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Cerebellum: Development and Medulloblastoma

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Abstract

In the last 20 years, it has become clear that developmental genes and their regulators, noncoding RNAs including microRNAs and long-noncoding RNAs, within signaling pathways play a critical role in the pathogenesis of cancer. Many of these pathways were first identified in genetic screens in *Drosophila* and other lower organisms. Mammalian orthologs were subsequently identified and genes within the pathways cloned and found to regulate cell growth. The GENES and pathways expressed during embryonic development, including the Notch, Wnt/β-Catenin, TGF-β/BMP, Shh/Patched, and Hippo pathways are mutated, lost, or aberrantly regulated in a wide variety of human cancers, including skin, breast, blood, and brain cancers, including medulloblastoma. These biochemical pathways affect cell fate determination, axis formation, and patterning during development and regulate tissue homeostasis and regeneration in adults.

Medulloblastoma, the most common malignant nervous system tumor in childhood, are thought to arise from disruptions in cerebellar development [reviewed by Marino, S. (2005). Medulloblastoma: Developmental mechanisms out of control. Trends Mol. Med. 11, 17–22]. Defining the extracellular cues and intracellular signaling pathways that control cerebellar neurogenesis, especially granule cell progenitor (GCP) proliferation and differentiation has been useful for developing models to unravel the mechanisms underlying medulloblastoma formation and growth. In this chapter, we will review the development of the cerebellar cortex, highlighting signaling pathways of potential relevance to tumorigenesis.

1. Introduction

In their classical treatise on brain tumors, Bailey and Cushing wrote, “the histogenesis of the brain furnishes the indispensable background for an understanding of its tumors” (Bailey and Cushing, 1926). The idea that tumors form from specific populations of immature neurons suggests that common mechanisms underlie development and tumor formation. In the developing cerebellum, precursors of the granule cell are thought to give rise to medulloblastomas (Bailey and Cushing, 1925) the most common childhood primary CNS tumor (Packer *et al.*., 1999). Medulloblastomas arise in the cerebellar vermis and spread rapidly through the cerebrospinal pathways, where they form tumors of variable size along the ventricles (Packer *et al.*, 1999). Medulloblastomas are a diverse set of tumors as evidenced by several criteria including differing histopathologies (Louis *et al.*, 2007). The most aggressive forms of the disease occur in infants and young children and long-term survivors are at significant risk of cognitive and psychological deficits (Levisohn *et al.*, 2000) due to the effects of current treatment protocols that include resection, irradiation, and chemotherapy.

2. Cerebellar Development

The cerebellar cortex is a remarkably simple laminar structure, with two principal neurons, the granule cell and the Purkinje cell, and a diverse set of interneurons, which modulate the output of the Purkinje cell to the cerebellar nuclei (Palay and Chan-Palay, 1974). The most prevalent neuronal subclass within the cerebellum, indeed within the entire mammalian central nervous system, is the cerebellar granule neuron. Granule neurons serve an essential role in coordinating afferent input to and motor output from the cerebellum through their excitatory connections with Purkinje neurons (Ito, 2006; Fig. 8.1). While the role of the cerebellum in sensorimotor functions, balance control, and the vestibular ocular reflex have long been appreciated (Ito, 2006), recent studies have revealed a role for the cerebellum in a wide range of cognitive functions, including feed-forward sensory-motor learning, speech, and spatial memory (Boyden *et al.*, 2004; De Zeeuw and Yeo, 2005; du Lac *et al.*, 1995; Fiez, 1996; Fiez and Petersen, 1998; Schmahmann and Caplan, 2006; Timmann *et al.*, 1999). Notably, a loss of spatial memory and other cognitive functions have been reported in children after successful tumor resection (Levisohn *et al.*, 2000). Although the function of the cerebellum in learning and memory is complex, the remarkably simple architectonics of the cerebellum make it an attractive model system for studying CNS tumors, especially developmental tumors such as medulloblastoma.

2.1. Embryonic cerebellar development: Neurogenesis and patterning in the cerebellar anlagen

Fate mapping and transplantation studies indicate that the cerebellar territory arises from rhombomere 1, delineated by the expression of *Hoxa2* (posterior) and *Otx2* (anterior) (Wingate and Hatten, 1999). Secreted proteins of the Wnt (McMahon and Bradley, 1990) and fibroblast growth factor (FGF) families (Chi *et al.*, 2003; Crossley *et al.*, 1996; Martinez *et al.*, 1999), including Wnt1, Fgf8, and Fgf15, control the expression of transcription factors that delineate rhombomere 1 (*Otx2* and *Gbx1*; Joyner *et al.*, 2000) and of genes required to establish the cerebellar territory, including the homeobox genes En1 and Pax2/5/8 (Joyner, 1996; Joyner *et al.*, 1989)

The cerebellum, like the cerebrum, consists of an outer cortical structure and set of subcortical nuclei, the cerebellar nuclei, which project to cerebellar targets (Fig. 8.2). During cerebellar histogenesis, a complex pattern of neurogenesis and cell movements generates the cerebellar cortex and cerebellar nuclei (Hatten and Heintz, 1995; Morales and Hatten, 2006). Classical studies indicate that the dorsomedial ventricular zone (VZ) along the fourth ventricle gives rise to the principal output neuron of the cerebellar cortex, the Purkinje cell, neurons of the cerebellar nuclei, and more than half a dozen types of cerebellar interneurons, including Golgi, basket, and stellate cells (Dino *et al.*, 2000; Laine and Axelrad, 2002; Palay and Chan-Palay, 1974). A secondary germinal zone forms along the anterior aspect of the rhombic lip, which generates the cerebellar granule neuron as well as a subpopulation of neurons of the cerebellar nuclei (Fink *et al.*, 2006; Wang *et al.*, 2005) and neurons of several precerebellar nuclei of the “cerebellar system” (Dymecki and Tomasiewicz, 1998; Machold and Fishell, 2005; Wang *et al.*, 2005; Wingate, 2001; Wingate and Hatten, 1999).

In the mouse, the earliest cerebellar progenitors exit the cell cycle in the VZ at approximately embryonic (E) day E10.25 and generate neurons of the cerebellar nuclei (Fig. 8.3). Studies on transcription factor expression during cerebellar histogenesis show that postmitotic precursors of neurons of the cerebellar nuclei express the transcription factors *Lhx2/9*, *Meis 1/2*, and *Irx3* (Morales and Hatten, 2006). By E11.2, this pool of progenitors migrates radially along the emerging glial fiber system to form a superficial zone across the dorsal surface of the cerebellar anlagen. Between E11 and E14, postmitotic precursors of the Purkinje neuron, identified by expression of the LIM transcription factors LHX1 and LHX5,

migrate away from the VZ along radial glial fibers and assemble into a broad zone in the core of the anlagen (Morales and Hatten, 2006). Recent genetic loss of function studies and fate mapping analyses demonstrate that *Ptf1a* expression is required to generate the progenitors of cerebellar GABAergic neurons (Purkinje cells and interneurons) in the cerebellar ventricular zone (Hoshino *et al.*, 2005; Pascual *et al.*, 2007). In humans, PTF1A mutations are associated with cerebellar agenesis (Aldinger and Elsen, 2008).

2.2. The rhombic lip, a secondary germinal zone

By E12.5, a secondary neurogenic zone appears along the anterior aspect of the rhombic lip. Cells in this zone express the basic helix-loop-helix (bHLH) transcription factor *Atoh1/Math1*, (Alder *et al.*, 1996; Machold and Fishell, 2005; Wang *et al.*, 2005) the zinc finger protein *Zic1* (Aruga, 2004), and *Meis1* as well as the markers *Pde1c*, and *Pcsk9* (Morales and Hatten, 2006). At this stage, proliferating pools of progenitors migrate out of the rhombic lip and spread across the surface of the cerebellar anlagen to form the external granule layer (EGL). As this morphogenetic out of the rhombic lip begins, postmitotic precursors of the cerebellar nuclei along the surface of the anlagen migrate toward the rostral aspect of the anlage (E12.5–E15.5) and inward to a position beneath the emerging zone of Purkinje cell precursors to form the cerebellar nuclei. Fate mapping experiments confirm that a subpopulation of *Math1*-positive progenitors in the rhombic lip migrate with the pool of cerebellar nuclei progenitors into the deeper aspect of the anlagen as a subpopulation of the neurons of the cerebellar nuclei (Fink *et al.*, 2006; Morales and Hatten, 2006; Wang *et al.*, 2005). The anterior rhombic lip also generates neuronal precursors that migrate ventrally where they form the lateral pontine nucleus and cochlear nucleus, hindbrain nuclei of the “cerebellar system” (Dymecki and Tomasiewicz, 1998; Machold and Fishell, 2005; Morales and Hatten, 2006; Wang *et al.*, 2005; Wingate, 2001; Wingate and Hatten, 1999).

The vast majority of rhombic lip derivatives migrate onto the dorsal surface where they form the EGL (Miale and Sidman, 1961; Palay and Chan-Palay, 1974; Ramon y Cajal, 1995), a zone of proliferating GCPs that generates the cerebellar granule cell (Alder *et al.*, 1996; Alder *et al.*, 1999), the most abundant neuron in the brain (Fig. 8.4). In man, some 45 billion granule neurons are produced during cerebellar development, out of approximately 110 billion neurons in the human brain. Molecular genetic studies demonstrate that *Math1* (Ben-Arie *et al.*, 1997) and *MycN* (Knoepfler *et al.*, 2002) are required for granule cell specification and the expansion of the pool of granule cerebellar progenitors (GCPs) in the early postnatal period of development. Studies on the transcriptional regulation of *Math1*, by Johnson and colleagues show that ZIC1 binds a conserved site within the sequence of the *Math1* enhancer region, and represses *Math1* transcription by blocking the autoregulatory activity of *Math1* (Ebert *et al.*, 2003; Fig. 8.4).

The specification of granule cell identity also depends on extracellular signals that dorsalize the neural tube, including the BMP family members GDF, Bmp6, and Bmp7 (Hogan, 1996). To analyze the role of BMPs in granule cell specification, we examined the pattern of expression of genes encoding BMP family members in the anterior rhombic lip and adjacent tissues (Alder *et al.*, 1999; Fig. 8.5). These experiments showed that Bmp7 was expressed in the anterior rhombic lip and that Bmp6 and Gdf7 were expressed in cells of the dorsal neuroepithelium adjacent to the rhombic lip (Alder *et al.*, 1999). Treatment of progenitors from the ventral region of the mes/met region where the cerebellar territory forms with Bmp7 induced the expression of both *En1* and *Math1*. In addition, transplantation studies showed the generation of GCPs from neural tube explants treated with BMP7. These studies support the hypothesis that in the developing cerebellum, BMP signaling pathways play a critical role in GCP differentiation (Alder *et al.*, 1999). This view is consistent with recent genetic analyses showing that mice with a targeted deletion of the BMP type 1 receptor

genes *Bmpr1a* and *Bmpr1b* have a severe loss of granule neurons, resulting in a smaller cerebellar cortex without foliation (Qin *et al.*, 2006).

Other studies, employing both gain of function and loss of function genetic approaches in mice, provide evidence that Notch1 activation increases Math1 expression by antagonizing BMP receptor signaling (Machold *et al.*, 2007). These studies further suggest that the level of Notch1 activity in cerebellar rhombic lip progenitors regulates their fate. These and other studies that have identified changing patterns of gene expression during GCP development (Hatten and Heintz, 1995; Kuhar *et al.*, 1993) that include cell cycle regulators (Cyclins D1,D2/Ccnd1,2), genes required for glial-guided migration of GCPs (Zheng *et al.*, 1996), the GABAβ6 receptor, and the Lynx family of proteins involved in modulating the activity of a subset of parallel fiber-Purkinje cell circuits (Ibanez-Tallon *et al.*, 2002; Miwa *et al.*, 1999, 2006).

2.3. Cerebellar radial glia

Although radial glia are required for the migration of several populations of neuronal progenitors, including Purkinje cell progenitors, in embryonic cerebellar histogenesis (Morales and Hatten, 2006) and Bergmann glia guide the migration of postmitotic GCPs in postnatal cerebellar development (Edmondson and Hatten, 1987; Hatten, 2002; Rakic, 1971), relatively little attention has been given to the role of neuron–glial interactions in cerebellar neurogenesis (Fig. 8.6). Early studies on neuron–glia interactions in cultured GCPs and in cultured glioblastoma cells showed that neurons regulate the proliferation of cerebellar glial cells and of human glioblastoma cell lines (Hatten *et al.*, 1984). Molecular genetic studies on transgenic lines of mice expressing the radial glial gene Blbp (Anthony *et al.*, 2004; Feng *et al.*, 1994) indicate that radial glia are neurogenic in all brain regions, including the cerebellum (Anthony *et al.*, 2004). Biochemical and molecular genetic studies by Heintz and colleagues further demonstrate that Blbp is a target of Notch signaling (Anthony *et al.*, 2005). Recent genetic studies on mice lacking *MycN*, enhanced proliferation of GCPs and impaired differentiation of cerebellar glial cells (D'Arca *et al.*, 2010). These studies raise the question as to whether neuron–glial interactions regulate cerebellar neurogenesis, especially granule cell neurogenesis, as well as the formation and growth of medulloblastoma and glioblastoma.

