Brain as a Paradigm of Organ Growth: Hedgehog–Gli Signaling in Neural Stem Cells and Brain Tumors

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Received 22 December 2004; accepted 3 January 2005

ABSTRACT: The Hedgehog–Gli (Hh–Gli) signaling pathway is essential for numerous events during the development of many animal cell types and organs. In particular, it controls neural cell precursor proliferation in dorsal brain structures and regulates the number of neural stem cells in distinct embryonic, perinatal, and adult niches, such as the developing neocortex, the subventricular zone of the lateral ventricle of the forebrain, and the hippocampus. We have proposed that Hh–Gli signaling regulates dorsal brain growth during ontogeny and that its differential regulation underlays evolutionary change in the morphology (size and shape) of dorsal brain structures. It is also critically involved in sporadic brain tumorigenesis — as well as several other human cancers — suggesting that tumors derive from stem cells or progenitors maintaining an inappropriate active Hh–Gli pathway. Importantly, we and others have demonstrated that human sporadic tumors from the brain and other organs require sustained HH–GLI signaling for sustained growth and survival. Modulating HH–GLI signaling thus represents a novel rational avenue to treat, on one hand, brain degeneration and injury by inducing controlled HH–GLI-mediated regeneration and growth, and on the other hand, to combat cancer by blocking its abnormal activity in tumor cells. © 2005 Wiley Periodicals, Inc. J Neurobiol 64: 476–490, 2005

Keywords: **Hedgehog; Gli; brain; growth; stem cell; cancer; medulloblastoma; glioma; cyclopamine; pattern**

THE HEDGEHOG–GLI SIGNALING PATHWAY

The *hedgehog (hh)* gene was originally identified in flies, where it is first required for patterning of the early embryo (Nusslein-Volhard and Wieschaus, 1980; Mohler, 1988; Lee et al., 1992; Tabata and Kornberg, 1994). In mammals, the Hh family consists of three different members, Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) (Riddle et al., 1993; Krauss et al., 1993; Echelard et al., 1993; Roelink et al., 1994). Shh is the most broadly expressed member and is involved in the patterning and growth of a large variety of organs, including the brain, skin, lung, prostate, gastrointestinal tract, and skeletal system (reviewed in Ruiz i Altaba, et al., 2002; Pasca di Magliano and Hebrok, 2003).

Hedgehogs are secreted glycoproteins, which undergo posttranslational modifications, including autocatalytic cleavage and lipid modification, before binding to a transmembrane receptor in responding cells (reviewed in Hooper and Scott, 2005). Hh ligands act through the transmembrane proteins Patched1 (Ptch1) and Smoothened (Smo) to trigger an intracellular signal transduction pathway that results in the activation of the Gli zinc finger transcription factors (Fig. 1). The current model of ligand receptor signaling proposes that in the absence of Hh ligands, Ptch1 blocks the function of Smo. The

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Contract grant sponsor: NIH-NINDS.
Contract grant sponsor: NIH-NCI.
Contract grant sponsor: HFSP.
Contract grant sponsor: Pew Trusts.
Contract grant sponsor: Hirschl and Jeantet Foundations.

Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/neu.20160

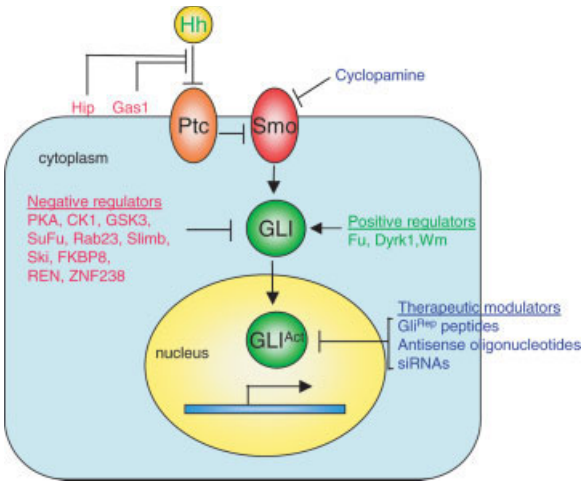

Figure 1 Diagram of the generalized HH–GLI signaling pathway, derived from knowledge in different systems. The drawing shows the cellular location of critical Hh pathway components and the action of negative regulators (red), positive regulators (green), and possible therapeutic modulators (blue) negatively affecting positive GLI function. (See text for discussion). T bars illustrate inhibitory interactions and arrows positive ones. Abbreviations and names: Hh = Hedgehog; Hip = Hedgehog-interacting protein; Gas1 = growth arrest specific 1; GLI act = GLI activator; GLI rep = GLI repressor; Ptc = Patched 1; Smo = Smoothened.

binding of Hhs to Ptch1 releases this basal repression of Smo. As a consequence, Smo initiates an intracellular signaling cascade that is regulated by a multimolecular complex, leading to the action of the Gli proteins.

THE GLI PROTEINS MEDIATE HH SIGNALING

The three Gli transcription factors behave differently and have context-dependent repressor and activator functions. Gli1 seems to act mostly as a transcriptional activator and is consistently transcribed in Hh-responding cells in all contexts examined, whereas Gli2 and Gli3 have also dominant negative function (Ruiz i Altaba, 1999; Sasaki et al., 1999; Shin et al., 1999; reviewed in Ruiz i Altaba et al., 2003). Hh acts to regulate the three Gli proteins in different ways. For example, Gli1 appears to be a strong constitutive activator of target genes (Lee et al., 1997; Hynes et al., 1997), whereas Gli2 activator function seems to be enhanced by Hh signaling (Aza-Blanc et al., 2000). In contrast, Hh signaling inhibits the formation of Gli3, but not Gli2, repressor forms and inhibits Gli3 transcription (Ruiz i Altaba, 1999; Sasaki et al., 1999; Dai et al., 1999; Wang et al., 2000; Aza-Blanc et al., 2000).

The function of the Gli proteins is complex. For example, in the frog neural plate, Gli1 mediates the effects of Shh, inducing floor plate differentiation, whereas both Gli2 and Gli3 inhibit this function (Lee et al., 1997; Ruiz i Altaba, 1998). In the spinal cord, Gli1 and Gli2 induce motor neurons, but Gli3 has an opposite function (Ruiz i Altaba, 1998). In zebrafish Gli1 is required for normal ventral central nervous system (CNS) development (Karlstrom et al., 2003) and seems to be the main activator of Hh target genes, whereas Gli2 and Gli3 can act as repressors or activators (Karlstrom et al., 2003; Tyurina et al., 2005). In mice, Gli1 has been reported to be redundant (Park et al., 2000), whereas Gli2 and Gli3 have specific and partially overlapping functions. Nevertheless, Gli1/Gli2 double mutants have stronger defects than the single Gli2 mutants (Park et al., 2000) and Gli1 can rescue the *in vivo* function of Gli2 (Bai and Joyner, 2001). In contrast, Gli2, but not Gli1, appears to be mainly involved in floor plate development (Matise et al., 1998) and is required for initial Shh signaling (Bai et al., 2002), but Gli2 cannot compensate for the defects in dorsal brain seen in Gli3 mutant mice and vice versa (Mo et al., 1997; Theil et al., 1999; Tole et al., 2000) (Palma and Ruiz i Altaba, 2004). Moreover, the phenotype of double Gli2/Gli3 mutant mice, which do not express *Gli1* at any appreciable levels, indicates a requirement of Gli proteins for proper ventral patterning in the spinal cord (Bai et al., 2004; Lei et al., 2004). This is consistent with previous results in frogs (Brewster et al., 1998) and chicks (Persson et al., 2002) showing that overall positive Gli function is required for neural plate and neural tube patterning.

Gli1 and Gli2 can mediate Shh signaling (Lee et al., 1997; Hynes et al., 1997; Ruiz i Altaba, 1998; Aza-Blanc et al., 2000; Sasaki et al., 1999; Matise et al., 1998; Ding et al., 1998), whereas Gli3 has been proposed to function primarily as an inhibitor of Shh-Gli1 function (Ruiz i Altaba, 1998; Litingtung and Chiang, 2000). Strikingly, the loss of ventral spinal cord cell types seen in *Shh* mutant mice can be partially rescued by abrogating Gli3 function (Litingtung and Chiang, 2000), indicating that in the ventral neural tube Gli3 acts mainly as a repressor and that Shh is required to inhibit Gli3. However, in different contexts Gli3 can have also an activating function, as suggested by its ability to induce neurogenesis in the frog early neural plate (Brewster et al., 1998) and its requirement in mouse and chick spinal cord patterning (Persson et al., 2002; Motoyama et al., 2003; Bai et al., 2004). Moreover, antisense experiments in frog embryos demonstrate the requirement of the Gli proteins, including Gli3, in primary neurogenesis

and indicate that Gli proteins act in a cooperative manner, likely forming multimers (Nguyen et al., 2005).

