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Suppressors of Hedgehog Signaling

Linking Aberrant Development of Neural Progenitors and Tumorigenesis

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Abstract

Subversion of signals that physiologically suppress Hedgehog pathway results in aberrant neural progenitor development and medulloblastoma, a malignancy of the cerebellum. The Hedgehog antagonist *REN*^{KCTD11} maps to chromosome 17p13.2 and is involved in the withdrawal of the Hedgehog signaling at the granule cell progenitor transition from the outer to the inner external germinal layers, thus promoting growth arrest and differentiation. Deletion of chromosome 17p, the most frequent genetic lesion observed in this tumor, is responsible for the loss of function of *REN*^{KCTD11}, resulting in upregulated Hedgehog signaling and medulloblastoma. Persistence of signals that limit Hedgehog activity is also associated with malignancy. Hedgehog signaling-induced downregulation of ErbB4 receptor expression is attenuated in medulloblastoma subsets in which the extent of Hedgehog pathway activity is limited, thus favoring the accumulation of ErbB4 with imbalanced alternative splice CYT-1 isoform over the CYT-2. This is responsible for both Neuregulin ligand-induced CYT-1-dependent prosurvival activity and loss of CYT-2-mediated growth arrest.

Index Entries: Hedgehog; Gli; cerebellum; neural stem cells; granule cell progenitors; medulloblastoma; REN^{KCTD11}; ErbB4.

Introduction

Hedgehog (Hh) signaling pathway is a crucial developmental regulator and is evolutionary conserved from *Drosophila* to mammals. As originally described in *Drosophila (1)*, the Hh pathway is critical for the proper embryonic patterning and acts as a cell fate and body-segment polarity determination factor, thus regulating the development of numerous tissues in mammals *(2,3)*. In the central nervous system (CNS), the Hh pathway is one of the most

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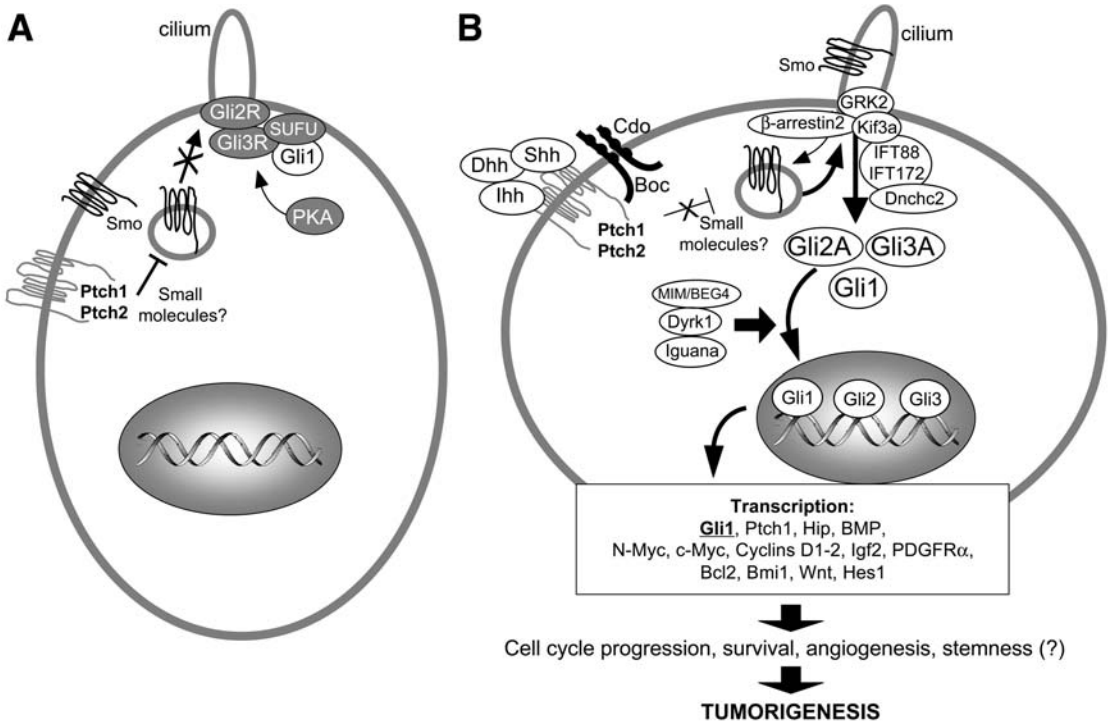

Fig. 1. Mechanisms of activation of Hegehog signaling. **(A)** Inactive Hh signaling in the absence of ligands. **(B)** Binding of ligands (Shh, Dhh, Ihh) to Ptch receptor triggers signaling activation. Gli2R and Gli3R are repressor forms, Gli2A and Gli3A are transcriptionally active forms. For other details and references, *see* the text.

important developmental regulators, behaving as a morphogen, mitogenic, and differentiation factor *(2)*. Most importantly, Hh signaling also controls tissue repair, a property that is extended to adult tissues *(3,4)*. Indeed, the Hh pathway has been reported as a part of the tissue repair process in response to tissue damage (e.g., in neurodegenerative or ischemic damage; recently reviewed in ref. *5*). Therefore, if Hh signaling is aberrantly regulated, it underlies several pathological conditions, ranging from cancer to neurodegenerative disorders. A fine-tuning of the Hh signaling pathway is required to maintain physiological cell functions during development and after. This article reviews signals that restrain the Hh pathway, focusing mainly on conditions that link aberrant development to tumorigenesis.