2.4. Postnatal cerebellar development: GCP proliferation and migration

After birth, between the second and fourth days postnatal (P2–P4), a number of signaling pathways promote GCP proliferation (Wechsler-Reya and Scott, 1999). At peak periods of proliferation (P5–8) more than a million GCPs can be isolated from a single mouse, providing an unparalleled resource for studies of primary neural progenitor populations (Hatten, 1985). Between birth and the end of the second postnatal week, GCPs exit the cell cycle and move into the inner regions of the EGL where they extend parallel fibers and migrate along the radial fibers of Bergmann glial cells (Edmondson and Hatten, 1987; Rakic, 1971) to a position beneath the Purkinje cells, where they form the inner granule layer (IGL). The spatiotemporal dynamics of GCP proliferation in the superficial zone of the EGL, cell cycle exit, formation of parallel fibers and migration into the IGL are potentially relevant to tumorigenesis. Classical [^{3}H]-thymidine labeling studies by Fujita (Fujita, 1967; Fujita *et al.*, 1966) show temporal regulation of the step-wise progression of GCPs through phases of rapid proliferation to cell cycle exit and differentiation. In the mouse, the transit time of postmitotic GCPs across the EGL and the molecular layer (ML) is 21 and 4 h, respectively. As GCPs exit the cell cycle, they down regulate expression of the bHLH gene Atoh1/Math1 (Ben-Arie *et al.*, 1996; Helms *et al.*, 2000) and upregulate expression of NeuroD1 (Lee *et al.*, 2000; Pan *et al.*, 2009), Zic1,3 (Aruga, 2004; Aruga *et al.*, 1996), and the tumor suppressor cyclin-dependent kinase inhibitory protein p27^{Kip1} (Ayrault *et al.*,

2009). During their movement from the mantle of the EGL into the deeper layers of the EGL, GCPs extend long, parallel fiber axons that express the GPI-linked axonal glycoprotein TAG1 (Furley *et al.*, 1990), among others. Recent studies by Luo *et al.* (Espinosa and Luo, 2008), discussed below, suggest that clones of GCPs stack their parallel fibers in a chronological order that relates to the timing of their penultimate cell division. The importance of correlated timing of axon outgrowth and gene expression is underscored by the anatomical studies of Rivas, who demonstrated TAG1 immunoreactive of parallel fibers peaks during the first 3 days after the GCPs become postmitotic. TAG1 levels decrease dramatically with the differentiation of Purkinje into the ML and formation of parallel fiber synapses with Purkinje cell dendrites (Stottmann and Rivas, 1998).

The timing of GCP migration away from the EGL is another important determinant of cerebellar patterning. GCPs first begin to exit the cell cycle and migrate across the ML into the IGL in the late embryonic period. Several classes of genes that modify the timing of glial-guided GCP migration out of the EGL have potential importance to medulloblastoma. First, in mice lacking the Gi Protein-coupled chemokine CXCR4, which is broadly expressed in both the CNS and immune system, GCPs migrate along Bergmann glial fibers prematurely, with large numbers of cells pouring down the Bergmann glia in the late embryonic period. The premature migration of GCPs from the EGL into the cerebellar anlagen, prevents the massive expansion of GCPs that normally occurs in the postnatal period. Several studies indicate a potential relevance of CXCR4 to medulloblastoma, as blocking CXCR4-mediated cyclin AMP suppression inhibits tumor growth (Yang *et al.*, 2007). Further support for the idea that CXCR4 is critical for the progression of brain tumors comes from studies showing that small molecule inhibitors of CXCR4 inhibit intracranial tumor growth (Rubin *et al.*, 2003).

Molecular genetic studies on genes involved in glial-guided migration, including *Astn1* and *Pex5*, raise the possibility that slowed migration or altered patterns of GCP survival and differentiation cause ectopic zones of GCPs that fail to migrate into the IGL. In the case of mice lacking *Astn1* or *Pex5*, GCP migration along Bergmann glial fibers is slowed markedly, causing the dendritic arbors of young Purkinje neurons to tilt out of the sagittal plane (Adams *et al.*, 2002; Faust, 2003; Faust and Hatten, 1997). In addition, the slowed migration results in ectopic zones of GCPs in the EGL that persist into late childhood, a time by which the EGL has normally dissipated. Since Purkinje neurons provide the mitogen sonic hedgehog (SHH) for GCP expansion in the EGL, it is possible that the growth rates of GCPs depend on the spatiotemporal dynamics of GCP and Purkinje neuron maturation and the assembly of the cerebellar circuit. Finally, it is worth noting that Purkinje cells also provide BDNF, a neurotrophin that promotes the stepwise differentiation of postmitotic GCPs, including migration along Bergmann fibers (Borghesani *et al.*, 2002). Indeed, mice lacking BDNF also have defects in cerebellar patterning that include ectopic zones of immature GCPs in the superficial aspect of the cerebellar cortex (Schwartz *et al.*, 1997). All of these perturbations of the normal spatiotemporal program of GCP expansion and differentiation could be relevant to medulloblastoma formation in childhood.

Although a number of studies have demonstrated that the EGL gives rise to a single class of neurons, the granule cells (Alder *et al.*, 1996, 1999; Hallonet *et al.*, 1990; Zhang and Goldman, 1996), recent studies by Luo and colleagues using the mosaic analysis with double markers (MADM) show that GCPs undergo predominantly symmetric divisions during EGL development and that clonally related granule cells exit the cell cycle within a narrow time frame (Espinosa and Luo, 2008). In agreement with the earlier studies by Fujita (Fujita, 1967; Fujita *et al.*, 1966), these studies showed a progressive slowing of GCP proliferation just before birth, and a rapid expansion of clonally related GCPs just prior to cell cycle exit in the postnatal period (Espinosa and Luo, 2008). These studies show that

GCP expansion produces distinct clones of granule cells (GCs). Studies on a large number of BAC transgenic lines of mice in the GENSAT Project also provide molecular genetic evidence for subpopulations of GCPs and of cerebellar granule cells (Hatten and Heintz, unpublished; www.gensat.org). These subpopulations of GCPs may be relevant to some of the subtypes of medulloblastomas (Gilbertson and Ellison, 2008).

2.5. Differentiation of ES cells toward a granule cell identity

As a proof of principle of the role of key developmental regulators in granule cell development (Hatten and Heintz, 1995), we treated E14 embryonic stem cells, as well as ES cell lines derived from *Tg*(*Pde1c–Egfp*) transgenic mice generated using BAC methodology (Gong *et al.*, 2003), with "cerebellar organizers" (Fgf8, RA), followed by the local signals that "dorsalize" the cerebellar anlagen (*Wnt1, Wnt3a, Bmp7, Gdf7, Bmp6*), and mitogens that expand the cerebellar granule cell progenitor cell population (*Shh, Jag1*) (Salero and Hatten, 2007). To achieve terminal differentiation, we cultured differentiated ES cells in medium conditioned by purified cerebellar glial cells. After these treatments, which parallel known steps of GCP differentiation in vivo, treated ES cells expressed multiple markers of granule neurons (*En1, Math1, Zic1, Zic2, Pax6*, and $GABAa_6$). To test the behavior of these differentiated *Tg*(*Pde1c–Egfp*) ES cells into the EGL of the cerebella of living, neonatal mice, using a stereotaxic injection system, and followed the migration and development of EGFP-positive ES cells *in vivo*. After 3 days to 2 weeks, the cells migrated into the IGL, the position where mature granule neurons function in the cerebellar cortex. Thus, ES cell differentiation confirms the role of local signals in normal cerebellar neuron specification (Salero and Hatten, 2007), and provides an initial strategy for CNS cell replacement therapy.

2.6. Mitogenic pathways that promote GCP proliferation

A number of mitogenic pathways maintain the rapid expansion of the pool of GCPs in the EGL. A role for Shh as a GCP mitogen was suggested by studies of *Patched* (*Ptch*), a gene that encodes a Shh-binding protein that functions as an antagonist of Shh signaling (Fig. 8.7). Since mutations in Ptch occur in sporadic human medulloblastoma and promote medulloblastoma in mouse models, Wechsler-Reya and Scott tested the idea that Shh signaling regulates cerebellar growth. Their studies identified Shh as a key mitogen for GCP expansion (Wechsler-Reya and Scott, 1999), and numerous subsequent studies indicate that Shh signaling is defective in ~25% of medulloblastoma (reviewed in Gilbertson and Ellison, 2008). Activation of the SHH signaling pathway leads to increased transcription of the primary downstream mediator of Shh signaling, the zinc finger protein Gli. In the embryonic cerebellar development, Shh signaling regulates midbrain/hindbrain development through positive regulation of the Gli activators (GliA) and inhibition of the Gli3 repressor (Gli3R). The levels of Gli3R control the overall growth of the midbrain/hindbrain territory and play a critical role in sustaining the activity of the anterior posterior (AP) organizer Fgf8 in the isthmus region (Blaess *et al.*, 2006). In the early postnatal period, Shh signaling is a primary driver of the dramatic expansion of the GCP precursor pool. Analysis of the role of Gli family members in medulloblastoma, by inactivation of Gli alleles in *Pcth1*(+/−) mice suggests that Gli1 both provides a marker of Shh pathway activation and functions in medulloblastoma formation from GCPs (Kimura *et al.*, 2005).

Shh appears to regulate GCP proliferation by several mechanisms (Fig. 8.8). First, Shh signaling regulates the expression of the cell cycle regulators cyclin D1 (Ccnd1), cyclin D2 (Ccnd2), and cyclin E (Ccne) (Kenney and Rowitch, 2000). During cerebellar development, early post-natal GCPs express cyclin D1 while GCPs generated during the peak of GCP neurogenesis express both Ccnd1 and Ccnd2. Mice lacking *Ccnd1* have slowed GCP proliferation and cerebellar development (Pogoriler *et al.*, 2006). Interestingly mice lacking both *Ccnd1* and *Ptc1* had a reduced incidence of medulloblastoma, suggesting that a loss of

Ccnd1 may suppress medulloblastoma formation (Pogoriler *et al.*, 2006). Second, activation of the SHH pathway upregulates the expression of the proto-oncogene MycN that when overexpressed, promotes cell autonomous upregulation of *Ccnd1* mRNA and protein independently of Shh signaling (Kenney *et al.*, 2003). Thus, Shh controls cerebellar growth and GCP proliferation by multiple mechanisms.

The importance of SHH to cerebellar histogenesis is also underscored by elegant genetic analyses of Joyner and colleagues, who demonstrated that the levels of Shh signaling control the foliation patterning of the cerebellar cortex (Corrales *et al.*, 2006). At present, the regulation of Purkinje cell expression and release of Shh is not as well understood as the mitogenic Shh signaling pathways in GCP neurogenesis. Gene expression studies show that Purkinje neurons are the primary source of Shh in the neonatal cerebellum (Wechsler-Reya and Scott, 1999). The role of the Purkinje neuron in GCP expansion is of particular interest, given the increasing ratio of granule cells to Purkinje cells during evolution (750:1 in the mouse; 3300:1 in humans; Lange, 1975; Fig. 8.9). This suggests the possibility that the PC as the primary output neuron of the cerebellar cortex, regulates the production of interneurons in the cerebellar circuitry (Hatten, 1999) by a feedback loop that involves Shh.

A number of studies have defined a role for Shh pathway activation in desmoplastic medulloblastoma that represent ~25% of all medulloblastoma in children. Major effort has been spent identifying genes that are specifically expressed in embryonal tumors and medulloblastoma (Pomeroy *et al.*, 2002; Thompson *et al.*, 2006), especially the targets of the Gli1 expressed in medulloblastoma (Yoon *et al.*, 2009). Their studies suggest that Gli1 affects medulloblastoma growth and survival via targets that include p53, SGK1, MGMT, and NTRK2.

Notch2 signaling also stimulates granule cell proliferation (Solecki *et al.*, 2001), as activated *Notch2* maintains GCP proliferation and inhibits granule neuron differentiation. Exposure of GCPs to the Notch2 ligand, JAG1, or a constitutively activated form of the Notch2 receptor results in increased (3–5×) cell proliferation. In these cells, expression of the downstream transcription factor, HES1, is upregulated and HES1 overexpression has a similar ability to maintain proliferation in granule cell progenitor populations. HES1 expression is also induced by the SHH pathway, suggesting that it is a common downstream effector of these two pathways (Solecki *et al.*, 2001). This hypothesis is consistent with gene expression studies in medulloblastoma from *Ptch1*(+/−) mice with elevated Shh signaling, showing increased expression of components of the Notch and Wnt pathways. In addition, genetic studies on mice with reduced Shh signaling report a downregulation of expression of Notch2, Jag1, and Hes1 (Dakubo *et al.*, 2006). Studies on the expression of Notch family members in medulloblastomas and other tumors also report a link between Notch2 activation and tumorigenesis. Immunocytochemical studies indicate expression of Notch2, but not Notch 1,3,4, is detected in medulloblastoma, with increasing numbers of immunopositive cells with tumor (Xu *et al.*, 2009). Direct studies on the effect of expressing truncated, constitutively active forms of Notch1 or Notch2 support the idea that activation of Notch2, but not Notch1, has oncogenic effects on tumor formation. Examination of the levels of Notch2, and its downstream target Hes1, mRNAs in 40 embryonal tumors showed enhanced expression in 15% of the tumors. Taken together, these findings suggest that Notch1 and Notch2 have different effects on GCP proliferation, as well as on human medulloblastoma (Fan *et al.*, 2004).

Cell cycle regulators are also important regulators of normal GCP proliferation. In the postnatal EGL, $p18^{Ink4c}$ is transiently expressed in GCPs during cell cycle exit (Uziel *et al.*, 2005). N-Myc is also required for the rapid expansion of GCP proliferation in the postnatal cerebellum (Knoepfler *et al.*, 2002). Loss of MycN increases expression of two cyclin-

dependent kinase inhibitory proteins, $p27^{Kip1}$ and $p18^{Ink4c}$ in the cerebel lum, and the D-type cyclins dramatically expand the GCP population in the EGL. Studies by Zindy *et al.* (2006) further demonstrate that MycN expression and downregulation of $p18^{Ink4c}$ and $p27^{Kip1}$ are both critical for GCP expansion during cerebellar development.

