Modeling medulloblastoma with genetically engineered mice

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Medulloblastoma is a malignant tumor that arises in the cerebellum in children, presumably by transformation of granule neuron precursor cells. In vivo models of medulloblastoma in genetically engineered mice have shown that activation of signal transduction pathways that stimulate proliferation and inhibit differentiation of neural progenitor cells during cerebellar development initiate medulloblastoma formation. Activation of the Sonic hedgehog (Shh)/Patched signaling pathway in the postnatal cerebellum is sufficient to induce medulloblastoma in mice. Activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway by insulin-like growth factor-II, inactivation of the p53 tumor suppressor protein, loss of DNA damage repair mechanisms, and ectopic expression of Myc oncoproteins cooperate with Shh/Patched signaling to enhance tumor formation in mice. Ectopic expression of alpha and beta interferons in the developing brain also induces Shh-mediated medulloblastoma formation, suggesting a possible role for antiviral response in the genesis of medulloblastoma. By revealing which cell signaling proteins can initiate medulloblastoma formation, mouse models have enabled investigators to identify molecular targets for designing new therapies. Small-molecule inhibitors of the Shh/Patched and PI3K pathways are potential chemotherapeutic agents for patients with medulloblastoma.

KEY WORDS • medulloblastoma • *Sonic hedgehog* gene • phosphatidylinositol 3-kinase • signal transduction • mouse

OVERVIEW

Over the past several decades, rapid expansion in our understanding of the molecular biology of cancer has dispelled any hope that this disease might be explained by mutations in a small number of genes. On the contrary, cancer cells have many mutant genes and dysfunctional proteins. Although this fact may pique the interest of basic scientists who challenge themselves by solving complex problems, it makes it difficult for clinical scientists to select targets for new cancer therapies. Therefore, we look to animal models of human cancer to help identify key proteins that are physiologically relevant to tumor formation. In this review, I will discuss how scientists are using genetically engineered mice to decipher, at the molecular level, the origins of the pediatric brain tumor, medulloblastoma, and how mouse models are used to design novel therapeutic strategies.

Medulloblastoma is the most common solid tumor afflicting children, and the need for improved treatments for patients with this disease is pressing. Current treatment regimens, which combine surgery with craniospinal radiation

and multiagent chemotherapy, offer 5-year survival rates of 75% and result in cures in half of newly diagnosed patients.⁴² Nevertheless, long-term survivors experience skeletal growth retardation, endocrine dysfunction, and progressive cognitive impairment.^{43,57} They are also at increased risk for psychiatric disturbances and social difficulties.⁵² These devastating side effects are attributed to collateral damage to the developing nervous system by anticancer agents, particularly radiation, rather than to the disease itself. Thus, the central practical objective of modern medulloblastoma research is to find ways of increasing treatment specificity for cancer cells and thereby to minimize damage to the developing brain.

What can experiments in mice tell us about human cancer? First, the mouse models discussed in this review have made it possible to determine which cell signaling pathways are required to initiate formation of the tumor and which ones are needed to maintain its growth. Knowing which signaling pathways are activated in mouse tumors can reveal molecular targets for therapeutic intervention in humans, in addition to providing insights into tumor origins. Second, mouse models of human cancer are valuable experimental systems for preclinical testing of novel therapies. Their utility includes testing new chemotherapy drugs and novel uses of existing drugs or combined regimens. Third, mouse models generate an abundant supply of tumor specimens for molecular analysis. This is particularly important for comparatively uncommon types of tumors like

Abbreviations used in this paper: EGL = external granule layer; GFAP = glial fibrillary acidic protein; GNP = granule neuron precursor; IFN = interferon; IGF-I and -II = insulin-like growth factor-I and -II; IGF-IR = IGF-I receptor; IRS-1 = insulin receptor substrate-1; mRNA = messenger RNA; mTOR = mammalian target of rapamycin; PI3K = phosphatidylinositol 3-kinase; PTCH = human homolog of the *Patched* gene; Rb = retinoblastoma; Shh = Sonic hedgehog.

medulloblastomas. Finally, mouse models are facilitating the development of sensitive and noninvasive imaging modalities.