Hh Signaling Mechanisms

The overall Hh signaling machinery (Fig. 1) is conserved between *Drosophila* and vertebrates, although it diverged at a number of points. The first difference is the nature of the ligands of the pathway that in mammals includes three distinct proteins—Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh), which share the signaling machinery but also display distinct functional properties *(6)*. Hh ligand binds to two distinct but related 12-pass membrane receptors, Patched1 and 2 (Ptch), in concert with two recently identified additional coreceptors, Cdo and Boc (homologs of *Drosphila Ihog* and *Boi*) *(7–9)*. This interaction relieves the Ptch-dependent inhibition of the 7-pass G protein-coupled

receptor-like transmembrane Smoothened (Smo) transducer.

In turn, Smo triggers downstream transcription factors belonging to the Gli family (Gli1, -2, and -3), the vertebrate homologs of *Drosophila Cubitus interruptus* (Ci). Because Ptch does not bind Smo, the nature of the Ptch-dependent regulation of Smo is unclear, but it may involve the distribution of a small hydrophobic molecule Smo ligand, in turn regulating its activity. Indeed, several small molecules acting as either agonists or antagonists of Smo, have been described (reviewed in ref. *5*). Accordingly, oxysterols, specific derivatives of cholesterol, have recently been suggested as the endogenous regulatory small molecules *(10)*. Smo is localized in plasma membrane and intracellular vescicles. Activation of Smo modulates its trafficking and involves G protein-coupled receptor kinase 2 (GRK2)-dependent phosphorylation. This event allows the recruitment of β-arrestin-2, a scaffold protein that diverts Smo to endocytic clathrin-coated pits, and results in Hh-dependent Smo internalization *(11–13)*. It is not clear whether this process is linked to Smo signaling or desensitation. However, recruitment of Smo to specialized membranes has also been described in response to ligand activation. These specialized structures are represented by cilia, underscoring a role for transport proteins in Smo activity *(14–16)*.

In *Drosophila*, the kinesin-like molecule Costal-2 (Cos2) forms a complex with Ci that is bound to microtubules in the absence of Hh signal and prevents the Ci processing that allows the nuclear translocation of the activator form of Ci in response to Hh. However, although Kif7 has been reported to be active in zebrafish *(17)*, mammalian Cos2 homologs Kif7 and Kif27 do not play any role in Hh pathway signaling *(18)*. Such a role has been instead reported for intraflagellar transport (IFT) proteins, in which IFTs sustain the transport of proteins along microtubules, a process required for the assembly and maintenance of cilia *(14–16,19,20)*. The recently identified requirement of cilia for Hh signaling is supported by defective Hh pathway function resulting from the absence of *Inturned* and *Fuzzy* genes, both of which are required for ciliogenesis *(21)*. This would exert a secondary effect on Hh signaling, presumably by inhibiting the presentation of Gli factors to Smo. Several components of the IFT machinery (e.g., Polaris/IFT88, IFT172; the anterograde motor subunit Kif3a and the retrograde motor subunit Dnchc2) are required for Smo-dependent regulation of activator or repressor Gli functions *(14–16,19,20)*. Therefore, in response to Ptch ligands, Smo is accumulated in cilia where Gli1, -2, and -3 factors have also been described *(15,16)*.

The final output of the Hh/Smo-triggered signal transduction cascade is represented by the activation of the transcriptional function of Gli proteins. This results in the translocation of Gli proteins into the nucleus and in the enhancement of the expression of numerous target genes, including Gli1 itself *(22)*. Whereas Gli2 and Gli3 are also processed onto a transcription-repressor form, Gli1 behaves exclusively as a strong transcriptional activator that sustains and also amplifies the Hh pathway-induced response.

Negative Regulators

The inhibition exerted by the upstream inhibitor Ptch is relieved by its interaction with the ligand. Several negative regulators of Hh signaling have also been described, which can switch off the response at different levels (Fig. 2). The endocytic trafficking of Hh pathway signals is negatively regulated by Rab23 *(23)*. Proteolytic processing and degradation of Gli factors is also a critical step by which Hh signaling output in limited. The process was first described in *Drosophila*, where the Gli-homolog *Cubitus interruptus* (Ci) was shown to be ubiquitinated by Slimb (*Supernumerary limbs*), an F-box protein of the SCF E3 ligase complex *(24)*. Recent studies have shown that this pathway has been conserved in mammals, where Gli1, Gli2, and Gli3 undergo ubiquitination mediated by the Slimb-homolog βTrCP. This results in their subsequent processing

