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# THE DEVELOPMENTAL BIOLOGY OF BRAIN TUMORS

Robert Wechsler-Reya and Matthew P. Scott
*Departments of Developmental Biology and Genetics and Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California, 94305-5329; e-mail: rwreya@cmgm.stanford.edu; scott@cmgm.stanford.edu*

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■ **Abstract** Tumors of the central nervous system (CNS) can be devastating because they often affect children, are difficult to treat, and frequently cause mental impairment or death. New insights into the causes and potential treatment of CNS tumors have come from discovering connections with genes that control cell growth, differentiation, and death during normal development. Links between tumorigenesis and normal development are illustrated by three common CNS tumors: retinoblastoma, glioblastoma, and medulloblastoma. For example, the retinoblastoma (Rb) tumor suppressor protein is crucial for control of normal neuronal differentiation and apoptosis. Excessive activity of the epidermal growth factor receptor and loss of the phosphatase PTEN are associated with glioblastoma, and both genes are required for normal growth and development. The membrane protein Patched1 (Ptc1), which controls cell fate in many tissues, regulates cell growth in the cerebellum, and reduced Ptc1 function contributes to medulloblastoma. Just as elucidating the mechanisms that control normal development can lead to the identification of new cancer-related genes and signaling pathways, studies of tumor biology can increase our understanding of normal development. Learning that Ptc1 is a medulloblastoma tumor suppressor led directly to the identification of the Ptc1 ligand, Sonic hedgehog, as a powerful mitogen for cerebellar granule cell precursors. Much remains to be learned about the genetic events that lead to brain tumors and how each event regulates cell cycle progression, apoptosis, and differentiation. The prospects for beneficial work at the boundary between oncology and developmental biology are great.

## INTRODUCTION

More than a century ago, in trying to understand the origin of cancer, the German pathologist Julius Cohnheim noted the similarities between tumors and embryonic cells (Rather 1978). Both types of cells are morphologically simple, both can differentiate into cells of various shapes and sizes, and, most importantly, both have the capacity for extensive growth. Based on these observations, Cohnheim proposed that cancer might originate from embryonic cells. During development,

he suggested, more cells might be produced than are necessary for the construction of a particular tissue. These excess cells (which he called "embryonic rests") would persist in that tissue until later in life and, because they were embryonic in origin, would retain the capacity for growth. Tumorigenesis, then, would result from abnormal activation of a growth program in these cells.

Although some of the details of Cohnheim's theory differ from our current understanding of tumorigenesis, his recognition of the relationship between development and cancer was remarkably prescient. In the last few years, striking parallels between cancer and normal development have begun to emerge. Molecules originally discovered based on their role in cancer—oncogenes and tumor suppressors—have now been shown to function as fundamental regulators of cell growth and differentiation during development. Similarly, genes identified as regulators of pattern formation in invertebrates and vertebrates have been implicated in a variety of human cancers. In light of these findings, the fields of developmental biology and tumor biology, which were distinct for many years, have begun to converge and inform each other in fascinating ways.

In the context of the nervous system, the interface between development and cancer is the study of brain tumors. Although brain tumors are rare compared with many other types of cancer, they are not uncommon; about 20,000 new primary brain tumors are diagnosed in the United States each year. That they often affect children and young adults and frequently lead to mental impairment or death makes them particularly devastating. This area of investigation offers a unique opportunity for synergistic interactions between basic scientists and clinicians. For developmental neurobiologists, brain tumors represent a kind of natural genetic screen, which can provide valuable information about genes that regulate proliferation, differentiation, and death in the nervous system. For neurooncologists, exploring the mechanisms that control cell fate in the developing nervous system can yield important insights into the mechanisms of tumorigenesis and potentially yield new targets for therapy.

We focus here on three of the most common and widely studied central nervous system (CNS) tumors: retinoblastoma, glioblastoma, and medulloblastoma. Each of these tumors arises from a distinct cell type, and each has important implications for our understanding of how neurons and glia grow, differentiate, and die. For each tumor, we (*a*) briefly review what is known about normal development of the cell type from which the tumor is thought to originate, (*b*) highlight the molecules implicated in transformation of this cell type, and (*c*) discuss the insights gained and the questions raised by comparing the mechanisms of development and tumorigenesis.

## RETINAL DEVELOPMENT AND RETINOBLASTOMA

### Overview of Retinal Development

The retina, a highly ordered array of neurons and glia, is optimized for sensing, transducing, and transmitting visual information. It is derived from the rostral neural tube, from a region that evaginates early in embryonic development to form

a pouch called the optic vesicle (Robinson 1991). As the optic vesicle grows and comes into contact with the overlying ectoderm, which will give rise to the lens, it forms a concave structure called the optic cup. Neuroepithelial cells in the optic cup initially undergo symmetric divisions to generate a large pool of retinoblasts. Retinoblasts then begin to divide asymmetrically, producing a variety of neurons and glia that migrate outward to form distinct layers. The mature retina consists of an outer nuclear layer containing photoreceptors (rods and cones), an inner nuclear layer made up of interneurons (horizontal, bipolar, and amacrine cells) and Müller glial cells, and a ganglion layer, containing the retinal ganglion cells whose axons form the optic nerve.

Retroviral lineage-tracing experiments have demonstrated that retinoblasts are multipotent; they each have the capacity to give rise to all of the different neurons and glia in the retina (Holt et al 1988, Turner et al 1990, Wetts et al 1989). The cells that retinoblasts actually generate change during the course of development (Reh 1992, Reh & Kljavin 1989). Initially they produce primarily ganglion cells, cones, and horizontal cells; later they give rise to amacrine cells and rods. During the last phase of retinal development, retinoblasts generate rods, bipolar cells, and Müller glia. This sequential pattern of cell generation is believed to result from changes in cell-cell interactions and soluble factors in the retinoblast microenvironment (Cepko 1999, Reh 1992, Watanabe & Raff 1990).

A variety of soluble and cell-bound factors influence retinoblast growth and differentiation (reviewed in Reh & Levine 1998, Cepko 1999, Levine et al 2000). For example, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factors alpha (TGF-$\alpha$) and beta-3 (TGF-$\beta$3), and Sonic hedgehog (Shh) all induce retinal precursors to enter mitosis. Many of these factors have optimal activity at particular stages of retinal development and regulate not only cell growth but also the types of neurons that retinoblasts generate. Thus, bFGF is a potent mitogen early in embryogenesis and favors production of ganglion cells, TGF-$\alpha$ promotes proliferation of later retinoblasts and causes them to produce more amacrine cells, and TGF-$\beta$3 is most active on postnatal retinoblasts and promotes proliferation of Müller glia. In addition to soluble factors, integral membrane proteins of the Notch and Delta families have been shown to promote growth of retinoblasts and inhibit their differentiation into various cell types (Austin et al 1995, Perron & Harris 2000). The timing and direction of differentiation of each retinoblast is determined by the sum of the signals to which it is exposed at a particular stage of development.

Transcription factors that integrate these signals and control retinal growth and development have also been identified [reviewed by Cepko (1999), Mathers & Jamrich (2000), and Perron & Harris (2000)]. The homeobox gene *pax6* is mutated in the mouse mutant "*small-eye*" and in humans with aniridia (Glaser et al 1992, Quinn et al 1996). *Chx10* is disrupted in the "ocular retardation" mouse (Burmeister et al 1996). *Rx* plays important roles in early eye development, controlling specification of the retinal primordium and growth of retinoblasts in the optic cup (Mathers et al 1997). Loss of any of these homeobox genes leads to severe defects in formation of the retina and surrounding eye structures.

## Retinoblastoma and Identification of the *Rb* Tumor Suppressor Gene

Retinoblastoma is one of the best-studied tumors of the CNS and perhaps the best example of a tumor that has taught us about the molecular mechanisms of development. Retinoblastoma occurs most commonly in children. Among 200 to 300 new cases of retinoblastoma each year, 90% occur before the age of 5 (Brodeur 1995). Untreated retinoblastoma is almost always fatal, but with early detection and treatment most cases can be cured. Unfortunately, treatment often results in loss of vision, and survivors have a relatively high incidence of other tumors.

The cell type from which retinoblastoma originates has been debated. Analysis of primary tumors indicates that the majority of cells have a morphology and antigenic profile reminiscent of photoreceptors (Nork et al 1995, Tajima et al 1994), suggesting transformation of a cell with relatively restricted potential. On the other hand, retinoblastoma cells can differentiate in vitro into cells resembling conventional neurons, photoreceptors, glia, and pigment epithelial cells (Kyritsis et al 1984, 1986; Tsokos et al 1986), consistent with transformation of a more primitive neuroectodermal precursor.

The discovery of the genetic basis of retinoblastoma began with the observation that 30%–40% of cases of retinoblastoma are hereditary. These cases tend to be more severe; often both eyes are affected (bilateral disease) and multiple tumor foci occur in each eye. In the early 1970s, Knudson (Knudson 1971, Knudson et al 1975) compared the age of onset of bilateral (hereditary) vs unilateral (sporadic) retinoblastoma and noted that bilateral cases arise significantly earlier than unilateral ones. Based on the age of onset and estimates of mutation rates, he proposed that bilateral cases result from one genetic event, whereas unilateral cases result from two. His two-mutation (or two-hit) hypothesis suggested that retinoblastomas result from loss or inactivation of both alleles of a gene; in hereditary cases, one mutant allele is inherited and the other is disrupted by somatic mutation, whereas in sporadic cases both alleles are inactivated by somatic events.

The implication of the Knudson model was that complete inactivation of a single gene might result in a tumor. The gene, later called a tumor suppressor, would normally act as a brake on cell growth, and loss of the gene would therefore lead to uncontrolled growth. The concept of a tumor suppressor was based on theoretical data, at a time when limited molecular techniques were available. Cytogenetic studies indicated that many retinoblastoma patients had abnormalities of chromosome 13, and improved molecular techniques made it possible to compare chromosomal changes among patients and narrow down the region that was most commonly affected (Dryja et al 1986). In 1986, the first tumor suppressor gene was isolated and named *Rb-1* (Friend et al 1986). *Rb-1* mutations were found in all patients with hereditary retinoblastoma and in the majority of retinoblastoma patients with nonhereditary disease (Friend et al 1986, Fung et al 1987, Lee et al 1987).

Although *Rb* was first identified for its function in the retina, it is expressed in most tissues. *Rb* encodes a phosphoprotein that plays a critical role in cell cycle

regulation (reviewed in Lipinski & Jacks 1999). Rb regulates the earliest checkpoint in the cell cycle, progression into late $G_1$ phase (Figure 1). Members of the E2F family of transcription factors, whose targets include many genes required for cell cycle progression, normally mediate passage through this checkpoint (DeGregori et al 1997, Nevins et al 1997). In nondividing cells, E2Fs form complexes with Rb, which renders the E2Fs inactive. In response to mitogens, cells increase expression of D- and E-type cyclins, which activate cyclin-dependent kinases (Cdk4 and Cdk2, respectively). Cdks phosphorylate Rb, causing it to dissociate from E2Fs and freeing E2Fs to activate transcription and cell cycle progression. Rb can also associate with a number of viral proteins, including simian virus 40 (SV40), large-T antigen (TAg), adenovirus E1a protein, and papillomavirus E7 protein (DeCaprio et al 1988, Dyson et al 1989, Nevins 1994, Whyte et al 1988). Binding of any of these oncogenic viral proteins dissociates Rb from E2Fs, thus promoting cell cycle progression and tumor formation. TAg and E1A can also associate with the p53 tumor suppressor and with p300/CBP (CREB binding protein) transcriptional coactivators, and this contributes to their transforming activity as well (Giordano & Avantaggiati 1999, Ludlow & Skuse 1995, Snowden & Perkins 1998).