Expressing and Silencing Genes in Genetically Engineered Mice

To identify genes that play key roles in medulloblastoma formation, cancer geneticists have used two general classes of genetically engineered mice: transgenic and knock-out mice. Transgenic mice carry an exogenous gene (transgene) with attached control sequences (promoter/enhancer elements) in every cell, both germ cells and somatic cells (Fig. 1A). The promoter/enhancer elements determine the range of tissues and cell types in which the exogenous gene is expressed as well as the developmental timing of gene expression. In transgenic mice, the transgene is overexpressed compared with normal, endogenous genes because of the activity of the attached promoter/enhancer sequences.

In knock-out mice, by contrast, expression of a gene is silenced by replacing an endogenous gene (target gene) with a defective version (Fig. 1B). If both copies (homologs) of the target gene are knocked out, the mice are said to be homozygous defective for the target gene. If only one homolog is defective, the mice are heterozygous defective. If the target gene plays a vital role in embryonic development, then knocking out that gene in the germ line may cause fetal death. Because embryonic lethality is usually uninformative to the mouse geneticist, an alternative strategy has been developed to inactivate a gene at a later point in embryonic development, after it has played its essential role in embryogenesis. Such mice are called conditional knock-outs.

The genetic engineering required to create a conditional knock-out mouse involves flanking the target gene with a set of DNA sequences (LoxP sites; Fig. 1C). These sites are recognized by a bacterial enzyme called Cre recombinase, which excises all DNA sequences interposed between two LoxP sites. In conditional knock-out mice, *Cre*, the gene encoding Cre recombinase, is expressed as a transgene under control of a promoter/enhancer that is active only in certain cell or tissue types and only at specific times during embryonic development. These conditional knock-out mice, then, contain two transgenes: 1) the target gene, whose expression will be knocked out; and 2) *Cre*, whose expression is driven by a tissue-specific promoter/enhancer. Thus, when the *Cre* promoter is switched on in a cell-type-specific manner, the encoded recombinase enzyme removes the target gene through its flanking LoxP sites.

When using genetically engineered mice to design a model of medulloblastoma, one must answer two questions. First, what is the presumed cell of medulloblastoma origin? Second, what signal transduction pathways must be activated in those cells to initiate tumor formation? A substantial body of experimental evidence implicates the GNP as the cell of origin for medulloblastoma.^{38,64} During embryogenesis, undifferentiated GNPs originate from a hindbrain structure called the rhombic lip. A stream of GNPs migrates away from the rhombic lip to the cortical surface of the cerebellum, where these precursors form a secondary germinal zone called the EGL. In mice and in humans, GNPs undergo a rapid postnatal expansion phase, after which they migrate inward, where they differentiate to generate the abundant granule neurons of the internal granule layer.²⁶ The

rapid proliferation of GNPs during the postnatal expansion phase of the EGL presents a vulnerable setting for transformation events leading to medulloblastoma formation.

Germ Line Inactivation of the Patched Gene Causes Medulloblastoma

In an attempt to create mouse models that accurately recapitulate the origin of human medulloblastoma, scientists have endeavored to activate signaling pathways that function normally to stimulate proliferation and inhibit differentiation of GNPs. Considerable attention has been focused on the role of the Shh/Patched signaling pathway in the genesis of medulloblastoma (Fig. 2A). Sonic hedgehog is a secreted protein that is a crucial determinant of embryonic pattern formation in the central nervous system; Shh binds the transmembrane protein, Patched, at the cell surface. This interaction activates a signal transduction pathway, leading to transcription of genes that govern various aspects of neural development.²⁸ The Gli transcription factors are the terminal effectors of Shh/Patched signaling. In the developing cerebellum, Shh stimulates the proliferation of GNPs and blocks their differentiation into neurons.^{14,61,65}

Fig. 1. Schematic drawings showing protocols for using genetically engineered mice to express or knock out a gene in a specific cell type. A: In transgenic mice, a tissue-specific promoter/enhancer is used to drive expression of the transgene (Gene X). In the example shown, promoter/enhancer sequences from the gene encoding GFAP (gfap) are used to overexpress Gene X in astrocytes. B: In knock-out mice, a sequence (for example, the gene conferring neomycin resistance) is inserted into a target gene (Gene X) to disrupt expression of the latter. The gene knock-out cassette is propagated from the germ line to all cells of the body. C: In conditional knock-out mice, the target gene (Gene X) is deleted only in specific cell types (for example, astrocytes). The promoter/enhancer for the *GFAP* gene is used to drive expression of Cre recombinase, which recognizes LoxP sites and excises Gene X positioned between these sites.

