

Morphing into Cancer: The Role of Developmental Signaling Pathways in Brain Tumor Formation

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ABSTRACT: Morphogens play a critical role in most aspects of development, including expansion and patterning of the central nervous system. Activating germline mutations in components of the Hedgehog and Wnt pathways have provided evidence for the important roles morphogens play in the genesis of brain tumors such as cerebellar medulloblastoma. In addition, aberrant expression of transforming growth factor- β

(TGF- β) superfamily members has been demonstrated to contribute to progression of malignant gliomas. This review summarizes our current knowledge about the roles of morphogens in central nervous system tumorigenesis. © 2005 Wiley Periodicals, Inc. *J Neurobiol* 64: 458–475, 2005

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INTRODUCTION

Morphogens are secreted signaling molecules that play essential roles in pattern formation. To be classified as a morphogen a molecule must be expressed in a temporally and spatially regulated manner during development, it must be released from a localized source and capable of forming a concentration gradient, and it must affect target cells in a concentration-dependent manner (Gurdon and Bourillot, 2001). Molecules that satisfy these criteria include members of the Wnt, Hedgehog (Hh), and bone morphogenetic protein (BMP) families, all of which have been found to play important roles in embryonic development and patterning. These molecules are particularly critical for proper development of the central nervous system. While many other secreted molecules (e.g., fibroblast growth factors, epidermal growth factors, and neurotrophins) also play important morphogenetic roles during CNS development (Novak et al.,

2001; Dono, 2003), due to space restrictions this review will focus on the “classic” morphogens (originally defined in *Drosophila*): Hedgehog, Wnt, and BMP.

Given the central role of morphogens in the highly regulated process of development, it is not surprising that aberrant expression of these molecules might play a role in tumorigenesis. Indeed, there is increasing evidence that Sonic Hedgehog and Wnt signaling contribute to the growth and maintenance of a variety of cancers (reviewed in: Taipale and Beachy, 2001; Pasca di Magliano and Hebrok, 2003; Moon et al., 2004). In the case of CNS malignancies, studies of inherited tumor syndromes such as Turcot’s and Gorlin’s syndromes have implicated the Wnt and Hedgehog signaling pathways in the etiology of one of the most common and aggressive brain tumors, medulloblastoma (Hamilton et al., 1995).

It has become increasingly clear that a strong link exists between the biology of development and the biology of cancer. Therefore, a deeper understanding of the mechanisms that govern normal development is likely to provide insight into the molecular basis of cancer. This review aims to summarize the evidence for the involvement of morphogens in CNS tumors

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and to discuss the implications of these findings for brain tumor biology. Particular emphasis will be placed on the Hedgehog and Wnt pathways, because mutations in these pathways have been demonstrated to predispose to CNS tumors. We will begin by describing the roles of these morphogens in normal neural development, and then discuss the evidence for their involvement in tumors of the CNS.

HEDGEHOG SIGNALING IN DEVELOPMENT

One of the most potent morphogens involved in both invertebrate and vertebrate development is Hedgehog. First discovered in *Drosophila*, Hedgehog was determined to regulate segment polarity in the fly embryo (Nusslein-Volhard and Wieschaus, 1980). Based on its vital roles in fly embryogenesis, investigators hypothesized that there might be homologous Hh proteins in vertebrates. Indeed, three mammalian homologues—Sonic Hedgehog (Shh), Desert Hedgehog (Dhh), and Indian Hedgehog (Ihh)—were discovered in the early 1990s, and homologues were also found in fish and chick (Echelard et al., 1993; Krauss et al., 1993; Riddle et al., 1993). Studies preceding the discovery of Shh had indicated that in the early embryo, an unknown ventralizing signal in the notochord and floor plate provided patterning signals for the development of the neural tube (Yamada et al., 1993). The discovery of mammalian homologues of the Hh gene led to the recognition that the ventralizing signal was Shh (Echelard et al., 1993; Roelink et al., 1995). This initial role for Shh soon broadened as it became clear that Shh plays roles in almost all aspects of development, ranging from regulation of proliferation and differentiation to cell fate specification.

Sonic Hedgehog Pathway

Due to its importance in the developing animal, elucidating the mechanisms of Shh signaling has been of prime interest. While much of the pathway has been identified and studied in *Drosophila*, many components are highly conserved in vertebrates as well (Goodrich et al., 1996; Stone et al., 1996, 1999). Shh itself is a secreted protein that undergoes a series of modifications in the signaling cell, including autocleavage, cholesterol modification, and palmitoylation, to produce the active form capable of triggering downstream signaling (Porter et al., 1996). One of the key players in this pathway is the Shh receptor

Patched (Ptc). Ptc is a twelve-pass transmembrane protein expressed on the surface of the receiving cell. In the absence of Shh, Ptc represses the activity of a seven-pass transmembrane protein called Smoothed (Smo), thereby preventing transcription of target genes (Alcedo et al., 1996). In the presence of Shh ligand, Ptc is internalized and its activity is inhibited, alleviating repression of Smo (Denef et al., 2000; Zhu et al., 2003). As a consequence Smo becomes activated, recruits other components of the signaling pathway, and promotes expression of target genes.

The mechanisms by which Ptc represses Smo, and the manner in which derepression of Smo leads to signaling and target gene expression, remain unclear. Recent studies have suggested that Ptc repression of Smo may involve transport of molecules or vesicles within the receiving cell. Structurally, Ptc is homologous to members of the resistance nodulation cell-division (RND) family of bacterial transporters, which pump ions and toxins across membranes. This has led to the proposal that Ptc represses Smo by transporting a small molecule into the cell, and that Hedgehog binding may function to prevent this transport (Taipale et al., 2002). A role for vesicle trafficking in the Hedgehog pathway is implied by the observation that mice with mutations in Rab23—a GTPase involved in vesicle transport—have a phenotype characteristic of overactive Shh signaling (Eggenchwiler et al., 2001). Because Hedgehog binding causes internalization of Ptc and relocation of Smo to the cell surface (Denef et al., 2000; Zhu et al., 2003), it is tempting to speculate that Rab23 participates in moving vesicles containing one of these proteins. Further studies of the mechanism and functional significance of Ptc and Smo localization will be necessary to resolve this issue.