Based on its regulation of cell cycle progression, one function of Rb in tumorigenesis is clear: loss or inactivation of both copies of the Rb gene unleashes E2F proteins to initiate cell cycle progression even in the absence of mitogens. However, the Rb story has become much more complex. First, proteins with structures and functions similar to Rb are made in many cell types. The proteins p107 and p130 resemble Rb, especially in a "pocket domain" that is crucial for binding E2Fs and viral proteins such as TAg, E1A, and E7 (Lipinski & Jacks 1999). The levels of Rb, p107, and p130 differ among cell types and at different stages in the cell cycle, but their binding of E2F proteins and their growth-regulating activities overlap substantially. In addition, a structurally unrelated protein called Necdin, which is expressed at high levels in neurons, shares many properties of the pocket proteins including the ability to bind E2Fs and viral proteins and to suppress cell growth (Aizawa et al 1992, Hayashi et al 1995, Taniura et al 1998, Yoshikawa 2000). Second, Rb regulates cell survival and differentiation in addition to cell cycle progression (Lipinski & Jacks 1999). Much of this information has come from studies of the expression and function of Rb and other pocket proteins in development. Here we focus on roles of the Rb family in neural development.

## Functions of Rb in Neural Development

Rb and the other pocket proteins are widely produced in the developing nervous system (Jiang et al 1997). Rb is found in the ventricular zone, where neuroblasts divide, as well as in regions that contain only postmitotic cells. Expression of *p107* overlaps with that of *Rb*, but it is restricted to proliferating cells in the ventricular zone. *p130* is expressed at low levels in the nervous system throughout embryogenesis. Members of the E2F family are also present in developing neurons,

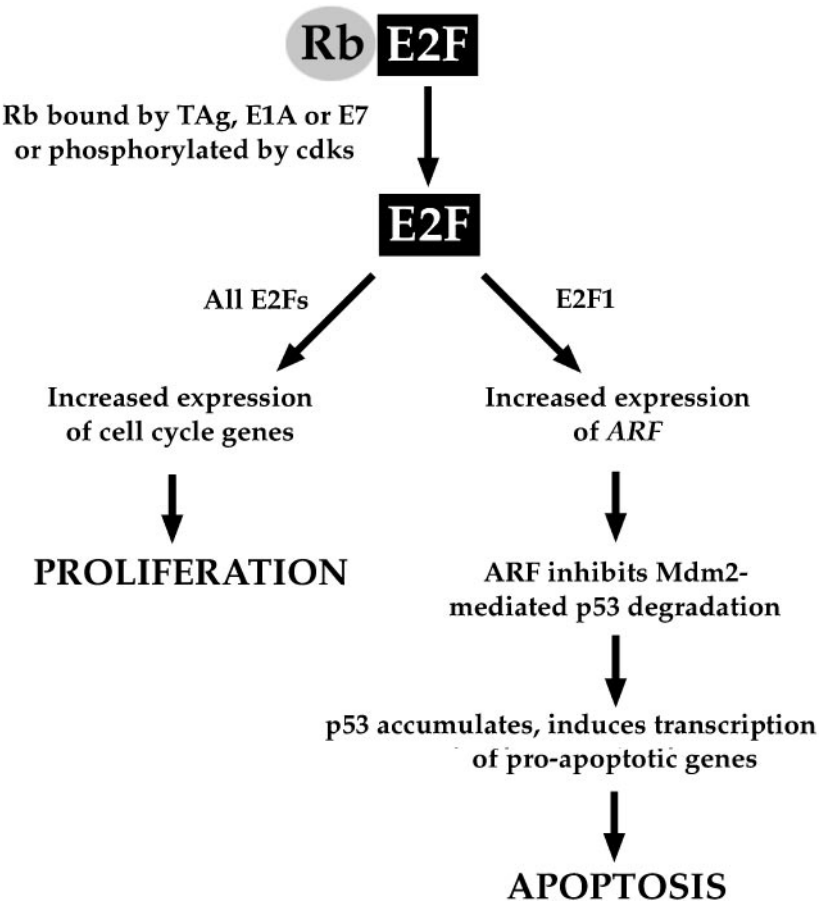


**Figure 1** Regulation of proliferation and apoptosis by the Rb/E2F pathway. In non-dividing cells, E2F transcription factors are bound to the Retinoblastoma (Rb) protein and are thereby rendered inactive. Phosphorylation of Rb by cyclin dependent kinases (cdks) or binding of Rb by viral oncoproteins such as SV40 T antigen (TAg), adenovirus E1A, or papillomavirus E7 leads to dissociation of Rb from E2Fs. E2Fs can then activate transcription of genes necessary for cell cycle progression, allowing cells to proliferate. In some cells, inactivation of Rb can also lead to apoptosis. This may be mediated by E2F1, which can promote transcription of the ARF tumor suppressor. ARF binds to a complex of Mdm2 and p53, and in so doing, prevents the Mdm2-mediated degradation of p53. The resulting accumulation of p53 allows transcription of pro-apoptotic genes, causing cells to undergo apoptosis. The fact that Rb can inhibit apoptosis as well as proliferation means that loss of Rb may lead to cell death rather than tumorigenesis; however, loss of other apoptotic regulators (such as p53) can synergize with loss of Rb to promote tumor formation.

with maximal expression in the ventricular zone (Dagnino et al 1997). This expression pattern suggests that the Rb family might play an important role in regulating proliferation of neuronal precursors.

Studies of mice lacking *Rb* function ("knockout mice") support this notion (Clarke et al 1992, Jacks et al 1992, Lee et al 1992). These mice die between embryonic days 13 (E13) and 15, and they have defects in hematopoietic, lens, and neural development. Abnormal patterns of cell division occur in the central and peripheral nervous systems of homozygous $Rb^{-/-}$ mice (Lee et al 1992, Lee et al 1994). Dividing CNS cells are normally restricted to the ventricular zone, but in *Rb* mutants, cells well outside this region divide. Extra cell division was expected, given the role of Rb in cell cycle regulation. What was not expected was the dramatic neuronal cell death seen in the knockout mice. This death, most prominent in the hindbrain, spinal cord, and sensory ganglia, often affected the ectopic proliferating cells. The surviving neurons in the mutant mice were also not normal; many had abnormal morphology and failed to express differentiation markers such as neuronal $\beta$II tubulin. Similar effects have been seen in some strains of *p130* knockout mice (LeCouter et al 1998). *p107* knockouts do not have significant neuronal defects, but *Rb/p107* double homozygotes die earlier than the *Rb* knockout mice and have even more severe apoptosis in the CNS, which suggests that *p107* and *Rb* may be partially redundant (Lee et al 1996). These results indicate that Rb and other pocket proteins are essential not only for proliferation of neuronal precursors but also for survival and differentiation of postmitotic neurons.

## Rb as a Regulator of Cell Cycle Exit and Survival

The antiapoptotic function of Rb family proteins is most apparent as neurons exit the cell cycle and begin to differentiate. In many cases, increased Rb levels accompany cell cycle exit. For example, Rb and p130 levels increase dramatically in embryonal carcinoma cells that have been induced to differentiate into neurons (Gill et al 1998, Kranenburg et al 1995a, Slack et al 1993). Similarly, in the quail retina, cell cycle exit at E6-E7 is accompanied by a sudden rise in the amount of E2F-1/Rb complexes (Kastner et al 1998). Pocket proteins appear to be necessary for both cell cycle exit and survival. The pocket protein inhibitor E1A both prevents cell cycle exit and increases cell death in cultured retinal precursors (Kastner et al 1998). Similarly, introduction of E1A into embryonal carcinoma cells, cortical neuronal precursors, or striatal stem cells inhibits cell cycle exit and promotes apoptosis (Callaghan et al 1999; Slack et al 1995, 1998).

The contributions of Rb, p107, and p130 to cell cycle exit and survival may differ. Cultured neuronal precursors from *Rb* knockout mice have delayed terminal mitosis, but they survive (Callaghan et al 1999, Slack et al 1998). However, these cells have increased levels of p107 compared with wild-type cells, suggesting that p107 may compensate for Rb in protection from apoptosis. In support of this notion, inactivation of all pocket proteins by expression of E1A does result in increased apoptosis of $Rb^{-/-}$ cells (Callaghan et al 1999). Similar compensation

is presumably behind the increased apoptosis seen in *Rb/p107* double knockouts compared with the *Rb* single knockouts (Lee et al 1996). The molecular basis of apoptosis in $Rb^{-/-}$ mice has been elucidated by crossing these mice to other knockout strains. Apoptosis in the central—but not in the peripheral—nervous system of $Rb^{-/-}$ mice is dependent on E2F1 and p53, because it is dramatically reduced in mice lacking either of these genes (Macleod et al 1996, Tsai et al 1998). It is interesting to note that the ectopic cell proliferation in the *Rb* knockout is not suppressed by p53 deficiency, indicating that apoptosis and cell cycle exit are separately regulated. The involvement of E2F1 and p53 suggests how apoptosis is controlled (Figure 1). Loss of Rb leads to increased E2F1 transcriptional activity. Although E2F proteins can induce cell cycle progression, E2F1 is unique in that it can also promote apoptosis (DeGregori et al 1997, Field et al 1996, Hsieh et al 1997, Kowalik et al 1998, Phillips et al 1997, Qin et al 1994, Wu & Levine 1994). It does this, in part, by inducing expression of the tumor suppressor gene *ARF* (encoded by an alternative reading frame in the *CDKN2A/p16* locus; Bates et al 1998, Zhu et al 1999). ARF binds to the oncoprotein Mdm2 (murine double minute 2) and prevents Mdm2-mediated p53 degradation (Chin et al 1998, Kamijo et al 1998, Pomerantz et al 1998, Zhang et al 1998). The resulting increase in p53 then promotes apoptosis by activating transcription of proapoptotic genes such as *bax*, *fas*, and *killer/dr5* (el-Deiry 1998).

Another potential mediator of apoptosis in *Rb*-deficient mice is a nuclear protein called N5 (Doostzadeh-Cizeron et al 1999). N5 contains a region of sequence similarity to death domain proteins involved in apoptosis. Overexpression of N5 can promote apoptosis in certain cells. Rb can associate with N5 and inhibit N5-induced apoptosis, so loss of Rb might lead to increased apoptosis via N5.