2.7. Negative regulators of GCP proliferation

In addition to signaling pathways that promote growth, GCP cell cycle exit and differentiation depend on signaling pathways that provide negative growth regulation. Recent studies have described several signaling molecules that antagonize the SHH-mediated proliferation of GCPs, including bFGF (Fogarty *et al.*, 2007) and members of the BMP family. Among the latter, BMP2 and BMP4 are expressed in granule cell progenitors, and in postmitotic, differentiating GCPs in the EGL. *In vitro* assays indicate that Bmp2 and Bmp4, but not Bmp7, inhibit Shh-induced GCP proliferation (Rios *et al.*, 2004) via the Smad signaling pathway (Zhao *et al.*, 2008). Recent studies showed that Bmp4 antigonizes Shh signaling and induce differentiation of GCPs by rapid posttranscriptional turnover of Math1/Atoh1 (Ayrault *et al.*, 2010). Recently, we have used a combination of genetic, cell, molecular, and biochemical methods to identify Wnt3 as a novel negative regulator of GCP proliferation (Kim *et al.*, 2010, submitted). Our experiments show that Wnt3, which is provided by Bergmann glia in the developing cerebellum, blocks SHH-induced stimulation of GCP growth and slows medulloblastoma growth through a noncanonical Wnt signaling pathway.

In addition to changes in the regulation of cell cycle regulator RNAs and proteins, cell-cycle transitions are driven by Ubiquitin-dependent degradation of key cell-cycle regulators. SCF (Skp1/Cullin/F-box protein) complexes and anaphase-promoting complexes (APC) represent two major classes of ubiquitin ligases whose activities are thought to regulate the G1/S and metaphase/anaphase cell-cycle transitions, respectively (Ayad *et al.*, 2005; Rankin *et al.*, 2005; Wei *et al.*, 2004). The APC complex is an E3 ubiquitin ligase required for the metaphase-to-anaphase transition and mitotic exit. GCPs provide a unique model for understanding cell cycle transitions. Recent studies by Harmey *et al.* (2009) indicate that the APC is essential for GCP proliferation and differentiation. Inhibition of APC activity by either a dominant negative approach or shRNAi depletion of the APC activator Cdh1 reduced GCP neurite outgrowth and migration in organotypic slice cultures. Subsequent studies using high-throughput over-expression screens identified ubiquitin ligases, which are similar to Cdh1, in controlling GCP migration (Simanski *et al*., submitted). A clearer understanding of the role of ubiquitin-dependent degradation of GCP cell cycle regulators in normal cerebellar development could have implications for medulloblastoma growth and treatment.

2.8. Addendum: Timing of human cerebellar development

In humans, between 24 and 40 weeks of gestation, the cerebellum undergoes a remarkable rate of growth as reviewed by Volpe (2009). During this period, as assessed by 3-D volumetric ultrasound, the volume of the cerebellar territory increases fivefold (Limperopoulos *et al*., 2005). In humans, the major embryonic histogenic events occur by 20 weeks gestation. Between 20 and 30 weeks, the EGL forms and development accelerates as evidenced by surface foliation (Volpe, 2009). At 25 weeks, the EGL reaches peak thickness, 6–8 cells deep. GCP proliferation and migration continue between 30 and 40 weeks. A number of features of GCP expansion, including a role for Shh, provided by Purkinje cells have been documented in human cerebellar development (Carletti and Rossi, 2008). During this period, although the thickness of the EGL remains constant, the surface expands horizontally via a more than 30-fold increase in the surface area of the cerebellar cortex. In human infants, the granule cell population accounts for more than 95% of the neurons of

cerebellar cortex (Andersen *et al*., 1992), with the ratio of granule cells to Purkinje neurons being 3300:1. During the first postnatal year, as the EGL dissipates, the IGL continues to increase in size as the cerebellar circuitry matures (Volpe, 2009).

3. Medulloblastoma

Medulloblastoma is the most common malignant pediatric brain tumor with about 1000 new cases every year worldwide and a mean age between 3 and 7 years (Fogarty *et al*., 2005). Medulloblastoma, a cancer of the cerebellum, (Fig. 8.10), is a heterogenous class of embryonal tumors, that include subgroups with genetic anomalies in developmental pathways that are critical for normal cerebellar development. In the last 10 years a variety of studies, including analyses of primary human patient medulloblastoma samples as well as cell culture and mouse models have identified signaling pathways that promote or suppress medulloblastoma. As we refine our understanding of the disease at the molecular level, we will no doubt improve diagnostic tools and develop new and improved targeted therapies.

3.1. Current therapy and its consequences and targeted therapy

Current therapies for all subgroups of MB regardless of the subgroup include surgical resection, radiation therapy, and chemotherapy. Although advances in the radiation oncology and chemotherapy have led to dramatic increases in survival rates, ~70% of patients with average risk MBs, current treatments have significant morbidity and adverse secondary effects including loss of hearing, due mainly to the use of ototoxic drugs, and cognitive impairment (Gajjar *et al*., 2006). Such toxicities have profound effects in children that even if cured, face many years of posttreatment disabilities. High risk patients fair less well and ~30% of children die of advanced disease High risk MBs having the large cell anaplastic variants with *MYC* amplification and those associated with metastasis are associated with a poor outcome (Finlay *et al*., 2007; Gajjar *et al*., 2006). In contrast, a subgroup of MBs with mutations in the Wnt signaling pathway (see below), found primarily in adolescents and invariably are associated with high survival rates.

Our understanding of Shh/Ptch signaling has led to the discovery of small molecule inhibitors of Smoothened function that worked remarkably well to suppress MBs in allografts and mouse models of medulloblastoma (Berman *et al*., 2002; Romer and Curran, 2005). These compounds are currently in clinical trials. Although these small molecule inhibitors were very effective in reducing and eliminating tumor cells in mice, a case report indicates that one patient with remarkable remission of the disease later had a recurrence of his MB associated with a mutation in Smo, which made the tumor cells insensitive to the drug (Rudin *et al*., 2009). This emphasizes the need to discover novel targets and small molecule antagonists to the Shh pathway. A nasal antifungal, itracodazole, was recently found to block Smo function while arsenic trioxide was shown to antagonize Shh signaling by targeting Gli2 rather than Smoothened. Like cyclopamine and analogs, itracodazole and arsenic block proliferation of tumor cells. However, arsenic has the advantage to be active against MBs in which Smo is mutated, suggesting that it could be used therapeutically in patients in which tumors express a mutated Smo protein (Kim *et al*., 2010, Phillip Beachy, personal communication). Ongoing molecular analysis of primary MBs combined with the discovery of new biomarkers for histopathology studies should provide physicians with better diagnostic and therapeutic tools. In patients with MBs with a good prognosis, including those with mutations in the Wnt pathway, the treatment could be less aggressive thus lessening the negative secondary effects of the treatment. Such approaches are currently being tested in clinical trials.

3.2. Histopathology

The 2007 WHO classification of medulloblastomas defines five histopathologic subgroups or variants, including the classic subgroup with some differentiated neurons, the desmoplastic variant in which tumor cells show some differentiation and are surrounded by extracellular matrix, MBs with nodularity, and the anaplastic and large cell anaplastic forms, the most aggressive of the disease that invariably connotes poor prognosis (Fig. 8.11; Louis *et al*., 2007; reviewed in Gilbertson and Ellison, 2008; Northcott *et al*., 2009). Molecular genetic analyses have identified only four subgroups that overlap the WHO pathological classification (Kool *et al*., 2008; Thompson *et al*., 2006) Whereas medulloblastoma with mutations in the Shh/Ptch pathway are characterized as desmoplastic and represent ~25% of all MBs and the classic type is the most represented while the aggressive large cell anaplastic MBs are found within each molecularly distinct subgroup and represent ~10% of all MBs (Thompson *et al*., 2006). MBs with a good prognosis include desmoplastic MBs and those with increased nodularity characterized with monosomy 6, mutations in the Wnt pathway, and high TrkC expression. In contrast, high risk patients with medulloblastoma often have metastases and amplification or high expression of *C-MYC* or *MYCN* which is associated with the large cell anaplastic variants, 17p loss and 1q gain (Eberhart *et al*., 2004; von Hoff *et al*., 2010). MBs also can spread within the cerebrospinal fluid and outside the cerebral nervous system (CNS) in bone and bone marrow in very few cases conferring an adverse prognosis (reviewed in Pizer and Clifford, 2009). The identification of new biomarkers that define subgroups of MB's and the development of antibodies to these markers will likely lead to better diagnostic tools.

3.3. Origin of medulloblastomas

Like other embryonal tumors in children, MBs are thought to arise from neuronal progenitors with defects in gene regulation or genetic anomalies in genes and proteins that regulate normal growth and development. To date, one subgroup of MBs with constitutive activation of the Shh/Ptch pathway has been shown to originate from granule cerebellar progenitors (GCPs) in the external granule layer (EGL) (reviewed in Behesti and Marino, 2009; Gilbertson and Ellison, 2008). The question remains as to whether the other subgroups of MBs arise from subpopulations of GCPs in the cerebellar EGL, from ectopic GCPs at developmental stages after the EGL dissipates, or from other types of cerebellar neurons. Recently, Gilbertson and colleagues developed a mouse model for MBs that recapitulate human MBs from the Wnt subgroup by targeting neuronal progenitors in the ventricular zone distinct from the GCPs using a mouse line in which the Cre recombinase is expressed in neuronal progenitors located in the ventricular zone (Blbp-Cre). By breeding this mouse line with a conditional form of a constitutively activated β-Catenin protein in a p53-null background, they were able to generate a mouse model that mirror the human subgroup with a Wnt signature (Gibson *et al*., in revision). This is especially interesting because progenitors that express the glial gene Blbp give rise to both neurons and glial cells (Anthony and Heintz, 2008) and Blbp is a direct target of Notch1 in radial glial cells (Anthony *et al*., 2005). Thus, different subgroups of MB may derive from different subpopulations of cerebellar progenitors, including embryonic radial glial cells, although animal models will be required to test this hypothesis.

3.4. Molecular characterization of MBs

Transcriptional profiling of messenger mRNAs in human medulloblastomas (Kool *et al*., 2008; Thompson *et al*., 2006) and more recently microRNAs profiling (Ferretti *et al*., 2008; Northcott *et al*., 2009) have identified four subgroups of medulloblastoma with distinct RNA signatures. It is likely that more tumor suppressors and possibly oncogenic mutations and amplifications will be identified as we continue to analyze an increasing number of tumors (reviewed in Lindsey *et al*., 2005). Genetic and molecular genetic analyses of

medulloblastoma have identified changes in the regulation of signaling pathways that are important for cerebellar progenitor cell neuro-genesis, including pathways that promote growth (Shh/Patched, the Wnt, Notch, IGF/PTEN/mTOR) and negative growth regulators (BMP). Molecular changes in these pathways associated with MB include both loss of function and gain of function mutations, as well as alterations in the mRNA or protein levels of regulators of these pathways.

3.4.1. Shh/Patched signaling—The Shh/Patched signaling pathway is the most studied in medulloblastoma (Fig. 8.12). The mitogen Sonic hedgehog (Shh) drives proliferation of granule cerebellar progenitors (GCPs) by binding to the 12 transmembrane receptor Patched (Ptch) that exists in two forms, Ptch1 and Ptch2 (Hatten, 1999). In the absence of Shh, Ptch represses the function of Smoothened (Smo), a seven transmembrane G-protein-coupled receptor-like protein that activates the Gli1 and Gli2 transcription factors and inactivates the transcriptional repressor Gli3 that together regulate the transcriptional program in the cell nucleus (reviewed by Wechsler-Reya and Scott, 2001). The primary cilium, a cellular structure essential to cell proliferation and function, was recently found to concentrate components of Shh signaling in cerebellar GCPs and to be required for Shh signaling in GCP expansion (Fig. 8.13; Rohatgi *et al*., 2007; Spassky *et al*., 2008). While the three Gli transcription factors and their partner SuFu are present in the cilia together with Ptch1 in the absence of Shh stimulation, in the presence of the mitogen, Smo is recruited to the cilia while Ptch is degraded in the cytoplasm (Wen *et al*., 2010).

Patients with Gorlin syndrome (also called Nevoid Basal Cell Carcinoma Syndrome) sustain germline mutations in *PTCH1* and *SUFU* that predisposes them to multiple cancers including medulloblastomas (Hahn *et al.*, 1996; Johnson *et al.*, 1996; Pastorino *et al.*, 2009). Loss of function mouse models in which *Ptch1* is deleted in the germline or conditionally demonstrates that loss of *Ptch1* induces medulloblastoma with histopathologic features of desmoplastic human MBs (Goodrich *et al.*, 1997; Lee *et al.*, 2003; Marino *et al.*, 2000; Oliver *et al.*, 2005). Molecular analysis of sporadic human MB primary samples revealed activation of the SHH/PTCH pathway from the loss of PTCH, and mutations in SUFU (Brugieres *et al.*, 2010), and SMO (reviewed by Rubin and Rowitch, 2002; Thompson *et al.*, 2006). Mice lacking *Sufu* develop medulloblastomas in conjunction with p53 loss, demonstrating that *Sufu* functions as a tumor suppressor gene (Lee *et al.*, 2007).