HH–GLI SIGNALING IN BRAIN DEVELOPMENT

During early neural tube formation, Shh secreted from midline cells of the notocord and the floorplate, directs the development of specific ventral cell types in a dose-dependent manner (Krauss et al., 1993; Echelard et al., 1993; Roelink et al., 1994; Ericson et al., 1996; reviewed in Jessell, 2000). For example, it induces dopaminergic and serotonergic neuronal differentiation in the ventral midbrain and hindbrain (e.g., Hynes et al., 1995; Wang et al., 1995; Ye et al., 1998) and motoneurons or oligodendrocytes in the spinal cord (e.g., Ericson et al., 1992; Poncet et al., 1996; Pringle et al., 1996; Orentas et al., 1999; Lu et al., 2000; Soula et al., 2001). Humans and mice lacking Shh signaling develop holoprosencephaly and cyclopia due to defective ventral patterning and the failure of separation of the bilateral lobes of the forebrain (Roessler et al., 1996; Belloni et al., 1996; Chiang et al., 1996). After an early period in which Shh is indispensable for ventral differentiation (reviewed in Ruiz i Altaba et al., 2003), in late embryonic and early postnatal stages Shh is prominently expressed in the dorsal brain, including the cerebellum (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999), neocortex, tectum (Dahmane et al., 2001) and hippocampus (Dahmane et al., 2001; Lai et al., 2003; Machold et al., 2003), as well as in other areas, including the amigdala and septum (e.g., Dahmane et al., 2001; Lai et al., 2003). Here we focus on the dorsal brain.

The Cerebellum

The cerebellum develops from the dorsal region of the posterior midbrain/anterior hindbrain and is involved in multiple tasks, including motor and balance control (Altman and Bayer, 1997). These functions are highly regulated by granule neurons (granule cells; GCs), the most numerous neurons in the brain. GCs originate in the rhombic lip and expand in the external region of the cerebellum, in contrast to other types of neurons, such as Purkinje neurons, which arise in the ventricular zone. The cerebellum contains a cortical region in which distinct cell types are found in an evolutionarily conserved, stereotyped layered fashion. The outermost layer, the external germinal layer (EGL), contains dividing granule cell precursors (GCPs). These cells undergo a dramatic proliferative expansion during neonatal and early postnatal nervous system development. Developing GCPs then exit the cell cycle, differentiate, and migrate to a deeper layer past the Purkinje cell bodies in the Purkinje layer (PL), to form the internal granule layer (IGL). The whole process of proliferation, differentiation, and migration is completed by the third week of age in the mouse and by the ninth postnatal month in humans (Altman and Bayer, 1997).

The mechanisms and the genetic regulation of cerebellum development have been studied in great detail (e.g., Wang and Zoghbi, 2001). Among the several factors that have been found to contribute to the growth of the cerebellum, recent findings have highlighted the role of Shh in controlling proliferation of GCPs during late embryogenesis and after birth (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999).

Shh signaling controls the development of the cerebellum in different ways. First, it is produced by Purkinje neurons and regulates the proliferation of GCPs (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). Treatment of chick cerebellum cortical explants or mouse purified GCPs with recombinant Shh induces proliferation and maintains them in a undifferentiated state, whereas inhibition *in vitro* with blocking anti-Shh antibody causes a decrease in proliferation. *In vivo*, inhibition of Shh signaling results in a marked decrease of proliferation in the EGL (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). Second, it induces the differentiation of Bergmann glia (Dahmane and Ruiz i Altaba, 1999). Moreover, blocking Shh signaling *in vivo* leads to the development of hypoplastic cerebellum with abnormal foliation and disorganized PL (Dahmane and Ruiz i Altaba, 1999). Consistent results have been recently obtained by genetic ablation of *Shh* in the cerebellum, which shows that loss of Shh impairs GCPs proliferation, Purkinje cell development and compromises the total volume of the cerebellum and its foliation (Lewis et al., 2004).

The three *Gli* genes are expressed in the developing cerebellum. *Gli1* is found in the PL and EGL (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999). *Gli2* and *Gli3* are also expressed in the EGL and PL, although the level of *Gli3* is low (Dahmane and Ruiz i Altaba, 1999). This suggests an action of Shh at distance from its source: Shh producing cells are located in the PL, and it regulates the proliferation of cells in the distant germinative zone that expresses *Gli1* in the EGL (Fig. 2). The mitogenic effect of Shh *in vitro* can be suppressed by FGF (Wechsler-Reya and Scott, 1999), suggesting

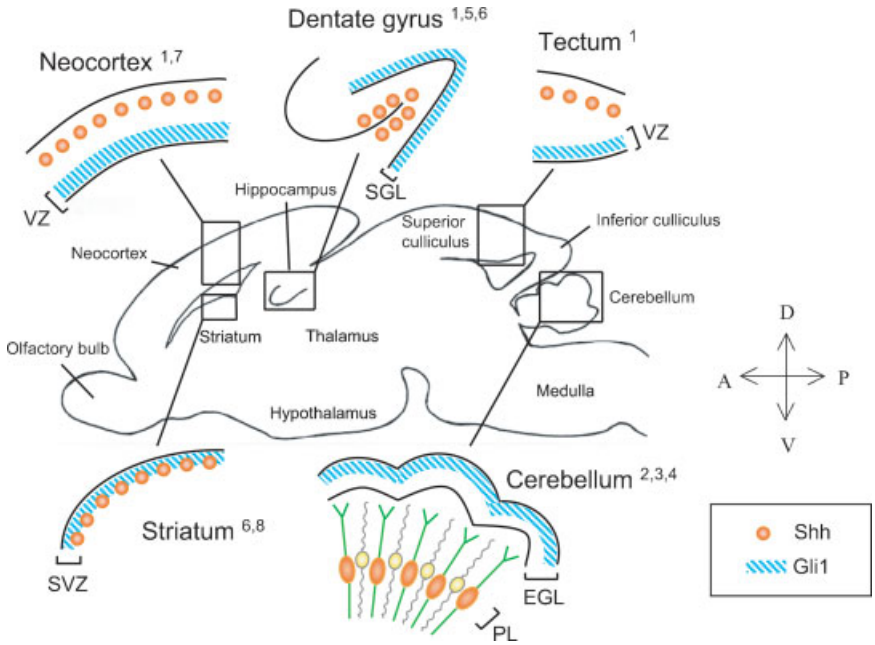

Figure 2 Schematic representation of *Shh* and *Gli1* expression in the rodent perinatal/adult brain in different dorsal regions. Expression of *Gli1* (blue hatched areas) in germinative zones and of *Shh* in differentiated cells and stem cell niches (orange dotted areas) is shown to give approximate location of signaling and responding sites. References: (1) Dahmane et al., 2001; (2) Dahmane and Ruiz i Altaba, 1999; (3) Wallace, 1999; (4) Wechsler-Reya and Scott, 1999; (5) Lai et al., 2003; (6) Machold et al., 2003; (7) Palma and Ruiz i Altaba, 2004; (8) Palma et al., 2005. Abbreviations: VZ = ventricular zone; SVZ = subventricular zone of the lateral ventricle of the forebrain; SGL = subgranular zone of the dentate gyrus of the hippocampus; EGL = external germinal layer of the cerebellar cortex; PL = Purkinje layer. The source of Shh affecting the SVZ is not clearly determined. Similarly, the Shh source affecting cell behavior in the hippocampus may lie in the hilus (Dahmane et al., 2001) or in the septum, being transported by its axons to the SGL (Lai et al., 2003). Expression of *Gli1* and *Shh* in several other areas, including the septum and the amigdala, is not depicted here.

a possible interaction between different pathways to strictly control granule cell precursors proliferation *in vivo*. Indeed, it is possible that Bergman glial cells maturing after receiving Shh send a negative signal that inhibits Shh-driven GCP proliferation, forming a Shh loop that may determine the timing of GCP proliferation and thus cell number (Dahmane and Ruiz i Altaba, 1999). This idea is consistent with the inhibitory effect of Bergman glia on GCPs demonstrated *in vitro* (Gao et al., 1991). *Shh* is still expressed in the Purkinje cell layer of the adult brain well after the last GCP ceased to proliferate (Traiffort et al., 1999; Wallace, 1999). Here it could play a role related to its described neurotrophic and survival activities in other contexts (e.g., Miao et al., 1997; Thibert et al., 2003).