Modeling medulloblastoma with genetically engineered mice

The first clue that hyperactive Shh/Patched signaling might play a causal role in human medulloblastoma formation came from an analysis of a rare, hereditary condition called Gorlin syndrome, characterized by neural tube defects, craniofacial abnormalities, and predisposition to various neoplasms, including medulloblastomas.¹⁹ Genetic analysis revealed that inherited mutations in *PTCH* segregate in families with Gorlin syndrome.^{21,30} In a subsequent analysis of sporadic medulloblastomas in patients with these lesions, somatically acquired loss-of-function mutations in *PTCH* were discovered in 3 to 14% of cases.^{44,46,70} In addition, a hereditary form of medulloblastoma is caused by germ line mutations in the *SUFU* gene, which encodes a suppressor of the Shh/Patched signaling pathway.⁵⁹ Taken together, these findings have implicated *PTCH* as a tumor suppressor gene, inactivation of which could promote medulloblastoma formation by activating the Shh/Patched signaling pathway during cerebellar development.

Definitive proof that *Patched* was a medulloblastoma suppressor gene came from the creation of *Patched* knock-out mice. In mice that are homozygous defective for *Patched*, severe defects develop in the neural tube, heart, and other organs, and the animals die at 9 to 10 days of gestation.^{18,23} Heterozygous defective mice are viable, however, and in approximately 14% aggressive cerebellar tumors develop that closely resemble human medulloblastomas.^{18,68}

Activation of the Shh/Patched Signaling Pathway in Developing Cerebellum Induces Medulloblastomas

The fact that knocking out the *Patched* gene, which encodes the inhibitory component of the Shh/Patched signaling pathway, induces medulloblastoma predicts that overexpression of Shh itself or a downstream, positive effector of the pathway would also induce tumor formation. Indeed, two experimental strategies in which retroviruses are used to transfer and express *Shh* in the developing cerebellum induce medulloblastomas. One team of investigators used an ultrasound-guided microinjection technique to transfer an *Shh*-expressing murine leukemia retrovirus to the developing cerebellum in utero on embryonic Day 13.5.⁶⁶ Medulloblastomas appeared in 76% of animals, indicating that ectopic expression of Shh is sufficient for medulloblastoma induction. Interestingly, in utero transfer of Shh was still capable of inducing medulloblastomas in mice that were homozygous defective for the *Gli1* gene, which encodes a positively responding, downstream component of the Shh/Patched signaling pathway.

Another group of investigators used an avian retroviral vector to transfer and express Shh to nestin-expressing neural progenitor cells in the cerebella of newborn mice. They found that ectopic expression of Shh induced tumors in 9% of mice and that tumor induction increased to 23% by coexpression of the oncogenic transcription factor c-Myc.⁴⁷ The c-Myc gene is overexpressed in human medulloblastomas,⁸ and accumulation of c-Myc mRNA is an unfavorable prognostic indicator for patients with medulloblastoma.^{20,27}

Further evidence that activation of the Shh/Patched signaling pathway induces medulloblastoma was provided by scientists who created a transgenic mouse in which an activated version of the *Smoothed* gene was expressed in the developing cerebellum.²⁴ In this mouse model, transgene expression was driven by promoter/enhancer elements

from the *NeuroD2* gene, which is expressed at high levels during normal cerebellar development in GNPs within the EGL. Cerebellar tumors closely resembling human medulloblastomas were found in 48% of mice at a median age of 25.7 weeks.