The two Shh-dependent transcription factors, Gli1 and Gli2, activate the transcription of several proproliferative genes, including MycN, cyclins D1, and D2, and cyclin E (Fig. 8.5; Ciemerych *et al.*, 2002; Hatton *et al.*, 2006; Kenney *et al.*, 2003; reviewed in Katoh and Katoh, 2009). In turn, MycN, a b-HLH-transcription factor induces the expression of many genes and microRNAs, among which is the miR-17~92 cluster (see below). Conversely, MycN suppresses the expression of two cyclin-dependent kinase inhibitory proteins, $p18^{Ink4c}$ and $p27^{Kip1}$ to induce Rb and p107 phosphorylation and cell cycle progression (Fig. 8.14; Knoepfler *et al.*, 2002). While Rb and p107 are required for normal cerebellar development and GNP survival, their loss in mice in conjunction with loss of p53 induces MBs (Marino *et al.*, 2003). We found that in mice $p18^{Ink4c}$ is transiently expressed in GCPs to time their exit from the cell cycle, whereas $p27^{Kip1}$ is turned on in postmitotic GCPs in the inner EGL and in the IGL (Uziel *et al.*, 2005). Loss of *Ink4c* or *Kip1* indeed collaborates with the loss of *Ptch1* to induce medulloblastoma in mice (Uziel *et al.*, 2005). Similarly, *Ink4c* and the tumor suppressor *p53* co-repress the induction of tumors (Zindy *et al.*, 2003). Although TP53 mutations are seldom found in human MBs that represent only ~10%, Li-Fraumeni patients with familial *TP53* mutations are prone to medulloblastoma development (Malkin *et al.*, 1990; Shrivastava *et al.*, 1990). For reasons that are not entirely clear, GCPs are exquisitely sensitive to DNA damage and rely heavily on an intact p53 pathway to eliminate cells that have acquired mutations during their division cycle. Several experiments

illustrate this point: first, mouse models of medulloblastoma with a Shh signature were generated by mutations of genes in the DNA repair pathways, including Ligase 4, PARP1, XRCC4 in combination with p53 loss (Lee and McKinnon, 2002; Tong *et al.*, 2003; Yan *et al.*, 2006); second, 5 days old mice irradiated with nonlethal doses (~4 Gy) all develop medulloblastoma by 3 months of age (Zindy *et al.*, 2007).

Initial karyotyping and recent CGH analysis revealed that the genes of the MYC family, c-MYC on chromosome 8q24, MYCN on chromosome 2p, and MYCL1 on chromosome 1p are frequently amplified in medulloblastomas (reviewed in Northcott *et al.*, 2010). Indeed, enforced expression of C-Myc or MycN in GCPs either from *p53*-null or *Ptch1*+/− mice induce medulloblastoma with full penetrance (100%) after orthotopic transplants in naïve recipient animals (Zindy *et al.*, 2007).

3.4.2. Collaboration between Shh and insulin-like growth factor—Shh and IGF promote GCP growth and constitutive activation of the Shh/Ptch and IGF pathway are found in medulloblastomas in mouse and man (Kenney *et al.*, 2004). IGF-I and IGF-II are potent survival factors that bind to IGF-receptors (IGF-R). IGFRs in turn activate PI3K, Akt, and mTOR, to promote mRNA translation (Fig. 8.15). Most growth factors activate mTOR by suppressing the tuberous sclerosis complex (TSC) that include TSC1 (hamartin) and TSC2 (tuberin) that normally restrain mTOR activity. TSC1 stabilizes TSC2, and TSC2 upregulates $p27^{Kip1}$ levels by preventing its degradation, promoting its nuclear localization and preventing cell cycle progression. Although TSC inactivation alone has no effects, in collaboration with *Ptch1* mutation, it drives MB formation in mice by increasing translation of mRNAs and possibly affecting $p27^{Kip1}$ localization (Bhatia *et al.*, 2009, 2010). Similar results were found using an IGF-1 transgene which expression was driven in GNPs and in a *Ptch1*+/− background (Tanori *et al.*, 2010). Several inhibitors of the mTOR pathway (rapamycin) are currently being tested as a treatment for MBs.

The 3.4.3. Collaboration between Shh and Hippo pathways—Hippo pathway is thought to limit cell proliferation and promote apoptosis in differentiating cells (reviewed in Saucedo and Edgar, 2007). Moreover, mutations in this pathway have been proposed to provide cancer cells with a competitive advantage in genetically mosaic tumors, thereby promoting proliferation. The Hippo pathway contains two serine-threonine kinases Mst1 and Mst2 that redundantly phosphorylate the serine-threonine kinases Lats1 or Lats2. Activity of these kinases is enhanced by scaffolding proteins Sav1 (Ww45) for Mst 1 and 2 and Mob1–4 for Lats proteins. Lats proteins phosphorylate two related transcriptional adaptor proteins Yes-associated protein (YAP) and TAZ. Phosphorylation of YAP and TAZ induces their interaction with the chaperone protein 14-3-3 and retention into the cytoplasm thus inhibiting their function. In the absence or the presence of reduced Hippo signaling, YAP/TAZ phosphorylation is absent or low, respectively, inducing their translocation to the nucleus where they interact with DNA binding TEA domain transcription factor TEAD (1–4), as well as several other transcription factors including RUNX2, Smad7, p73, and p53BP2 to activate the transcription of genes involved in cell proliferation and survival (reviewed in Saucedo and Edgar, 2007).

Recent studies implicate the Hippo pathway in medulloblastomas in concert with the Shh/Ptch signaling pathway (Fernandez *et al.*, 2009). Fernandez and collaborators found that YAP expression is regulated by Shh in granule neuron progenitors, that its translocation is mediated by Shh and that it regulates GCPs proliferation (Fig. 8.16). Consistent with its expression in GCPs, YAP is upregulated by overexpression and amplification of the locus on chromosome 11q22 in human medulloblastomas in which the Shh signaling pathway is activated. YAP is also highly overexpressed in human medulloblastomas with activation of the Wnt signaling pathway. TEAD, the major partner of YAP1 is similarly overexpressed in

human MBs with constitutive activation of the Shh or Wnt signaling pathways. In a mouse medulloblastoma model that recapitulates the human tumors with a Shh "signature," YAP is expressed in the perivascular niche, a region of the tumor surrounding blood vessels, in which cancer stem cells reside. These YAP expressing cells were resistant to irradiation that otherwise eradicated all other cell types (Fernandez *et al.*, 2009). Since YAP may maintain "stemness" and protect tumor cells from DNA damage-induced apoptosis, it is a potential new therapeutic target in the treatment of medulloblastomas with Shh and Wnt pathway activation. However, experiments in animal models will be required to validate the function of YAP1 in MBs.

3.4.4. The Wnt signaling pathway—The canonical Wnt signaling pathway is activated when Wnt binds to the seven transmembrane receptor Frizzled that activates the Disheveled protein which has two functions: one to block β-Catenin activation and nuclear localization and the other to regulate actin stress fibers (Fig. 8.17). β-Catenin protein is part of a complex of proteins containing the kinase GSK-3β, Axins 1 and 2, the adenomatous polyposis coli (APC) and Caseine kinase a, CK1a. β-Catenin levels are normally regulated by phosphorylation by GSK-3β leading to ubiquitination and proteasome-dependent degradation. Mutations in APC or in the phosphorylation site of β-Catenin that can no longer be degraded by the proteasome, induces the constitutive activation of nuclear β-Catenin that by binding to the transcription factors TCF/LEF induces unabated transcription of proproliferative target genes including cyclin D1 and c-Myc.

Deregulated Wnt signaling occurs in ~10–15% of human medulloblastomas. It was first identified in patients with Turcot's syndrome in whom colon cancer and malignant neuroepithelial brain tumors including medulloblastomas were associated with mutations in *APC* (reviewed in Gilbertson and Ellison, 2008; Marino, 2005). Subsequently mutations in *BETA-CATENIN/CTNNB1* were also found in human medulloblastomas (Thompson *et al.*, 2006). Besides activated mutations of *B-CATENIN/CTNNB1*, these MBs harbor a single copy loss of chromosome 6, also called monosomy 6. Monosomy 6 represents the most significant prognostic factor for the WNT subgroup of tumors that correlates with a good outcome (Gajjar *et al.*, 2006). Finally, epigenetic silencing of the secreted frizzled-related proteins (SFRP) family of Wnt inhibitors, SFRP-1,-2, and -3 is thought to elevate Wnt signaling in MBs (Fig. 8.17). In addition, forced expression of these SFRP proteins reduces the proliferation and anchorage-independent growth of medulloblastoma cells, limits tumor burden, and prolongs survival in xenografts of MB in mice (Kongkham *et al.*, 2010). The recent mouse model that recapitulates the WNT MB human variant will provide a preclinical model for potential novel therapeutic intervention in patients afflicted with this subgroup of MB (Gibson *et al.*, 2010).

3.4.5. The Notch signaling pathway—Notch signaling is involved in many cellular processes, including stem cell renewal, cell fate specification, and proliferation. Intercellular Notch signaling is mediated by expression of the membrane-bound ligands Delta (1–4) and Jagged (Jag1, 2) on one cell and of the transmembrane receptors Notch (1–4) on the other cell (Fig. 8.18). Upon ligand binding, the intracellular domain (ICD) is clipped by the γ-secretase and translocated to the nucleus where it enters in complexes to activate the transcription of several targets, in particular the basic-helix-loop-helix (bHLH) transcription factors, Hes1 and Hes5. Activated Notch 2 stimulates GCP proliferation (Solecki *et al.*, 2001). Consistent with a role for Notch2 in GCP proliferation, high levels of Notch 2 expression, but not of other Notch family members, was detected in human medulloblastoma (Fan *et al.*, 2004). In addition to Notch2, Hes1 and Jag1 were found to be highly expressed in mouse medulloblastoma models by several groups (Dakubo *et al.*, 2006; Hallahan *et al.*, 2004). In addition, genetic studies do not support a role for Notch1 in medulloblastoma formation in collaboration with the Shh/Ptch pathway (Julian *et al.*, 2010)

Thus, the components of the Notch1 and Notch2 signaling pathways may delineate important differences among subgroups of MBs

3.4.6. The $BMP_{2/4}$ signaling pathway—Unlike the growth promoting signaling pathways described above, the BMP signaling pathway inhibits proliferation and induces the differentiation of GCPs postnatally and GCP-like tumor cells. BMPs, 1–7, belong to the TGF-β family of cytokines (reviewed in Massague, 1996; Massague *et al.*, 2005). BMP-2,-4, and 7 bind as dimers to promote the heterodimerization and transphosphorylation of serine/threonine BMP receptors type I (Alk3) and II (Alk6) (Fig. 8.19). Upon receptor activation, Smad1is recruited to and phosphorylated by the receptor type II (Alk6), inducing its conformational change and heterodimerization with Smad4. The heterodimer is then translocated to the nucleus where it directs the transcription of several genes including the Inhibitors of division (Ids), Id1-4, and TGF-B-early inducible gene 1 (TEIG-1) (Fig. 8.19).

Several studies found that in postnatal cerebellum development and in MBs with a Shh signature BMPs oppose Shh-induced proliferation and induce irreversible neuronal differentiation. In 2004, Rios and collaborators showed that BMP2 antigonizes Shh-dependent proliferation of GCPs (Rios *et al.*, 2004). In 2007, the same group found that BMP2 antigonizes Shh-dependent proliferation by expressing TIEG-1 which directly represses *MycN* transcription. Conversely, they found that TIEG-1 overexpression promotes cell cycle arrest and apoptosis of GCPs in the absence of other differentiation signals (Alvarez-Rodriguez *et al.*, 2007). In 2008, we found that cell cycle exit and terminal differentiation of GCPs and GCP-like tumor cells is mediated by the rapid posttranscriptional downregulation of Math1/Atoh1, a b-HLH- transcription factor that expression marks proliferating GNPs. Enforced Math1/Atoh1 expression in turn prevented differentiation (Fig. 8.20). Finally, Math1/Atoh1 was recently demonstrated to be essential for MB development (Flora *et al.*, 2010) and to collaborate with Gli1 to transform normal GCPs into tumor-initiating cells (Ayrault *et al.*, 2010).

Gene profiling of mouse and human MBs with a constitutively activated SHH pathway revealed that most genes within the BMP signaling pathway are downregulated in tumors compared to proliferating GCPs and neurons, whereas Atoh1 levels are high (Zhao *et al.*, 2008). Epigenetic studies of human medulloblastoma samples identified a tumor suppressor called HIC1, for hypermethylated in cancer-1, the normal function of which is to suppress ATOH1/MATH1. In human medulloblastoma, the promoter of HIC1 is hypermethylated leading to inhibition of its expression and to the induction of high ATOH1/MATH1 protein levels (Lindsey *et al.*, 2004; reviewed by Briggs *et al.*, 2008a,b). Current CGH analysis confirmed that the most frequently affected chromosome in MBs with a SHH signature is 17 that encodes the tumor suppressor HIC1 (Ferretti *et al.*, 2005). However, it is likely that other genes are affected as the result of the frequent loss of heterozygocity on 17p.

3.5. Epigenetic silencing in MBs

Besides loss of function by gene deletions or mutations, epigenetic silencing also plays an important role in the development of MBs (reviewed in Lindsey *et al.*, 2005). Survey of the methylation status of tumor suppressors or oncogenes in human MBs has revealed several genes the disregulation of which is associated with MBs. They include SFRPs that negatively regulate the Wnt pathway (Kongkham *et al.*, 2010), the S100 gene family (Lindsey *et al.*, 2007), the Kruppel-like factor 4 (Nakahara *et al.*, 2010), and HIC1 that regulates Atoh1/Math1 expression (Briggs *et al.*, 2008a,b). As these studies are expanded to an increasing number of primary tumors in the future, it is likely that more genes will be found to be regulated epigenetically and to be relevant to MB formation.