Neocortex and Tectum

The neocortex derives from the most anterior part of the neural tube and is the most prominent brain structure in apes and humans. The superior and inferior culliculi (the tectum) form part of the roof of the midbrain and appear behind the posterior part of the cerebral cortex (Bayer and Altman, 1991). Given the correlation between cerebral cortical size and brain capacity across species, it is clear that regulating cortical cell number is important. As in the cerebellum, it is critical that progenitor cells in the neocortex and in the tectum proliferate to the correct extent before differentiating and that the resulting number of progenitors produced be strictly regulated in a species-dependent fashion.

The size of the neocortex has been shown to be affected by many factors (reviewed in Monuki and Walsh, 2001; Gupta et al., 2002; Ross et al., 2003), including FGF ligands (Vaccarino et al., 1999). Unexpectedly, the major structures of the brain, the cerebral cortex and the tectum, share a common mechanisms for growth regulation (Dahmane et al., 2001): in parallel with the cerebellum, *Shh* is expressed in a layer-specific manner in the perinatal mouse neocortex and tectum, whereas the *Gli* genes are expressed in the periventricular proliferative zones (Fig. 2). *In vitro* and *in vivo* analyses suggest that Shh is an endogenous mitogen for neocortical

and tectal Nestin-positive precursors, thus modulating cell proliferation in the dorsal brain (Dahmane et al., 2001). Consistently, the brains of $Shh^{-/-}$ late embryos show decreased proliferation in the ventricular/subventricular zones of the forebrain (Dahmane et al., 2001).

As in the cerebellum, but in an inverse topology, the layer-specific expression of Shh in the dorsal brain suggests that it may regulate $Gli1^{+}$ precursor proliferation in the germinative zones (Fig. 2). There seems to be a common mechanism by which Shh secreted from early differentiated neurons in the neocortex, tectum, and cerebellum regulates precursors proliferation and the number of later-born cells. Such mechanism has been proposed to regulate brain size and shape during ontogeny and to be modified independently in the different brain modules. This strategy would therefore allow the control of organ size and the rapid change in overall brain size as well as shape and size of each dorsal brain structure independently during evolution (Dahmane et al., 2001; Ruiz i Altaba, et al., 2002).

HH–GLI SIGNALING IN NEURAL STEM CELLS

Normal tissue stem cells are broadly defined by three common properties: (i) the presence of an extensive capacity for self-renewal; (ii) strict regulation of their number, and (iii) the ability to undergo a range of differentiation events to reconstitute the functional elements within a tissue. They are present in many developing vertebrate organs, and continue into adulthood in tissues that are regenerated throughout life, such as blood and skin. Stem cells have been described also in several regions of the developing and adult central nervous system (reviewed in Temple, 1999; Gage, 2000; Alvarez-Buylla et al., 2001; Temple, 2001; Panchision and McKay, 2002; Morshead and van der Kooy, 2004). In mammals, they contribute to active neurogenesis in stem cells niches: the subventricular zone (SVZ) of the lateral ventricles of the forebrain and the subgranular zone (SGZ) of the hippocampus (reviewed in Temple and Alvarez-Buylla, 1999; Alvarez-Buylla and Lim, 2004).

The lack of specific, prospective neural stem cell markers for *in situ* analysis does not yet make feasible a direct and general study of stem cells *in vivo*, therefore they are usually identified retrospectively on the basis of their behavior and surface properties after isolation. For example, CNS stem cells can be cultured as a cluster of floating cells called neurospheres (Reynolds and Weiss, 1996; Tropepe et al., 1999),

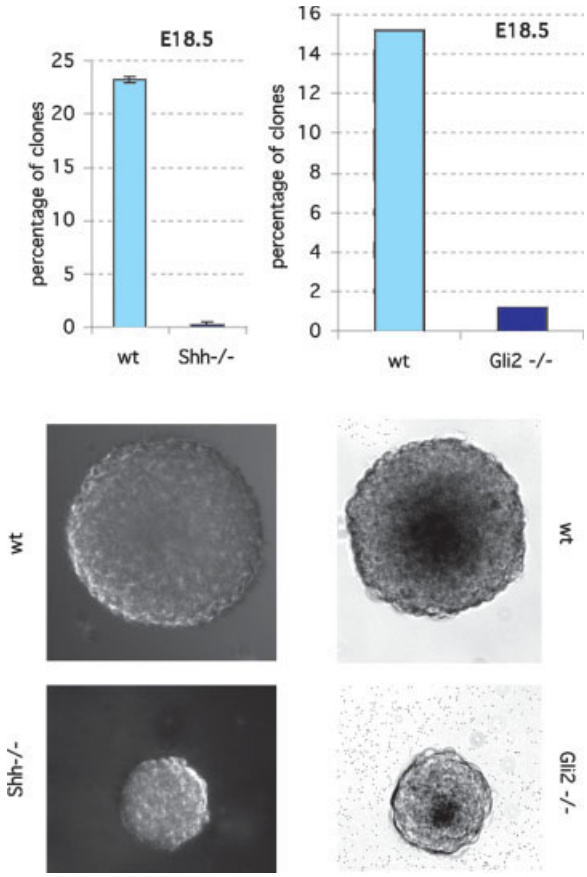

Figure 3 Hh–Gli signaling pathway regulates the behavior of neural stem cells in the mouse developing neocortex. Neocortical cells from embryonic (E18.5) *Shh*−/− and *Gli2*−/− mice show fewer and smaller neurospheres in comparison with wild-type. From Palma and Ruiz i Altaba (2004).

which have multipotent properties and can differentiate into neurons, astrocytes and oligodendrocytes. Adult and perinatal stem cell niches express the *Gli* genes (Dahmane et al., 2001; Palma et al., 2005). Based on this observation, Hh–Gli signaling was shown to regulate the behavior of cells with stem cell properties in the mouse developing neocortex (Palma and Ruiz i Altaba, 2004). In particular, $Shh^{-/-}$, $Gli2^{-/-}$, and $Gli3^{-/-}$ E15-E18 mutant mice show a reduced capacity to form neocortical neurospheres and the ones that form are smaller (Palma and Ruiz i Altaba, 2004; Fig. 3). Furthermore, Shh–Gli signaling was shown to regulate the number of embryonic and postnatal cells with stem cell properties able to from neurospheres, and to control the proliferation of neural precursors in a concentration-dependent manner in cooperation with EGF signaling (Palma and Ruiz i Altaba, 2004). Because EGFR is expressed by precursors (Doetsch et al., 2002), the data suggests a dual effect of Shh on stem cells and derived early precursors.

Shh signaling is also required for the maintenance of neural stem cells in the SVZ. Shh signaling affects both neural stem cells with clonogenic properties and precursors, and *Gli1* is expressed in periventricular $GFAP^+$ astrocytes (Palma et al., 2005), a bona fide *in vivo* neural stem cells (Doetsch et al., 1999), as well as in derived precursors. This is the first, and so far best, evidence that *in vivo* stem cells respond to Shh signaling (Palma et al., 2005). Genetic ablation of *Smo* or *Shh* in $Nestin^+$ neural progenitors (starting from ~E8.5–9) showed that loss of Shh signaling results in a dramatic reduction in the number of cells forming neurospheres from the postnatal and adult SVZ and also in massive cell death (Machold et al., 2003). Pharmacological inhibition of Hh signaling with cyclopamine similarly compromises neurosphere forming ability of SVZ cells, further indicating that Shh signaling is absolutely required for normal proliferation and self-renewal of SVZ stem cells (Palma et al., 2005).

As in the SVZ, Shh is required for the maintenance of progenitors in the subgranular zone (SGZ) of the hippocampus (Lai et al., 2003; Machold et al., 2003), where it was found to be a potent mitogen for adult hippocampal progenitors *in vitro* (Lai et al., 2003). *In vivo* delivery of Shh to the hippocampus through the use of adenoviral vectors also leads to significant increase in cell proliferation whereas cyclopamine treatment markedly reduced neural progenitor proliferation in the hippocampus (Lai et al., 2003). This result is mimicked by loss of *Shh* or *Smo* function (Machold et al., 2003).