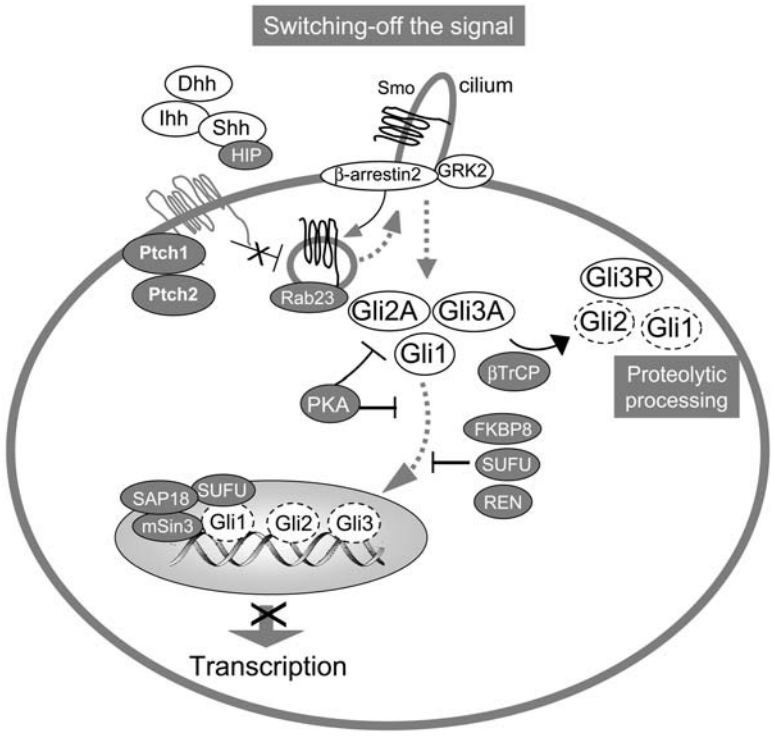

Fig. 2. Switch-off of the Hh signaling induced by inhibitory signals (gray symbols). For other details and references, *see* the text and Fig. 1 legend.

and/or degradation by a PKA-, casein kinase- and GSK3β-dependent phosphorylation event that is inhibited by Hh signaling *(25–27)*. Interestingly, proteolytic processing of Gli3 to a repressor form requires IFT protein function *(20)*. PKA-dependent phosphorylation has also been reported to impair nuclear transfer of Gli1 *(28)*. A powerful antagonist of Gli is suppressor of fused (SUFU), also localized in cilia *(16)*. SUFU binds Gli proteins, thereby inhibiting nuclear transfer and interfering with its transcriptional activity *(29–32)*. The Gli1 nuclear transfer is also inhibited by REN^{KCTD11}, encoded by a gene frequently deleted in medulloblastoma *(33)*.

A detailed description of the Hh pathway has been reported in several recent reviews *(34–36)*.

Hedgehog Signaling in Neural Stem Cells: Development Meets Tumorigenesis

Hh Signaling and Neural Stem Cells

Developmental morphogenetic processes and tissue repair events have been recently suggested to be sustained by the regulated growth of progenitor cells with stem cell properties. Stem cell population undergoes cell developmental processes in combination with self-renewal. Self-renewal maintains a pool of noncommitted cells to allow subsequent waves of tissue development or repair. Hh signaling has been described as playing a critical role in brain morphogenesis by regulating the ventral patterning of the neural tube, as well as the proliferation of precursor cells in the dorsal

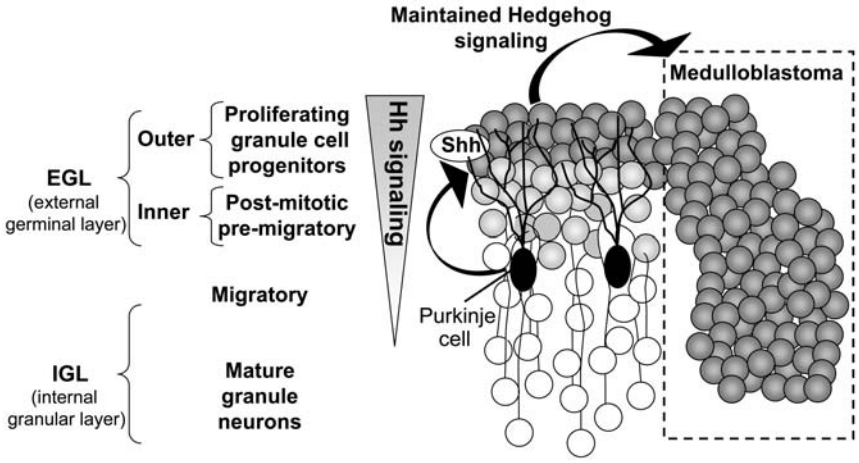

Fig. 3. Model of development of granule cell progenitors in the mouse cerebellum. Granule cell progenitors of the outer EGL proliferate and subsequently exit cell cycle and start a migration and differentiation program as soon as they enter the inner EGL. They subsequently migrate into the IGL, where they terminally differentiate. Purkinje cells secrete Shh, which triggers Hedgehog (Hh) signaling in granule cell progenitors of the outer EGL and maintains them in an undifferentiated and proliferating status *(44-46)*. A gradient of Hh signaling activity (gray) occurs with highest level in outer EGL and lowest level in IGL. If Hh signaling is not interrupted at the outer to inner EGL transition and is instead maintained at high levels, continous overgrowth eventually leads to medulloblastoma formation.