3.6. Other genes involved in medulloblastoma

A number of other genes have been associated with human medulloblastoma. However, the mechanism of action for these genes in MB or their association with specific subgroups of MBs remains to be determined. Among these, high expression of the neurotrophin TrkC receptor is associated with a good prognostic and a favorable outcome in patients with medulloblastoma. TrkC is found in desmoplastic nodular medulloblastoma and is suggested to be responsible for neuronal differentiation. Unlike TrkC, other proteins are correlated with poor prognosis and metastasis. These include ERBB2, a member of the epidermal growth factor receptor, the platelet-derived growth factor receptor, PDFGR and C-Met, the receptor tyrosine kinase for the hepatocyte growth factor (reviewed in Guessous *et al.*, 2008).

In addition, REN^{KCTD11} that maps to 17p13.2 and is deleted in human medulloblastoma with a SHH "signature" is a suppressor of Hedgehog signaling and was shown by the same group to regulate proliferation of GCPs (Argenti *et al.*, 2005; Di Marcotullio *et al.*, 2006). On the other hand, expression of OTX1 and OTX2, two developmentally regulated transcription factors was found to correlate with two subgroups of medulloblastoma; whereas overexpression of OTX1 was found in nodular/desmoplastic human tumors, OTX2 corresponded to the classic subgroup in man and mice (Adamson *et al.*, 2010; de Haas *et al.*, 2006).

3.7. MicroRNAs and medulloblastoma

In the last 10 years, microRNAs and long noncoding RNAs have emerged as novel regulators of many aspects of cellular biology and development. MicroRNAs are small noncoding 22 nucleotides long RNAs the sequence of which is complementary to those in the 3′-UTR of targeted messenger RNAs. MicroRNAs are thought to regulate hundreds of mRNA targets (reviewed in Bartel, 2004).

MicroRNAs are encoded on human and mouse chromosomes as long pri-miRNAs that are processed in the nucleus as pre-miRNAs by a processing enzyme called Drosha (Fig. 8.21). Pre-miRNAs are processed by Dicer in the cytoplasm as mature microRNAs that form complexes, called RISC with argonaute proteins. The RISC complex recognizes the complementary sequences on the messenger RNA targets within its 3′ end, 5′ end and often coding sequences. Several microRNAs were found to be overexpressed and possibly act as oncogenes in tumors whereas others were shown to be underexpressed in tumors suggesting that their expression might suppress tumor development (reviewed in Garzon *et al.*, 2006). As such, microRNAs are being considered as targets for therapy.

Two groups identified the microRNA miR-17~92 cluster as an oncogene in MBs with a Shh signature (Northcott *et al.*, 2009; Uziel *et al.*, 2009). The mir-17~92 cluster belongs to a family of a three clusters encoded by different chromosomes in mouse and man (Ventura *et al.*, 2008) (Fig. 8.22). It encodes six unique microRNAs, miR-17, 18, 19, 20, and 92, that share common seed sequences with microRNAs encoded by the two other clusters of the family, miR-106b~25 and miR-106a-363. MiR-17~92 was found to be a direct target of C-Myc and MycN (O'Donnell *et al.*, 2005). Consistent with this finding, the miR-17~92 cluster is found at high levels in human medulloblastoma with high expression or amplification of C-MYC or MYCN. Although several important targets of the miR-17~92 cluster were found in B cells including E2F-1, PTEN, and Bim that all induce apoptosis (Fig. 8.23), specific bona fide targets in MBs have not yet been identified. Whereas ectopic expression of miR-17~92 in GCPs is not sufficient to drive tumorigenesis on its own, it does so in collaboration with *Ptch1* loss in an orthotopic transplant model in mice (Uziel *et al.*, 2009). If this cluster is required for MB development, anti-gomiRs to this cluster may prove to be

an efficacious and novel therapeutic approach for MBs in which this cluster is overexpressed.

3.8. Mouse models of medulloblastoma and preclinical testing of novel targeted therapies

Remarkably, all mouse models of medulloblastoma that have been developed to date mirror only one subgroup of human MBs, with a Shh signature, even though they were generated with different founding mutations (Fig. 8.24; Lee *et al.*, 2003). These include mutations in *Sufu*, *Smo*, *loss* of *Ptch1* alone or together with *Ink4c* or *Kip1*, *Rb*, hypermethylation of the *HIC1* promoter, loss of *Ligase 4*, *XRCC4*, *Brca2*, and *PARP1*, often in combination with *p53* loss (reviewed in Behesti and Marino, 2009). In contrast, forced expression of MycN, C-Myc, and miR-17~92, induced MB development in combination with loss of *Ptch1* or loss of *p53* (Uziel *et al.*, 2009; Zindy *et al.*, 2007). Comparison of the gene profile of GCPs and tumors with that of differentiated neurons revealed that in all these mouse models, the cell of origin is a GCP (Lee *et al.*, 2003). Although p53 mutations are not common in human MBs (~10%), most of the mouse models, except those from *Ptch1*+/−, *Ink4c*−/− and *Ptch1*+/−, *Kip1*−/−, required the loss of p53. This may reflect the extreme sensitivity of GCPs to DNA damage that must rely on the p53 checkpoint to eliminate all cells that fail to properly replicate their DNA. It is plausible that mutations upstream of downstream of p53 affect its function without a requirement for its elimination in human tumors.

These recent findings suggest that successful development of mouse models that recapitulate the two other subgroups of human MBs, will require a better understanding of the location and identity of the GCPs as well as the alterations in gene or/and microRNAs associated with these specific tumor subgroups. This will enable the targeting of the right mutations into the right cell type at the right time.

3.9. What does the future hold?

An understanding of the Shh signaling pathway and the development of a mouse model that recapitulates the human disease has led to a therapeutic drug targeting the Shh pathway currently in clinical trial. A better understanding of the signaling pathways that are disrupted in each subgroup of medulloblastoma will enable the development of mouse models for all subgroups of human medulloblastomas and the screening of small molecules that ultimately will be used as novel therapeutic drugs. The complete sequencing of human primary medulloblastoma samples and the complete molecular analysis of these tumors will provide additional information that hopefully will lead to better targeted therapies.

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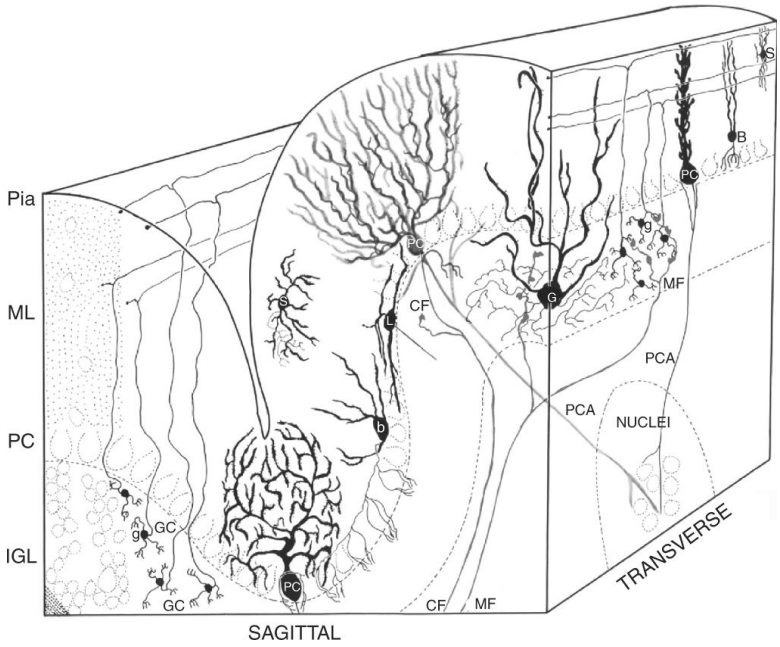

Figure 8.1.

Neurons of the cerebellar cortex. Four granule cells (GC) are shown at the lower left of the sagittal view of the cerebellar cortex. Short, claw-like dendrites extend from the granule cell soma, which projects an ascending axon that bifurcates in a T. The resultant branches extend through the molecular layer (ML) parallel to the long axis of the folium in the transverse plane and form synaptic connections with the Purkinje cell (PC). The dendritic arbors of the PC extend in the sagittal plane. Mossy fiber (MF) afferents from the pontine nucleus and other sources synapse with the dendrites of the granule cells (in the transverse plane). The axons of the Golgi (G) interneuron, the mossy fiber axons, and granule cell dendrites form the synaptic glomeruli of the internal granule cell layer (IGL). PCs align with the cell soma of Bergmann glia (B) in the Purkinje cell layer (PC), as well as several classes of cerebellar interneurons, including basket (b), Lugaro (L), and candelabra (not shown) neurons. Stellate interneurons (S) are located among the parallel fiber axons and Purkinje cell dendrites in the molecular layer (ML). Afferent axons from the olivary nucleus form single climbing fiber projections (CF) onto the Purkinje neurons. Purkinje neurons, the sole output neuron of the cerebellar cortex, project axons (PCA) to the neurons of the cerebellar nuclei (Nuclei). Modified from Palay and Chan-Palay (1974).

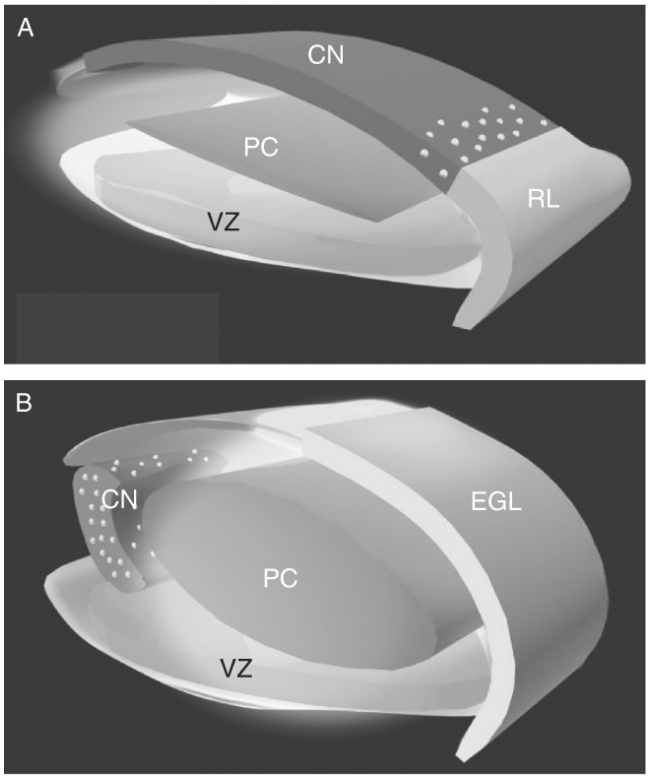

Figure 8.2.
A model for the generation and migratory pathways of progenitors of the cerebellar cortex and the cerebellar nuclei. In the mouse, progenitors of the cerebellar nuclei (CNP) are generated in the VZ of the emerging cerebellar anlagen. Between E10 and E12.5, this progenitor population migrates radially along the glial fiber system (data not shown) to establish a superficial layer (dark gray) Subsequently, progenitors of the Purkinje cell (PCP) are generated in the VZ (E11–E14.5) and migrate radially to form a zone (PC, medium gray) beneath the CNPs. A second germinal zone, the anterior rhombic lip (RL, light gray), generates a subpopulation of precursors of neurons of the cerebellar nuclei, precerebellar nuclei (data not shown), and the granule neurons of the cerebellar cortex. At E12.5, a layer of CNPs (dark gray) generated in the VZ occupy the surface of the embryonic cerebellum. Between E12.5 and E14.5, several populations of RL progenitors move up onto the surface of the anlagen (RL, light gray). These include the bulk of the progenitor population, the precursors of the granule neuron (light gray), and a subpopulation of cells that intercalate among the VZ-derived CNPs (gray zone with white dots). By E14.5, the CNPs (containing both VZ and RL derived progenitors) have migrated off of the surface and formed the nascent cerebellar nuclei. By E16, the cerebellar nuclei (gray zone with white dots) settle beneath the emerging cerebellar cortex (containing the precursors of the granule neurons (EGL, light gray)), the Purkinje neuron (PC, medium gray zone), and the interneurons. Thus, a complex pattern of histogenesis and migration set forth the architecture of the murine cerebellum. From Morales and Hatten (2006).

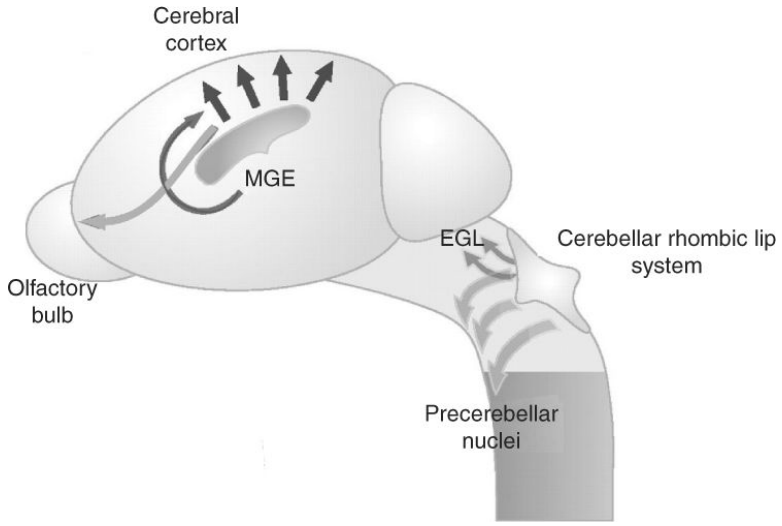

Figure 8.3.