Whatever the mechanisms of Hedgehog signal transduction at the membrane, it is clear that the major consequence of pathway activation is regulation of Ci/Gli family transcription factors. The *Drosophila* Cubitus interruptus (Ci) protein can function as both a repressor and activator of Shh target genes. In the absence of Shh, Ci is found in a cytoplasmic complex containing the kinesin-like protein Costal2 (Cos2), the serine-threonine kinase Fused (Fu), and Suppressor of Fused (SUFU), a protein required for cytoplasmic retention of Ci (Monnier et al., 1998; Delattre et al., 1999). This complex is anchored to microtubules, and in the resting state, promotes the ubiquitination of Ci by the E3 ubiquitin ligase Slimb/ β -transducin repeat containing protein (β -TRCP). Ubiquitination promotes proteolytic cleavage of Ci into an N-terminal fragment that enters the nucleus and represses target gene expression (Aza-Blanc et al., 1997; Maniatis, 1999; Wang et al., 1999). In

the presence of Shh ligand, Smo is stabilized and able to recruit Cos2 and Fu to its cytoplasmic tail (Jia et al., 2003; Lum et al., 2003; Ogden et al., 2003; Ruel et al., 2003). This allows full-length Ci to enter the nucleus and activate target genes.

In vertebrates, there are three homologues of Ci: Gli1, Gli2, and Gli3 (Yoon et al., 1998; Aza-Blanc et al., 2000). Of these, Gli2 is most closely related to Ci, in that it can be cleaved and function as both a repressor and an activator of Shh target genes. In contrast, Gli1 cannot be cleaved and can only function as an activator. Because it is often found at low levels in Hh-responsive cells, and is transcriptionally induced by the pathway, it may function more as an amplifier than as a primary transducer of the Hh signal (Park et al., 2000; Bai et al., 2002). Gli3 has both activation and repression domains, and has been shown to be cleaved in the presence of Shh; however, in most tissues it functions as a repressor of Shh signaling (Marigo et al., 1996; Wang et al., 2000). Thus, all three Gli family members may cooperate to regulate expression of Shh target genes.

Shh in CNS Development

Through activation of its target genes, Shh signaling regulates a variety of cellular responses including proliferation, differentiation and survival. For example, Shh is involved in left-right asymmetrical patterning of the embryo, limb bud formation, formation of the olfactory system, and prostate growth (Riddle et al., 1993; Meyers and Martin, 1999; Podlasek et al., 1999; LaMantia et al., 2000). Among the best characterized effects of Shh signaling is its activity in the embryonic spinal cord (neural tube). Acting in a morphogen gradient along the ventral neural tube, Shh specifies the fate of neural precursors by activating a specific pattern of homeobox genes throughout the dorso-ventral axis of the embryo (Briscoe et al., 2000; Sander et al., 2000). Shh also plays a role in specification of the dopaminergic neurons in the developing midbrain and forebrain (Hynes et al., 1995; Ye et al., 1998). In late embryonic and postnatal stages, Shh is expressed dorsally and regulates proliferation of precursors in the cerebral cortex, tectum, and cerebellum (Dahmane et al., 2001).

In the cerebellum, Shh controls the generation of granule cells, the most abundant class of neurons. Granule cell precursors (GCPs) originate from a dorsal hindbrain structure known as the rhombic lip. During late embryonic life, these cells stream around the surface of the cerebellar anlage to form the external germinal layer (EGL). Around the time of birth,

the EGL contains a small number of GCPs. These cells express Ptc, Smo, and Gli-2, and are therefore competent to respond to Hedgehog signals (Corrales et al., 2004). At the same time, Purkinje cells arrayed beneath the EGL are secreting Shh protein, which induces robust proliferation of GCPs (Dahmane and Ruiz-i-Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). During the first 2–3 weeks after birth, GCPs proliferate rapidly at the surface of the cerebellum, and then exit the cell cycle and migrate inward to the internal granule layer, where they differentiate into mature granule neurons (Wang and Zoghbi, 2001). Shh appears to be a critical signal for proliferation of GCPs, because mice lacking Shh or Gli-2 in the cerebellum show severe reductions in granule cell generation (Corrales et al., 2004; Lewis et al., 2004). However, while it has been shown that Shh acts as a mitogen for GCPs, there is no evidence that Shh acts in a morphogen gradient in the cerebellum. The signals that allow cell cycle exit and differentiation of these cells are less well understood, but may include fibroblast growth factors, BMPs, pituitary adenylate cyclase activating polypeptide (PACAP), and extracellular matrix molecules such as vitronectin (Wechsler-Reya and Scott, 1999; Pons et al., 2001; Nicot et al., 2002; Rios et al., 2004).

WNT SIGNALING IN DEVELOPMENT

Wnt genes encode a large family of well-conserved secreted glycoproteins, of which there are 24 known vertebrate members (reviewed in Cadigan and Nusse, 1997). The founding member of the family, Wingless (Wg), was identified in *Drosophila* (Sharma and Chopra, 1976), where it was demonstrated to be essential for establishment of segment polarity and for specification of the wing (Couso et al., 1993). The vertebrate counterpart, Int-1 (now called Wnt-1), was identified independently in the mouse as a protooncogene activated by integration of mouse mammary tumor virus (Nusse and Varmus, 1982). It was not until 5 years later that Wg was identified as an ortholog of Wnt-1 (Cabrera et al., 1987; Rijsewijk et al., 1987). Since the discovery of Wnt-1, homologous genes have been found in organisms ranging from the nematode *C. elegans* to mammals (Cadigan and Nusse, 1997). These proteins play essential roles in embryonic development, including regulation of patterning, proliferation, and cell fate determination (McMahon and Bradley, 1990; Thomas and Capecchi, 1990; Lee et al., 2000; Wilson et al., 2001). There is evidence that Wg functions as a morphogen during *Drosophila* development, altering gene

expression in a concentration-dependent manner and eliciting different responses depending on the distance from Wg-secreting cells (Zecca et al., 1996; Neumann and Cohen, 1997). However, it remains unclear whether other Wnt proteins act as “classical” morphogens or whether they mediate their effects indirectly by a relay-type mechanism.