STEM CELLS AND CANCER STEM CELLS

There are intriguing parallels between stem cells and cancer cells. For instance, both types have the capacity to self-renew, although stem cells do it in a regulated manner in which cell number is highly controlled to generate or regenerate organs and tissues, whereas in cancer an ever-increasing cell number is a *sine qua non*. The first evidence for the existence of cancer stem cells derives from experiments showing the ability of teratocarcinoma cells to participate in blastocyst development and normally differentiate into a wide range of somatic tissues in the derived mouse (e.g., Mintz and Illmensee, 1975; Martin and Evans, 1975; Papaioannou et al., 1975; Illmensee and Mintz, 1976). Additional studies in nonsolid tumors provide strong support for the existence of cancer stem cells (reviewed in Reya et al., 2001; Pardal et al., 2003). For example, human leukemia cells, but not differentiated cells coming from the same tumor, can recapitulate the tumor after transplantation (Lapidot et al., 1994; Bonnet and Dick, 1997). Recent experiments extended this model to solid tumors, including brain and breast cancers. For instance, a minority of cells within breast tumors were prospectively isolated, based on cell surface marker expression ($CD44^+$/$CD24^-$), and these cells alone were found to drive the formation and growth of tumors (Al-Hajj et al., 2004). Similar results have been observed for brain tumors. Several groups (Ignatova et al., 2002; Hemmati et al., 2003; Singh et al., 2003) have described the existence of a putative cancer stem cell population in human brain tumors of different phenotypes from both children and adults, based on the expression of neural stem cell markers, including *CD133*, *Sox2*, *musashi-1,* and *bmi-1,* and their ability to self-renew *in vitro*. Moreover, another group has described the isolation of a subset of human glioblastoma multiforme cells, which possess characteristics of neural stem cells *in vitro* and can establish glioblastoma multiforme-like tumors *in vivo* (Galli et al., 2004). A recent paper further proved that a small fraction of human $CD133^+$ brain tumor cells is sufficient and required to initiate brain tumorigenesis in mice (Singh et al., 2004).

The existence of cancer stem cells in solid tumors could have important implications for cancer therapy. The current available anticancer therapies can reduce or eliminate the mass of the tumor, but these effects are usually transient, indicating that they might spare putative cancer stem cells, allowing tumor recurrence. Therefore, therapies targeting cancer stem cells might result in more durable responses.

The Hh–Gli pathway regulates the number of cells with stem cells properties in distinct neural niches in the brain, such as the neocortex, SVZ, and hippocampus (Lai et al., 2003; Machold et al., 2003; Palma and Ruiz i Altaba 2004; Palma et al., 2005) in addition to controlling neural precursors proliferation (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999; Dahmane et al., 2001). These data, together with the results on the involvement of the Hh–Gli pathway in tumorigenesis (see below; reviewed in Ruiz i Altaba et al., 2004), suggest the hypothesis that many cancers derive from cells with stem or progenitor character that maintain an inappropriate active HH–GLI pathway, long after their normal requirement or that acquire sustained HH–GLI activation *de novo*, perhaps perverting wound-healing or regenerative mechanisms.

HH–GLI SIGNALING IN BRAIN TUMORS

The first correlation between SHH signaling and tumor formation came from the finding that patients with Gorlin's or basal-cell nevus syndrome, a familial

Table 1 Vertebrate Animal Models of Hh–GLI Induced/Dependent CNS Tumors

Animal	Defect	Tumor	References	Inhibitors	References
Tadpole	GLI1 misexpression	CNS tumors	Dahmane et al., 2001	Gli-antisense	Dahmane et al., 2001
Mouse	$Ptc1^{+/-}$	Medulloblastoma	Goodrich et al., 1997 Hahn et al., 1998		
	$Ptc1^{+/-}$; $p53^{-/-}$	Medulloblastoma	Wetmore et al., 2001	Hh-Antag691 Cyclopamine	Romer et al., 2004 Sanchez and Ruiz i Altaba, 2005

condition with a predisposition to medulloblastomas, rhabdomyosarcomas, and basal cell carcinomas (Gorlin, 1995), carry *PTCH1* mutations (Hahn et al., 1996; Johnson et al., 1996). Similarly, mice with only one functional allele of the *Ptch1* gene have an abnormally high frequency of tumors in the cerebellum and muscle (Goodrich et al., 1997; Hahn et al., 1998). Loss of Ptch1 function leads to the activation of the Hh pathway (Goodrich et al., 1997, 1998) and consequently to activation of Gli1 function (Lee et al., 1997).

GLI1 was originally identified in a human glioma cell line, where it was found amplified more than 50 fold (Kinzler et al., 1987) and it was shown to transform fibroblasts *in vitro* in cooperation with E1A (Ruppert et al., 1991). However, its possible role in sporadic tumorigenesis was subsequently denied (Salgaller et al., 1991; Xiao et al., 1994), ignored for years and demonstrated only recently (Dahmane et al., 2001; reviewed in Ruiz i Altaba et al., 2004).

The first evidence of the involvement of Gli1 in initiating brain tumorigenesis derived from findings that human GLI1 can induce CNS and skin tumors in developing tadpoles (Dahmane et al., 1997, 2001; Table 1). Even if the comparison of a tadpole CNS or skin tumor with human gliomas or basal cell carcinoma is difficult histologically, these tumors display a molecular signature reminiscent of their human counterparts. For example, they show upregulation of platelet-derived growth factor receptor-α (PDGFRα; Dahmane et al., 2001), which is a marker of oligodendrocyte precursors and of human gliomas (e.g., Richardson et al., 1988; Maxwell et al., 1990), and PDGFRα is also upregulated in BCCs (Xie et al., 2001). Several mouse models have further demonstrated that the activation of the Hh–Gli pathway by misexpression of Shh (Oro et al., 1997), GLI1 (Nilsson et al., 2000), or Gli2 (Grachtchouk et al., 2000), activating mutations of SMOH (Xie et al., 1998; Grachtchouk et al., 2003), or by reduced repression of Ptch1 combined with irradiation (Azsterbaun et al., 1999) is sufficient to induce tumor formation in the skin.

Importantly, the formation of GLI-induced tumors in tadpoles is dependent on the activation of endogenous Gli function because antisense oligonucleotides specific for the endogenous frog *Gli1* block tumor formation by human GLI1 (Dahmane et al., 2001). This idea fits with studies with conditional mouse systems showing that tumors initiated by oncogenes are dependent on their continuous activity (e.g., Chin et al., 1999; Felsher and Bishop, 1999; Fisher et al., 2001; Pelengaris et al., 1999). The initial results in tadpoles suggested that an active Hh–Gli pathway is required not only for tumor initiation, but also for tumor maintenance (Dahmane et al., 2001) and this critical result led us to test the hypothesis that sustained Hh–Gli signaling is required for the viability of human tumors.

Direct experimental evidence that HH–GLI signaling activity contributes to the maintenance of human tumors comes from the use of cyclopamine, a plant-derived alkaloid (Keeler, 1970; Keeler and Baker, 1989) that specifically inhibits the Hh–Gli pathway (Cooper et al., 1998; Incardona et al., 1998; Taipale et al., 2000) by suppressing the function of Smo (Chen et al., 2002). Our group showed for the first time that primary human brain tumors and a number of human brain tumor cell lines consistently express the *GLI* genes and that cyclopamine inhibits growth of human medulloblastoma (Dahmane et al., 2001) and glioma (Dahmane et al., 2001). Subsequently, it was shown that cyclopamine inhibits the growth of medulloblastoma mouse allografts (Berman et al., 2002) (Table 2). Together, these experiments revealed very important because they first provided a basis for a therapeutic approach for brain tumors and, by extension, other tumors that depend on an active HH–GLI pathway. Indeed, cyclopamine has been shown to inhibit the effect of oncogenic mutations in Smoothened and Patched (Taipale et al., 2000) and is expected to shut down the pathway activated by mutation affecting Smo or upstream components.

The discovery of Shh-induced growth of GCPs in the normal cerebellum (Dahmane and Ruiz i Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott,

Table 2 Sporadic Human Brain Tumors Dependent on HH–GLI Signaling

Tumor	Antagonists	Target	References
Medulloblastoma	Cyclopamine	PC, CL	Dahmane et al., 2001
	Cyclopamine	PC, CL, MA	Berman et al., 2002
Glioma	Cyclopamine	PC, CL	Dahmane et al., 2001

Abbreviations: PC, primary culture; CL, cell line; MA, mouse allograft.

1999) and the finding that *Ptch1*$^{+/-}$ mice and humans can develop medulloblastoma (Johnson et al., 1996; Hahn et al., 1996, 1998; Goodrich et al., 1997) were cornerstones in our understanding of the development of this tumor type. Murine and human medulloblastomas could thus arise from granule–neuron precursors maintaining an inappropriately active Shh–Gli pathway. These studies suggest that the inability to downregulate SHH signaling, as a result of activating mutations, epigenetic events, or the absence of endogenous factors that antagonize the mitogenic effect of SHH, may initiate medulloblastoma formation. In this regard, it is interesting to note that cerebellar GCPs are a population of CNS precursors that proliferates over a protracted postnatal period. Thus, it is possible that the long period of GCP proliferation may facilitate the events required for tumor formation in response to Hh–Gli pathway activation. It is also likely that tumor induction is dependent on a specific genetic backgrounds as different mouse strains show a variable penetrance of *Ptch1*$^{+/-}$-induced tumors (reviewed in Wetmore, 2003). Similar strain-dependent influences were noted for glioma formation in *Nf1/Trp53* double mutant mice (Reilly et al., 2000).