## Rb and Prevention of Cell Cycle Reentry

In addition to ensuring proper cell cycle exit and survival, Rb and other pocket proteins may prevent postmitotic neurons from resuming cell division. The most striking evidence for this comes from studies of transgenic mice in which SV40 TAg is targeted to cerebellar Purkinje cells (Feddersen et al 1992). These mice develop ataxia as a result of Purkinje cell degeneration. The mechanisms of Purkinje cell death in the TAg mice have been studied in detail (Athanasiou et al 1998; Feddersen et al 1995, 1997). TAg expression induces inappropriate cell cycle entry, followed by apoptosis. The apoptosis is dependent on binding of TAg to Rb, because mutant forms of TAg that cannot bind Rb are unable to induce apoptosis. Purkinje cells expressing TAg have elevated E2F in their nuclei, suggesting that E2F is involved in the apoptosis of these cells. Although transgenic mice overexpressing E2F1 in their Purkinje cells do not have Purkinje cell degeneration or ataxia, crossing these mice to the TAg mice results in accelerated Purkinje cell loss and ataxia. Together, these studies suggest that Rb/E2F function may prevent postmitotic neurons from reentering the cell cycle.

## Rb as a Regulator of Neuronal Differentiation

*Rb* knockout mice, in addition to ectopic proliferation and apoptosis, have defects in expression of differentiation markers such as $\beta$II tubulin and the neurotrophin receptors TrkA, TrkB, and p75 (Lee et al 1994). Primary cultures of sensory ganglion cells from *Rb* mutant embryos have reduced neurite outgrowth, even in the presence of appropriate neurotrophins (Lee et al 1994). A role for Rb in differentiation is also suggested by studies of some neuronal cell lines. In these cells, decreased Cdk activity and dephosphorylation of Rb accompany differentiation, and overexpression of Rb or Cdk inhibitors can promote differentiation and neurite outgrowth (Dobashi et al 1995, Kranenburg et al 1995b).

The mechanisms by which Rb promotes differentiation are unknown. One potential mediator of Rb's effects on neurite outgrowth is Cdk5, a kinase that has structural homology to cyclin-dependent kinases but does not control the cell cycle (Lee et al 1997b, Tang et al 1996). Cdk5, in conjunction with its activators p35 and p39, plays a critical role in neuron migration and axon growth. In cultured cortical and hippocampal neurons, overexpression of Cdk5, p35, or p39 stimulates growth of neurites, whereas dominant-negative mutants of Cdk5 inhibit neurite growth (Nikolic et al 1996, Tang & Wang 1996, Xiong et al 1997). Cdk5 can bind and phosphorylate Rb (Lee et al 1997a), so Rb may affect neurite growth by binding to Cdk5.

Another protein that may be involved in Rb-mediated neurite outgrowth is NRP/B (nuclear restricted protein/brain) (Kim et al 1998). NRP/B is a nuclear matrix protein whose expression increases during neuronal differentiation. NRP/B overexpressed in neuroblastoma cells can promote neurite growth. Antisense NRP/B oligonucleotides can inhibit neurite development in primary hippocampal neurons and in PC12 cells. During differentiation of neuroblastoma cells induced by retinoic acid, NRP/B associates with hypophosphorylated Rb. Thus, NRP/B interaction with Rb may be necessary for neuronal differentiation.

## The Limits to Growth: Why Do Rb-Related Tumors Usually Form in the Retina?

It has been almost 15 years since Rb was first isolated. In light of what we have learned about Rb function in neurons and other cells, do we now understand the mechanisms of tumorigenesis in retinoblastoma? One great mystery is why *Rb* mutations in humans cause tumors in the retina but not in the many other cell types in which *Rb* is expressed. *Rb*-heterozygous mice, in contrast to people, do not develop retinoblastoma (Clarke et al 1992, Jacks et al 1992, Lee et al 1992). However, retinal tumors do occur in transgenic mice that overexpress SV40 TAg (which inactivates all pocket proteins) in the retina and in chimeric mice derived from *Rb/p107* knockout embryonic stem cells (al-Ubaidi et al 1992, Robanus-Maandag et al 1998). These studies suggest that in the mouse, loss of Rb does not cause retinoblastoma because other pocket proteins in the retina can compensate.

In people, similar compensation may be absent from the retina but present in other tissues, thus accounting for the focus of human tumors in the retina.

However, pocket protein redundancy may not be the whole story. SV40 TAg can bind and inactivate pocket proteins, but it can also inactivate the p53 tumor suppressor. p53 is necessary for apoptosis, so tumors may arise in TAg transgenic mice because both p53 and pocket protein functions are reduced. To address this, mice expressing the human papillomavirus E7 protein in photoreceptors were generated (Howes et al 1994). E7 shares with TAg the ability to bind pocket proteins, but it cannot bind p53. Strikingly, the E7 mice do not develop retinal tumors but instead exhibit retinal apoptosis. If the E7 mice are crossed to *p53*-knockout mice, they develop tumors. Thus, the status of p53 may determine whether retinoblastoma develops. *p53* mutations have been observed in human retinoblastoma, although they are not common (Emre et al 1996, Kato et al 1996). This suggests that other components of the apoptotic machinery may need to be inactivated for retinoblastoma to develop.

In fact, genes other than *Rb* are likely to play a role in the development of retinoblastoma. The frequency of mouse retinoblastoma induced by overexpression of papilloma virus *E6* and *E7* genes (which together inactivate both Rb and p53) is strain dependent (Griep et al 1998). In families that carry *Rb* mutations, the penetrance of retinoblastoma varies from 20% to 95% (Griep et al 1998, Hamel et al 1993). The majority of retinoblastomas contain at least one other genetic change besides inactivation of *Rb* (Benedict et al 1983, Hamel et al 1993, Kusnetsova et al 1982, Potluri et al 1986, Squire et al 1985). By far the most common changes are extra copies of the long arm of chromosome 1, also seen in other tumors, and extra copies of the short arm of chromosome 6, which are unique to retinoblastoma. Cloning the genes at these loci is likely to provide important insights into the basis of retinoblastoma and the regulation of normal neural development.

## ASTROCYTE DEVELOPMENT AND GLIOBLASTOMA

### Overview of Astrocyte Development

Astrocytes perform diverse functions in the CNS, including regulating neuronal growth and survival (Arenander & de Vellis 1992, Kornblum et al 1998, Richardson 1994), guiding cell migration and axon growth during development (Bentivoglio & Mazzarello 1999, Hatten & Mason 1990, Komuro & Rakic 1998, Mason & Sretavan 1997, Powell et al 1997, van den Pol & Spencer 2000), promoting synapse formation and modulating synaptic transmission (Araque et al 1999, Bacci et al 1999, Pfrieger & Barres 1996, Vesce et al 1999), and orchestrating inflammatory and immune responses during brain infection and injury (Aschner 1998, Montgomery 1994). In most regions of the brain, astrocytes begin to develop later than neurons and are still being generated long after neurons have stopped being produced (Altman 1966; Schubert & Rudolphi 1998; Sturrock 1982, 1987). Many

astrocytes or their precursors retain the capacity for division throughout life. This makes them uniquely susceptible to transformation and is presumably one reason that astrocytic tumors are the most common brain tumors (Collins 1998, Rasheed et al 1999, Salcman 1995).

Astrocytes arise from multipotent neural stem cells (NSCs) (Pringle et al 1998) that have the capacity to self-renew and to produce a variety of classes of neurons, astrocytes, and oligodendrocytes (Davis & Temple 1994, Kalyani et al 1997, Quinn et al 1999, Rao 1999, Reynolds et al 1992). Differentiation along each of these lineages involves generation of progressively more restricted precursor cells (Lee et al 2000). Thus, NSCs give rise to neuron-restricted precursors that produce only neurons (Luskin et al 1993, Mayer-Proschel et al 1997) and glial-restricted precursors, which cannot generate neurons but can produce astrocytes and oligodendrocytes (Rao & Mayer-Proschel 1997, Rao et al 1998). Glial-restricted precursors, in turn, generate even more restricted precursors, which produce either astrocytes or oligodendrocytes but not both. Oligodendrocyte precursor cells and astrocyte precursor cells (APCs) (Mi & Barres 1999, Noble et al 1995, Seidman et al 1997, Tang et al 2000) are presumed to be direct progenitors of glia in the CNS.

Progenitor cell proliferation and astrocytic differentiation are controlled, in part, by extracellular signals (Lee et al 2000). NSCs isolated from various parts of the CNS at various stages of development undergo self-renewal in response to fibroblast growth factors (FGFs) and epidermal growth factors (EGFs) (Ben-Hur et al 1998, Kalyani et al 1997, Quinn et al 1999, Reynolds et al 1992, Weiss et al 1996). EGF may also promote astrocyte differentiation, since infection of progenitors with a retrovirus that increases expression of EGF receptor (EGFR) results in increased generation of astrocytes in vitro and in vivo (Burrows et al 2000, Lillien 1995). Morever, EGFR-knockout mice exhibit delayed astrocyte differentiation and reduced numbers of astrocytes in certain parts of the brain (Kornblum et al 1998, Sibilia et al 1998).

Other signals that regulate differentiation of NSCs into astrocytes include cytokines of the ciliary neurotrophic factor (CNTF)/leukemia inhibitory factor (LIF) family, whose signals are transduced through the gp130 receptor chain and the Jak/Stat signaling pathway (Segal & Greenberg 1996, Touw et al 2000). CNTF and LIF can promote astrocytic differentiation of NSCs and more restricted progenitors in culture (Bonni et al 1997, Hughes et al 1988, Mi & Barres 1999, Park et al 1999, Rajan & McKay 1998), and knockout mice lacking either the LIF receptor or gp130 have severe defects in astrocyte generation (Koblar et al 1998, Nakashima et al 1999a). Bone morphogenetic proteins (BMPs) can also promote astrocyte development (Kawabata et al 1998, Mehler et al 1997). BMPs activate a signaling pathway distinct from that triggered by LIF and CNTF and can promote astrocyte generation on their own or in synergy with LIF (D'Alessandro et al 1994; Gross et al 1996; Mabie et al 1997; Mehler et al 2000; Nakashima et al 1999b, 1999c). Whether CNTF/LIF, BMPs, and EGF act on the same precursors, and whether they generate the same types of astrocytes is not yet clear.

## Glioblastoma

Glioblastoma multiforme is the most common brain tumor in adults (Collins 1998, Salcman 1995). It is a highly malignant tumor that is thought to arise from astrocytes or astrocyte precursors, but the heterogeneity of tumor morphology and behavior (indicated by the term "multiforme") makes conclusions about its origin extremely difficult (Lopes et al 1995). Although retinoblastoma and medulloblastoma can often be treated successfully, glioblastoma is almost invariably fatal. Glioblastomas are often divided into two subtypes: progressive, which arises from lower-grade astrocytic tumors, and de novo, which does not (Collins 1998, Rasheed et al 1999).

The etiology of glioblastoma is complex, and almost always involves mutation or overexpression of multiple genes. Cytogenetically, glioblastomas exhibit losses of portions of chromosomes 6, 9, 10, 13, 17, 22, and Y and amplification or gain of material on chromosomes 7, 12, and 19 (Collins 1998, Rasheed et al 1999). Some of the relevant genes have been identified (Table 1), and they include growth factor receptors (e.g. EGFR), components of the cell cycle machinery (Rb, cdk4, and the cdk inhibitor p16), and regulators of apoptosis (p53, mdm2, ARF, and PTEN).