Wnt Signaling

Wnts exert their effects by binding to members of the Frizzled (Fz) family of seven-pass transmembrane receptors. Downstream of Fz proteins, Wnts can signal by at least two distinct mechanisms, termed the canonical and noncanonical pathways (Heisenberg et al., 2000; Kuhl et al., 2000; Pinson et al., 2000; Moon et al., 2002). It should be noted that the two pathways are not mutually exclusive, with several Wnts having both canonical and noncanonical properties.

Activation of the canonical Wnt pathway requires binding of ligands not only to Fz proteins, but also to the low density lipoprotein receptor related proteins, LRP-5 and LRP-6 (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). The central mediator of canonical Wnt signaling is β -catenin. In the absence of a Wnt stimulus, β -catenin is recruited to a cytoplasmic complex (the “destruction complex”) containing the serine-threonine kinase glycogen synthase kinase 3- β (GSK-3 β), the scaffold protein Axin, and the tumor suppressor protein adenomatous polyposis coli (APC). Within this complex, GSK-3 β phosphorylates the N-terminal domain of β -catenin and thereby targets it for degradation via the ubiquitin-proteasome pathway (Brown and Moon, 1998; Wodarz and Nusse, 1998). Binding of Wnt to Fz and LRP-5/6 induces a cascade of downstream events including activation and membrane recruitment of the phosphoprotein Dishevelled (DSH). DSH recruits Axin to the plasma membrane, where it binds directly to the cytoplasmic tail of LRP-5/6 (Cliffe et al., 2003; Moon et al., 2004). This results in degradation of Axin and inhibition of GSK-3 β , with subsequent dissociation of the destruction complex. Unphosphorylated and free from the complex, β -catenin accumulates and translocates to the nucleus, where it interacts with members of the T-cell factor/lymphocyte enhancer factor (TCF/LEF) family of transcription factors to induce expression of Wnt-responsive genes. Wnt target genes include regulators of cell cycle progression *c-myc* and *cyclin D1* (He et al., 1998; Shtutman et al., 1999). Antagonists of the Wnt pathway include members of the secreted frizzled-related

protein family (sFRP) and members of the Dickkopf family (reviewed in Kawano and Kypta, 2003).

Noncanonical (β -catenin independent) Wnt pathways are less well understood but are generally believed to regulate cell movement and behavior during development. (Moon et al., 2004). They include the Wnt/calcium (Ca^{++}) pathway (Kuhl et al., 2000), whereby receptor ligation causes increased intracellular Ca^{++} with subsequent activation of protein kinase C (PKC), and the planar cell polarity (PCP) pathway, which involves activation of the c-jun N-terminal kinase (JNK) and has been shown to guide cell movement during gastrulation (Heisenberg et al., 2000). There is evidence that noncanonical Wnt signaling can antagonize canonical signaling in specific contexts. An example of this antagonism is seen with Wnt-5A, which is thought to act through the Wnt/ Ca^{++} pathway. Wnt-5A knockout mice (Topol et al., 2003) and Wnt-5A zebrafish mutants (Westfall et al., 2003) exhibit increased β -catenin signaling, consistent with the idea that Wnt-5A can function as an antagonist of β -catenin. Interestingly, a tumor suppressor role has been proposed for Wnt-5A. Mice that are hemizygous for Wnt-5A develop myeloid leukemia and B-cell lymphoma (Liang et al., 2003). In addition, Wnt-5A has been found to be deleted or down-regulated in most patients with these cancers (Liang et al., 2003). However, Wnt-5A levels are increased in other types of cancer including metastatic melanoma, and there is some evidence that Wnt-5A might play a role in cell invasiveness and motility in melanoma through the Wnt/ Ca^{++} pathway (Weeraratna et al., 2002).

Wnt Mediated Control of Neural Progenitor Growth

The Wnt pathway has been shown to regulate growth and patterning in the developing nervous system. For example, targeted disruption of Wnt-1 results in loss of cells at the midbrain-hindbrain boundary and severe abnormalities in midbrain and cerebellar development (Thomas and Capecchi, 1990; McMahon et al., 1992). Additionally, absence of Wnt-3a in the cortical hem (a transient neuron-containing structure that forms the boundary between the hippocampus and the telencephalic choroid plexus epithelium) results in a dramatic reduction of proliferation of hippocampal progenitors and complete absence of the dentate gyrus (Galceran et al., 2000; Lee et al., 2000).

Further support for the role of the Wnt pathway in regulating proliferation and differentiation of neural progenitors comes from recent studies by Zechner et al. (2003). These investigators generated two con-

ditional mutant alleles, one resulting in ablation of β -catenin in the CNS and the other resulting in CNS-specific expression of constitutively active β -catenin. In the absence of β -catenin they found that the tissue mass of the spinal cord and brain was markedly reduced. Conversely, the spinal cord and brain of mice expressing activated β -catenin were enlarged with a marked increase in the neuronal precursor population. These data concur with studies carried out by Chenn and Walsh (2002, 2003). Using transgenic mice expressing a stabilized form of β -catenin in neuronal progenitor cells, these authors demonstrated that adult mice develop enlarged forebrains with increased cerebral cortical surface area and enlarged hippocampi. The authors suggest that β -catenin influences the decision of neural precursors to re-enter the cell cycle instead of differentiating. These studies also provide evidence that regulation of β -catenin is critical for proper neuronal migration.

Wnt proteins continue to be expressed in the brain at postnatal stages and have been implicated in several aspects of cerebellar development. Expression of Wnt-3a is restricted to Purkinje cells in the postnatal cerebellum and is modulated by presynaptic granule cells, while Wnt-7a expression is restricted to the granule cells where it is thought to play a role in the formation of neural connections (Hall et al., 2000). Taken together, these results highlight the importance of Wnt signaling in normal development of the central nervous system.