The *Smoothed* gene encodes a transmembrane protein that acts as a transducer of extracellular input signals from Shh. The current paradigm is that Patched prevents Smoothed from relaying Shh signals to the interior of the cell and that binding of Shh to Patched stops Patched-mediated repression of Smoothed. The precise molecular mechanism whereby this interaction takes place remains the subject of intense scientific inquiry (see review by Cohen¹³). The *Smoothed* allele used in the transgenic mouse described

Fig. 2. Schematic drawings showing cell signaling pathways activated in medulloblastoma. A: The Shh/Patched signaling pathway is activated in the medulloblastoma. In the absence of Shh, Patched inhibits transmembrane protein, Smoothed. When Shh binds Patched, this inhibition is relieved. The intermediary reactions leading from ligand-receptor (Shh-Patched) binding at the cell surface to transcription activation in the nucleus are not completely understood. The effect of Shh is to activate the Gli family of transcription factors, which stimulate transcription of target genes. B: The IGFs signal through the PI3K pathway. Engagement of IGF-IR (IGF1R) by IGF-I or IGF-II activates the receptor tyrosine kinase. Phosphorylation of IRS-1 by IGF-IR creates a docking site for the regulatory subunit (p85) of PI3K. Consequent activation of the catalytic subunit (p110) generates phosphatidylinositol 3,4,5-trisphosphate (PI [3,4,5] P3), which recruits Akt to the plasma membrane through Akt's pleckstrin homology domain (PHD). Serine/threonine phosphorylation of Akt at the plasma membrane by 3-phosphoinositide-dependent kinases (PDKs) activates Akt. The PI3K signaling is suppressed by the phosphatase activity of tumor suppressor protein, PTEN.

earlier encodes a mutant protein with a single amino acid substitution (leucine substituted for the normal tryptophan at position 535). Structure–function studies suggest that this missense mutant of *Smoothed* is constitutively active, in that it continuously transduces an Shh-like signal, even in the absence of Shh. Missense mutations in the *Smoothed* gene occur in human neuroectodermal tumors, albeit rarely.^{49,72} Taken together, these studies indicate that activation of the Shh/Patched signaling pathway in the developing cerebellum is sufficient to initiate medulloblastoma formation and that undifferentiated neural progenitor cells, which are present in the cerebellum at birth, can act as the cells of origin for medulloblastoma.

Hyperplasia of the EGL: a Precursor Stage of Medulloblastoma

In the mouse models discussed earlier, in which medulloblastomas were induced by ectopic expression of Shh or *Smoothed*, examination of the cerebellar cortex showed abnormal thickening of the EGL, even in mice with no obvious tumors.^{24,47,66} This observation suggests that EGL hyperplasia may be a precursor stage of medulloblastoma. In support of this concept, foci of GNP-like cells have been identified on the cortical surface of the cerebella from *Patched*-deficient mice at time points in development after GNPs have normally migrated inward from the EGL to the internal granule layer.⁴¹ Isolation and molecular characterization revealed that these cells have activation of the Shh/Patched signaling pathway and that they proliferate in culture. Transcriptional profiling using DNA microarrays showed a gene expression pattern distinct from that of GNPs obtained in wild-type mice, suggesting that these cells mark a preneoplastic stage of medulloblastoma.

Overproduction of Immune Response Proteins can Initiate Medulloblastoma Formation

If overstimulation of GNPs by Shh is the proximate cause of medulloblastoma formation, then what environmental or developmental cues might trigger this process? The human neurotropic polyomavirus, JC virus, has been implicated as a cause of medulloblastoma because JC virus antigens are present in human medulloblastomas and because transgenic mice expressing the JC virus T antigen develop medulloblastomas.⁶³ Virus-induced carcinogenesis is not the topic of this review. Nevertheless, two transgenic mouse models have shown that IFNs, a class of cytokines that mediate a host's response to viral infection, can induce medulloblastoma formation when overexpressed in the developing brain. One team of investigators created a transgenic mouse in which IFN- α was expressed in the central nervous system via promoter/enhancer elements from the *GFAP* gene.³⁷ Highly aggressive medulloblastomas developed in these mice, and nearly 100% of the animals died by 1 month after birth. Tumors developed only in mice in which the gene encoding signal transducer and activator of transcription 2 was knocked out. Signal transducer and activator of transcription proteins are intracellular signaling molecules that become activated when cytokines like IFN- α engage their cognate receptors on the cell surface. Analysis of the tumors induced by IFN- α revealed that the Shh/Patched signaling pathway was activated (through a signal transducer and activator of transcription 1-dependent

mechanism), consistent with the idea that *Shh* plays an essential role in medulloblastoma formation. Furthermore, the authors showed that Shh/Patched pathway activation was a direct response to a different IFN, IFN- γ , which was produced by T lymphocytes migrating into the brain in response to IFN- α .