## Epidermal Growth Factor Receptor Gene Amplification

Amplification of the *EGFR* gene was among the first genetic abnormalities to be linked to glioblastoma (Libermann et al 1985a, 1985b). *EGFR* amplification

**TABLE 1** Genetic events that correlate with glioblastoma

| Chromosome | Amplified (+) or lost (−) | Candidate genes |
|---|---|---|
| 6 | − | ? |
| 7 | + | *EGFR* |
| 9 | − | *CDKN2A/p16*, *CDKN2B/p15*, *ARF* |
| 10 | − | *PTEN*, *DMBT1*, *LGI1*, *MxiI*, h-*neu* |
| 12 | + | *CDK4*, *SAS*, *MDM2*, *GLI*, *GAS41* |
| 13 | − | *Rb* |
| 17 | − | *p53* |
| 19 | + | ? |
| 22 | − | *NF2?* |
| *Y* | − | ? |

[1]Abbreviations: EGFR, epidermal growth factor receptor; CDKN2A/p16, cyclin dependent kinase inhibitor 2A; CDKN2B/p15, cyclin-dependent kinase inhibitor 2B; ARF, tumor suppressor encoded by alternative reading frame of CDKN2A/p16 exon 2; PTEN, phosphatase/tensin homolog on chromosome 10; DMBT1, deleted in malignant brain tumors 1; LGI1, Leucine-rich gene-Glioma Inactivated 1; Mxi1, Max interactor 1; h-*neu*, human homolog of Drosophila *neuralized* gene; CDK4, cyclin dependent kinase 4; SAS, sarcoma amplified sequence; MDM2, murine double minute 2; GLI, glioblastoma amplified gene; GAS41, glioma amplified sequence 41; Rb, retinoblastoma; p53, 53-kilodalton tumor suppressor protein; NF2, neurofibromatosis 2. "?" refers to chromosomal deletions or amplifications for which no strong candidates have been identified.

occurs in 40%–50% of glioblastomas and usually results in elevated levels of EGFR expression. The fact that many glioblastomas contain at least one ligand capable of activating the receptor (Ekstrand et al 1991, Mishima et al 1998) suggests that in some cases, tumor growth might be promoted by autocrine or paracrine stimulation of the EGFR-signaling pathway. However, another view of the significance of *EGFR* amplification came from sequencing of the amplified EFGR genes, which revealed that they are often mutated or rearranged to generate a protein that lacks part of the extracellular domain (Ekstrand et al 1992, Sugawa et al 1990, Wong et al 1992). The mutant EGFRs cannot bind ligand, but they have constitutive tyrosine kinase activity, promote increased growth of tumor cells in vitro, and enhance tumorigenicity of glioblastoma cells that are transferred into mice (Ekstrand et al 1994, Hoi Sang et al 1995a, Nagane et al 1998, Nishikawa et al 1994). Furthermore, overexpression of mutant EGFRs in astrocytes (or their precursors) can promote development of glioblastoma (Holland et al 1998). Thus, in glioblastoma cells EGFR signaling may be activated in a ligand-dependent or -independent manner.

The activation of EGFR in a large percentage of glioblastomas suggests an important role for EGFR in astrocyte development. Recent studies of mice lacking EGFR function support this idea. The mice often die before birth, but in some genetic backgrounds, they are able to survive for $\leq$3 weeks after birth (Sibilia & Wagner 1995, Threadgill et al 1995). In the surviving mice the effects on brain development are striking: a significant reduction in the number of glial fibrillary acidic protein-expressing astrocytes in many brain regions, and massive degeneration of neurons in the frontal cortex, olfactory bulb, and thalamus (Kornblum et al 1998, Sibilia et al 1998). Because the glial defects appear before the neuronal degeneration and because many of the affected neurons do not normally express EGFR, some of the neuronal death observed may result from the loss of trophic support from glia. In any case, EGFR signaling appears to be critical for normal development of astrocytes.

EGFR can be activated by a number of ligands, including EGF, TGF-$\alpha$, amphiregulin/schwannoma-derived growth factor, betacellulin, epiregulin, and heparin-binding EGF (Khazaie et al 1993, Kimura et al 1990, Komurasaki et al 1997, Prigent & Lemoine 1992, Riese et al 1996, Shoyab et al 1989). EGFR ligands have different affinities for EGFR and other receptors, yet the consequences of their binding to EGFR, at least in astrocytes, appear to be similar. At least four ligands—EGF, TGF-$\alpha$, amphiregulin, and heparin-binding EGF—promote proliferation of astrocytes in culture (Kimura et al 1990; Kornblum et al 1998, 1999; Leutz & Schachner 1981; Simpson et al 1982). Although glioblastomas are often presumed to arise from cells committed to the astrocyte lineage, some tumors may originate from less differentiated progenitors (Noble et al 1995, Shoshan et al 1999). In this regard, it is worth noting that EGFR ligands can also promote growth of astrocyte precursors (Ben-Hur et al 1998, Seidman et al 1997) and of multipotent CNS stem cells that give rise to neurons and glia (Kornblum et al 1999, Reynolds et al 1992). Thus, EGFR activation may contribute to glioblastoma by promoting proliferation of astrocytes or their precursors.

In addition to their growth-inducing effects, EGFR ligands promote process extension and migration in both normal astrocytes and astrocytic tumor cells (Faber-Elman et al 1996, Hoi Sang et al 1995b, Rabchevsky et al 1998, Westermark et al 1982). EGFR activation may therefore also contribute to tumor malignancy by promoting invasion and metastasis (Nishikawa et al 1994). Perhaps this explains why EGFR amplification and rearrangement are most commonly observed in high-grade, malignant astrocytic tumors (Collins 1998, Rasheed et al 1999). Whatever its mechanisms of action, EGFR activation in glioblastoma is extremely potent and has therefore become the target of a variety of pharmacologic, immunologic, and gene-transfer-based therapies (Halatsch et al 2000, Pfosser et al 1999, Pu et al 2000, Tian et al 1998).

## Impaired Control of Cell Cycle and Apoptosis

Disruption of the cell cycle machinery also contributes to glioblastoma. Changes in the genes encoding cyclins, cdks, cdk inhibitors, and Rb have been observed at high frequency in glioblastomas (Biernat et al 1998, Ichimura et al 1996, Schmidt et al 1994, Simon et al 1999). Altogether, two-thirds of glioblastomas have abnormalities in one of these genes (Collins 1998, Rasheed et al 1999). Homozygous deletions or mutations of chromosome 9, in a region that encodes the cdk inhibitors CDKN2A/p16 and CDKN2B/p15, are found in 30%–40% of tumors. At least 14% of tumors have inactivated both copies of the *Rb* gene on chromosome 13. About 15% of glioblastomas have amplifications of a segment of chromosome 12 that includes the *cdk4* gene. A small number of tumors have amplification and overexpression of *cyclin D1* or *cyclin D3* (Buschges et al 1999). The different cell cycle machinery changes are rarely seen in the same tumor, so any one of them may be sufficient for loss of cell cycle control.

Many cells that enter the cell cycle inappropriately, for example as a result of loss of Rb, subsequently undergo apoptosis (Lipinski & Jacks 1999). Thus, tumor progression may require not only cell cycle dysregulation but also loss of the ability to die. In glioblastoma this is often achieved by disruption of the p53 pathway (Biernat et al 1998, Ichimura et al 2000, Rasheed et al 1999, Simon et al 1999). At least 25% of glioblastomas have mutations or deletions of the p53 gene itself, and another 5%–12% have amplifications of the locus on chromosome 12 that encodes Mdm2, which promotes degradation of p53. ARF, which inhibits Mdm2-mediated p53 degradation, is generated by alternative splicing of a transcript from the *CDKN2A/p16* locus on chromosome 9. This locus is deleted or mutated in 30%–40% of glioblastomas, resulting in loss of functional ARF and decreased levels of p53. Overall, about 75% of glioblastomas have reduced ability to undergo apoptosis due to p53-related lesions (Ichimura et al 2000). The critical importance of *Rb* and *p53* defects in glioblastoma has raised hopes of treating tumors using gene therapy directed at components of the Rb and p53 pathways (Fueyo et al 1998, 1999; Gomez-Manzano et al 1999; Lang et al 1999).

## PTEN: A Multifunctional Tumor Suppressor

A significant advance in the understanding of glioblastoma etiology came with the discovery of the tumor suppressor gene *PTEN* (Besson et al 1999, Cantley & Neel 1999, Di Cristofano & Pandolfi 2000). A large percentage of glioblastomas have part of the long arm of chromosome 10 deleted (Rasheed et al 1992). This region is also frequently deleted in many other tumors, including breast, prostate, and endometrial carcinoma and melanoma (Cantley & Neel 1999, Di Cristofano & Pandolfi 2000). In 1997, three groups independently cloned a gene that was eliminated by the deletion (Li & Sun 1997, Li et al 1997a, Steck et al 1997). The gene was called *PTEN* (phosphatase/tensin homolog on chromosome 10), MMAC (mutated in multiple advanced cancers), or TEP-1 (TGF$\beta$- regulated and epithelial cell-enriched phosphatase 1). Both alleles of *PTEN* are inactivated in $\sim$30% of glioblastomas. *PTEN* is also the target of mutation in three rare inherited disorders: Cowden disease (CD), Lhermitte-Duclos disease, and the Bannayan-Zonana syndrome (Liaw et al 1997; Marsh et al 1997, 1998). Patients with these disorders develop widespread benign tumors (harmartomas) in the skin, mouth, thyroid, breast, intestine, and other tissues. They also have an increased risk of breast and thyroid cancer, cerebellar tumors, and mental retardation. The association of PTEN mutations with sporadic as well as inherited tumor syndromes suggested that PTEN might act as a regulator of cell growth, differentiation, or survival, but its mechanism of action was initially unclear.

***PTEN Protein: Structure and Function*** The PTEN protein has sequences similar to protein tyrosine phosphatases (Cantley & Neel 1999, Li & Sun 1997, Li et al 1997a, Steck et al 1997), specifically dual-specificity phosphatases which can dephosphorylate both tyrosine and threonine/serine residues (Neel & Tonks 1997, Tonks & Neel 1996). The significance of the sequence similarity was reinforced by mutations found in most tumors, which cluster in the putative phosphatase domain (Ali et al 1999, Marsh et al 1998). Surprisingly, in vitro assays indicated that PTEN was unable to dephosphorylate many conventional protein tyrosine phosphatase substrates (Li et al 1997b, Myers et al 1997). The proteins it could dephosphorylate did not appear to be critical for PTEN function because some disease-associated PTEN mutant proteins could still dephosphorylate them (Furnari et al 1998; Myers et al 1997, 1998). These observations suggested that PTEN might have a biological function that did not depend on protein phosphatase activity.