Bone Morphogenetic Proteins

BMPs are members of the transforming growth factor-beta (TGF- β) superfamily (Rosen et al., 1989; Celeste et al., 1990). Other members of this large family of cytokines include the TGF- β proteins, activins, inhibins, and Mullerian-inhibiting substance (Massague, 1998). BMPs comprise a growing family of more than 20 members that can be classified into distinct subgroups based on their primary amino acid sequences (Ebendal et al., 1998). These subgroups include the Decapentaplegic (Dpp) family (BMP-2 and 4), and the 60A family (BMP-5-8). BMPs were originally identified as bone-repair factors in vertebrates (Urist, 1965) and independently as dorso-ventral patterning agents in *Drosophila* (Spencer et al., 1982). Subsequent studies in vertebrate systems have shown that BMPs play crucial roles in many aspects of development—influencing gastrulation and neurogenesis, and regulating growth, differentiation, apoptosis, cell adhesion, and migration in many cell types (reviewed in Hogan, 1996; Mishina, 2003).

BMP Signaling

Like all members of the TGF- β superfamily, BMPs are synthesized with an amino terminal propeptide region. The mature protein derives from the carboxy-terminal region and is released upon proteolytic cleavage. BMPs exert their effects by binding distinct combinations of type I and type II serine/threonine kinase cell surface receptors, resulting in initiation of intracellular signaling (Attisano et al., 1992; Yamashita et al., 1994; Kawabata et al., 1995; Rosenzweig et al., 1995). BMP signals are transmitted to the nucleus via Smad proteins—cytoplasmic proteins that are activated by receptor-mediated phosphorylation.

To date, three type I receptors [activin type I receptor (ActR-I) and BMP receptors (BMPRs) IA and 2A] and three type II receptors (BMPR-II, ActR-II, and ActR-IIB) have been identified in mammals (Koenig et al., 1994; ten Dijke et al., 1994; Liu et al., 1995; Rosenzweig et al., 1995; Yamashita et al., 1995). Activation of BMP signaling requires binding of ligands to at least one type I receptor and one type II receptor. BMPs bind with differing affinities to distinct type I receptors, with BMP-2 and -4 preferentially binding BMPR-1A and BMPR-2A, and BMP-7 preferentially binding ActR-I and BMPR-2A (ten Dijke et al., 1994; Yamashita et al., 1995; Macias-Silva et al., 1998). Type II receptors possess a constitutively active kinase that activates type I receptors by phosphorylation. Type I receptors in turn phosphorylate and activate members of the pathway-restricted Smads (R-Smads-1, 5, and 8), initiating activation of intracellular signaling (Kretzschmar et al., 1997). R-Smads form a complex with Smad-4 (co-Smad) and move to the nucleus (Liu et al., 1997). Once in the nucleus, Smad proteins regulate transcription of BMP target genes either by directly binding DNA or through association with other DNA binding proteins (Yingling et al., 1997; Dennler et al., 1998; Zawel et al., 1998). BMP target genes include Runx2, JunB, and Id proteins (Jonk et al., 1998; Korchynskyi and ten Dijke, 2002; Lee et al., 2002). Given the broad range of biological effects exerted by BMPs it is not surprising that their activities are tightly regulated. One mode of regulation involves the antagonists Noggin and Chordin, which act by directly binding BMPs and blocking ligand activity (Balemans and Van Hul, 2002).

BMPs in CNS Development and Neural Fate Specification

BMPs have diverse effects in the development of the CNS, where they are involved in dorso-ventral

patterning of the neural tube (Liem et al., 1995), regulation of neuronal and glial differentiation in the spinal cord and cortex (Kalyani et al., 1998; Li et al., 1998; Wine-Lee et al., 2004), and control of apoptosis (Graham et al., 1994). Although expression of BMPs is relatively low in the adult CNS, expression increases rapidly following CNS injury and may have neuroprotective effects (Wang et al., 2001; Chang et al., 2003). BMP expression is tightly controlled in a complex spatio-temporal manner—BMP-2 shows peak expression at embryonic day 16 (E16) in the hippocampus, cerebellum, and cerebral cortex, while BMP-4 exhibits peak expression in all brain regions at postnatal day 4 (reviewed in Mehler et al., 1997).

As is the case for Shh, during development BMPs act as gradient morphogens, inducing dorso-ventral effects in a concentration-dependent manner (Furuta et al., 1997; Nguyen et al., 2000). BMP signaling, at first from the epidermal ectoderm and subsequently from roof plate cells located at the dorsal midline of the neural tube, mediates induction of the neural crest (Liem et al., 1997). In addition to this morphogenetic function, BMPs have also been demonstrated to play roles in specification of neurons and glia. For example, Alder et al. (1999) demonstrated that BMP-6, BMP-7, and BMP-12 were expressed in dorsal midline cells adjacent to the rhombic lip (where granule cell progenitors originate) and that these BMP family members induced expression of granule cell precursor markers in cultured neural tissue. Additionally, transplantation of BMP-treated neural cells into the EGL of the postnatal mouse cerebellum resulted in formation of mature granule neurons (Alder et al., 1999). BMPs also play a role in cell fate determination in the cortex, increasing neuronal differentiation early in development and inhibiting neurogenesis and oligodendroglialogenesis while promoting astroglialogenesis later in development (Li et al., 1998; Mabié et al., 1999; Mehler et al., 2000; Hall and Miller, 2004). Collectively these studies provide evidence that BMPs are important regulators of cell fate determination in the CNS.

TUMORS OF THE CNS

Tumors of the CNS are particularly challenging to treat due to their rapid growth and metastasis. In addition, therapeutic agents must consist of molecules that are small enough (<600 kDa) to cross the blood-brain barrier. Furthermore, most neurons of the CNS cannot regenerate. Consequently, therapeutic strategies must specifically target cancer cells while pre-

serving the integrity of nonmalignant neuronal and glial cells.