brain (reviewed in refs. *2* and *37*). Therefore, several loss-of-function genetic studies underscore the role of Hh-pathway-induced enhanced proliferation of neural progenitor cells, showing that Gli1 function is redundant, whereas Gli2 and Gli3 display specific defects as well as overlapping functions *(2,37)*. More recently, a specific essential function for Hh signaling has also been described in the maintenance and self-renewal of neural progenitors in stem cell niches located in several regions of the embryonic, postnatal, and adult brain *(38–42)*. For example, in the developing embryonic cortex, Hh signaling regulates the number and growth of cells with stem cell properties and maintains stem cell niches in which these cells exist and proliferate by acting in concert with specific microenvironmental niche factors (e.g., epidermal growth factor [EGF]) *(40)*. Similar observations have been reported in the postnatal forebrain subventricular zone, the stem cell niche of adult mammalian brain *(41)*. Brain tumors have been described to contain cancer stem cells that self-renew and, therefore, maintain a never-ending reservoir for the tumor mass *(43)*. The role of Hh signaling in sustaining the malignant conversion from normal to cancer stem cells and in their maintenance during tumor progression has important therapeutic implications *(35)* and is an intense field of investigation that has not been elucidated as yet.

Cerebellum Development and Tumorigenesis

An important site in which Hh properties have been characterized is the mammalian cerebellum. During cerebellum development, granule cell progenitors (GCPs) migrate from the rhombic lip over the outer layer of the cerebellar surface (external germinal layer [EGL]) *(2)*. After a first burst of cell proliferation in the outer EGL, GCPs start a differentiation program by exiting from the cell cycle and moving into the inner EGL and further migrate inward into the internal granule layer (IGL), where

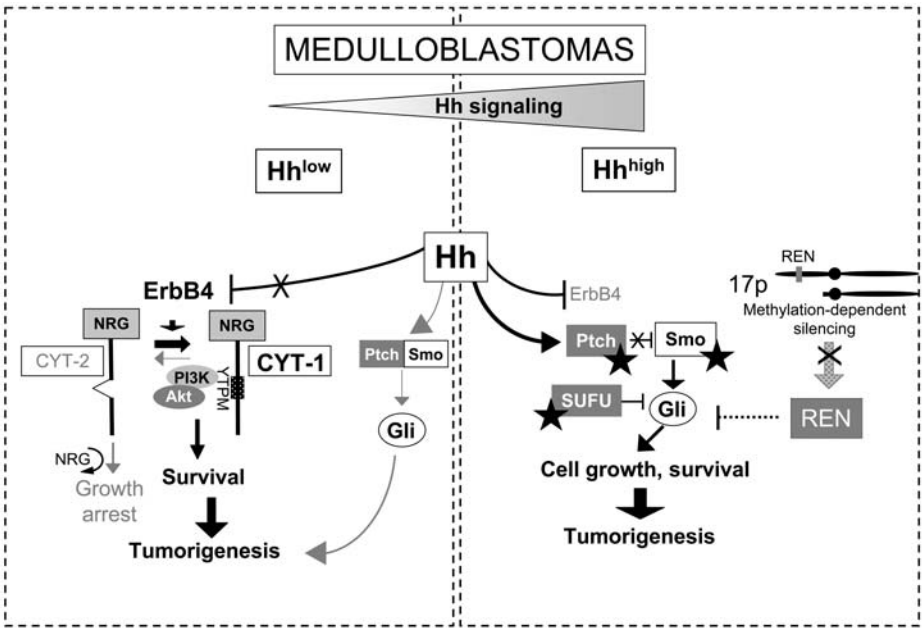

Fig. 4. Heterogeneity of human medulloblastomas with respect to the level of Hedgehog signaling (Hh^{high}, high signaling; Hh^{low}, low signaling). In the left panel, low Hedgehog signaling attenuates the inhibitory activity upon ErbB4 expression. The resulting high levels of ErbB4 are associated with the imbalance of its alternative splicing that favors the prosurvival CYT-1 isoform at the expense of the CYT-2 isoform promoting growth arrest. NRG, ErbB4 receptor ligand neuregulin. In the right panel, star symbols in Ptch, Smo, and SUFU indicate mutations in corresponding genes. Allelic deletion of chromosome 17p leading to reduced expression of *REN* is also indicated, which attenuates the inhibitory acitvity upon Gli function.