That function became clear with the discovery that PTEN is a more effective phosphatase for lipids than it is for proteins (Maehama & Dixon 1998, Myers et al 1998). One important reaction catalyzed by PTEN is the conversion of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (4,5)-bisphosphate (PIP2). This ability placed PTEN in a well-characterized signaling pathway linking growth factor and extracellular matrix signaling to cell survival (Downward 1998, Marte & Downward 1997, Stambolic et al 1999) (Figure 2).

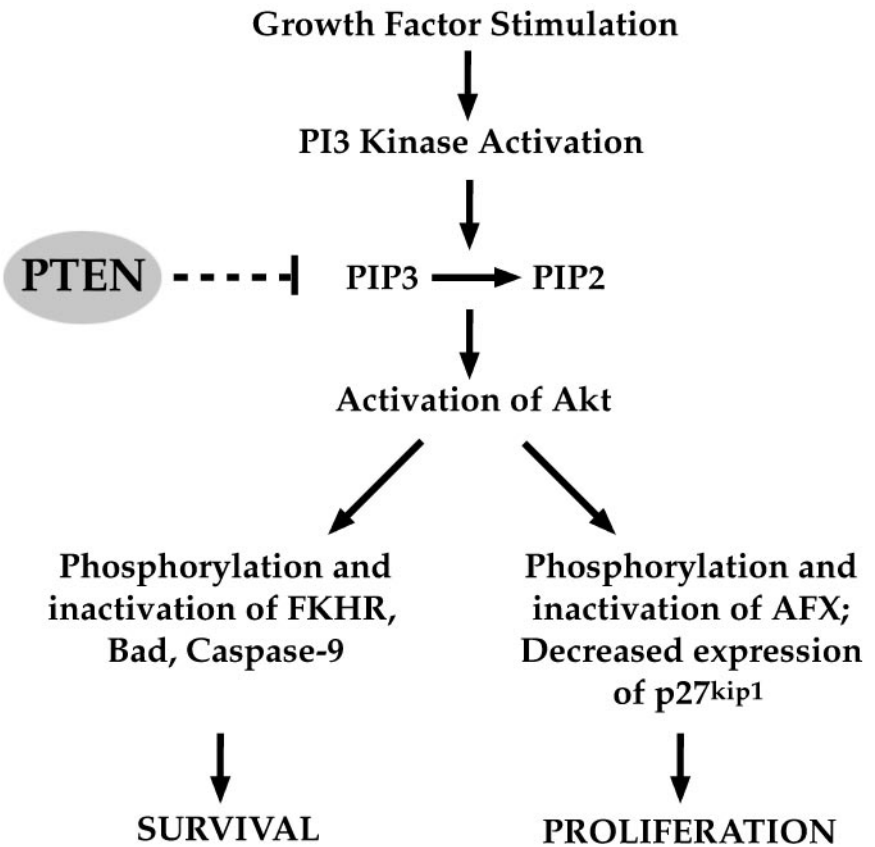


**Figure 2** Loss of PTEN promotes growth and survival by activating the PI3 kinase/Akt signaling pathway. In normal cells, growth factor stimulation causes activation of phosphatidylinositol-3′ kinase (PI3 Kinase), which catalyzes with conversion of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) into phosphatidylinositol (4,5)-bisphosphate (PIP2). Membrane-associated PIP2 attracts and activates the Akt kinase, which promotes survival by phosphorylating and inactivating pro-apoptotic proteins such as the transcription factor FKHR, the Bcl2-like protein Bad, and the cysteine protease caspase-9. Akt activation also promotes cell growth by phosphorylating and inactivating the transcription factor AFX, which would otherwise induce expression of the cdk inhibitor $p27^{kip1}$. PTEN is a lipid phosphatase that blocks the activation of Akt by converting PIP3 to PIP2 and thereby promotes cell cycle arrest and apoptosis. Loss of PTEN function increases cell growth and survival and can contribute to glioblastoma.

Growth factors activate phosphatidylinositol-3′ kinase (PI3K), which increases cellular levels of PIP3. PIP3 serves as a second messenger that attracts proteins containing pleckstrin homology domains to the plasma membrane. One such protein is the serine-threonine kinase Akt (also called protein kinase B), which binds to PIP3 and becomes activated. Akt then phosphorylates and inactivates a number

of proteins necessary for apoptosis (e.g. the Bcl2-like protein Bad, the apoptotic effector caspase 9, and the forkhead rhabdomyosarcoma transcription factor (FKHR), thereby promoting cell survival (Brunet et al 1999, Cardone et al 1998, Datta et al 1997, del Peso et al 1997). In this scheme, reduction of cellular PIP3 levels by PTEN would reduce Akt activation and make cells more likely to undergo apoptosis. Loss of PTEN would have the opposite effect: cells lacking wild-type PTEN would be resistant to apoptosis and would be much more likely to form a tumor.

***PTEN Regulates Cell Survival, Growth, and Adhesion*** The function of PTEN in the PIP3 pathway has now been validated by many studies. For example, fibroblasts from PTEN-deficient mice have elevated levels of PIP3, constitutively activated Akt and resistance to induction of apoptosis by a number of different stimuli (Stambolic et al 1998). Introduction of wild-type PTEN into these cells restores normal PIP3 levels and Akt activity and promotes apoptosis. Similarly, epithelial cells transfected with *PTEN* undergo apoptosis, and this can be inhibited by cotransfection of *Akt* (Li et al 1998). These findings indicate that PTEN is a regulator of the PI3K-Akt signaling pathway and is necessary to maintain the sensitivity of cells to apoptosis.

The loss of PTEN observed in many glioblastomas is consistent with this model in most respects. Glioblastoma cells containing mutant PTEN have abnormally high levels of PIP3 and elevated Akt activity, and expression of wild-type PTEN in these cells normalizes both of these parameters (Davies et al 1998, Haas-Kogan et al 1998). However, there is an important discrepancy between predictions of the model and the observed effects of PTEN in glioblastoma cells. Rather than inducing apoptosis, introduced PTEN causes most cells to arrest in the G1 phase of the cell cycle (Davies et al 1998, Furnari et al 1997, Tian et al 1999). Cells expressing exogenous PTEN have increased glial fibrillary acidic protein and develop long cytoplasmic processes, characteristics of astrocyte differentiation (Adachi et al 1999, Tian et al 1999). Glioblastoma cells do undergo apoptosis if they are prevented from binding to a substrate—a phenomenon termed "anoikis" (Davies et al 1998, Tamura et al 1999a).

The mechanisms of PTEN-mediated growth arrest are still not fully understood, but some potential mediators of the effect have recently been identified (Figure 2). The process requires the phospholipid phosphatase activity of PTEN (Furnari et al 1998) and appears to involve regulation of Akt because a constitutively active form of Akt can override PTEN-induced growth arrest (Ramaswamy et al 1999). An important substrate of Akt in this pathway may be the forkhead transcription factor AFX (acute lymphocytic leukemia fusion, X chromosome) (Kops et al 1999, Medema et al 2000, Takaishi et al 1999). AFX can induce expression of the cdk inhibitor $p27^{kip1}$, which promotes cell cycle arrest by inactivating cyclin E/cdk2 complexes. Phosphorylation by Akt prevents AFX from entering the nucleus and inducing $p27^{kip1}$ and thereby allows cells to proliferate. Since PTEN inhibits Akt activation, it is expected to increase AFX function and $p27^{kip1}$ expression. Indeed,

introduction of exogenous PTEN causes increased production of $p27^{kip1}$ in various cell types (Cheney et al 1999, Lu et al 1999, Sun et al 1999, Wu et al 2000a). This results in reduced phosphorylation of Rb, which in turn prevents cell cycle progression. In other words, PTEN inhibits growth by promoting activation of Rb, and loss of PTEN contributes to tumorigenesis, at least in part, by reducing Rb function (Paramio et al 1999).

In addition to its function in cell cycle control, PTEN can regulate cell adhesion and migration. PTEN contains extensive homology to tensin, a protein that interacts with actin filaments at focal adhesions (Li et al 1997a, Steck et al 1997). PTEN also contains a sequence that binds to PDZ domains, which are frequently involved in assembling multiprotein complexes at membrane/cytoskeletal interfaces (Wu et al 2000b). These features raise the possibility that PTEN associates with and regulates phosphorylation of cytoskeleton-associated molecules.

A number of studies indicate that PTEN can regulate cell shape and movement, and that this function depends primarily on its ability to dephosphorylate proteins rather than lipids. Although PTEN is inefficient at dephosphorylating most proteins, among the substrates it can act on are focal adhesion kinase (Fak) and the SH2-containing adaptor protein Shc. Fak and Shc mediate signal transduction by integrins and regulate cell adhesion and migration (Gu et al 1999; Tamura et al 1998, 1999b). Overexpression of PTEN inhibits cell spreading and cell migration induced by integrins, whereas reduction of PTEN levels by using antisense oligonucleotides has the opposite effect (Tamura et al 1998). The inhibitory effects of PTEN can be blocked by overexpression of Fak or Shc, suggesting that these substrates may be important mediators of PTEN's effects. Interestingly, the types of cell movement mediated by Fak and Shc appear to be different; Fak induces extensive reorganization of the actin cytoskeleton, formation of focal adhesions, and directional migration, whereas Shc causes less actin reorganization and more random cell movement (Gu et al 1999). Both forms of movement are inhibited by PTEN. Thus, cells lacking functional PTEN would be expected to display increased migration and an increased tendency to undergo metastasis. In keeping with this notion, PTEN mutations are found in glioblastoma but not in most lower-grade (less metastatic) astrocytic tumors.

The interaction of PTEN with Fak also provides a rationale for the ability of PTEN to induce growth arrest under some circumstances and apoptosis or anoikis under others (Davies et al 1998, Lu et al 1999, Tamura et al 1999b). Fak is normally phosphorylated in response to integrin ligation at sites of cell adhesion. This phosphorylation activates Fak, which in turn promotes survival by activating the PI3K/Akt-signaling pathway. When normal cells are prevented from attaching to the extracellular matrix, Fak becomes dephosphorylated, survival signaling is reduced, and cells undergo programmed cell death (anoikis). However, in cancer cells lacking wild-type PTEN, Fak cannot be effectively dephosphorylated upon cell detachment. Persistent phosphorylated Fak results in sustained PI3-K and Akt activity, and prolonged cell survival. Thus, loss of PTEN helps make tumor cells

anchorage-independent. Overexpression of PTEN inhibits cell growth by inducing $p27^{kip1}$, but it also makes cells anchorage dependent by inhibiting the PI3K/Akt pathway. If cells are allowed to attach to the extracellular matrix, the first pathway predominates, and they undergo cell cycle arrest. If they are unable to attach, the second pathway takes over, and they undergo anoikis. In this regard, PTEN can be seen as a critical integrator of cell adhesion, growth, and survival.