Mutations in other components of the SHH–GLI signaling pathway have also been detected in medulloblastomas and in primitive neuroectodermal tumors of the CNS (e.g., Pietsch et al., 1997; Raffel et al., 1997; Wolter et al., 1997; Reifenberger et al., 1998; Vortmeyer et al., 1999). It has been reported that a subset of children with medulloblastomas carry germline and somatic mutations in Suppressor of Fused (SUFU) (Taylor et al., 2002), a component of the pathway, which has been shown to function in directing the subcellular localization of Gli1 (Ding et al., 1999; Pearse et al., 1999; Stone et al., 1999; Kogerman et al., 1999). Some mutations of SUFU may result in mutant proteins that are unable to transport Gli1 out of the nucleus, resulting in the persistent activation of the Shh pathway (Taylor et al., 2002). In addition, new negative regulators of the Hh pathway have been reported, including REN (Di Marcotullio et al., 2004), which antagonizes nuclear localization of Gli1, a process promoted by Dyrk1 (Mao et al., 2000).

THERAPEUTIC APPROACHES OF HH–GLI SIGNALING MODULATORS

Manipulation of Stem Cell Number with Agonists of Hh–Gli Signaling

The controlled manipulation of HH signaling could be used to develop new strategies to improve neurodegenerative diseases, such as Parkinson's disease. Indeed, Shh itself has been shown to be able to induce formation of dopaminergic neurons (Wang et al., 1995; Hynes et al., 1995) in mice, injection of Shh into the striatum reduces behavior deficits in a rat model of Parkinson's disease (Tsuboi and Shults, 2002), and Shh protects neurons from toxic insults (Miao et al., 1997). Indeed, much effort has been put now to find long-acting forms of Shh (Frank-Kamenetski et al., 2002; Chen et al., 2002), with improved pharmacokinetic properties (Pepinski et al., 2002). The use of synthetic Hh agonists mimicking Shh activity could provide an alternative to the use of Shh (Frank-Kamenetski et al., 2002; Chen et al., 2002). For example, synthetic small molecules that activate the Hh–Gli pathway by interacting directly with Smo have been described. One such agonist can rescue developmental defects of Shh null mice (Frank-Kamenetski et al., 2002).

Furthermore, given the action of the Hh–Gli pathway on stem cells in the neocortex, SVZ, and hippocampus (Lai et al., 2003; Machold et al., 2003; Palma and Ruiz i Altaba, 2004; Palma et al., 2005), the manipulation of Shh signaling in humans might be used to expand cell populations with stem cell properties *ex vivo* in the attempt to improve areas of damage and cell loss in the brain. In addition, manipulation *in vivo* of the stem cell niches may be an attractive approach. SVZ progenitors generate large number of neurons in the recipient animal when transplanted to another SVZ, but not when transplanted to non-neurogenic brain regions (Gage, 2000; Lim et al., 2000). Thus, molecular modulation of the Shh–Gli pathway by altering the level of pathway function directly or

indirectly is likely to lead to a novel stem cells therapeutic approach to ameliorate neural degenerative disorders.

Inhibitors of the Hh–Gli Pathway as Anticancer Agents

In addition to brain tumors, a large variety of sporadic human cancers, such as basal cell carcinomas (Dahmane et al., 1997; Xie et al., 1998; Williams et al., 2003), small cell lung cancers (Watkins et al., 2003), pancreatic cancers (Thayer et al., 2003), oesophageal and stomach cancers (Berman et al., 2003), prostate cancers (Karhadkar et al., 2004; Sanchez et al., 2004; Sheng et al.., 2004) and possibly breast cancers (Kubo et al., 2004) have an active Hh–Gli pathway that is required for their continued growth. This unexpected unity raises the exciting possibility that understanding how Hh–Gli signaling initiate and maintain cancer growth will lead to a rational and broad-spectrum anticancer therapy. So far, inhibition of Hh signaling can be performed with different antagonists that block the pathway at least at three different levels: extracellularly blocking Hh ligands, inhibiting SMOH in the cell membrane, or repressing positive Gli action inside the cells.

Extracellular inhibition of ligand activity has been reported with anti-Hh antibodies (Marti et al., 1995; Ericsson et al., 1996). However, the therapeutic use of anti-Shh antibodies is only limited to tumors characterized by an overexpression of Shh that are shown to require continuous ligand activity for survival, as in the case of stomach (Berman et al., 2003) and prostate cancers (Sanchez et al., 2004).

At the membrane level, several specific Smoothened inhibitors have been identified. The first member of this class of small molecules to be identified was cyclopamine. It was originally identified as a teratogenic steroidal alkaloid that causes holoprosencephaly and cyclopia in mammalian and avian embryos (Keeler, 1970; Keeler and Baker, 1989). The possibility of a therapeutic use of cyclopamine seems very promising. The proliferation of a number of human brain tumor cell lines and primary tumors, including medulloblastomas (Dahmane et al., 2001), as well as medulloblastomas allografts (Berman et al., 2002) are inhibited by cyclopamine treatment. Moreover, systemic treatment of *Ptch1*+/−; *p53*−/− medulloblastoma-carrying mice with cyclopamine results in a beneficial and remarkable inhibition of tumor growth without serious side effects (Sanchez and Ruiz i Altaba, 2005). Very similar but more extensive results have been achieved using the same mouse system but with a different antagonist, which also blocks the function of Smo (Romer et al., 2004). Systemic treatment with cyclopamine does not appear to affect the health of treated mice over the period of the time of the experiment, suggesting that HH signaling inhibition may cause no serious toxic effects in mice (Romer et al., 2004; Sanchez and Ruiz i Altaba, 2005). However, whether this is also true in humans remains to be determined. The beneficial topical use of cyclopamine has been suggested for human psoriasis and BCCs (Tas and Avci, 2004a,b) begging the expansion of such tests. In addition, a small molecule with inhibitory properties similar to those of cyclopamine and also acting at the level of Smo, has been used to treat mouse BCCs explants (Williams et al., 2003). Therefore, cyclopamine and other small molecules acting similarly could represent good therapeutic agents for human tumors that arise from activation of the Hh signaling at the level of SMOH or above.

Agents blocking the positive action of Gli proteins, which are the ultimate targets of Hh pathway, may be used to treat better and more effectively a wide variety of Gli-dependent tumors (Fig. 1). For example, carboxy-terminally truncated repressor forms of GLI2/3 are potent pan-GLI inhibitors of the activating action of the HH signaling (Ruiz i Altaba, 1999; Sasaki et al., 1999; Shin et al., 1999), and these forms could be used as antagonists for the treatment of tumors. More specifically, GLI function could also be inhibited at the RNA level through antisense oligonucleotides (Dahmane et al., 2001). In addition, small interfering RNA (siRNAs) targeting *GLI1* has proven efficient to inhibit the growth of prostate tumor cells (Sanchez et al., 2004).

Taken together, the results discussed in this review indicate that there is every reason to be enthusiastic about the development of effective new drugs to improve human diseases resulting from cell loss, including neurodegenerative diseases, and diseases derived from hyperproliferation, including brain tumors and other cancers, in many tissues and organs that use the Hh–Gli pathway. In addition, the data suggest that the Hh–Gli pathway may be a key element in the control of organ size and organ shape. The recent demonstration of an interaction between Gli3 and Hoxd proteins (Chen et al., 2004) opens new possibilities for understanding the integration of pattern formation and cell number control both in normal development and in cancer.

We are grateful to N. Dahmane, P. Sanchez and present members of the Ruiz i Altaba lab for discussion. The readers are encouraged to perform PubMed searches for addi-

tional relevant literature. B. Stecca was a recipient of a postdoctoral fellowship from the Parkinson's Foundation, USA.

REFERENCES

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983–3988.

Altman J, Bayer SA. 1997. Development of the cerebellar system in relation to its evolution, structure, and function. New York: CRC Press.

Alvarez-Buylla A, Garcia-Verdugo JM, Tramontin AD. 2001. A unified hypothesis on the lineage of neural stem cells. Nat Rev Neurosci 2:287–293.

Alvarez-Buylla A, Lim DA. 2004. For the long run: maintaining germinal niches in the adult brain. Neuron 41:683–686.

Aszterbaum M, Epstein J, Oro A, Douglas V, LeBoit PE, Scott MP, Epstein EH Jr. 1999. Ultraviolet and ionizing radiation enhance the growth of BCCs and trichoblastomas in patched heterozygous knockout mice. Nat Med 5:1285–1291.

Aza-Blanc P, Lin HY, Ruiz i Altaba A, Kornberg TB. 2000. Expression of the vertebrate Gli proteins in Drosophila reveals a distribution of activator and repressor activities. Development 127:4293–4301.