differentiated granule cells reside (Fig. 3; ref. *2*). Cerebellar Purkinje cells produce Shh, acting upon EGL GCPs. In this way, Hh signaling keeps the EGL GCP population in an undifferentiated state while promoting cell expansion *(2,44-46)*. The cause of the interruption of Hh signaling and the subsequent growth arrest of GCPs in the inner EGL is much less understood. Although cerebellar neural stem cells have been recently identified *(47)*, the role of Hh signaling has not been elucidated. This is an important aspect because of the relationship between cerebellar tumorigenesis and aberrant Hh signaling. Indeed, inappropriate activation of Hh pathway resulting from failure to constrain Hh signaling is responsible for the development of several medulloblastomas (Fig. 3). This appears to be caused by the Hh signaling-dependent maintenance of cell cycle progression and arrest of differentiation of GCP, which results in the abnormal persistence of progenitor cells that are susceptible to malignant transformation *(37)*.

Heterogeneity of Meduloblastoma Subsets

The critical role played by Hh signaling in cerebellar tumorigenesis is sustained by the ability of Hh pathway antagonists to suppress the growth of virtually all medulloblastoma tested, both in cultured cells and in vivo *(48-51)*. However, the extent of Hh signaling appears heterogeneous in the various medulloblastomas. Indeed, a recent high-throughput complementary DNA (cDNA) microarray analysis of human primary medulloblastomas revealed different subsets of tumors based on clustered gene expression patterns *(52)*. Gene

expression signatures of one of these subsets includes upregulation of target genes of the Hh pathway, suggesting that inappropriate high activation of this signaling occurs in this specific subset. Conversely, distinct genetic and gene expression patterns (e.g., Wnt pathway) characterized tumors with low expression of Hh pathway target genes. Interestingly, tumors belonging to the high Hh signaling subset display known mutations of genes encoding components of the Hh pathway (Ptch1, SUFU) in only a fraction of cases *(52)*. This confirms previously reported frequencies of activatory mutations of *Smo* or loss-of-function mutations of *Ptch1* and *-2* and *SUFU*, all genes belonging to the pathway (20–25% of tumors, compared with the higher, approx 60% frequency of tumors displaying inappropriately high Hh signaling; Fig. 4; reviewed in ref. *35*). These findings suggest the presence of additional genetic or epigenetic hits in pathways that in some way control Hh signaling. A group of tumors that clustered with concurrent deletion of chromosome 17p also displayed high Hh signaling signature in the absence of known mutations of components of the Hh pathway *(52)*. On the other hand, tumor subsets missing Hh signaling gene expression signature, while still sensitive to the activity of the Hh pathway *(48,49)*, are likely to be mainly regulated by additional or alternative signals. Examples of these conditions and their relationships with Hh signaling are described in the following sections.

Disruption of Negative Regulation of Hedgehog Signaling in Neural Progenitor Development and Brain Tumorigenesis

Loss of negative regulation of Hh pathway has been reported to play a critical role in the maintenance of active Hh pathway signals and in sustaining the subsequent developmental effects. Loss of function resulting from homozygous mutation of both the Hh pathway inhibitory receptor Ptch1 and the Gli antagonist SUFU share a common phenotype in knockout mice. This phenotype is represented by early embryonic lethality (E9.5) with open fore-, mid-, and hindbrain as well as neural tube, which also adopted a ventralized identity *(53,54)*. In some aspects, the neuronal phenotype was different between the two heterozygous Ptch1 and SUFU mice, although mutations of both genes have been associated with medulloblastomas characterized by high Hh signaling (Fig. 4). Although $Ptch1^{-/+}$ mice develop medulloblastomas *(54,55)*, $SUFU^{-/+}$ mice only display a skin phenotype with hyperplastic lesions resembling human Gorlin's syndrome *(53)*. This disease results from activated Hh signaling consequently to mutations of Ptch1 gene, leading to basal cell carcinoma but also to medulloblastoma. The mild phenotype observed in Ptch1 heterozygous mutants suggests that loss of both allele is required to develop tumors. Indeed wild-type Ptch1 allele has recently been shown to be either epigenetically silenced by methylation *(49)* or lost even in preneoplastic lesions *(56,57)*, suggesting that it is an early event in tumorigenesis.

REN^{KCTD11}

Disruption of REN^{KCTD11} gene is an example of the link between unrestrained Hh signaling and chromosome 17p deletion in medulloblastoma (Fig. 4). This genetic alteration is most frequently observed in medulloblastoma (up to 50%), and it is believed to underly the loss of one or more putative tumor suppressors *(35)*. *REN* is a novel putative tumor suppressor that is upreguated by EGF, retinoic acid, and nerve growth factor and is expressed early during embryonic development, first in neural fold epithelium during gastrulation and subsequently throughout the neural tube and in postmitotic neuroblasts of the outer layer of the ventricular encephalic epithelium *(58)*. In the developing cerebellum (3- to 21-d-old mice), *REN* is expressed to a higher extent in nonproliferating cells of the inner EGL and in IGL dfferentiated granule neurons rather than