Transverse migratory pathways from the rhombic lip of the cerbellar territory establish the precerebellar nuclei in the brainstem. Progenitors of the cerebellum arise along the rhombic lip at the midbrain/hindbrain junction and migrate dorsally, (dark gray arrows). Progenitors of the precerebellar nuclei in the brainstem arise from more caudal portions of the rhombic lip and migrate ventrally along a netrin1 pathway (light gray arrows). In the forebrain, transverse neuronal migrations include dorsal migration of progenitors from the medial ganglionic eminence (MGE) into the cortex (dark gray arrow), and the posterior to anterior migration of progenitors in the rostral migratory stream (light gray arrow). Radial migrations in the cerebral cortex are also illustrated (black arrows). Adapted from Hatten (2002).

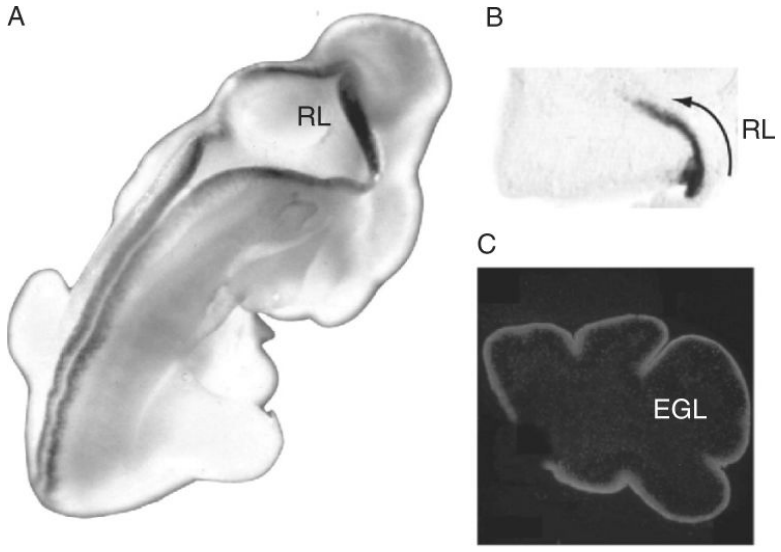

Figure 8.4.

Math1 Expression in the developing cerebellum. Math1 is expressed in the rhombic lip (RL) of the cerebellar territory and the dorsal ridge of the developing CNS in the E12.5 mouse embryo (A). At E14.5 *Math1* is expressed in RL progenitors forming the EGL of the developing anlagen (B). In the postnatal cerebellar cortex, *Math1* is expressed in proliferating GCPs located in the superficial aspect of the EGL. Photos in panels (A) and (C) were kindly provided by Dr. Jane Johnson.

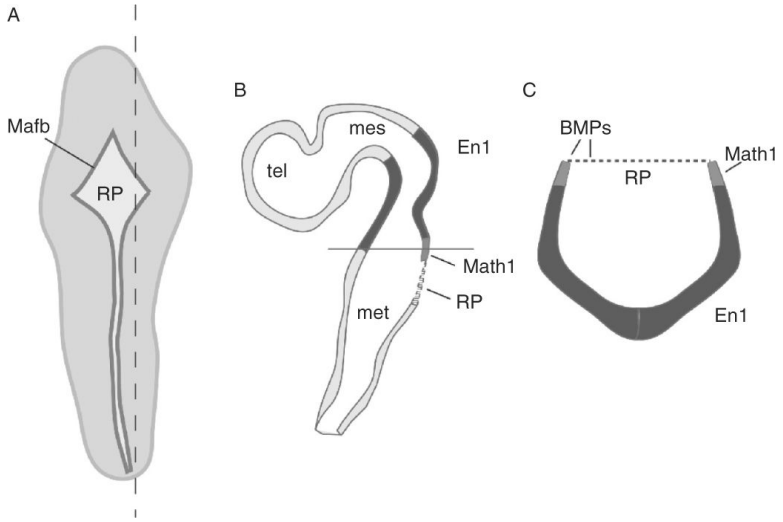

Figure 8.5.

Expression of Bmps in the embryonic cerebellar anlagen. Schematic diagram of an E11 mouse embryo (A), sagittal section (B) through level indicated by dashed line in (A), and a transverse section (C) at the level indicated by the black line in (B). In (A), the roof plate (RP), shown in dark gray, is revealed by expression of the roof plate marker Mafb (Millonig *et al*., 2000), across the rautenbreite and the dorsal ridge of the embryo. In (B) and (C) *En1/2* expression, which marks the cerebellar territory, is shown in black, and the RP is represented as a dotted line. *Gdf7*, *Bmp6*, and *Bmp7* are expressed along the dorsal edges of the neural tube and in the overlying RP. The only region of overlapping expression between *En1/2* (black) and *Math1* (gray) is in the rhombic lip, which is adjacent to the RP (for details see Alder *et al*., 1999). mes, mesencephalon; met, metencephalon; tel, telencephalon.

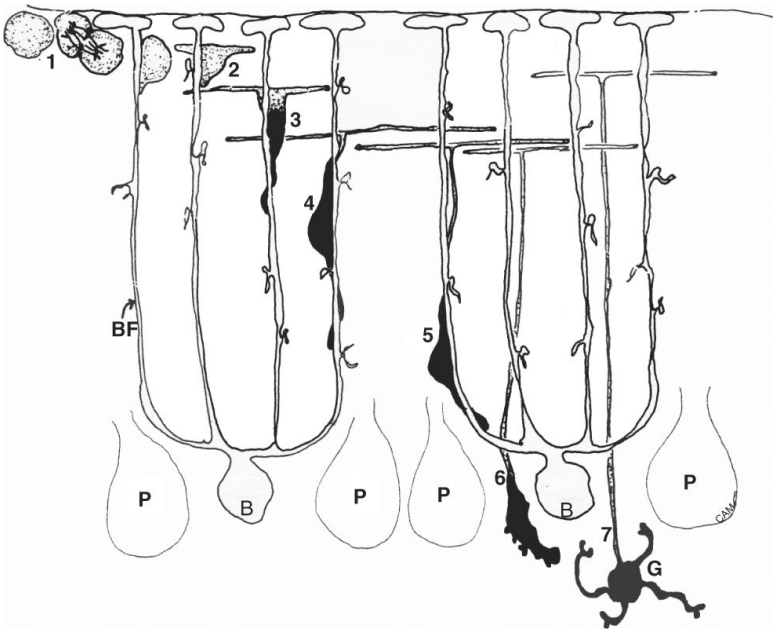

Figure 8.6.

GCP migration and development. GCPs divide in the superficial layer of the neonatal cerebellar cortex (1), after which postmitotic GCPs begin to extend axons (2) and a descending, leading process (black) (3) which migrates along the radial fibers of Bergmann glia (B) by closely opposing the cell soma to the glial fiber (4,5). At the level of the Purkinje neurons (P) GCPs detach from the glial fiber (6) and move into a deeper layer where they differentiate into mature granule neurons (G) with short, claw-like dendrites that form connections with mossy fiber afferents (not shown). Drawing kindly provided by Dr. Carol A. Mason.

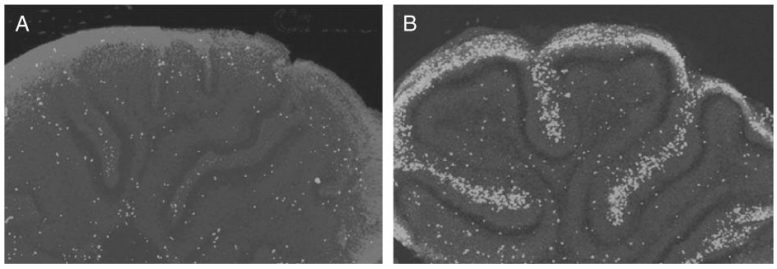

Figure 8.7.
Mitogenic effect of sonic hedgehog (Shh) on the proliferation of granule cell progenitors (GCPs) of the developing cerebellum. The addition of SHH to early postnatal cerebellar cortex (B) results in a large increase in the number of dividing cells, as measured by incorporation of bromodeoxyuridine (BrdU), a deoxythymidine analog (light gray). Adapted from Wechsler-Reya and Scott, (1999).

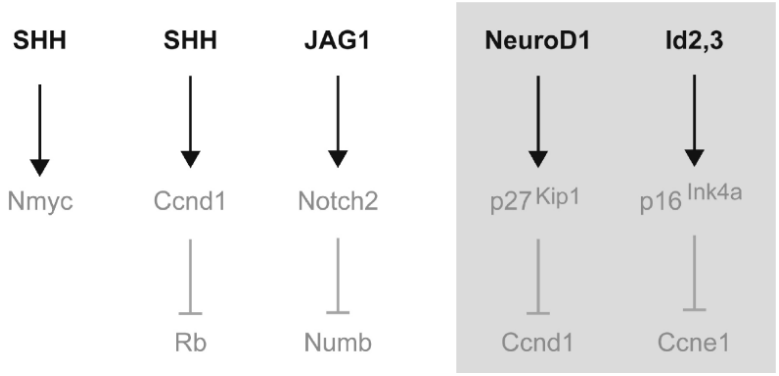

Figure 8.8.
Mitogenic pathway and their inhibitors in GCPs of the developing cerebellum. Schema of the primary mitogens (Shh and JAG1) in the early postnatal cerebellum.

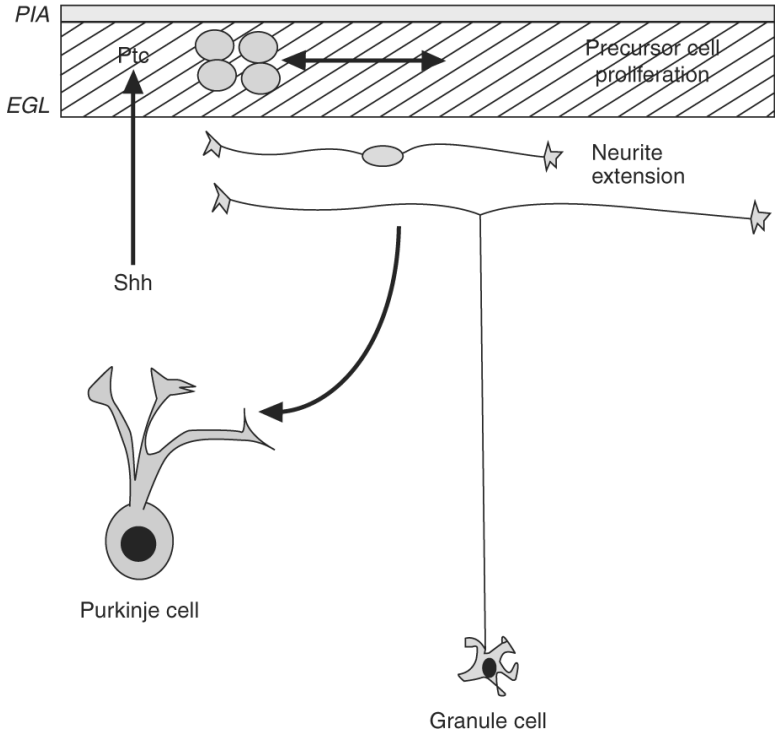

Figure 8.9.

Model for the action of sonic hedgehog in cerebellar development. Sonic hedgehog (Shh) is produced by Purkinje neurons during the developmental stage when they are extending their dendritic tree and beginning to form synaptic connections with the axons of granule neurons. Granule cell precursors bind Shh via the protein Patched (Ptc), which releases Ptc's inhibition of Smoothened and induces a cascade of signals leading to proliferation. Granule cells later exit the cell cycle, extend neurites, and form synaptic connections with Purkinje cells. Thus, Purkinje cells appear to control the number of granule cells entering the cerebellar circuit. Adapted from Hatten (1999).

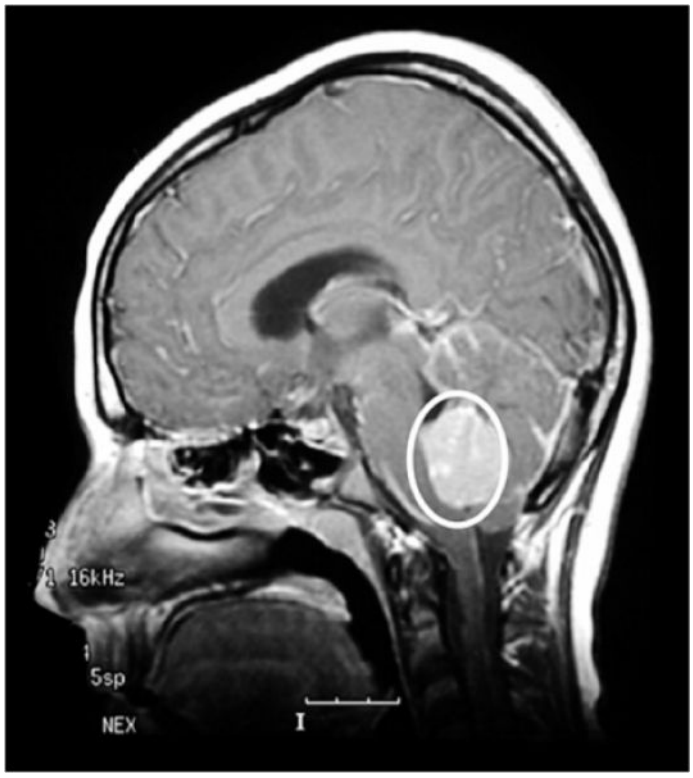

Figure 8.10.
Medulloblastoma. Sagittal MRI scan following gadolinium. The tumor appears dense and is outlined by a circle. Image provided by Dr. Richard Gilbertson.