The two most common classes of CNS tumors are primitive neuroectodermal tumors (PNETs) and gliomas. The most common and aggressive PNET is medulloblastoma, a highly malignant cerebellar tumor arising most frequently in children and to a lesser extent in young adults. It accounts for 20–30% of pediatric brain tumors, with peak onset between 5–10 years of age. Treatment to date has consisted of a combination of surgery, radiation, and chemotherapy. Although advances in treatment of medulloblastoma have been significant over the past 30 years, the current 5 year survival rate is only 70% in moderate risk patients treated with all three therapies and 25% in high risk patients (Ellison, 2002). In addition, the currently available treatments often result in cognitive and neuroendocrine deficits, and tumor recurrence is quite common.

Medulloblastoma has been divided into two major subtypes based on histological and molecular characteristics. The majority of tumors consist of a homogeneous array of small, rapidly dividing neuroblasts, and are termed “classic” medulloblastomas. Approximately 25% of tumors exhibit regions of densely packed cells surrounding pale islands (or nodules) of less dense and more differentiated cells. These are termed nodular or “desmoplastic” medulloblastomas. A major unanswered question regarding the biology of these tumors is the cell of origin. It has been suggested that classic medulloblastomas originate from multipotent precursors in the ventricular zone of the cerebellum, while desmoplastic medulloblastomas are thought to originate from granule cell precursors in the EGL (Katsetos et al., 1995; Buhren et al., 2000).

Gliomas encompass a number of tumor types, including astrocytomas, oligodendrogliomas, ependymomas, and choroid plexus tumors. The incidence of gliomas is 5–10 cases per 100,000 people (Legler et al., 1999). Astrocytomas are the most common type of gliomas, and are graded on a scale of I–IV based on degree of cellularity, nuclear and cellular pleomorphism, mitoses, endothelial proliferation, and necrosis (Kleihues et al., 1995). Astrocytic tumors range from benign and low-grade or diffuse astrocytomas (grades I and II) to anaplastic astrocytomas (grade III) and glioblastoma multiforme (GBM; grade IV).

Glioblastoma multiforme is a highly invasive astrocytic tumor with a mean survival time of only 10–12 months following treatment (Scott et al., 1998; Davis et al., 1999). It is the most common and devastating brain tumor in adults, with peak onset between

47 and 70 years of age, and with men more frequently affected than women (Behin et al., 2003). Current treatment consists of maximal surgical resection, radiotherapy, and chemotherapy. The dismal cure rate of this disease is partly due to the highly invasive and heterogeneous nature of the tumor and partly due to tumor hypoxia, which results in resistance to radiation and chemotherapy. The long-term survival rate of patients is low, and patients who do survive have significant disabilities that compromise quality of life (Remer and Murphy, 2004).

Glioblastoma can be divided into two subtypes: primary glioblastomas that develop de novo are the most common, whereas secondary tumors arising from a previously diagnosed tumor of lower malignancy grade are less frequent. These tumors are likely to develop from the mutation of different genes but affect the same cellular pathways (Wechsler-Reya and Scott, 2001). Glioblastomas are highly cellular tumors displaying nuclear and cellular pleomorphism, endothelial proliferation, mitotic figures, and often necrosis (Kleihues et al., 1995). These tumors are of a highly heterogeneous nature in terms of pathology and gene expression. Common signaling pathways that are altered in glioblastoma, either through mutation or overexpression, include the p53 pathway, the pRB pathway, and growth factor receptor pathways, including epidermal growth factor, insulin-like growth factors, vascular endothelial growth factor, and platelet derived growth factor (reviewed in: Wechsler-Reya and Scott, 2001; Rich and Bigner, 2004).

SHH SIGNALING IN CNS TUMORS

ptc Mutations

Interest in the link between Shh signaling and medulloblastoma began with the discovery that human patients with Gorlin's syndrome have inherited mutations in the *ptc* gene (Hahn et al., 1996; Johnson et al., 1996). Gorlin's syndrome presents with skeletal deformities, basal cell carcinomas, and a high incidence of sporadic medulloblastomas (Gorlin, 1995). In addition, mutations in the *ptc* gene were identified in numerous sporadic desmoplastic medulloblastomas (Pietsch et al., 1997; Raffel et al., 1997). This correlation between *ptc* mutations and medulloblastoma led to the theory that *ptc* may function as a tumor suppressor gene. This hypothesis makes particular sense in the case of the cerebellum, where Shh induces proliferation of GCPs (Dahmane and Ruiz-i-Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). Thus,

it follows that if *ptc* is mutated and is unable to repress *smo*, GCPs will overproliferate and perhaps become transformed.

Support for this theory comes from a mouse model of medulloblastoma. In mice, homozygous *ptc* mutations are lethal at the embryonic stage; however, heterozygous *ptc* mutants survive to adulthood and approximately 15–20% develop sporadic medulloblastomas (Goodrich et al., 1997; Hahn et al., 1998). Morphologically and molecularly, these tumors strikingly resemble human medulloblastomas.

Mutations in Additional Shh Pathway Genes

Although mutations in *ptc* account for a subset of medulloblastomas, mutations in other genes in the Hedgehog pathway may also promote medulloblastoma formation. One such gene currently under investigation is *SUFU*. In normal development, *SUFU* aids in the cytoplasmic retention of Gli, which results in Shh target genes remaining inactive. Thus, loss of *SUFU* could serve as a mechanism allowing excessive proliferation of GCPs and promoting tumorigenesis. Indeed, 9% of sporadic human medulloblastomas contain truncating mutations in *SUFU* (Taylor et al., 2002).