in high proliferating outer EGL GCPs, which instead express Gli1, a sensitive readout of active Hh signaling *(59)*. REN expression is also upregulated in GCPs differentiated in culture *(59)*. The pattern of REN expression is consistent with a role in the control of the differentiation and growth of GCPs. Indeed, REN is able to promote growth arrest, neuronal differentiation, and apoptosis of cultured GCPs *(59)*. Interestingly, the effects of REN appear to be result from the antagonism on the Hh pathway, as indicated by its ability to inhibit Gli-dependent transcriptional activation of target genes and the Shh-induced mitogenic activity and the Shh-suppressed neuronal differentiation of cultured GCPs *(59)*. Conversely, inactivation of REN function in cultured GCPs resulted in both enhancement of Hh signaling and increased cell proliferation, together with reduced neuronal differentiation *(59)*.

These observations suggest a role for REN as an inhibitory signal required for withdrawing GCP expansion at the outer-to-inner EGL transition in an early phase of development.

Interestingly, human REN maps to 17p13.2 and is both deleted and silenced in a consistent number (40%) of medulloblastomas *(33)*. Restoring high REN expression inhibits medulloblastoma growth by negatively regulating Gli function. Therefore, the loss of such an inhibitory signal, as a consequence of 17p deletion, may lead to the uncontrolled GCP overgrowth linked to medulloblastoma development (Fig. 4). Additional genetic changes in 17p (e.g., p53 deletion) are likely to cooperate with REN loss of function for GCP tumorigenesis (reviewed in ref. *35*).

Low Hh Signaling and Brain Tumorigenesis: The ErbB4 Alternative Splicing Pathway

However, persistence of Hh pathway suppressor signals is also associated with tumorigeneis. Indeed, based on the critical role played by Hh signaling in cerebellum development and tumorigenesis, an important question deals with medulloblastomas that develop in the presence of a low level of Hh signaling. Researchers recently investigated how these additional tumorigenic pathways occur and their relationships with Hh signaling. We have recently described a role for ErbB4 receptor alternative splicing in this context *(60)*. ErbB4 belongs to the ErbB family of tyrosine kinase membrane receptors, which play critical roles in cerebellar neural progenitor development. More specifically, the role of ErbB4 is reflected in the architectural anomalies observed in the cerebella of ErbB4$^{-/-}$/HER4^{heart} mice *(61)* and in the ability of Neuregulin (the ErbB4 ligand) to induce differentiation of cerebellar GCPs *(62)*. Accordingly, ErbB4 expression is restricted to GCPs of the inner EGL zone (characterized by low proliferation) in neonatal mouse cerebellum and is mutually exclusive with the outer EGL localization of Gli1 *(60)*. Interestingly, we recently observed reduced ErbB4 expression in cultured murine GCPs treated with Shh and in medulloblastomas from Ptch-deficient mice *(60)*. The reduced ErbB4 expression was rescued by the Hh pathway antagonist cyclopamine in human medulloblastoma cells *(60)*, suggesting that Hh signaling might also enhance tumorigenesis by suppressing ErbB4-generated differentiation signals. However, ErbB4 expression is high in a subset of medulloblastomas that display a particularly aggressive tumor phenotype *(60,63)*. How may this high expression be compatible with the tumor phenotype and with the role of Hh signaling in downregulating ErbB4 expression? A possible explanation involves the finding that ErbB4 overexpression was only detected in the medulloblastoma subset that did not upregulate Hh signaling (Hh^{low}) (Fig. 4; ref. *60*). Therefore, a low level of Hh signaling, although still required for sustaining the growth of tumor cells, would relieve an ErbB4 inhibitory signal, allowing upregulation of ErbB4 expression (Fig. 4). However, why do these ErbB4 signals not promote cell differentiate and allow tumorigenesis? A likely reason appears to be the alternative splicing of the

ErbB4 cytoplasmic domain, which generates two isoforms: CYT1 and CYT2 *(64)*. Interestingly, medulloblastomas only display ErbB4 CYT1 isoform accumulation *(60)*. The cytoplasmic domain of this isoform includes the YTPM consensus motif for PI3K binding (amino acids 1046 through 1061), which is missing in CYT2 *(65)*. Phosphorylation of the tyrosine in position 1056 (as a result of ligand-receptor interaction) creates a binding site with high affinity for the SH-2 domain of PI3K and subsequent coupling with the Akt anti-apoptotic signaling cascade *(64,65)*; this occurs in medulloblastoma cells *(60)*. Because ErbB4 CYT2 lacks this PI3K-binding domain, it is incapable of mediating neuregulin-induced survival *(59)*. The imbalance of CYT1/CYT2 isoforms favoring CYT1 would also favor tumor progression and correlate with medulloblastoma features predictive of unfavorable outcomes, such as the anaplastic histotype and ErbB2 expression *(60)*.