Bai CB, Joyner AL. 2001. Gli1 can rescue the in vivo function of Gli2. Development 128:5161–5172.

Bai CB, Auerbach W, Lee JS, Stephen D, Joyner AL. 2002. Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway. Development 129:4753–4761.

Bai CB, Stephen D, Joyner AL. 2004. All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. Dev Cell 6:103–115.

Bayer SA, Altman J. 1991. Neocortical development. New York: Raven Press.

Belloni E, Muenke M, Roessler E, Traverso G, Siegel-Bartelt J, Frumkin A, Mitchell HF, Donis-Keller H, Helms C, Hing AV, Heng HH, Koop B, Martindale D, Rommens JM, Tsui LC, Scherer SW. 1996. Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. Nat Genet 14:353–356.

Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK, Cooper MK, Taipale J, Olson JM, Beachy PA. 2002. Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 297:1559–1561.

Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA. 2003. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature 425:846–851.

Bonnet D, Dick JE. 1997. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737.

Brewster R, Lee J, Ruiz i Altaba A. 1998. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. Nature 393:579–583.

Chen JK, Taipale J, Cooper MK, Beachy PA. 2002. Inhibition of Hedgehog signal by direct binding of cyclopamine to Smoothened. Genes Dev 16:2743–2748.

Chen JK, Taipale J, Young KE, Maiti T, Beachy PA. 2002. Small molecule modulation of smoothened activity. Proc Natl Acad Sci USA 99:14071–14076.

Chen Y, Knezevic V, Ervin V, Hutson R, Ward Y, Mackem S. 2004. Direct interaction with Hoxd proteins reverses Gli3-repressor function to promote digit formation downstream of Shh. Development 131:2339–2347.

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA. 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383:407–413.

Chin L, Tam A, Pomerantz J, Wong M, Holash J, Bardeesy N, Shen Q, O'Hagan R, Pantginis J, Zhou H, Horner JW 2nd, Cordon-Cardo C, Yancopoulos GD, DePinho RA. 1999. Essential role for oncogenic Ras in tumour maintenance. Nature 400:468–472.

Cooper MK, Porter JA, Young KE, Beachy PA. 1998. Teratogen-mediated inhibition of target tissue response to Shh signaling. Science 280:1603–1607.

Dahmane N, Ruiz i Altaba A. 1999. Sonic hedgehog regulates the growth and patterning of the cerebellum. Development 126:3089–3100.

Dahmane N, Lee J, Robins P, Heller P, Ruiz i Altaba A. 1997. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. Nature 389:876–881.

Dahmane N, Sanchez P, Gitton Y, Palma V, Sun T, Beyna M, Weiner H, Ruiz i Altaba A. 2001. The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. Development 128:5201–5212.

Dai P, Akimaru H, Tanaka Y, Maekawa T, Nakafuku M, Ishii S. 1999. Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. J Biol Chem 274:8143–8152.

Di Marcotullio L, Ferretti E, De Smaele E, Argenti B, Mincione C, Zazzeroni F, Gallo R, Masuelli L, Napolitano M, Maroder M, Modesti A, Giangaspero F, Screpanti I, Alesse E, Gulino A. 2004. REN(KCTD11) is a suppressor of Hedgehog signaling and is deleted in human medulloblastoma. Proc Natl Acad Sci U S A 101:10833–10838.

Ding Q, Fukami S, Meng X, Nishizaki Y, Zhang X, Sasaki H, Dlugosz A, Nakafuku M, Hui C. 1999. Mouse suppressor of fused is a negative regulator of sonic hedgehog signaling and alters the subcellular distribution of Gli1. Curr Biol 9:1119–1122.

Ding Q, Motoyama J, Gasca S, Mo R, Sasaki H, Rossant J, Hui CC. 1998. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. Development 125:2533–2543.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97: 703–716.

Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A. 2002. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 36:1021–1034.

Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP. 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75:1417–1430.

Ericson J, Morton S, Kawakami A, Roelink H, Jessell TM. 1996. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. Cell 87:661–673.

Ericson J, Thor S, Edlund T, Jessell TM, Yamada T. 1992. Early stages of motor neuron differentiation revealed by expression of homeobox gene Islet-1. Science 256:1555–1560.

Felsher DW, Bishop JM. 1999. Reversible tumorigenesis by MYC in hematopoietic lineages. Mol Cell 4:199–207.

Fisher GH, Wellen SL, Klimstra D, Lenczowski JM, Tichelaar JW, Lizak MJ, Whitsett JA, Koretsky A, Varmus HE. 2001. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. Genes Dev 15:3249–3262.

Frank-Kamenetsky M, Zhang XM, Bottega S, Guicherit O, Wichterle H, Dudek H, Bumcrot D, Wang FY, Jones S, Shulok J, Rubin LL, Porter JA. 2002. Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. J Biol 1:10.

Gage FH. 2000. Mammalian neural stem cells. Science 287:1433–1438.

Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A. 2004. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64:7011–7021.

Gao WO, Heintz N, Hatten ME. 1991. Cerebellar granule cell neurogenesis is regulated by cell-cell interactions in vitro. Neuron 6:705–715.

Goodrich LV, Milenkovic L, Higgins KM, Scott MP. 1997. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 277:1109–1113.

Goodrich LV, Scott MP. 1998. Hedgehog and patched in neural development and disease. Neuron 21:1243–1257.

Gorlin RJ. 1995. Nevoid basal cell carcinoma syndrome. Dermatol Clin 13:113–125.

Grachtchouk M, Mo R, Yu S, Zhang X, Sasaki H, Hui CC, Dlugosz AA. 2000. Basal cell carcinomas in mice overexpressing Gli2 in skin. Nat Genet 24:216–217.

Grachtchouk V, Grachtchouk M, Lowe L, Johnson T, Wei L, Wang A, de Sauvage F, Dlugosz AA. 2003. The magnitude of hedgehog signaling activity defines skin tumor phonotype. EMBO J 22:2741–2751.

Gupta A, Tsai LH, Wynshaw-Boris A. 2002. Life is a journey: a genetic look at neocortical development. Nat Rev Genet 3:342–355.

Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. 1996. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85: 841–851.

Hahn H, Wojnowski L, Zimmer AM, Hall J, Miller G, Zimmer A. 1998. Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. Nat Med 4:619–622.

Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. 2003. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A 100:15178–15183.

Hooper JE, Scott MP. 2005. Communicating with Hedgehogs. Nat Rev Mol Cell Biol 6:306–317.

Hynes M, Porter JA, Chiang C, Chang D, Tessier-Lavigne M, Beachy PA, Rosenthal A. 1995. Induction of midbrain dopaminergic neurons by Sonic hedgehog. Neuron 15:35–44.

Hynes M, Stone DM, Dowd M, Pitts-Meek S, Goddard A, Gurney A, Rosenthal A. 1997. Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene Gli-1. Neuron 19:15–26.

Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. 2002. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro. Glia 39:193–206.

Illmensee K, Mintz B. 1976. Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. Proc Natl Acad Sci U S A 73: 549–553.

Incardona JP, Gaffield W, Kapur RP, Roelink H. 1998. The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. Development 125: 3553–3562.

Jessell TM. 2000. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nat Rev Genet 1:20–29.

Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH Jr, Scott MP. 1996. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 272:1668–1671.

Karhadkar SS, Steven Bova G, Abdallah N, Dhara S, Gardner D, Maitra A, Isaacs JT, Berman DM, Beachy PA. 2004. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 431:707–712.

Karlstrom RO, Tyurina OV, Kawakami A, Nishioka N, Talbot WS, Sasaki H, Schier AF. 2003. Genetic analysis of zebrafish gli1 and gli2 reveals divergent requirements for gli genes in vertebrate development. Development 130:1549–1564.

Keeler RF. 1970. Teratogenic compounds of Veratrum californicum (Durand) X. Cyclopia in rabbits produced by cyclopamine. Teratology 3:175–180.

Keeler RF, Baker DC. 1989. Oral, osmotic minipump, and intramuscular administration to sheep of the Veratrum alkaloid cyclopamine. Proc Soc Exp Biol Med 192:153–156.

Kinzler KW, Bigner SH, Bigner DD, Trent JM, Law ML, O'Brien SJ, Wong AJ, Vogelstein B. 1987. Identification of an amplified, highly expressed gene in a human glioma. Science 236:70–73.

Kogerman P, Grimm T, Kogerman L, Krause D, Unden AB, Sandstedt B, Toftgard R and Zaphiropoulos PG. 1999. Mammalian suppressor of fused modulates nuclear-cytoplasmic shuttling of Gli1. Nat Cell Biol 1:312–319.

Krauss S, Concordet JP, Ingham PW. 1993. A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell 75:1431–1444.

