]

The histogenesis of medulloblastomas has been controversial for many years. Some feel they arise primarily from primitive neuroectodermal cells in the germinal matrix surrounding the ventricle.[21,22] Others have argued that proliferating neuroblasts of the cerebellar EGL are the most likely progenitors.[23,24] It is also possible that cells from both of these locations give rise to medulloblastomas.[25] Whatever their origin, it is clear that many human medulloblastomas express the cerebellar developmental markers discussed above.

Medulloblastoma Genetics

In man, three inherited syndromes associated with medulloblastomas have been described: Turcot's, Gorlin's, and Li Fraumeni (reviewed in[3]). Gorlin's syndrome results

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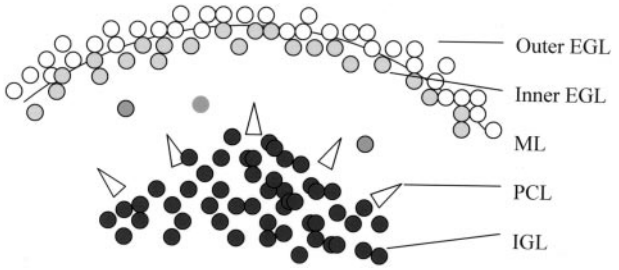

Figure 1. The developing cerebellum. EGL, external granule cell layer; ML, molecular layer; PCL, Purkinje cell layer; IGL, internal granule cell layer.

from inherited mutations in the Hedgehog pathway gene *PTCH*. Mutations in the Hedgehog pathway members *PTCH*, *PTCH 2*, *Smo*, or *Sufu* have been identified in approximately 25% of sporadic medulloblastomas as well.[26–30] In addition, mutation of the *PTCH* gene in mice causes medulloblastoma-like tumors to form in 10 to 15% of heterozygotes by 6 months of age.[31,32] These murine tumors derive from the EGL, providing further support for the histogenetic importance of this neuroblast layer in medulloblastomas.

Turcot's syndrome is caused by germline mutations in the gene *APC*, a member of the Wnt signaling pathway. The pathway contains several proteins (APC, Axin, GSK3) acting in concert to promote the proteosomal degradation of β-catenin.[33] Mutations in *APC, β-catenin*, or *Axin* have been identified in approximately 25% of sporadic medulloblastomas.[34–37]

Li Fraumeni syndrome is caused by inherited mutations in the *p53* tumor suppressor gene (reviewed in[38]). Affected individuals develop a large spectrum of CNS and extra-CNS tumors, including medulloblastomas.[39] Interestingly, alterations in *p53* are relatively rare in sporadic medulloblastomas, with an incidence of approximately 5%.[40–43] *MDM2* amplification can inhibit p53 function in many tumor types, but no such amplification has been detected in medulloblastomas.[40,44]

Mouse Medulloblastoma Models

Despite the paucity of human medulloblastomas with *p53* mutations, a growing number of investigators have reported that lack of p53 function plays an important role in the formation of medulloblastomas in rodent models. The first experiments to suggest this were performed in Syrian golden hamsters. Perinatal infection of EGL cells by JC virus resulted in medulloblastomas, presumably via the inactivation of p53 and Rb by virus-encoded T antigen.[45,46] Subsequent experiments using retrovirus-mediated transfer of SV40 T antigen or transgenic expression of JC virus T antigen in mice and rats confirmed the medulloblastoma-promoting effects of this protein.[47,48] Targeted deletion of both *p53* and *Rb* in the cerebellum also results in medulloblastoma.[49]

Loss of *p53* can enhance the medulloblastoma-promoting effects of *PTCH* mutation. Wetmore and colleagues[50] have demonstrated that *p53* inactivation markedly increases the number of medulloblastomas forming in *PTCH* heterozygous animals. Ionizing radiation also seems to strongly promote medulloblastoma development in *PTCH* heterozygotes when applied to newborn mice in which the EGL is still proliferating.[51] In all of these models, tumors developed months after the initial genetic insults, suggesting additional mutational events were required.