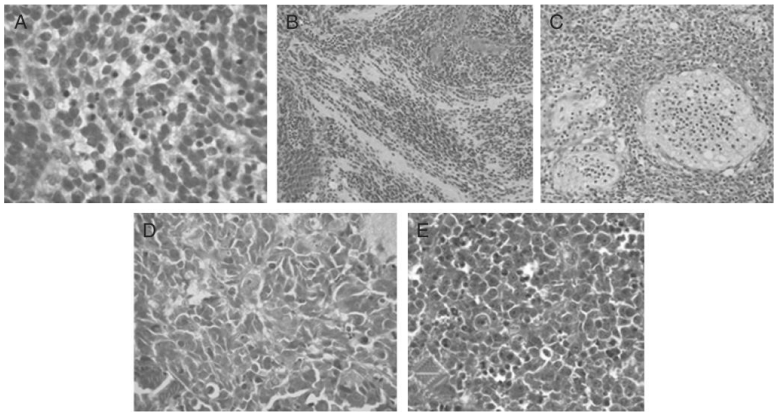

Figure 8.11.

Histopathology of the five medulloblastoma variants. (A) Classic medulloblastoma marked here by a syncytial arrangement of densely packed small uniform cells with hyperchromatic nuclei. (B) Desmoplastic/nodular medulloblastoma showing nodules of differentiated neurocytic cells separated by regions containing moderately pleomorphic cells with a high growth fraction. (C) Medulloblastoma with extensive nodularity with a large irregularly shaped nodules of neurocytic cells often forming linear patterns interspersed with small collections of pleomorphic cells. (D) Anaplastic medulloblastoma characterized with a large markedly pleomorphic cells with a high mitotic count and nuclear:cytoplasmic ratio mold to each other. (E) Large cell medulloblastoma showing large cells with a single nucleolus among more pleomorphic cells with an anaplastic phenotype.

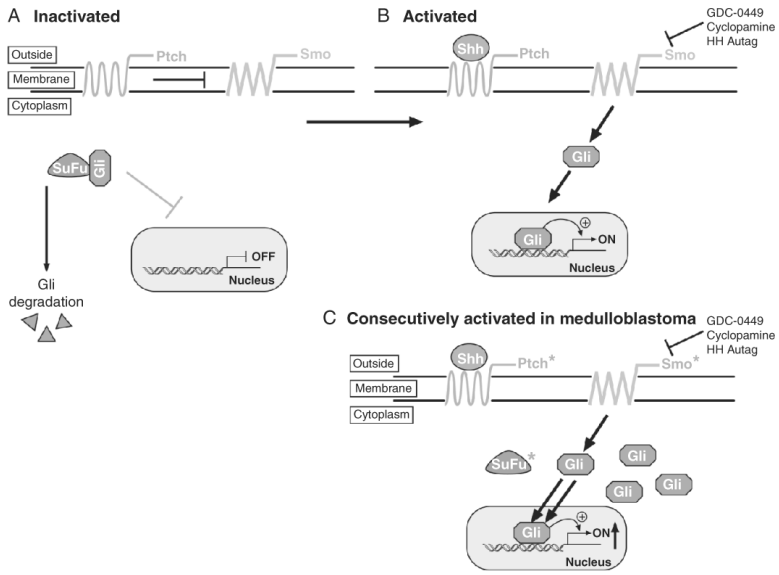

Figure 8.12.

Schematic of the sonic hedgehog signaling pathway. (A) In the absence of Sonic hedgehog (Shh), the 12 transmembrane receptor Patched (Ptch) suppresses the seven transmembrane G-coupled receptor Smoothened (Smo) and the transcription factor Gli is sequestered into the cytoplasm by the Suppressor-of-Fuse (SuFu) preventing it from translocating to the nucleus and is degraded leading to a block of transcription. (B) In the presence of Shh, the suppression by Ptch is relieved and Smo activates Gli1 that is translocated to the nucleus and induces the transcription of Gli targets, including many proproliferative genes. (C) In medulloblastoma, loss of Ptch and mutations in Smo or SuFu lead to the constitutive activity of Smo, accumulation of Gli and increased transcription of proproliferative Gli-dependent targets. Asterisk (*) identifies proteins mutated in medulloblastoma.

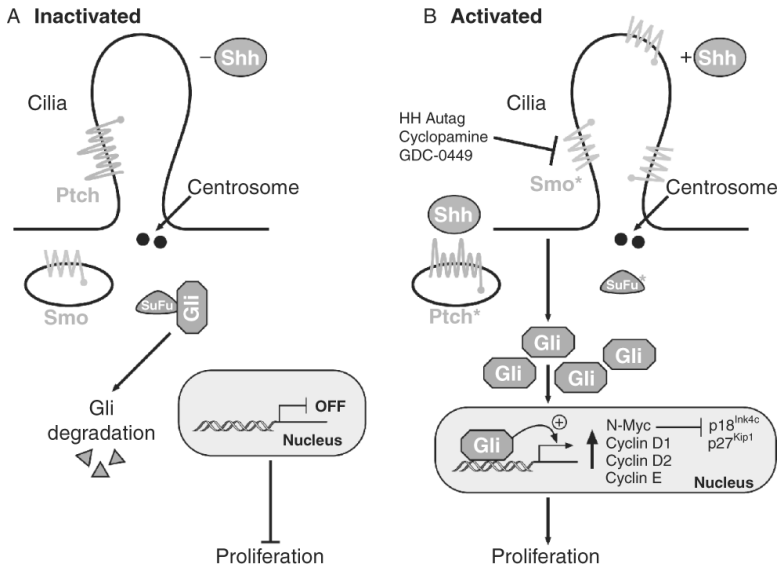

Figure 8.13.

Schematic of the sonic hedgehog signaling pathway in the primary cilium. (A) In the absence of Shh, Ptch resides at the base of the cilium whereas Smo is in the cytoplasm and the pathway is off. (B) In the presence of Shh or in medulloblastoma, Smo becomes located to the cilium. In the absence of Ptch or when Smo or SuFu are mutated, Gli and the levels of targets increase. Asterisk (*) identifies proteins mutated in medulloblastoma.

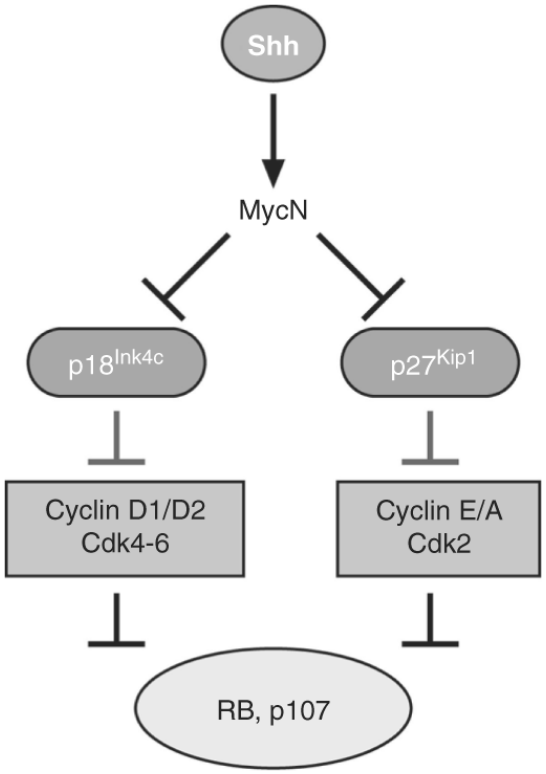

Figure 8.14.
Regulation of MycN and cell cycle proteins by sonic hedgehog signaling. Shh signaling directly induces the expression of MycN and Cyclins D1, D2, and E. MycN in turn suppresses the expression of two cyclin-dependent kinase (Cdk) inhibitory proteins, $p18^{Ink4c}$ and $p27^{Kip1}$. While $p18^{Ink4c}$ inhibits the activity of Cdk4 and Cdk6 in complex with cyclins D1 and D2, $p27^{Kip1}$ suppresses the activity of the Cyclin E/ and A/Cdk2 complexes. Cyclin/Cdk complexes phosphorylate Rb and p107 to allow the cells to progress through the first gap (G1) phase of the cell cycle and enter the DNA synthetic (S) phase when they replicate their DNA.

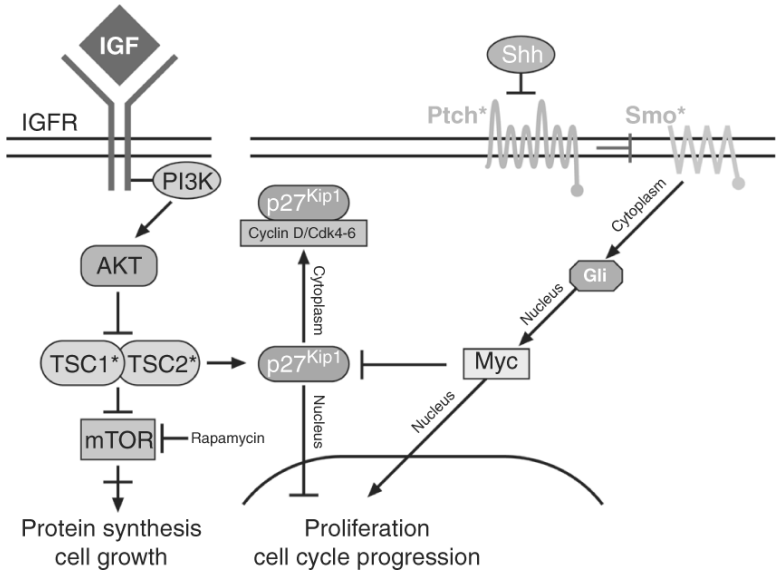

Figure 8.15.

Collaboration between insulin-like growth factor and Shh signaling. IGF activates the insulin-like growth factor (IGF) receptor (IGFR) which in turns activates the phosphoinositol 3-kinase (PI3K), the AKT kinase which suppresses the tumor suppressor TSC1 and TSC2 proteins. TSC1 and TSC2 proteins induce p27Kip1 that prevent G1 progression and block mTOR which is required for protein synthesis and cell growth. Rapamycin blocks mTOR activity. Shh signaling in turn activates Mycn that suppresses $p27^{Kip1}$. Asterisk (*) identifies proteins mutated in medulloblastoma. Adapted from Bathia *et al.* (2010).

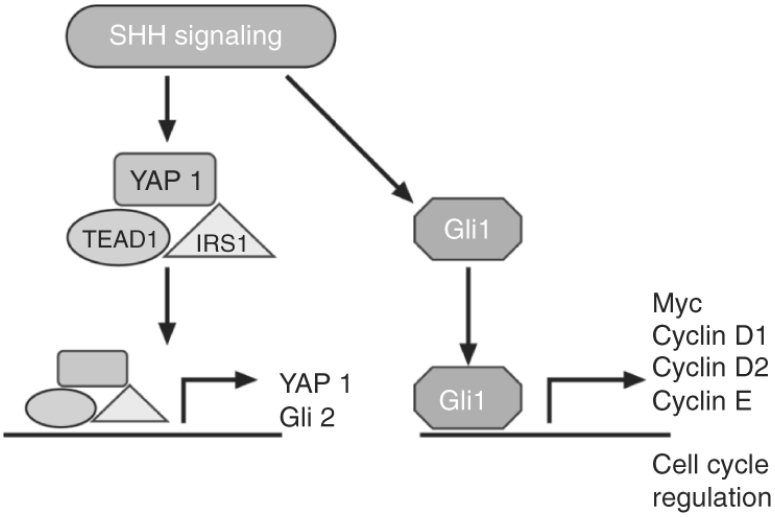

Figure 8.16.
Collaboration between Shh and Hippo signaling. Shh regulates YAP1 in complex with TEAD and IRS1 that is translocated to the nucleus where it regulates YAP1 itself and Gli2. Shh signaling regulates Gli1 and its direct targets. Adapted from Fernandez *et al*. (2009).

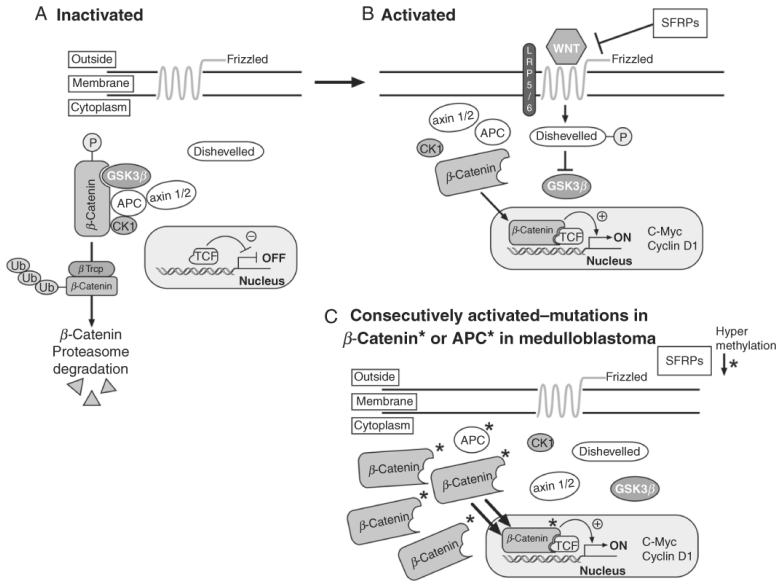

Figure 8.17.

The Wnt signaling pathway. (A) In the absence of ligand, the transmembrane frizzled receptor is inactive and β-Catenin is in complex with APC, axin 1 and 2, caseine kinase 1 (CK1), and GSK-3beta. This enables GSK-3beta to phosphorylate β-Catenin that is then ubiquitinaed by the E3 ligase β-Trcp and degraded by the proteasome. (B) Upon binding of Wnt ligands, lrp5/6 and Frizzell are activated, disheveled is phosphorylated and blocks GSK-3β kinase activity. β-Catenin is no longer in a complex with APC, Axin, and CK1 and β-Catenin is translocated to the nucleus where it transcribed cyclin D1 and C-Myc. SFRPs prevent Wnt from binding to Frizzell and inhibit Wnt signaling. (C) In medulloblastomas, the Wnt pathway is constitutively activated leading to the accumulation of β-Catenin levels and increase transcription.