Therefore, selective maintenance of high ErbB4 CYT1 expression in cerebellar GCPs may represent an additional pathway for medulloblastoma tumorigenesis that is particularly relevant in tumors characterized by low-level Hh signaling: upregulated expression of the CYT1 isoform resulting from the attenuation of Hh-pathway-dependent inhibitory signals would represent a potential advantage for tumor cells. With respect to the normal cell population, in which there is a more substantial excess of CYT2, medulloblastoma cells with higher levels of CYT1 are less susceptible to neuregulin/CYT2-induced inhibition of proliferation and more responsive to the CYT1/growth factor's protective effects during pro-apoptotic conditions (Fig. 4; ref. *60*).

Conclusions

We have discussed several aspects of a scenario in which aberrant regulation of Hh signaling pathway impairs neural progenitor development and leads to tumorigenesis. We have provided as an example medulloblastoma, a malignancy of the cerebellum, in which subversion of signals that physiologically suppress the Hh pathway results in aberrant neural progenitor development and tumor formation. A critical checkpoint appears to be the transition of the GCPs from the outer to the inner EGL; at this stage, the Hh pathway antagonist *REN* has been suggested to be involved in interrupting the Hh signaling, thus promoting growth arrest and differentiation. Deletion of chromosome 17p, the most frequent genetic lesion of this tumor, is responsible for the loss of function of *REN*, thus resulting in upregulated Hh signaling and medulloblastoma. However, persistence of signals that limit Hh activity is not incompatible with malignancy. For example, low Hh signaling fails to downregulate ErbB4 receptor expression in medulloblastoma subsets in which the extent of Hh pathway is limited, thus favoring the accumulation of ErbB4 with imbalanced alternative splice (CYT1 isoform over the CYT2). This is responsible for the neuregulin-ligand-induced CYT1-dependent prosurvival activity and the loss of CYT2-mediated growth arrest.

In addition to the various positive regulators of Hh signaling, the complexity of signals that limit the function of this pathway, and thus represent critical checkpoints of its activity, extends the molecular events that may underly the tumorigenic process. Furthermore, they may represent molecular targets for novel and rational therapeutic strategies.

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References

1. Nusslein-Volhard C. and Wieschaus E. (1980) Mutations affecting segment number and polarity in Drosophila. *Nature* **287**, 795–801.
2. Ruiz i Altaba A., Palma V., and Dahmane N. (2002) Hedgehog-Gli signaling and the growth of the brain. *Nat. Rev. Neurosci*. **3**, 24–33.
3. Pasca di Magliano M. and Hebrok M. (2003) Hedgehog signaling in cancer formation and maintenance. *Nat. Rev. Cancer*. **3**, 903–911.
4. Beachy P. A., Karhadkar S. S., and Berman D. M. (2004) Tissue repair and stem cell renewal in carcinogenesis. *Nature* **432,** 324–331.
5. Dellovade T., Romer J. T., Curran T., and Rubin L. L. (2006) The Hedgehog Pathway and Neurological Disorders. *Annu. Rev. Neurosci*. **29,** 539–563.
6. Hooper J. E., and Scott M. P. (2005) Communicating with Hedgehogs. *Nat. Rev. Mol. Cell Biol*. **6**, 306–317.
7. Yao S., Lum L., and Beachy P. (2006) The ihog cell-surface proteins bind Hedgehog and mediate pathway activation. *Cell* **125**, 343–357.
8. Tenzen T., Allen B. L., Cole F., Kang J. S., Krauss R. S., and McMahon A. P. (2006) The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. *Dev. Cell* **10**, 647–656.
9. Zhang W., Kang J. S., Cole F., Yi M. J., and Krauss R. S. (2006) Cdo functions at multiple points in the Sonic Hedgehog pathway, and Cdo-deficient mice accurately model human holoprosencephaly. *Dev. Cell* **10**, 657–665.
10. Corcoran R. B., and Scott M. P. (2006) Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc. Natl. Acad. Sci. USA*. **103**, 8408–8413.
11. Incardona J. P., Gruenberg J., and Roelink H. (2002) Sonic hedgehog induces the segregation of patched and smoothened in endosomes. *Curr Biol*. **12**, 983–995.
12. Chen W., Ren X. R., Nelson C. D., et al. (2004) Activity-dependent internalization of smoothened mediated by beta-arrestin 2 and GRK2. *Science* **306**, 2257–2260.
13. Wilbanks A. M., Fralish G. B., Kirby M. L., Barak L. S., Li Y. X., and Caron M. G. (2004) Beta-arrestin 2 regulates zebrafish development through the hedgehog signaling pathway. *Science* **306**, 2264–2267.
14. Huangfu D. and Anderson K. V. (2005) Cilia and Hedgehog responsiveness in the mouse. *Proc. Natl. Acad. Sci. USA*. **102**, 11,325–11,330.
15. Corbit K. C., Aanstad P., Singla V., Norman A. R., Stainier D. Y., and Reiter J. F. (2005) Vertebrate Smoothened functions at the primary cilium. *Nature* **437**, 1018–1021.
16. Haycraft C. J., Banizs B., Aydin-Son Y., Zhang Q., Michaud E. J., and Yoder B. K. (2005) Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS. Genet*. **1**, e53.
17. Tay S. Y., Ingham P. W., and Roy S. (2005) A homologue of the *Drosophila* kinesin-like protein Costal2 regulates Hedgehog signal transduction in the vertebrate embryo. *Development* **132**, 625–634.
18. Varjosalo M., Li S. P., and Taipale J. (2006) Divergence of hedgehog signal transduction mechanism between Drosophila and mammals. *Dev. Cell*. **10**, 177–186.
19. Huangfu D., Liu A., Rakeman A. S., Murcia N. S., Niswander L., and Anderson K. V. (2003) Hedgehog signaling in the mouse requires intraflagellar transport proteins. *Nature* **426**, 83–87.
20. Liu A., Wang B., and Niswander L. A. (2005) Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. *Development* **132**, 3103–3111.
21. Park T. J., Haigo S. L., and Wallingford J. B. (2006) Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat. Genet*. **38**, 303–311.
22. 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.
23. Eggenschwiler J. T., Bulgakov O. V., Qin J., Li T., and Anderson K. V. (2006) Mouse Rab23 regulates hedgehog signaling from smoothened to Gli proteins. *Dev. Biol*. **290**, 1–12.
24. Jiang J. and Struhl G. (1998) Regulation of the Hedgehog and Wingless signaling pathways by the F-box/WD40-repeat protein Slimb. *Nature* **391**, 493–496
25. Huntzicker E. G., Estay I. S., Zhen H., Lokteva L. A., Jackson P. K., and Oro A. E. (2006) Dual