These results suggested that T-cell-derived IFN- γ was capable of initiating medulloblastoma formation if present in sufficient quantity and during the appropriate window of cerebellar development. Direct proof for this concept was provided by Lin, et al.,³⁷ who created a transgenic mouse in which IFN- γ was expressed in the developing nervous system under control of the *GFAP* gene promoter. Through genetic engineering, the investigators were able to control the timing of IFN- α transgene expression. Medulloblastomas developed in 80% of mice in which IFN- γ was expressed at embryonic Day 16, but no tumors developed when IFN- α expression was switched on at birth. As in the IFN- α model, induction of medulloblastomas by IFN- γ correlated with activation of the Shh/Patched signaling pathway. Although IFN- γ was necessary for medulloblastoma initiation in this mouse model, continued IFN- γ expression was not required for tumor progression. Taken together, these two mouse models raise the intriguing possibility that increased expression of IFNs by viral infection in the cerebellum might be an inciting event that activates Shh/Patched signaling, thus leading to medulloblastoma formation.

Deficiency of p53 Cooperates With Shh to Accelerate Medulloblastoma Formation in Mice

Although the observations discussed earlier strongly implicate Shh/Patched signaling as a causative factor for medulloblastoma, mounting evidence indicates that this pathway is not the sole determinant of medulloblastoma formation. First, the frequency of mutations in Shh/Patched pathway genes in human medulloblastomas is very low.⁷² Second, medulloblastomas arise in only 14% of *Patched*-deficient mice within the 1st year of life,⁶⁸ and tumor incidence is highly dependent on the genetic background of the knock-out mice.⁹ What other molecular signals could subvert the normal process of neural differentiation in the cerebellum and bring about neoplastic transformation?

Medulloblastoma formation is dramatically accelerated in double knock-out mice that are not only heterozygous defective for *Patched* (*Patched*^{+/-}) but also homozygous defective for the *p53* gene (*p53*^{-/-}).⁶⁷ A well-known tumor suppressor gene, *p53* encodes a transcription factor that inhibits cell cycle progression and mediates cellular response to DNA damage by triggering apoptosis.²⁵ More than 95% of *Patched*^{+/-}/*p53*^{-/-} mice had tumors at 10 to 12 weeks of age, compared with a 14% incidence in *Patched*^{+/-} mice at 10 months. The fact that medulloblastomas from *Patched*^{+/-}/*p53*^{-/-} mice showed a higher rate of chromosome loss on cytogenetic analysis than tumors from *Patched*^{+/-} mice suggests that the effect of *p53* deficiency on tumor induction might be related to genomic instability and acquisition of secondary mutations in growth control genes. Despite the impact of *p53* deficiency on medulloblastoma formation in genetically engineered mice, loss-of-function mutations in the *p53* gene are rare in human medulloblastomas.^{1,3,54}

Modeling medulloblastoma with genetically engineered mice

Defective DNA Repair Programs Promote Medulloblastoma Formation

The high rate of chromosome loss in tumor cells from *Patched*^{-/-}/*P53*^{-/-} mice points to genomic instability as a driving force in medulloblastoma formation. This idea is supported by two other mouse models of medulloblastoma in which important genes governing DNA repair were knocked out. One mouse was a double knock-out of *p53* and *Lig4*, the gene encoding DNA ligase IV, an enzyme that is critical for repairing double-strand DNA breaks.³⁵ The second mouse model was created by knocking out *p53* in combination with the gene encoding poly(adenosine 5'-diphosphate-ribose) polymerase, a molecule that senses DNA strand breaks. Activation of poly(adenosine 5'-diphosphate-ribose) polymerase is an early response to DNA damage.⁶⁰ The *Lig4*^{-/-} mice die in early embryogenesis because of widespread neural apoptosis.³⁴ Additional loss of *p53* rescues normal neural development in *Lig4*^{-/-} mice, but it predisposes them to tumorigenesis. Loss of *p53* may be required to generate medulloblastomas in mice with defective DNA repair systems because *p53* functions normally to arrest cell cycle progression after DNA damage. In the absence of this control mechanism, DNA strand breaks in genes controlling cell growth are perpetuated because damaged cells are not eliminated via apoptosis.