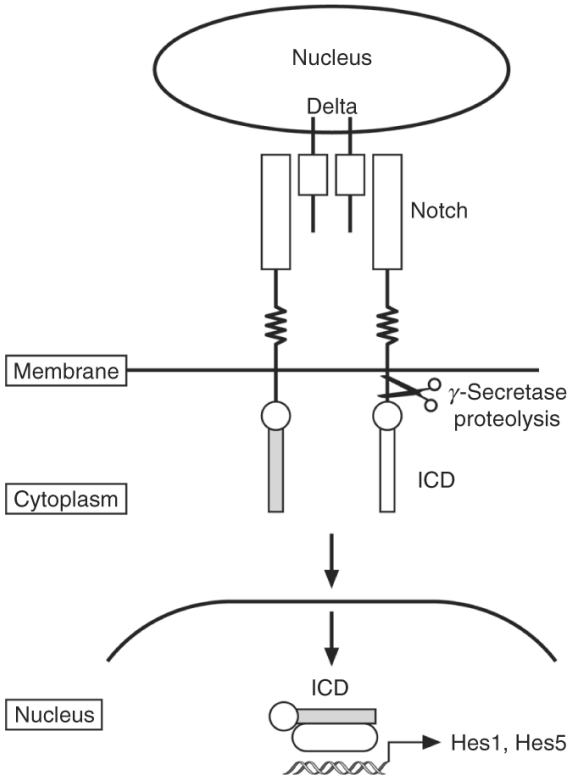

Figure 8.18.
The Notch signaling pathway. Notch signaling is mediated by two cells; one providing the transmembrane ligands Delta and Jagged, the other the single transmembrane receptor, Notch, that transmits the signals. Upon ligand binding, the intracellular domain (ICD) is clipped by the γ-secretase at the membrane, is translocated to the nucleus where it enters in a complex with other proteins, and induces transcription of specific targets including the basic-helix-loop-helix transcription factors, Hes1 and Hes5.

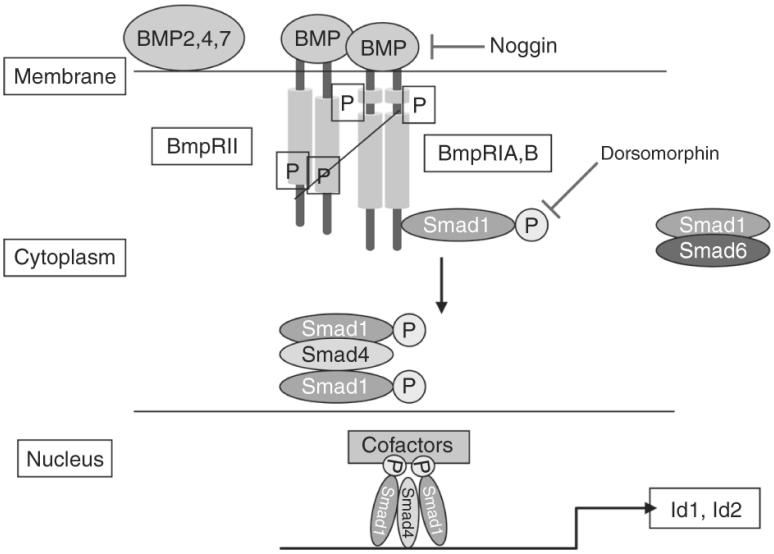

Figure 8.19.

Bone morphogenic protein (BMP) signaling. BMP-2, 4, 7 bind to heterodimeric serine/threonine kinase receptors BMP-RII and BMP-RIA/B to induce their activation and *trans*-phosphorylation. This induces the recruitment of Smad1 that is sequestered by Smad6 in the cytoplasm in the absence of BMPs, and its phosphorylation. Phosphorylated Smad1 enters in complexes with Smad4 and is translocated to the nucleus where is induces the transcription of several genes including the inhibitors of DNA binding, Id1 and Id2.

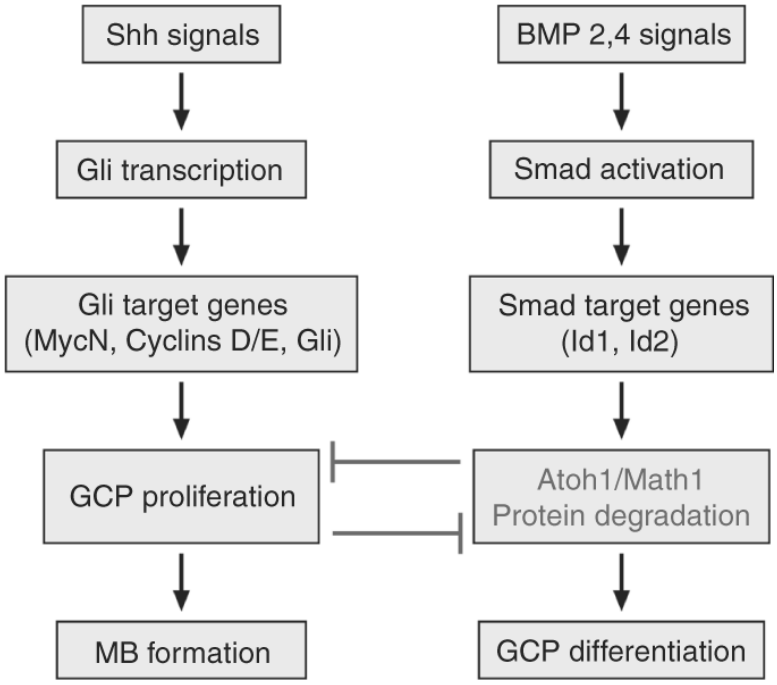

Figure 8.20.
Shh and BMP signaling. Both signaling pathways antagonize each other to regulate proliferation of GCPs by regulating the levels of the bHLH transcription factor Atoh1/Math1. Whereas Shh induces proliferation, BMP inhibits proliferation and induces the irreversible differentiation of GCPs. BMP induces cell growth arrest by the rapid posttranscriptional downregulation of Atoh1/Math1 protein by the proteasome. In medulloblastomas, Atoh1/Math1 levels are high whereas the genes regulating the BMP pathway are downregulated.

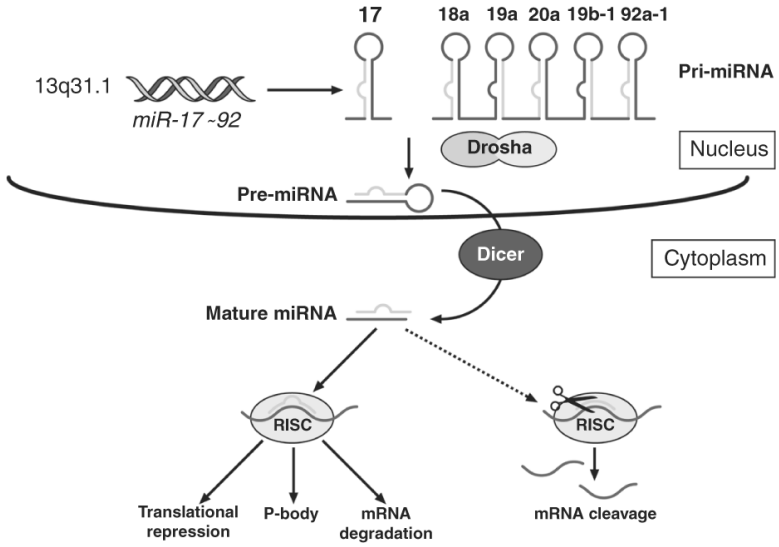

Figure 8.21.

MicroRNA biogenesis and miR-17~92 processing. MicroRNAs (miRs) are encoded by the genome on many chromosomes but do not encode proteins. The miR-17~92 cluster is encoded on human chromosome 13q31.1 as a long pri-miRNA and processed by the processing enzyme Drosha. Pre-miRNAs are further processed in mature miRs where they enter in a RISC complex with argonaute proteins. RISC complexes recognize the complementary sequence on the 3′ end, 5′ end or even the coding region of the messenger mRNA targets. This leads mainly to the inhibition of protein synthesis but the mRNA target could also be trapped in P bodies or degraded. Ultimately, gene expression is suppressed. Figure provided by Dr. Zindy.

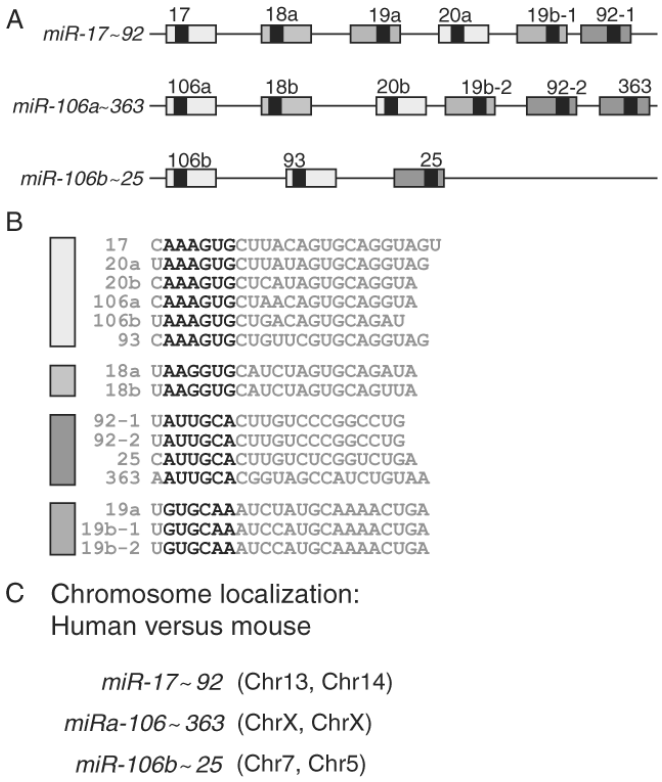

Figure 8.22.
The miR-17~92 cluster family. The miR-17~92 cluster family comprises three members (A) encoding unique miRs that share sequence homology in the 6 mer “seed” sequence labeled in bold letters (B) and encoded in different chromosomes in human and mice (C). Adapted from Ventura *et al.* (2008).

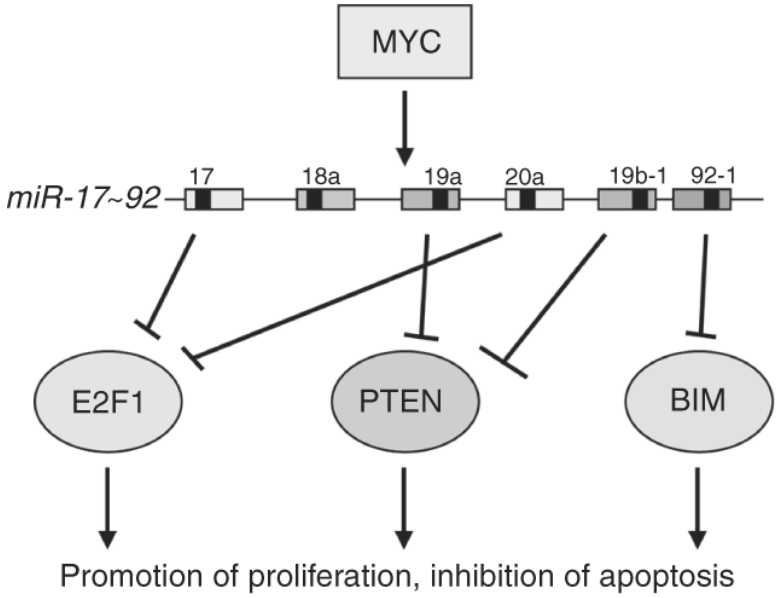

Figure 8.23.
The miR-17~92 cluster is an MYC target. Myc directly binds to the promoter of the cluster to induce its expression. The miR-17~92 encodes six individual miRs that target different cell death-inducing proteins, including E2F-1, PTEN, and BIM. Figure provided by Dr. Zindy.

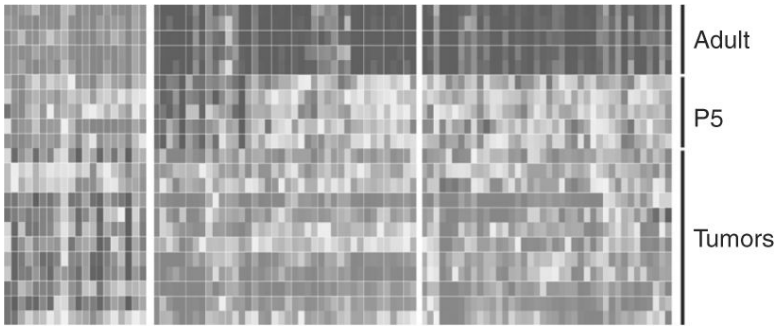

Figure 8.24.
Gene profile of mouse genes in the cerebellum and medulloblastoma. Upregulated (light gray) and downregulated (dark gray) gene expressions are compared between adult mouse cerebella, GCPs purified at postnatal day P5 and in medulloblastomas spontaneously arisen from genetically engineered mice, including *Ligase4−/−*, *p53−/−*, *Ptch1+/−*, *Ptch1+/−*, *P53−/−*, *P53−/−*, *Ink4c−/−*. Note that the profile of gene expression is similar between GCPs from P5 cerebella and tumors consistent with the GCPs as the cell of origin for this MB variant. Adapted from Lee *et al.* (2003).