While mutations in *ptc* and *SUFU* are associated with medulloblastoma, mutations in other Shh pathway genes appear to be much less prevalent. For example, a study by Lam et al. (1999) reported one case of medulloblastoma with an activating mutation in *smo*; however, a more recent study detected no mutations in *smo* (Zurawel et al., 2000). A second potential tumor suppressor is β -*TRCP*, a vertebrate homologue of *Drosophila Slimb*, which is thought to repress Hedgehog signaling by promoting degradation of Ci/Gli transcription factors (Stone et al., 1999; Wang et al., 1999). Loss of this gene would have the same effect as loss of *ptc*, in that Shh target genes would be activated. However, analysis of medulloblastomas revealed no evidence for mutation or decreased expression of β -*TRCP* (Wolter et al., 2003). *Gli3*, a negative regulator of Shh target genes, has also been proposed to function as a tumor suppressor, but there is no evidence for mutation of *gli3* in medulloblastoma, and one report even suggests that *gli3* mRNA levels are up-regulated (Dahmane et al., 2001; Erez et al., 2002). *Gli1* and *gli2* are also highly up-regulated in medulloblastoma, but this most likely reflects activation of the Shh pathway, because both genes have been suggested to be transcriptional targets (Dahmane et al., 2001). Consistent

Table 1 Shh Pathway Mutations in Sporadic Medulloblastomas

Gene Affected	Type of Mutation	% Mutation	No. of Cases	Study
<i>Ptc</i>	Loss of heterozygosity (LOH) accompanied by loss of function (LOF)	27%	68	(Pietsch et al., 1997)
<i>Ptc</i>	LOH or mutation accompanied by (LOF)	20%	24	(Raffel et al., 1997)
<i>Smo</i>	Activation accompanied by activation of Shh pathway	4%	21	(Lam et al., 1999)
<i>Smo/Shh</i>	–	No observed mutations	37	(Zurawel et al., 2000)
<i>β-TRCP</i>	–	No observed mutations	62	(Wolter et al., 2003)
<i>SUFU</i>	Missense mutation accompanied by activation of Shh pathway	9%	46	(Taylor et al., 2002)
<i>Gli3</i>	–	No observed mutations	2	(Erez et al., 2002)
<i>Gli1/Gli2/Gli3</i>	Up-regulation	100%	22	(Dahmane et al., 2001)

with this, other targets of the pathway, such as N-myc and cyclinD1, are also highly up-regulated in murine and human medulloblastoma (Pomeroy et al., 2002; Kenney et al., 2003; Oliver et al., 2003).

Based on these data (summarized in Table 1), it is clear that proper regulation of the Shh pathway is critical for normal development of the cerebellum and that dysregulation of one or more genes in the pathway can lead to tumor formation. This body of evidence led investigators to examine whether blocking the Shh pathway could lead to tumor regression. Cyclopamine, an inhibitor of Shh signaling, was used to treat both murine and human medulloblastoma cells, and in both cases, cells no longer proliferated and exhibited decreased expression of Shh target genes (Berman et al., 2002). Even more strikingly, *in vivo* treatment of *ptc1^{+/-}p53^{-/-}* mice with a small molecule antagonist of Shh (HhAntag) caused reversal of tumor cell programming (tumor cells differentiated, decreased Shh target gene expression, or died), and at high doses, caused tumor regression (Romer et al., 2004). Thus, while the complex molecular mechanisms of medulloblastoma formation still largely remain a mystery, it is clear that the Shh pathway plays as critical a role in tumorigenesis as it does in normal cerebellar development.

While the Shh pathway can promote tumor progression in the cerebellum, it is important to note that only 15–20% of *ptc^{+/-}* mice, and only 2–5% of patients with Gorlin's syndrome, develop medulloblastoma (Evans et al., 1991; Goodrich et al., 1997). Thus, disruption of Shh signaling alone may not be sufficient to promote tumorigenesis. In light of the similarities between normal cerebellar development and tumorigenesis (Lee et al., 2003; Kho et al., 2004), it is reasonable to consider that other genes, such as those involved in differentiation, migration,

and apoptosis, may play important roles in the transition from normal development to malignancy.

Shh and Other CNS Tumors

Although the importance of Shh signaling in medulloblastoma is widely acknowledged, much less is known about its role in other tumors of the CNS. To date, there have been no reports of *ptc* or *smo* mutations in extra-cerebellar tumors. However, several pieces of evidence suggest that the Shh pathway could contribute to gliomagenesis. First, *gli1* was originally isolated as a gene that was amplified in a glioblastoma cell line (Kinzler et al., 1987). In addition, analysis of primary CNS tumors and tumor cell lines indicates that nearly all tumors tested—including astrocytoma, oligodendroglioma, glioblastoma multiforme, and PNETs—consistently express *gli1* (Dahmane et al., 2001). Finally, the Hedgehog pathway inhibitor Cyclopamine inhibits the growth of several human glioma cell lines (Dahmane et al., 2001), suggesting that these tumors depend on this pathway for their growth or survival. Interestingly, a recent study reports that high expression of *ptc* and *smo* in a subset of astrocytic tumors correlates with decreased tumor malignancy (Katayam et al., 2002). The authors of this study found no correlation between *gli* gene expression and malignancy. Thus, many questions remain to be answered regarding the involvement of Shh signaling in glioma occurrence and progression.

A number of recent studies have suggested that brain tumors may arise from neural stem cells (Hemmati et al., 2003; Oliver and Wechsler-Reya, 2004; Singh et al., 2004). This notion is supported by the observation that primary brain tumors—including

Table 2 Wnt Pathway Mutations in Sporadic Medulloblastomas

Gene Affected	Type of Mutation	% Mutation	No. of Cases Examined	Study
<i>β-catenin</i>	Missense mutation with nuclear accumulation of <i>β-catenin</i>	5%	84	(Koch et al., 2001)
<i>APC</i>	Missense mutation	2.5%		
<i>β-catenin</i>	Missense mutation	8.7%		
<i>APC</i>	Missense mutation	4.3%	46	(Huang et al., 2000)
<i>β-catenin</i>	Missense mutation	4%	367	(Zurawel et al., 1998)
<i>β-catenin</i>	Missense mutation	4%		
<i>Axin1</i>	Missense mutation	4%		
	Both mutations accompanied by nuclear accumulation of <i>β-catenin</i>		23	(Yokota et al., 2002)
<i>β-catenin</i>	Missense mutation in 5/9 examined accompanied by nuclear accumulation of <i>β-catenin</i>	18%	51	(Eberhart et al., 2000)
<i>Axin1</i>	Missense mutation	1%	89	(Dahmen et al., 2001)
<i>Axin1</i>	Missense mutation	5%	39	(Baeza et al. 2003)