Activation of the PI3K Signaling Pathway by IGF-II Synergizes With Shh to Enhance Medulloblastoma Formation

Molecular oncology studies in humans and mice indicate that cell survival signals from IGFs are additional, causative factors for medulloblastoma. For example, transcriptional profiling of human medulloblastomas by using DNA microarrays has shown increased expression of IGF-II.⁴⁵ Homozygous deletion of the *Igf2* gene completely eliminates medulloblastoma formation in *Patched*^{-/-} mice, suggesting a requirement for IGF-II or downstream signaling molecules in Shh-induced medulloblastoma formation.²²

The physiological effects of IGF-II are mediated by IGF-IR, a receptor tyrosine kinase, activation of which triggers an important signal transduction pathway known as the PI3K pathway.³³ Salient features of the PI3K signaling pathway are illustrated schematically in Fig. 2B. In brief, phosphorylation of IRS-1 by activated IGF-IR creates a docking site for the regulatory subunit of PI3K. Consequent activation of the catalytic subunit of PI3K generates the membrane phospholipid, phosphatidylinositol (3,4,5)-trisphosphate, which recruits the serine/threonine kinase Akt (protein kinase B) to the plasma membrane, where phosphorylation of this molecule activates the Akt kinase.¹⁰

The first clues that IGF-mediated PI3K signaling might play a role in medulloblastoma formation came from studies of medulloblastoma cell lines derived from transgenic mice expressing JC virus T antigen.⁶² These cell lines showed increased expression of IRS-1, and exogenous IGF-I stimulated them to proliferate in culture. Furthermore, immunohistochemical studies of primary human medulloblastomas have shown expression of IRS-1 and phosphorylated (activated) IGF-IR.¹⁵

To evaluate the role of IGF signaling in medulloblastoma formation in vivo, we used the RCAS/*tv-a* mouse system, which allows postnatal gene transfer in a cell-type-specific

manner.^{16,29} The RCAS/*tv-a* system (Fig. 3) uses an avian retroviral vector, called RCAS, derived from avian leukemia virus, ALV (subgroup A), and a transgenic mouse line (*Ntv-a*) that produces TV-A (the receptor for ALV-A) under control of the *Nestin* gene promoter. Nestin is an intermediate filament protein expressed by neuronal and glial progenitor cells.³⁶ When RCAS retroviruses infect mammalian cells, virus replication does not occur. Instead, retroviral RNA is reverse-transcribed to generate a complementary copy of DNA (proviral DNA), which then integrates into the host cell genome. The integrated gene is expressed as a spliced message under control of the constitutive retroviral promoter LTR, or long terminal repeat. This system makes it possible to transfer and express exogenous genes in nestin-expressing neural progenitor cells (the presumed cells of origin for medulloblastoma) inside the brains of live mice. Furthermore, combinations of genes can be transferred to individual cells by infection with multiple RCAS vectors carrying different genes. This makes it possible to determine whether different oncogenes cooperate to induce tumor formation in vivo.

Using the RCAS/*tv-a* system, we transferred genes encoding IGF-II and Shh, alone and in combination, to nestin-expressing neural progenitors by injecting virus producer cells into the cerebella of newborn *Ntv-a* mice. Combined expression of IGF-II + Shh induced medulloblastomas in 39% of mice, a significantly higher incidence than in those with Shh alone, which induced tumors in 9 to 15% of animals.⁴⁸ Tumors induced by Shh or Shh + IGF-II showed increased expression of activated IGF-I receptor as well as downstream signaling proteins, IRS-1 and Akt. This finding suggests that sustained IGF signaling is required to maintain growth of tumors once their development is initiated by Shh.