***Role of PTEN in Normal Development and Tumorigenesis*** PTEN is expressed throughout the embryo, and has critically important functions in early embryonic development (Luukko et al 1999, Podsypanina et al 1999). Three different lines of *PTEN*-knockout mice have been established, and all exhibit early (6.5–9.5 days of gestation) embryonic lethality (Di Cristofano et al 1998, Podsypanina et al 1999, Suzuki et al 1998). Homozygous *PTEN*-knockout mice apparently die because they fail to establish normal connections with the maternal circulation (Suzuki et al 1998). At the time of death they also have disorganized germ layers and severe overgrowth of cephalic and caudal regions. Embryonic stem cells derived from these mice are impaired in their ability to differentiate into endoderm, ectoderm, and mesoderm in vitro and after implantation into normal blastocysts (Di Cristofano et al 1998).

PTEN also functions at later stages of development, as an important regulator of proliferation and survival. These functions have been investigated using heterozygous ($PTEN^{+/-}$) mice and using chimeras made with $PTEN^{+/-}$ embryonic stem cells (Di Cristofano et al 1998, Podsypanina et al 1999, Suzuki et al 1998). Both types of mice are viable and fertile, but they have marked hyperplasia in multiple tissues including the colon, skin, testes, and prostate. Full-blown tumors of the colon, testes, prostate, thyroid, liver, and immune system (leukemias and lymphomas) occur in 12%–15% of the mice. These tumors occur at an early age, often by 3.5 months in heterozygotes (Di Cristofano et al 1998), and their appearance is accelerated by exposure to gamma irradiation (Suzuki et al 1998). Although the tumors occur in heterozygous mice, in many cases the wild-type allele of the *PTEN* locus has been deleted or mutated in the tumor cells (Podsypanina et al 1999, Suzuki et al 1998). *PTEN* heterozygotes also have a high incidence of autoimmune disease (Di Cristofano et al 1999). This may reflect an inability of self-reactive lymphocytes to undergo apoptosis through a Fas-mediated pathway.

Given the frequency of *PTEN* mutations in human glioblastoma, it is surprising that *PTEN* heterozygotes have no defects in the nervous system and that none develop brain tumors. One possible explanation is that PTEN has a different function in human vs mouse astrocytes. Alternatively, defects in glial cells might require inactivation of the second *PTEN* allele, and this event may be less common in murine brain than in other tissues. Finally, the loss of *PTEN*, on its own, may not be sufficient to promote glial hyperplasia or tumor formation. Most human glioblastomas have mutations or amplifications of multiple genes that regulate

proliferation, differentiation, and cell death, so it may not be surprising that loss of *PTEN* alone does not cause glioblastoma in mice.

### Other Genes on Chromosome 10 May Also Contribute to Glioblastoma

Chromosome 10 deletions in glioblastoma frequently disrupt genes other than *PTEN*. A gene called *DMBT* (deleted in malignant brain tumors), located at chromosome 10q25, is lost from 20%–40% of glioblastomas and from many lower-grade astrocytic tumors (Lin et al 1998, Mollenhauer et al 1997, Somerville et al 1998). The *DMBT* gene product (also called hensin or collectin-binding protein/gp-340) is an extracellular matrix protein of the scavenger receptor cysteine-rich superfamily, and it has been implicated in epithelial-cell differentiation and immune responses (Mollenhauer et al 2000). However, the protein has no known function in the nervous system. Although it is frequently lost in glioblastoma, this loss does not correlate with changes in tumor cell growth in vitro or with increased severity of tumors in vivo (Lin et al 1998, Steck et al 1999).

*Mxi1* (Max interactor 1), also located on chromosome 10q25, is a more promising candidate for a tumor suppressor gene deleted in glioblastoma (Fults et al 1998). *Mxi1* encodes an antagonist of the Myc oncoprotein, and suppresses growth in a number of tissues (Foley & Eisenman 1999, Schreiber-Agus & DePinho 1998). In glioblastoma cells that lack endogenous *Mxi1* expression, introduction of an exogenous gene inhibits proliferation and induces cell cycle arrest (Wechsler et al 1997). Thus loss of *Mxi1* could contribute to excessive growth of tumor cells. Two other genes in this region—*LGI1* (leucine-rich gene-glioma inactivated), which encodes a putative adhesion molecule (Chernova et al 1998), and h-*neu*, a human homolog of the *Drosophila* neuralized gene which encodes a transcription factor (Nakamura et al 1998) – have also been suggested to play a role in glioblastoma.

## CEREBELLAR GRANULE CELL DEVELOPMENT AND MEDULLOBLASTOMA

### Granule Cell Development

The cerebellum plays an important role in motor coordination and learning, and it has been implicated in a variety of cognitive and affective functions (Altman & Bayer 1997, Leiner et al 1993). The cerebellar cortex contains intricate circuitry composed of five different types of neurons. Granule cells are by far the most abundant. They regulate the activity of Purkinje cells and thereby control the output from the cerebellum to other parts of the brain. The murine cerebellum contains $\sim 10^8$ granule cells, more than the number of all neurons in the rest of the brain. The critical importance of granule cells is evident from mutant mice in which the loss of granule cells causes severe ataxia (Mullen et al 1997) and

from patients with congenital granule cell degeneration, who have severe deficits in motor coordination, language use, and cognitive function (Pascual-Castroviejo et al 1994).

Granule cell development has extraordinary features that set it apart from other kinds of neurogenesis. Most neurons are born around the ventricles and then migrate outward toward the surface of the brain. In contrast, granule cells are generated on the outside of the cerebellum and then migrate inward. They arise from a dorsal hindbrain structure called the rhombic lip. During late embryogenesis, neural precursor cells leave the rhombic lip and stream across the surface of the early cerebellum to form the external germinal layer (EGL). At birth (in rodents), the EGL consists of a single layer of undifferentiated cells. During the next few days, these cells undergo extensive proliferation to generate a large pool of granule cell precursors (GCPs). As new GCPs are generated, older cells begin to exit the cell cycle and differentiate. The differentiating cells extend axons that contact Purkinje cell dendrites, and then their cell bodies migrate inward past the Purkinje cell bodies to their final destination, the internal granule layer. The waves of GCP proliferation and differentiation continue until ~3 weeks of age, at which time the EGL dwindles, and all GCPs mature into granule cells.

The molecular mechanisms that regulate the proliferation, differentiation, and migration of GCPs are not well understood. However, some insight into these mechanisms has come from recent studies of the cerebellar tumor medulloblastoma.

## Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children. There are ~350 new cases in the United States each year, most occurring at between 5 and 10 years of age (Novakovic 1994). Most medulloblastoma cases are treated with surgery followed by radiation and/or chemotherapy. Although these approaches are often effective at shrinking the primary tumor, recurrence and metastasis are common, and only ~50% of patients survive for 5 years after diagnosis (Packer et al 1994).

Medulloblastoma cells are small and round with little cytoplasm. The cells usually appear to be undifferentiated, although they may express markers of neurons and glia. The tumor cells are frequently found near the surface of the cerebellum. These properties are not completely consistent with any normal cerebellar cell, but they are most similar to GCPs. For this reason, many investigators believe that medulloblastomas originate from GCPs that become transformed and fail to undergo normal differentiation.

Genetic factors clearly affect the rate of medulloblastoma formation, because a number of chromosomal abnormalities have been associated with the disease (Rasheed & Bigner 1991, Thapar et al 1995). The most common of these abnormalities is loss or mutation of a portion of chromosome 17. The medulloblastoma gene on chromosome 17 appears to be distinct from p53 (which is also on that

chromosome) and has not yet been identified. In addition to chromosome loss, medulloblastomas overexpress certain genes, including c-*myc*, *pax5*, and *zic*, all of which encode transcription factors (Kozmik et al 1995, Rasheed & Bigner 1991, Yokota et al 1996). Whether these genes contribute to the initiation or progression of medulloblastoma or whether their expression is a consequence of the transformed cell type remains to be determined.

## *Patched1* Mutations in Medulloblastoma

New insight into the molecular basis of medulloblastoma has come from recent studies of the Hedgehog-Patched signaling pathway (Figure 3), which was first discovered for its role in *Drosophila* embryo segmentation. Sonic hedgehog (Shh), a vertebrate homolog of the *Drosophila* segment polarity gene product Hedgehog, is a secreted protein that plays a critical role in patterning of the nervous system, the limbs, the skin, and other tissues (Goodrich & Scott 1998, Hammerschmidt et al 1997). Patched1 (Ptc1) is a transmembrane protein that can bind Shh and appears to serve as a receptor. In the absence of Shh, Ptc1 actively represses target gene expression. In the presence of Shh, Ptc1-mediated repression is relieved, and the transcription of target genes is induced. The signaling cascade involves the transmembrane protein Smoothened (Smo) and a family of zinc finger transcription factors, Gli1, Gli2, and Gli3. Gli proteins have activating and repressing effects on transcription, and their effects may depend on proteolytic processing (Dai et al 1999, Ruiz i Altaba 1997, Sasaki et al 1999). Current research is directed at learning how proteolysis, subcellular location, and functional activation of Gli proteins are controlled. The net effect of Shh is to influence Gli proteins to induce target gene transcription. One common target gene is *ptc1* itself, so Shh induces production of its antagonist. The induced build-up of Ptc1 may limit the duration of the Shh effect on a cell. In flies, at least, Ptc also slows the movement of Hedgehog across cells, perhaps by binding and sequestering it (Chen & Struhl 1998).

The first evidence that Shh signaling might be involved in medulloblastoma came from studies of the human *PATCHED1* (*PTCH1*) gene (Hahn et al 1996, Johnson et al 1996). The gene is mutated in basal cell nevus syndrome (or Gorlin's syndrome), a disease characterized by widespread skin tumors, craniofacial and skeletal abnormalities, and an increased incidence of medulloblastoma (Gorlin 1987). Of sporadic medulloblastomas, 10%–20% contain mutations at the *PTCH* locus (Pietsch et al 1997, Raffel et al 1997, Xie et al 1997), suggesting that errors in Shh/Ptc signaling contribute to the etiology of cerebellar tumors.