astrocytomas and oligodendrogliomas—often express neural stem cell markers and can undergo self-renewal and multilineage differentiation *in vitro*. Because Shh signaling has been shown to be critical for maintenance of neural progenitor cells in the subventricular zone and hippocampus (Lai et al., 2003; Machold et al., 2003), it seems reasonable to speculate that the pathway may be involved in tumorigenesis outside the cerebellum. Clearly, further studies will be necessary to determine whether mutation of specific elements of the pathway is required for tumor initiation, or whether pathway activity is simply required for normal growth or survival of tumor cells (as has been shown in tumors of the lung and GI tract; Berman et al., 2003; Watkins et al., 2003). In either case, targeting the pathway with pharmacologic inhibitors may prove to be an effective mode of therapy.

WNT SIGNALING IN CNS TUMORS

APC Mutations in Turcot's Syndrome

Evidence for the involvement of Wnt signaling in brain tumors has come from studies of Turcot's syndrome. This familial tumor syndrome is characterized by multiple colorectal adenomas and an increased frequency of primary CNS tumors, in particular medulloblastomas and glioblastomas (Turcot et al., 1959). Turcot's can be further divided into two major inherited syndromes—familial adenomatous polyposis, resulting from germline mutations in the *APC* gene (an element of the Wnt pathway), and hereditary non-

polyposis, resulting from germline mutations in one of a group of DNA mismatch repair genes (Paraf et al., 1997). A study by Hamilton et al. (1995) analyzed 14 families with Turcot's syndrome and found that out of 10 families displaying *APC* mutations, the predominant brain tumor type was medulloblastoma (79%). Glioblastoma accounted for 13% of tumors. Further analysis of the cases exhibiting mutations in *APC* revealed loss of the second *APC* allele in one patient (Hamilton et al., 1995), arguing for a tumor suppressor function of *APC* in this hereditary tumor syndrome. In addition, the occurrence of ependymomas (glial tumors arising from ependymal cells lining the ventricular system of brain or spinal cord) has been reported in Turcot's syndrome patients with germline *APC* mutations (Torres et al., 1997; Mullins et al., 1998).

Dysregulation of Wnt Signaling in Sporadic Medulloblastoma

The finding of *APC* mutations associated with CNS tumors in Turcot's syndrome raised the possibility that aberrant Wnt signaling might also contribute to sporadic CNS tumors. A myriad of studies have now examined expression of Wnt pathway genes in brain tumors (summarized in Table 2), and for the most part, only PNETs have been found to harbor mutations in this pathway. For example, *APC* mutations have been demonstrated in 3–4% of sporadic medulloblastomas (Huang et al., 2000; Koch et al., 2001). These were all missense mutations, whereas those found in Turcot's syndrome generally result in pre-

mature truncation of the APC protein. Interestingly, Huang et al. (2000) demonstrated that the APC mutations they found in sporadic medulloblastoma were in regions containing β -catenin or Axin binding sites, raising the possibility that these mutations may impair APC binding to β -catenin or Axin. On the other hand, the mutations observed by Koch et al. (2001) failed to lead to nuclear accumulation of β -catenin, suggesting that APC mutations in medulloblastoma may not directly activate Wnt signaling.

In addition to APC mutations, PNETs have been found to harbor mutations in β -catenin itself. Koch et al. (2001) analyzed 80 patients with medulloblastomas and four patients with supratentorial PNETs (PNET arising in the cerebral hemispheres). Single strand conformational polymorphism (SSCP) and sequencing analysis revealed point mutations in exon 3 of the β -catenin (*CTNNB1*) gene in three cases of medulloblastoma and one case of PNET. All mutations resulted in loss of serine residues that serve as targets for phosphorylation by GSK-3 β and were associated with nuclear accumulation of β -catenin protein in tumor cells. The occurrence of β -catenin missense mutations at serine residues in exon 3 has also been observed in other studies (Zurawel et al., 1998; Eberhart et al., 2000; Huang et al., 2000).

Mutations in Axin have also been observed in medulloblastoma. Axin functions as a scaffold protein in the destruction complex that brings β -catenin into proximity with APC and GSK-3 β , and facilitates its phosphorylation and subsequent degradation (Hsu et al., 1999; Kishida et al., 1999). Two studies have identified *Axin1* point mutations in sporadic medulloblastomas, with a frequency of 1–5% (Dahmen et al., 2001; Baeza et al., 2003). These mutations occur in the APC-binding domain and thus may result in destabilization of the complex and accumulation of nuclear β -catenin. Dahmen et al. (2001) also report large deletions of *Axin* in 12% of examined cases. However, Baeza et al. (2003) detected similar deletions in normal brain tissue and suggested that these deletions may be artifacts of RT-PCR within the *Axin* gene. Baeza et al. (2003) also reported that a common *Axin1* polymorphism (G/A at nucleotide 16 in intron 4) was significantly over-represented in medulloblastoma compared to brain tissue from normal subjects. While the significance of this polymorphism remains to be clarified, collectively, point mutations observed in APC, β -catenin, and *Axin* are found in approximately 15% of sporadic medulloblastomas (Table 2). These mutations are mutually exclusive, suggesting that somatic mutation of any one of these genes may be sufficient to promote tumor formation.

While the studies described above support an oncogenic role for Wnt signaling in a subset of medulloblastomas, further analysis will be necessary to delineate the precise role of the Wnt pathway in tumorigenesis. Studies carried out by Fults et al. (2002) and Rao et al. (2003, 2004) have utilized the RCAS-TVA system to deliver oncogenic retroviruses to nestin-expressing precursor cells in the postnatal cerebellum. Although transduction of Shh and c-myc (or insulin-like growth factor) retroviruses into these cells resulted in increased proliferation and tumor formation, β -catenin retroviruses had no effect. These studies suggest that medulloblastomas harboring Wnt pathway mutations may arise from transformation of a distinct cell type or a distinct stage of cerebellar development. Further studies will be necessary to define the relevant target cell in these tumors.