To demonstrate a functional role for PI3K signaling in medulloblastoma formation, we transferred an activated, transforming allele of *Akt*. We found that Akt had an enhancing effect on Shh-induced medulloblastoma formation comparable to that of IGF-II (48% tumor incidence).⁴⁸ Nevertheless, neither IGF-II nor Akt was sufficient for tumor induction when expressed independently. These results with modeling medulloblastoma by using the RCAS/*tv-a* system indicate that combined activation of the Shh/*Patched* and IGF/PI3K signaling pathways is an important oncogenic mechanism leading to medulloblastoma formation.

Loss of Cell Cycle Control: a Driving Force in Medulloblastoma Oncogenesis

Mouse models of medulloblastoma have shown that an essential ingredient for tumor formation is a signal that drives GNPs through the cell cycle. The presence of Shh stimulates progression of GNPs through the G₁ phase of the cell cycle by increased expression of D-type cyclins, phosphorylation (and hence inactivation) of the Rb gene product, and activation of E2F transcription factors.³² Direct evidence for the role of Rb signaling in the genesis of medulloblastoma came from conditional knock-out of the *Rb* gene in mice that were homozygous defective for *p53*.³⁹ To generate these mice, the investigators drove expression of Cre recombinase via the promoter for GFAP, which is normally expressed in mature astrocytes. The fact that tumor formation and GFAP-driven expression of the *Cre*

Fig. 3. Schematic drawings showing the RCAS/*tv-a* system. The ALV receptors (TV-A) are produced in nestin-expressing neural progenitor cells in *Ntv-a* transgenic mice. The RCAS vectors carrying an exogenous gene (Gene X) replicate in avian cells to produce live virus capable of infecting TV-A⁺ mouse cells. Virus production occurs only in avian cells. In mouse cells, the exogenous gene is expressed as a spliced message from which viral genes (*gag*, *pol*, *env*) are removed. LTR = long terminal repeat.

transgene both occurred in the EGL in these mice further supports the idea that GNPs are the cells of origin for medulloblastoma. Conditional inactivation of *Rb* was not sufficient, however, to induce tumors in mice with normal levels of p53. Cooperative effects of *Rb* and p53 signaling on central nervous system tumor formation were also demonstrated by an earlier, transgenic mouse model expressing SV40 large T antigen, which functionally inactivates both *Rb* and p53. Primitive neuroectodermal tumors developed in the brainstem in these mice.¹⁷

Another set of cell cycle control proteins that plays an inhibitory role in medulloblastoma formation is the *Ink4* family (p16^{Ink4a}, p15^{Ink4b}, p18^{Ink4c}, and p19^{Ink4d}). These proteins normally prevent cells from entering the DNA synthetic phase (S phase) of the cell cycle.⁵⁶ They do this by blocking phosphorylation of the *Rb* protein, thereby maintaining it in its active, growth-inhibitory state. The important role of *Ink4a* proteins in restraining cell cycle progression predicts that inactivation of these proteins might promote tumor formation in mice. Indeed, mice that are homozygous defective for both *Ink4c* and *P53* develop medulloblastomas, as well as hemangiosarcomas, lymphomas, and pituitary adenomas.⁷¹

Mouse Models of Medulloblastoma can Guide Development of Molecular Targeted Therapy

A discouraging result of molecular cancer research has

been the discovery that cancer cells have many defective growth control programs, which makes selecting the best targets for therapeutic intervention an overwhelming task. How can mouse models be exploited to simplify this task? The central role of activated Shh/Patched signaling in the initiation and maintenance of medulloblastoma in mice has exposed a potential Achilles heel of this tumor for therapeutic intervention. Small-molecular-weight compounds that inhibit Shh/Patched signaling have been derived from steroidal alkaloids found in the range plant *Veratrum californicum*.³¹ Veterinary scientists recognized that this plant might contain compounds capable of perturbing neural development after observing that pregnant ewes that ingested *V. californicum* gave birth to lambs with holoprosencephaly, a midline craniofacial deformity whose most striking feature is the presence of a single eye (cyclopia).⁶ In humans, loss-of-function mutations in the *Shh* gene, which would suppress Shh/Patched pathway activity, cause holoprosencephaly.^{4,50} One of the plant alkaloids, cyclopamine, was subsequently shown to inhibit Smoothened, the positively responding component of the Shh/Patched signaling pathway.^{12,58} When medulloblastoma cells derived from *Patched*-deficient mice are exposed to cyclopamine in culture, these cells stop proliferating and differentiate into neurons.⁵