A causal link between *PTCH1* mutations and medulloblastoma was demonstrated using *ptc1*-knockout mice. Homozygous $ptc1^{-/-}$ mice have multiple defects in the neural tube, heart, and other tissues, and die at 9–10 days of gestation. Heterozygotes are viable and initially the only phenotypes are increased body size and a low incidence of polydactyly (Goodrich et al 1997). Between 4 and 6 months of age, however, 15%–25% of the heterozygotes develop aggressive cerebellar

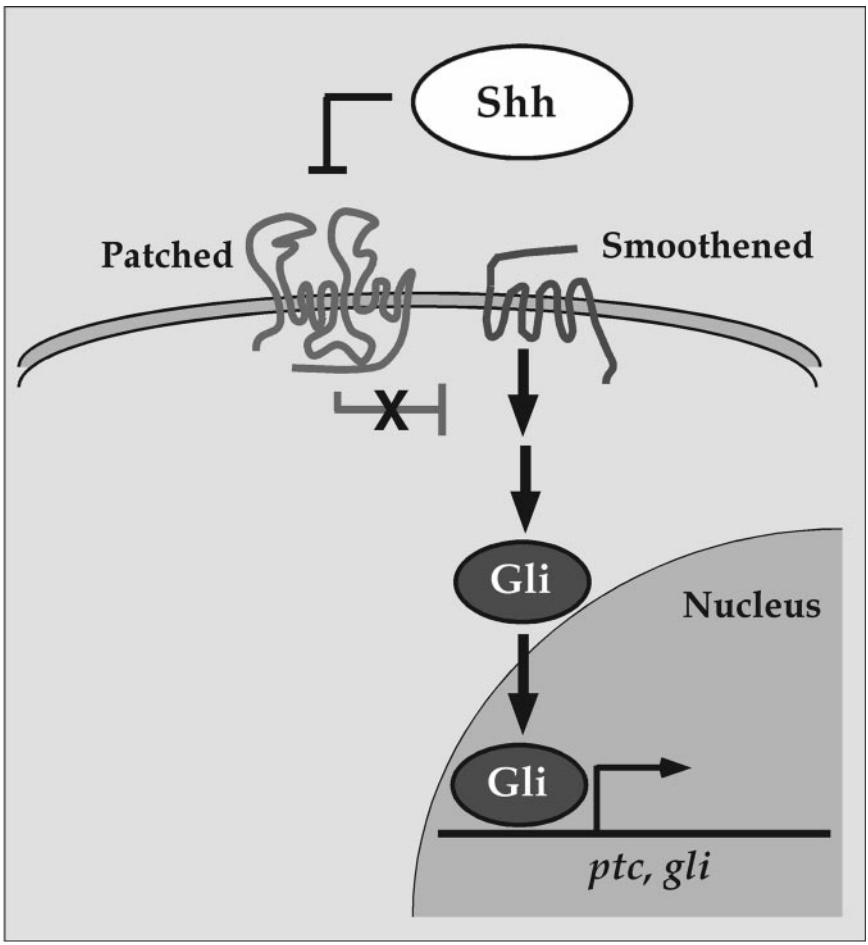


**Figure 3** The Sonic hedgehog/Patched1 signaling pathway. Sonic hedgehog (Shh) is a secreted signaling protein, Patched1 is a receptor for Shh (predicted to have 12-transmembrane-domains), and Smoothened is another membrane protein (predicted to have 7-transmembrane-domains). The bulk of Patched and Smoothened are found in intracellular organelles. The Gli proteins are transcription factors with activating or repressing activities for Shh target genes. In the absence of Shh signal, Patched1 inhibits Smoothened and Gli proteins remain in the transcriptional repression mode. The Shh signal inactivates Patched1, allowing Smoothened to become active. Active Smoothened transduces signals that convert Gli proteins into transcriptional activators, which turn on target genes. Among the targets commonly induced are those encoding components of the Shh pathway, *patched1* and *gli1*. Inactivating mutations in *patched1*, or activating mutations in *smoothened*, can lead to medulloblastoma because target genes are inappropriately active.

tumors (Goodrich et al 1997, Wetmore et al 2000; Figure 4). The tumors consist of small, round cells on the surface of the cerebellum and are morphologically similar to human medulloblastoma. *ptc1* heterozygotes also develop muscle tumors (rhabdomyosarcomas) (Hahn et al 1998, 1999), and they have an increased sensitivity to radiation-induced basal cell carcinomas of the skin (Aszterbaum et al 1999).

## Role of Shh Signaling in Granule Cell Development

The association of *ptc1* mutations with cerebellar tumors suggested that Shh signaling might play a role in normal cerebellar development. Shh had been studied for its role in patterning the ventral neural tube—the floor plate, motor neurons, and ventral interneurons—but its role in the dorsal neural tube, from which the cerebellum forms, was unknown. Initial studies indicated that *shh* was expressed by Purkinje cells (Millen et al 1995, Traiffort et al 1998). Components of the Shh signaling pathway—the membrane proteins Ptc1, Ptc2, and Smo and the transcription factors Gli1 and Gli2—were found to be produced in GCPs in the EGL (Dahmane & Ruiz i Altaba 1999, Wechsler-Reya & Scott 1999). Finally, ablating Purkinje cells leads to local failure of GCP proliferation, suggesting that a Purkinje cell-derived signal is necessary for GCP proliferation (Smeyne et al 1995). Together, these findings raised the possibility that Shh from Pukinje cells might be required for GCP proliferation (Figure 4).

The mitogenic function of Shh signaling was tested by adding Shh to dissociated cerebellar cells or to slices of cerebellum in culture (Dahmane & Ruiz i Altaba 1999, Wallace 1999, Wechsler-Reya & Scott 1999). Soluble Shh protein induced proliferation of GCPs in culture and could inhibit their differentiation and migration into the internal granule layer in cerebellar slices. Shh also promoted the differentiation of glial cells in the postnatal cerebellum (Dahmane & Ruiz i Altaba 1999). The mitogenic effects of Shh were found to be critical for granule cell development, because Shh-blocking antibodies produced in the brain during postnatal development reduced GCP proliferation and caused a thinning of the EGL (Wechsler-Reya & Scott 1999).

The fact that Shh could promote proliferation of GCPs raised another important question: How do the progeny of GCPs stop proliferating and differentiate into mature granule cells? GCPs exit the cell cycle in the middle of the EGL just as they approach the source of Shh, Purkinje cells. Thus, the signal that stops GCPs from dividing must overcome the effects of an increasing concentration of Shh. Two stimuli—bFGF and forskolin, an activator of protein kinase A—have this capacity. Each of them, added to GCPs together with Shh, can prevent the Shh-induced proliferative response (Wechsler-Reya & Scott 1999). In the developing cerebellum, bFGF is made by astrocytes and Purkinje cells (Hatten et al 1988, Matsuda et al 1994) and can stimulate granule cell differentiation and neurite extension (Hatten et al 1988, Saffell et al 1997). Pituitary adenylate cyclase-activating polypeptide (PACAP), a potent physiologic activator of protein kinase A, is made by Purkinje cells (Nielsen et al 1998, Skoglosa et al 1999). PACAP

promotes granule cell survival and neurite outgrowth (Basille et al 1993, Gonzalez et al 1997). Thus, FGF and PACAP are good candidates for regulators of GCP cell cycle exit and differentiation.

## Contributions of Shh/Ptc Signaling to Tumorigenesis

The mitogenic effect of Shh on GCPs is consistent with a role for *ptc1* mutations in medulloblastoma. Shh and Ptc1 are antagonists, so loss of Ptc1 is equivalent to overstimulation with Shh. The tumors can arise long after the EGL has disappeared, so either EGL-like cells persist when Ptc1 function is insufficient or a new type of rapidly dividing cell forms later. Loss of *ptc1* alone is unlikely to be sufficient to cause tumors. Among mouse *ptc1* heterozygotes, only 15%–25% develop medulloblastomas. Tumorigenesis could require loss of the second copy of *ptc1*, and that might occur only in a subset of the animals. However, recent studies of the *ptc1* heterozygous mice indicate that a wild-type (nonmutated) *ptc1* allele is expressed in the majority of tumors. Therefore, complete loss of *ptc1* function is not required for tumor formation (Wetmore et al 2000, Zurawel et al 1998). *ptc1* haploinsufficiency predisposes to medulloblastoma, but additional genetic alterations are probably necessary to produce a full-blown tumor.

## Wnt Signaling in Medulloblastoma and in Cerebellar Development

Just as studies of basal cell nevus syndrome led to an appreciation of the role of Shh signaling in medulloblastoma, studies of another hereditary disease—Turcot's syndrome—suggest a role for the Wnt signaling pathway. Patients with Turcot's syndrome have a high incidence of both colorectal cancers and brain tumors, especially medulloblastomas (Hamilton et al 1995). The genetic lesion in many cases of Turcot's syndrome is a mutation in the adenomatous polyposis coli (*APC*) gene (Kadin et al 1970), which was originally identified for its role in familial and sporadic colon cancers. Subsequent studies have revealed that APC is an important element of the Wnt signaling pathway (Peifer & Polakis 2000, Wodarz & Nusse 1998).

Wnt proteins, like Hedgehogs, are secreted molecules that play a critical role in pattern formation in invertebrates as well as vertebrates. In vertebrates, they are required for cell growth and cell fate determination in the nervous system, limbs, and other organs and tissues. Wnts bind to receptors called Frizzleds and modulate the cellular levels of $\beta$-catenin (Figure 5). In the absence of Wnt signaling, $\beta$-catenin is phosphorylated and degraded by a complex of proteins that includes the serine-threonine kinase GSK3$\beta$ (glycogen synthase kinase 3$\beta$), a scaffolding protein called Axin, and APC. Binding of Wnts to Frizzleds causes inactivation of GSK3$\beta$, which in turn results in reduced phosphorylation and degradation of $\beta$-catenin. The consequent release of $\beta$-catenin from the destruction complex leads to $\beta$-catenin accumulation and its translocation to the nucleus. There it interacts with transcription factors of the LEF/TCF family, and converts them from

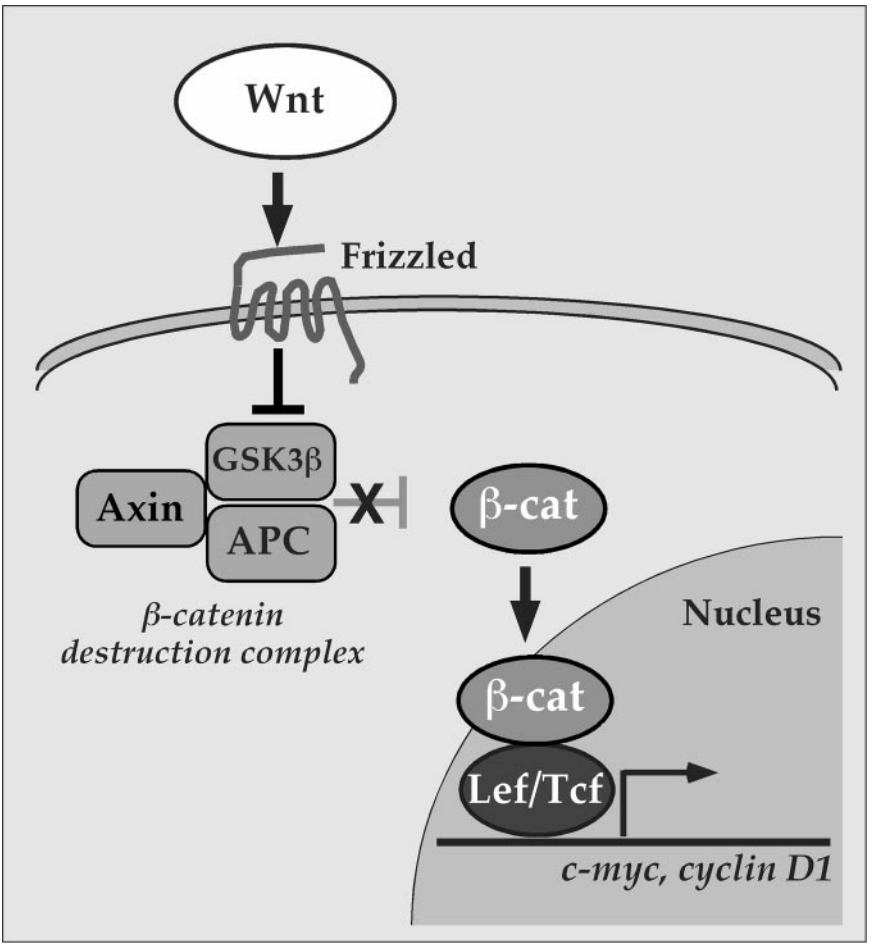


**Figure 5** The Wnt signaling pathway. Secreted proteins of the Wnt family act by binding to 7-transmembrane-receptors called Frizzleds and by regulating the levels of intracellular beta-catenin ($\beta$-cat). In the absence of Wnt signal, $\beta$-cat is phosphorylated and targeted for degradation by a complex of proteins (the "destruction complex") that includes the serine-threonine kinase Glycogen synthase kinase 3$\beta$ (GSK3$\beta$), the scaffolding protein Axin, and the tumor suppressor protein adenomatous polypsis coli (APC). Binding of Wnts to Frizzleds inactivates GSK3$\beta$, reducing the phosphorylation and degradation of $\beta$-cat and allowing it to accumulate in the cell. As $\beta$-cat builds up, it translocates to the nucleus, where it binds to transcription factors of the Lef/Tcf family and converts them from transcriptional repressors into activators. $\beta$-cat-Lef/TCF complexes induce expression of target genes such as c-myc and cyclin D1, which promote cell cycle progression. Mutations that inactivate APC or Axin, or render $\beta$-cat resistant to degradation, are associated with a wide variety of different tumor types.

transcriptional repressors to transcriptional activators. Activated LEF/TCF transcription factors induce transcription of *c-myc* and *cyclin D1*, among other genes. C-myc and cyclin D1 promote cell cycle progression and thereby contribute to tumorigenesis.