Role of SUFU in Wnt-Mediated Medulloblastoma

In addition to functioning as a negative regulator of the Hedgehog pathway, recent studies have suggested that SUFU may also regulate the activity of β -catenin. This is significant because, as mentioned above, Taylor et al. (2002) identified mutations of *SUFU* in a subset of medulloblastomas. The first evidence for a role of SUFU in Wnt signaling came from Meng et al. (2001), who reported that *SUFU* exists in a complex with β -catenin and that overexpression of SUFU in colon cancer cells leads to a reduction in nuclear β -catenin levels and TCF-dependent transcription.

An interesting subsequent study by Taylor et al. (2004) provides evidence that *SUFU* mutations may lead to activation of both the Wnt and Shh pathways. The study was centered on a mutant of *SUFU* (*SUFU- α ex8*) that was cloned from a desmoplastic medulloblastoma. The authors had previously reported that *SUFU- δ ex8* had lost its ability to bind and suppress *Gli*-mediated transcription and caused activation of the Shh pathway (Taylor et al., 2002). The present study demonstrates that the same mutant, while retaining its ability to bind β -catenin, has lost the capacity to export β -catenin from the nucleus and to inhibit β -catenin/TCF-mediated transcription. Given the evidence implicating both Shh and Wnt signaling in medulloblastoma, the concept that SUFU might mediate activation of both pathways is provocative. Further investigation of the link between these two pathways could prove valuable for identifying novel and highly specific therapeutics for targeting medulloblastoma development.

Wnt and Gliomas

Although a small percentage of Turcot's syndrome patients develop gliomas, to date, there has been little evidence for involvement of the Wnt pathway in sporadic gliomas. For example, in contrast to medulloblastoma, nuclear accumulation of β -catenin has not been observed in glial tumors (Eberhart et al., 2000). There is some evidence that β -catenin may be important in regulating intracellular adhesion and cellular locomotion, factors that are important in tumor invasion and metastasis. However, these effects likely involve membrane-associated β -catenin rather than the cytoplasmic/nuclear pool that is involved in Wnt signaling. Interestingly, a recent study has demonstrated that expression of the Wnt target gene *MARK4* (which is normally restricted to glial precursor cells) is elevated in human gliomas and glioma cell lines (Beghini et al., 2003). Whether *MARK4* expression in these cells depends on activity of the Wnt pathway remains to be determined. However, down-regulation of *MARK4* expression using antisense oligonucleotides results in decreased proliferation of glioblastoma cell lines, suggesting that the gene may be important for tumor cell growth. Thus, a role for Wnt signaling in sporadic gliomas cannot be ruled out and requires more investigation.

TGF- β SUPERFAMILY SIGNALING IN CNS TUMORS

The effects of TGF- β superfamily members on medulloblastoma have not been extensively studied, but there is some evidence that BMPs may play anti-proliferative roles in medulloblastoma. Rios et al. (2004) report that BMP-2 acts to suppress Shh-induced proliferation of GCPs, the putative cells of origin in some cases of medulloblastoma. In addition, BMP-2 has been demonstrated to cause cell death in both primary and cultured medulloblastoma cells (Hallahan et al., 2003). This study demonstrated that retinoids cause apoptosis of medulloblastoma cells by inducing secretion of BMP-2. This BMP-2 not only kills retinoid-sensitive cells, but also causes apoptosis of retinoid-resistant cells through a paracrine effect. Furthermore, treatment with a BMP-2 antagonist, Noggin, blocks both retinoid- and BMP2-induced cell death. Therefore, BMP-2 plays an antiproliferative role in medulloblastoma cells and may be useful as a therapeutic treatment. Further studies will be necessary to elucidate the role of BMPs and other TGF- β s in medulloblastoma.

There have been few studies of BMP family members in other CNS tumors. On the other hand, TGF- β s have been implicated in many aspects of malignant glioma progression, including proliferation, infiltrative growth, angiogenesis, and immune suppression (reviewed in Rich, 2003). Paradoxically, TGF- β acts as a growth inhibitor among normal glial cells (Jennings and Pietenpol, 1998). However, at later stages of tumorigenesis the growth inhibitory function of TGF- β is lost and TGF- β stimulates tumor progression by increasing invasiveness and survival of tumor cells (Jennings and Pietenpol, 1998). Because of the growth regulatory and immunomodulatory properties of TGF- β , it has become an important target for treatment of malignant gliomas. Many therapeutic strategies involving inhibition of TGF- β signaling are currently under investigation (reviewed in Rich and Bigner, 2004). For example, small molecule ATP mimetics have been designed to block protein phosphorylation induced by TGF β s. In addition, antisense oligonucleotides directed against TGF- β ligands have shown some promise in the treatment of gliomas. Specifically, immunization of mice with glioma cells in which TGF- β expression was inhibited using antisense technology resulted in increased tumor cell immunogenicity and increased survival time of animals (Fakhrai et al., 1996). Thus, TGF- β -based therapies may be valuable for treatment of malignant glioma.

CONCLUDING REMARKS

Recent studies have increased our understanding of the signals that control normal development and the manner in which these signals are dysregulated in cancer. The finding of mutations in morphogen signaling pathways in both familial cancer syndromes and sporadic brain tumors has pointed to the importance of these pathways in tumorigenesis, and has yielded useful biomarkers for diagnosis of specific tumor types. Moreover, the identification of Shh, Wnt, and TGF- β pathways as critical regulators of tumor growth and maintenance has begun to suggest new targets for therapy of brain tumors. However, given the complex nature of these tumors and the variation in tumor behavior from patient to patient, it is unlikely that any one therapeutic strategy will be sufficient to completely eradicate a tumor. Rather, a multifaceted approach involving disruption of multiple signaling pathways is likely to be required. Further studies of the role of morphogens in normal development and tumorigenesis will undoubtedly yield more effective and reliable approaches to therapy.

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