Another exciting step toward pharmacological inhibition of Shh/Patched signaling in medulloblastoma treatment was taken by Romer, et al.,⁵¹ who used the *Patched*^{+/-}/*p53*^{-/-} mouse model of medulloblastoma for preclinical testing of a cyclopamine derivative, HhAntag-691. They showed significant reduction in tumor volume and prolonged tumor-free survival in treated animals. The fact that animals receiving even the highest drug doses showed no toxic side effects has raised hopes of using cyclopamine analogs in human clinical trials.

The fact that activation of the PI3K signaling pathway by IGF-II synergizes with Shh to enhance medulloblastoma formation in mice suggests that pharmacological blockade of the PI3K signaling pathway might be a rational therapeutic approach.⁴⁸ For clinicians, this is an exciting prospect because several nodes along the pathway can be selectively blocked by small molecules.¹¹ The most strategic target for therapeutic intervention might be mTOR, a serine/threonine kinase, activation of which promotes mRNA translation and hence cell cycle progression. The precise biochemical mechanism whereby PI3K signaling activates mTOR is currently the focus of intense research, and is a topic beyond the scope of this review. Nevertheless, mTOR is a phosphorylation substrate for Akt, and therefore functional interference with mTOR can significantly impede PI3K signaling.^{40,55}

Exposure of tumor cells to rapamycin, a macrolide antibiotic drug, markedly suppresses growth of tumor cells in culture by interfering with mTOR's ability to activate mRNA translational machinery.⁷ Rapamycin was first developed as an antifungal drug. It was subsequently found to be a potent immunosuppressor, and this discovery led to approval by the US Food and Drug Administration for the use of rapamycin for preventing rejection of transplanted organs. Rapamycin and several chemical analogs are now in human clinical trials, and evidence is accumulating that they are effective anticancer agents.⁵³ For example, measurable response to the Wyeth Ayerst compound CCI-779 was 44 and 7% in heavily pretreated patients with mantle

cell lymphoma and renal cell carcinoma, respectively.^{2,69} Moreover, Phase I and II cancer trials have shown that these drugs are well tolerated by patients, a surprising result considering the importance of PI3K signaling in maintaining cell growth and survival. Reported toxic effects have been mild, and include cutaneous reactions, transitory myelosuppression, and reversible hepatic dysfunction. Chronic immunosuppression has not been reported with the dose schedules used in current clinical trials. Although mTOR inhibitors have not yet been used in clinical trials for patients with medulloblastoma, the fact that PI3K signaling is frequently activated in mouse and human medulloblastomas, coupled with the need for small-molecular-weight compounds to penetrate the blood–brain barrier and target migratory brain tumor cells, indicates that mTOR is a rational target for medulloblastoma chemotherapy.

Preclinical testing of mTOR inhibitors in rodent xenograft models of several types of human cancers has shown that delay in tumor growth occurs more commonly than tumor regression.^{7,53} This raises the possibility that, for maximum efficacy, rapamycin analogs should be used in conjunction with cytotoxic chemotherapeutic agents or with radiation therapy. Development of the best combinatorial drug regimens will require mouse models of human tumors that accurately reflect the molecular pathogenesis of human disease.

CONCLUSIONS

Models of human medulloblastoma in genetically engineered mice have shown that aberrant activation of signal transduction pathways that promote proliferation and inhibit differentiation of neural progenitor cells during normal cerebellar development is the proximate cause of medulloblastoma. The Shh/Patched pathway stands out as the principal mediator of tumor formation, with accessory or permissive roles played by IGF-mediated PI3K signaling, IFNs, Myc oncoproteins, and loss of DNA damage repair mechanisms. Mouse models have revealed molecular targets within the Shh/Patched and PI3K signaling pathways for therapeutic intervention, and these models will remain sturdy platforms for preclinical testing of novel chemotherapies.

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