It is unclear to what extent the murine medulloblastomas with loss of p53 or Rb function accurately model human tumors. While these neoplasms appear similar to human medulloblastomas, many have viewed them with skepticism because human cases largely lack mutations in these genes. The data presented above suggest that inactivation of p53, Rb, and other genes controlling DNA repair and apoptosis may promote medulloblastoma formation in mice by fostering the accumulation of genetic defects in other cellular pathways. If genetic instability during a defined developmental window is responsible for tumor formation, it is the additional mutational events in the tumors that will best define the genetic similarity between murine and human medulloblastomas. In their paper, Tong and colleagues[5] show that increased genetic instability caused by abrogation of p53 and PARP function results in murine medulloblastomas with activation of the Hedgehog pathway. This represents the first examination of Hedghog function in murine medulloblastomas without underlying *PTCH* mutations. The selection for molecular events activating Hedgehog signaling suggests the medulloblastomas arising in mice with genomic instability may indeed accurately model human medulloblastomas.

Mice Lacking PARP and p53 Develop Medulloblastomas

Poly(ADP-ribose) polymerase (PARP) binds DNA breaks and facilitates their repair. In earlier work, Tong and colleagues[52] showed that p53 and PARP interact to maintain genome integrity. Others have demonstrated that loss of PARP in neurons causes a resistance to cell death.[53] Deletion of both *PARP* and *p53* in transgenic mice results in embryonal brain tumors not seen with *p53* loss alone, suggesting that cooperation of DNA end-processing and cell cycle checkpoint molecules is required to suppress malignant transformation of neuronal cells.[52]

The paper in this issue characterizes the embryonal tumors arising in *p53*, *PARP* null mice more closely.[5] CNS tumors developed in approximately half of the animals with a median age of onset of 16 weeks. Interestingly, more than twice as many males developed tumors as females, a ratio similar to that observed in humans. The increased frequency and somewhat more aggressive biology of medulloblastomas in boys have never been explained, and this new mouse model may prove useful in examining the phenomenon. All but one of the tumors were centered in the cerebellum, with the final lesion detected in the cerebral cortex. The tumors appeared highly similar to human medulloblastomas, with sheets of

embryonal cells and "neuroblastic" rosettes. In eight animals early lesions were observed in the EGL.

Immunohistochemical analysis supported the similarity to human medulloblastoma. Tumors were positive for the neuronal markers NeuN, MAP-2, and synaptophysin. GFAP-positive tumor cells were also occasionally seen. MATH-1, a neuron-specific basic helix-loop-helix transcription factor required for the proliferation of granule cells in the cerebellum, was expressed in the tumors. As is the case in human medulloblastomas, numerous chromosomal aberrations were detected. Most intriguingly, the Hedgehog pathway appeared to be activated in all tumors examined, with markedly increased expression of the Hedgehog effector Gli, possibly resulting from PTCH deficiency.

This work raises several questions. First, is *PARP* mutated in human medulloblastomas? It is clear that many genes involved in medulloblastoma pathogenesis remain to be discovered, and *PARP* may be one of these. The tumor aneuploidy caused by *PARP* deficiency in mice is similar to that seen in human medulloblastomas, supporting a possible causal association. Alternatively, loss of *PARP* could facilitate mutagenesis in mice by promoting additional DNA damage and chromosomal aberrations, but not be involved in human lesions. Loss of heterozygosity and sequence analysis of the *PARP* gene in human tumors will be required to further evaluate these issues.

A second question is whether the single tumor arising in the cerebral cortex was generated through the same genetic mechanism as the cerebellar medulloblastomas. This cortical lesion seems similar to the supratentorial PNET found in humans. As discussed above, no EGL exists during cerebral cortical development, thus the tumor must have developed from different precursors. Human supratentorial PNET are considerably rarer than medulloblastoma, making them difficult to study. By examining similar lesions in mice, we may be able to better define the precursor cells and genetic pathways involved in their formation.

Finally, it remains to be seen how PTCH expression is lost in the tumors reported by Tong and colleagues. While it was initially proposed that loss of only one *PTCH* allele was sufficient for medulloblastoma formation in mice, recent reports suggest that the second allele is also inactivated by methylation or mutation.[4,51,54,55] *PTCH* could be inactivated by one of these methods in the *p53*, *PARP* null tumors, or its expression might be down-regulated by other means. The loss of PTCH expression and activation of Gli in all of the tumors examined is particularly interesting in light of the recent finding that 100% of human medulloblastomas tested respond to Hedghog inhibitors *in vitro*, while only a quarter of the cases should have mutations in the pathway.[4] Taken together, these data suggest that Hedgehog activity could be critical in most, if not all, medulloblastomas. The analysis of additional cellular pathways in this new murine tumor model may identify other genes commonly mutated on the road to medulloblastoma formation.

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