Kubo M, Nakamura M, Tasaki A, Yamanaka N, Nakashima H, Nomura M, Kuroki S, Katano M. 2004. Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer. Cancer Res 64:6071–6074.

Lai K, Kaspar BK, Gage FH, Schaffer DV. 2003. Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. Nat Neurosci 6:21–27.

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. 1994. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367:645–648.

Lee J, Platt KA, Censullo P, Ruiz i Altaba A. 1997. Gli1 is a target of Sonic hedgehog that induces ventral neural tube development. Development 124:2537–2352.

Lee JJ, von Kessler DP, Parks S, Beachy PA. 1992. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene hedgehog. Cell 71:33–50.

Lei Q, Zelman AK, Kuang E, Li S, Matise MP. 2004. Transduction of graded Hedgehog signaling by a combination of Gli2 and Gli3 activator functions in the developing spinal cord. Development 131:3593–3604.

Lewis PM, Gritli-Linde A, Smeyne R, Kottmann A, McMahon AP. 2004. Sonic hedgehog signaling is required for expansion of granule neuron precursors and patterning of the mouse cerebellum. Dev Biol 270:393–410.

Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A. 2000. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 28:713–726.

Litingtung Y, Chiang C 2000. Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. Nat Neurosci 3:979–985.

Lu QR, Yuk D, Alberta JA, Zhu Z, Pawlitzky I, Chan J, McMahon AP, Stiles CD, Rowitch DH. 2000. Sonic hedgehog-regulated oligodendrocyte lineage genes encoding bHLH proteins in the mammalian central nervous system. Neuron 25:317–329.

Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, Gritli-Linde A, Dellovade T, Porter JA, Rubin LL, Dudek H, McMahon AP, Fishell G. 2003. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39:937–950.

Mao J, Maye P, Kogerman P, Tejedor FJ, Toftgard R, Xie W, Wu G, Wu D. 2002. Regulation of Gli1 transcriptional activity in the nucleus by Dyrk1. J Biol Chem 277:35156–35161.

Marti E, Bumcrot DA, Takada R, McMahon AP. 1995. Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature 375:322–325.

Martin GR, Evans MJ. 1975. Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc Natl Acad Sci U S A. 72:1441–1445.

Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL. 1998. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. Development 125:2759–2770.

Maxwell M, Naber SP, Wolfe HJ, Galanopoulos T, Hedley-Whyte ET, Black PM, Antoniades HN. 1990. Coexpression of platelet-derived growth factor (PDGF) and PDGF-receptor genes by primary human astrocytomas may contribute to their development and maintenance. J Clin Invest 86:131–140.

Miao N, Wang M, Ott JA, D'Alessandro JS, Woolf TM, Bumcrot DA, Mahanthappa NK, Pang K. 1997. Sonic hedgehog promotes the survival of specific CNS neuron populations and protects these cells from toxic insult in vitro. J Neurosci 17:5891–5899.

Mintz B, Illmensee K. 1975. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci U S A. 72:3585–3589.

Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH, Joyner AL, Hui C. 1997. Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development. Development 124:113–123.

Mohler J. 1988. Requirements for hedgehod, a segmental polarity gene, in patterning larval and adult cuticle of Drosophila. Genetics 120:1061–1072.

Monuki ES, Walsh CA. 2001. Mechanisms of cerebral cortical patterning in mice and humans. Nat Neurosci 4: 1199–1206.

Morshead CM, van der Kooy D. 2004. Disguising adult neural stem cells. Curr Opin Neurobiol 14:125–131.

Motoyama J, Milenkovic L, Iwama M, Shikata Y, Scott MP, Hui CC. 2003. Differential requirement for Gli2 and Gli3 in ventral neural cell fate specification. Dev Biol 259:150–161.

Nguyen V, Chokas AL, Stecca B, Ruiz i Altaba A. 2005. Development. In press.

Nilsson M, Unden AB, Krause D, Malmqwist U, Raza K, Zaphiropoulos PG, Toftgard R. 2000. Induction of basal cell carcinomas and trichoepitheliomas in mice overexpressing GLI-1. Proc Natl Acad Sci U S A 97:3438–3443.

Nusslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in Drosophila. Nature 287:795–801.

Orentas DM, Hayes JE, Dyer KL, Miller RH. 1999. Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. Development 126:2419–2429.

Oro AE, Higgins KM, Hu Z, Bonifas JM, Epstein EH Jr, Scott MP. 1997. Basal cell carcinomas in mice overexpressing sonic hedgehog. Science 276:817–821.

Panchision DM, McKay RD. 2002. The control of neural stem cells by morphogenic signals. Curr Opin Genet Dev 12:478–487.

Palma V, Ruiz i Altaba A. 2004. Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex. Development 131:337–345.

Palma V, Lim DA, Dahmane N, Sanchez P, Brionne TC, Herzberg CD, Gitton Y, Carleton A, Alvarez-Buylla A, Ruiz i Altaba A. 2005. Sonic hedgehog signaling regulates the behavior of stem cells and early precursors in the postnatal and adult brain. Development 132:335–344.

Papaioannou VE, McBurney MW, Gardner RL, Evans MJ. 1975. Fate of teratocarcinoma cells injected into early mouse embryos. Nature 258:70–73.

Pardal R, Clarke MF, Morrison SJ. 2003. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 3:895–902.

Park HL, Bai C, Platt KA, Matise MP, Beeghly A, Hui CC, Nakashima M, Joyner AL. 2000. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. Development 127:1593–1605.

Pasca di Magliano M, Hebrok M. 2003. Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer 3:903–911.

Pearse RV 2nd, Collier LS, Scott MP, Tabin CJ. 1999. Vertebrate homologs of Drosophila suppressor of fused interact with the gli family of transcriptional regulators. Dev Biol 212:323–336.

Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. 1999. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. Mol Cell 3:565–577.

Pepinsky RB, Shapiro RI, Wang S, Chakraborty A, Gill A, Lepage DJ, Wen D, Rayhorn P, Horan GS, Taylor FR, Garber EA, Galdes A, Engber TM. 2002. Long-acting forms of Sonic hedgehog with improved pharmacokinetic and pharmacodynamic properties are efficacious in a nerve injury model. J Pharm Sci 91:371–387.

Persson M, Stamataki D, te Welscher P, Andersson E, Bose J, Ruther U, Ericson J, Briscoe J. 2002. Dorsal-ventral patterning of the spinal cord requires Gli3 transcriptional repressor activity. Genes Dev 16:2865–2578.

Pietsch T, Waha A, Koch A, Kraus J, Albrecht S, Tonn J, Sorensen N, Berthold F, Henk B, Schmandt N, Wolf HK, von Deimling A, Wainwright B, Chenevix-Trench G, Wiestler OD, Wicking C. 1997. Medulloblastomas of the desmoplastic variant carry mutations of the human homologue of Drosophila patched. Cancer Res 57:2085–2088.

Poncet C, Soula C, Trousse F, Kan P, Hirsinger E, Pourquie O, Duprat AM, Cochard P. 1996. Induction of oligodendrocyte progenitors in the trunk neural tube by ventralizing signals: effects of notochord and floor plate grafts, and of sonic hedgehog. Mech Dev 60:13–32.

Pringle NP, Yu WP, Guthrie S, Roelink H, Lumsden A, Peterson AC, Richardson WD. 1996. Determination of neuroepithelial cell fate: induction of the oligodendrocyte lineage by ventral midline cells and sonic hedgehog. Dev Biol 177:30–42.

Raffel C, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, James CD. 1997. Sporadic medulloblastomas contain PTCH mutations. Cancer Res 57:842–845.

Reifenberger J, Wolter M, Weber RG, Megahed M, Ruzicka T, Lichter P, Reifenberger G. 1998. Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 58:1798–1803.

Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T. 2000. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 26:109–113.

Reya T, Morrison SJ, Clarke MF, Weissman IL. 2001. Stem cells, cancer, and cancer stem cells. Nature 414: 105–111.

Reynolds BA, Weiss S. 1996. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev Biol 175:1–13.

Richardson WD, Pringle N, Mosley MJ, Westermark B, Dubois-Dalcq M. 1988. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 53:309–19.

Riddle RD, Johnson RL, Laufer E, Tabin C. 1993. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell 75:1401–1416.

Roelink H, Augsburger A, Heemskerk J, Korzh V, Norlin S, Ruiz i Altaba A, Tanabe Y, Placzek M, Edlund T, Jessell TM, et al. 1994. Floor plate and motor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. Cell 76:761–775.

Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. 1996. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. Nat Genet 14:357–360.

Romer JT, Kimura H, Magdaleno S, Sasai K, Fuller C, Baines H, Connelly M, Stewart CF, Gould S, Rubin LL, Curran T. 2004. Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/−)p53(−/−) mice. Cancer Cell 6:229–240.