Mutations in different Wnt pathway components have been implicated in a wide variety of cancers. The founding member of the Wnt family, Wnt-1, was originally identified as an oncogene activated by the mouse mammary tumor virus in murine breast cancer (Nusse & Varmus 1982). Deletion or inactivation of APC, which normally promotes degradation of $\beta$-catenin, is associated with colon cancer. Inactivation of Axin, another component of the $\beta$-catenin destruction complex, has been observed in hepatocellular carcinoma (de La Coste et al 1998). $\beta$-catenin mutations that render the protein resistant to phosphorylation or degradation have been identified in colon, prostate, and ovarian cancers (Polakis 1999).

The association of Wnt signaling with medulloblastoma was first suggested by the identification of APC mutations in Turcot's syndrome. Since then, a number of investigators have looked for APC mutations in sporadic medulloblastomas and in most cases have not found any (Yong et al 1995). However, a recent study identified APC mutations in 4% of the sporadic medulloblastomas examined (Huang et al 2000). Activating $\beta$-catenin mutations have been found in 8%–15% of sporadic medulloblastomas (Eberhart et al 2000, Huang et al 2000, Zurawel et al 1998).

By analogy with the Shh/Ptc signaling pathway, Wnt signaling might well have important functions in cerebellar growth or differentiation. Wnt-1 is required during early embryogenesis for specification of the midbrain/hindbrain boundary (isthmus) from which the cerebellum arises (McMahon & Bradley 1990, Thomas et al 1991). Wnt-1 mutant mice have serious defects in the midbrain and little or no discernable cerebellum. Wnt-7a has been implicated in axon branching and synapse formation in granule cells and mossy fibers (Lucas et al 1998). These early events may or may not be related to the formation of tumors much later. On the other hand, lithium chloride, which can inhibit GSK3$\beta$ and thereby mimic Wnt signaling, promotes proliferation of cultured granule cell precursors (Cui et al 1998). If this effect is indicative of a physiologic function of Wnt signaling, it might provide an explanation for the association of APC and $\beta$-catenin mutations with medulloblastoma.

## Chromosome 17 Deletions

One of the most important genes involved in medulloblastoma has yet to be identified. Of human medulloblastomas, 30%–50% have a deletion or rearrangement of part of chromosome 17 (Bigner et al 1997, Burnett et al 1997, Cogen & McDonald 1996). In most cases the short arm (17p) is lost, and head-to-head apposition of the long arms (17q) occurs, which is referred to as isochromosome 17q [i(17q)]. This rearrangement is frequently detected in leukemias, lymphomas, and cancers of the stomach, colon, and cervix. The loss of 17p in a number of types of cancer suggests that at least one potent tumor suppressor gene is located there. Fine mapping of deletions from different tumors has narrowed the region of interest

considerably. Most investigators now believe the putative tumor suppressor is located at 17p13.3, a region of ~20 known genes including those encoding the lissencephaly-associated protein Lis1, the breakpoint cluster region (BCR)-related gene *ABR*, and the Max-binding protein Mnt. Despite considerable effort, none of these genes has been clearly implicated in the etiology of medulloblastoma. When the chromosome 17 tumor suppressor is identified, it is likely to provide important insights into the basis of cerebellar tumors as well as into normal cerebellar development.

## CONCLUSIONS

Consistent themes emerge from the developmental biology and genetics of brain tumors.

1. Brain tumor cells bear some resemblance to certain normal cell types, often rapidly dividing cell types, but there is little reason to think that the tumor cells are exactly the same as any normal cell type. Ideas about "cell type of origin" are especially relevant to defining when and where the tumors arise. The properties of the normal cells that are most similar to the tumor cells may also be informative for devising therapeutic interventions. Much more needs to be learned about the extent to which the earliest tumor cells resemble particular types of normal cells. The normal function of Rb may be to control proliferation of neuronal precursors and promote differentiation. In the human retina, Rb family members do not adequately replace lost Rb function, and some type of neuronal precursor divides when it should not. EGF signaling is critical for normal astrocyte development. Glioblastomas often have constitutively active EGF receptors, so astrocyte precursor cells that are potentially responsive to EGFR ligands are inappropriately entering or remaining in the cell cycle. Ptc1 normally restricts growth of cerebellar granule cells; for them to grow, an Shh signal from Purkinje cells blocks the action of Ptc1. Reduced Ptc1 function allows excess growth. In medulloblastoma as in glioblastoma and retinoblastoma, the normal developmental regulation systems are informative about tumorigenesis.
2. Multiple genetic events are usually necessary for tumorigenesis. Prospective tumor cells must remain in the cell cycle beyond the appropriate time (or reenter the cycle). Differentiation into a nondividing cell type must be blocked, and apoptosis must be avoided, either by specific inactivation of the apoptotic machinery or by attaining a differentiation state that is resistant to programmed cell death. In retinoblastoma, loss of Rb function promotes cell cycle progression, but may also predispose cells to apoptosis; other genetic changes may be necessary for retinal tumors to occur. In glioblastoma, mutations that cause aberrant proliferation (e.g. CDK4 amplication, CDKN2A/B deletion) are invariably accompanied by genetic events that prevent differentiation and block apoptosis (e.g. *EGFR*

amplification, *PTEN* mutation, loss of *p53*). Although medulloblastoma occurs in people and mice that are heterozygous for mutations in *patched1*, other factors clearly contribute. For example, the chromosome 17 gene that is a common correlate with the tumor has yet to be identified.

3. The effect of a particular regulator (or its absence) is highly dependent on the cell type or stage of development. During normal development, cells that are powerfully affected by a regulator at one moment may ignore it at another time. That particular mutations transform only cells with certain properties seems likely to reflect normal limitations on responses to regulators. Rb deficiency may cause inappropriate growth of cells that are in a differentiation state with limited ability to die, even in the absence of Rb. Understanding what makes cells receptive to signals or mutations, such as the history of the cell or a convergence of regulators, will be critical for understanding how particular genetic changes lead to tumors.

Viewing brain tumors as an aberration of normal development has helped to put the events of tumorigenesis in proper context. Studies of developmental biology have enlarged our knowledge of the pathways that are affected by tumorigenic mutations and have led to the recognition of new oncogenes and tumor suppressors. The construction of better mouse models of human disease, guided by human genetics and knowledge of developmental pathways, has led to promising new ways to search for and test therapies. Deeper knowledge of the relationship between tumor cells and normal cells will help guide us to still hidden susceptibilities of tumor cells.

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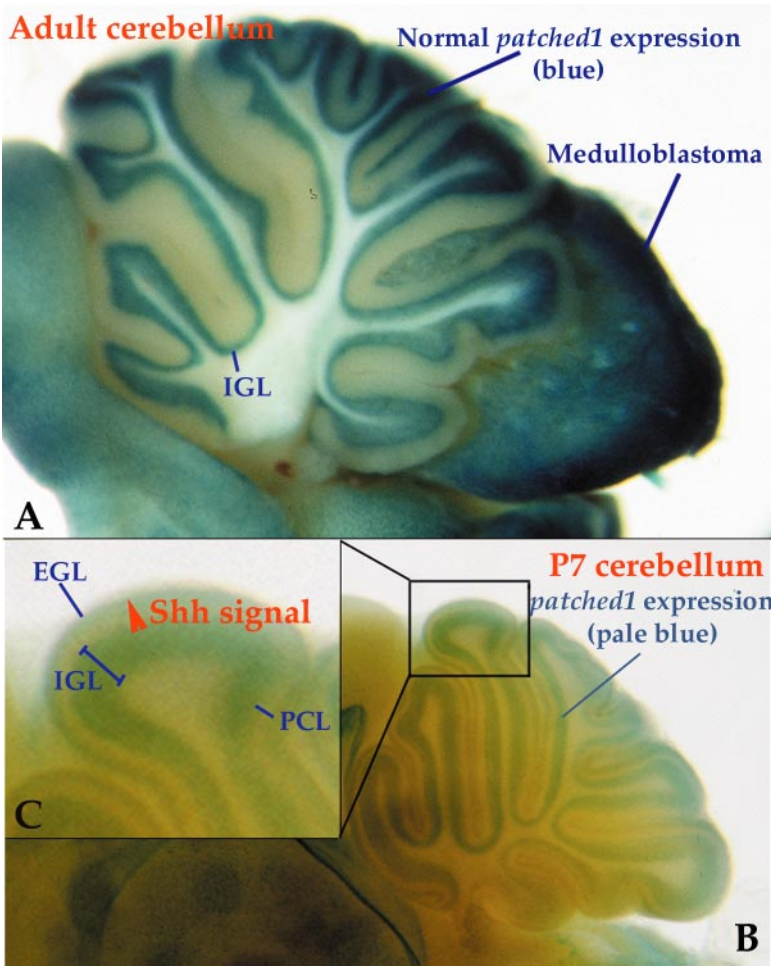


**Figure 4** Mouse model of medulloblastoma. (*A*) Saggital section of adult cerebellum from heterozygous *patched1*/+ mouse, showing normal cerebellum adjacent to a tumor. (*B*) Normal cerebellum at seven days after birth. (*C*) Enlargement of box in C, showing the layers of the cerebellum and the normal signaling process. Shh, originating from the Purkinje cell layer (PCL), signals to the external germinal layer (EGL) where granule cell precursors (GCPs) proliferate in response (red arrowhead). The blue stain in all three panels shows the pattern of patched1 expression, primarily in the internal granule cell layer (IGL) to which differentiating granule cells migrate from the EGL.