Ross SE, Greenberg ME, Stiles CD. 2003. Basic helix-loop-helix factors in cortical development. Neuron 39:13–25.

Ruiz i Altaba A. 1998. Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog. Development 125:2203–2212.

Ruiz i Altaba A. 1999. Gli proteins encode context-dependent positive and negative functions: implications for development and disease. Development 126:3205–3216.

Ruiz i Altaba A, Palma V, Dahmane N. 2002. Hedgehog-Gli signalling and the growth of the brain. Nat Rev Neurosci 3:24–33.

Ruiz i Altaba A, Nguyen V, Palma V. 2003. The emergent design of the neural tube: prepattern, SHH morphogen and GLI code. Curr Opin Genet Dev 13:513–521.

Ruiz i Altaba A, Sanchez P, Dahmane N. 2002. Gli and hedgehog in cancer: tumours, embryos and stem cells. Nat Rev Cancer 2:361–372.

Ruiz i Altaba A, Stecca B, Sanchez P. 2004. Hedgehog-Gli signaling in brain tumors: stem cells and paradevelopmental programs in cancer. Cancer Lett 204:145–157.

Ruppert JM, Vogelstein B, Kinzler KW. 1991. The zinc finger protein GLI transforms primary cells in cooperation with adenovirus E1A. Mol Cell Biol 11:1724–1728.

Salgaller M, Pearl D, Stephens R. 1991. In situ hybridization with single-stranded RNA probes to demonstrate infrequently elevated gli mRNA and no increased ras mRNA levels in meningiomas and astrocytomas. Cancer Lett 57: 243–253.

Sanchez P, Ruiz i Altaba A. 2005. In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. Mechanisms of Development 122:223–230.

Sanchez P, Hernandez AM, Stecca B, Kahler AJ, DeGueme AM, Barrett A, Beyna M, Datta MW, Datta S, Ruiz i Altaba A. 2004. Inhibition of prostate cancer proliferation by interference with SONIC HEDGEHOG-GLI1 signaling. Proc Natl Acad Sci U S A 101:12561–12566.

Sasaki H, Nishizaki Y, Hui C, Nakafuku M, Kondoh H. 1999. Regulation of Gli2 and Gli3 activities by an amino-terminal repression domain: implication of Gli2 and Gli3 as primary mediators of Shh signaling. Development 126:3915–3924.

Shin SH, Kogerman P, Lindstrom E, Toftgard R, Biesecker LG. 1999. GLI3 mutations in human disorders mimic Drosophila cubitus interruptus protein functions and localization. Proc Natl Acad Sci U S A 96:2880–2884.

Sheng T, Li C, Zhang X, Chi S, He N, Chen K, McCormick F, Gatalica Z, Xie J. 2004 Activation of the hedgehog pathway in advanced prostate cancer. Mol Cancer 3:29.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. 2003. Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. 2004. Identification of human brain tumour initiating cells. Nature 432:396–401.

Soula C, Danesin C, Kan P, Grob M, Poncet C, Cochard P. 2001. Distinct sites of origin of oligodendrocytes and somatic motoneurons in the chick spinal cord: oligodendrocytes arise from Nkx2.2-expressing progenitors by a Shh-dependent mechanism. Development 128:1369–1379.

Stone DM, Murone M, Luoh S-M, Ye W, Armanini MP, Gurney A, Philips H, Brush J, Goddard A, de Sauvage FJ, Rosenthal A. 1999. Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli. J Cell Sci 112:4437–4448.

Tabata T, Kornberg TB. 1994. Hedgehog is a signaling protein with a key role in patterning Drosophila imaginal discs. Cell 76:89–102.

Taipale J, Chen JK, Cooper MK, Wang B, Mann RK, Milenkovic L, Scott MP, Beachy PA. 2000. Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 406:1005–1009.

Tas S, Avci O. 2004a. Rapid clearance of psoriatic skin lesions induced by topical cyclopamine. A preliminary proof of concept study. Dermatology 209:126–131.

Tas S, Avci O. 2004b. Induction of the differentiation and apoptosis of tumor cells in vivo with efficiency and selectivity. Eur J Dermatol 14:96–102.

Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, Agatep R, Chiappa S, Gao L, Lowrance A, Hao A, Goldstein AM, Stavrou T, Scherer SW, Dura WT, Wainwright B, Squire JA, Rutka JT, Hogg D. 2002. Mutations in SUFU predispose to medulloblastoma. Nat Genet 31:306–310.

Temple S. 1999. CNS development: the obscure origins of adult stem cells. Curr Biol 9:R397–R399.

Temple S. 2001. The development of neural stem cells. Nature 414:112–117.

Temple S, Alvarez-Buylla A. 1999. Stem cells in the adult mammalian central nervous system. Curr Opin Neurobiol 9:135–141.

Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernandez-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. 2003. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 425:851–856.

Theil T, Alvarez-Bolado G, Walter A, Ruther U. 1999. Gli3 is required for Emx gene expression during dorsal telencephalon development. Development 126:3561–3571.

Thibert C, Teillet MA, Lapointe F, Mazelin L, Le Douarin NM, Mehlen P. 2003. Inhibition of neuroepithelial patched-induced apoptosis by sonic hedgehog. Science 301:843–846.

Tole S, Ragsdale CW, Grove EA. 2000. Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J). Dev Biol 217:254–265.

Traiffort E, Charytoniuk D, Watroba L, Faure H, Sales N, Ruat M. 1999. Discrete localizations of hedgehog signalling components in the developing and adult rat nervous system. Eur J Neurosci 11:3199–3214.

Tropepe V, Sibilia M, Ciruna BG, Rossant J, Wagner EF, van der Kooy D. 1999. Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. Dev Biol 208:166–188.

Tsuboi K, Shults CW. 2002. Intrastriatal injection of sonic hedgehog reduces behavioral impairment in a rat model of Parkinson's disease. Exp Neurol 173:95–104.

Tyurina OV, Guner B, Popova E, Feng J, Schier A, Kohtz JD, Karlstrom RO. 2005. Zebrafish Gli3 functions as both an activator and a repressor in Hedgehog signaling. Dev Biol 277:537–556.

Vaccarino FM, Schwartz ML, Raballo R, Nilsen J, Rhee J, Zhou M, Doetschman T, Coffin JD, Wyland JJ, Hung YT. 1999. Changes in cerebral cortex size are governed by fibroblast growth factor during embryogenesis. Nat Neurosci 2:246–253.

Vortmeyer AO, Stavrou T, Selby D, Li G, Weil RJ, Park WS, Moon YW, Chandra R, Goldstein AM, Zhuang Z. 1999. Deletion analysis of the adenomatous polyposis coli and PTCH gene loci in patients with sporadic and nevoid basal cell carcinoma syndrome-associated medulloblastoma. Cancer 85:2662–2667.

Wallace VA. 1999. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. Curr Biol 9:445–448.

Wang MZ, Jin P, Bumcrot DA, Marigo V, McMahon AP, Wang EA, Woolf T, Pang K. 1995. Induction of dopaminergic neuron phenotype in the midbrain by Sonic hedgehog protein. Nat Med 1:1184–1188.

Wang B, Fallon JF, Beachy PA. 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. Cell 100:423–434.

Wang VY, Zoghbi HY. 2001. Genetic regulation of cerebellar development. Nat Rev Neurosci 2:484–491.

Watkins DN, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. 2003. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature 422:313–317.

Wechsler-Reya RJ, Scott MP. 1999. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron 22:103–114.

Wetmore C, Eberhart DE, Curran T. 2001. Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. Cancer Res 61:513–516.

Wetmore C. 2003. Sonic hedgehog in normal and neoplastic proliferation: insight gained from human tumors and animal models. Curr Opin Genet Dev 13:34–42.

Williams JA, Guicherit OM, Zaharian BI, Xu Y, Chai L, Wichterle H, Kon C, Gatchalian C, Porter JA, Rubin LL, Wang FY. 2003. Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. Proc Natl Acad Sci U S A 100:4616–4621.

Wolter M, Reifenberger J, Sommer C, Ruzicka T, Reifenberger G. 1997. Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 57:2581–2585.

Xiao H, Goldthwait DA, Mapstone T. 1994. A search for gli expression in tumors of the central nervous system. Pediatr Neurosurg 20:178–182.

Xie J, Aszterbaum M, Zhang X, Bonifas JM, Zachary C, Epstein E, McCormick F. 2001. A role of PDGFRalpha in basal cell carcinoma proliferation. Proc Natl Acad Sci U S A 98:9255–9259.

Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, Rosenthal A, Epstein EH Jr, de Sauvage FJ. 1998. Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature 391:90–92.

Ye W, Shimamura K, Rubenstein JL, Hynes MA, Rosenthal A. 1998. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93:755–766.