degradation signals control Gli protein stability and tumor formation. *Gen. Dev*. **20,** 276–281.
26. Wang B. and Li Y. (2006) Evidence for the direct involvement of βTrCP in Gli3 protein processing. *Proc. Natl. Acad. Sci. USA*. **103,** 33–38
27. Pan Y., Bai C. B., Joyner A. L., and Wang B. (2006) Sonic hedgehog signaling regulates Gli2 transcriptional activity by suppressing its processing and degradation. *Mol. Cell Biol.* **26,** 3365–3377.
28. Sheng T., Chi S., Zhang X., and Xie J. (2006) Regulation of Gli1 localization by the cAMP/ protein kinase A signaling axis through a site near the nuclear localization signal. *J. Biol. Chem*. **281**, 9–12.
29. Kogerman P., Grimm T., Kogerman L., et al. (1999) Mammalian suppressor-of-fused modulates nuclear-cytoplasmic shuttling of Gli-1. *Nat. Cell Biol.* **1**, 312–319.
30. Cheng S. Y. and Bishop J. M. (2002) Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18-mSin3 corepressor complex. *Proc. Natl. Acad. Sci. USA.* **16**, 5442–5447
31. Stone D. M., Murone M., Luoh S., et al. (1999) Characterization of the human suppressor of fused, a negative regulator of the zinc-finger transcription factor Gli. *J. Cell Sci.* **112**, 4437–4448.
32. Ding Q., Fukami S., Meng X., et al. (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
33. Di Marcotullio L., Ferretti E., De Smaele E., et al. (2004) A. REN(KCTD11) is a suppressor of Hedgehog signaling and is deleted in human medulloblastoma. *Proc. Natl. Acad. Sci. USA*. **101**, 10,833–10,838.
34. Huangfu D. and Anderson K. V. (2006) Signaling from Smo to Ci/Gli: conservation and divergence of Hedgehog pathways from *Drosophila* to vertebrates. *Development* **133**, 3–14.
35. Ferretti E., De Smaele E., Di Marcotullio L., Screpanti I., and Gulino A. (2005) Hedgehog checkpoints in medulloblastoma: the chromosome 17p deletion paradigm. *Trends Mol. Med*. **11**, 537–545
36. Osterlund T. and Kogerman P. (2006) Hedgehog signaling: how to get from Smo to Ci and Gli. *Trends Cell Biol*. **16,** 176–180.
37. Ruiz i Altaba A., Sanchez P., and Dahmane N. (2002) Gli and hedgehog in cancer: tumours, embryos and stem cells. *Nat. Rev. Cancer*. **2**, 361–372.
38. Lai K., Kaspar B. K., Gage F. H., and Schaffer D. V. (2003) Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. *Nat. Neurosci*. **6**, 21–27
39. Machold R., Hayashi S., Rutlin M., et al. (2003) Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. *Neuron* **39**, 937–950
40. Palma V. and 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
41. Palma V., Lim D. A., Dahmane N., et al. (2005) Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development* **132**, 335–344
42. Ahn S. and Joyner A. L. (2005) In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. *Nature* **437**, 894–897
43. Singh S. K., Hawkins C., Clarke I. D., et al. (2004) Identification of human brain tumour initiating cells. *Nature* **432**, 396–401
44. Wechsler-Reya R. J. and Scott M. P. (1999) Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* **22,** 103–114.
45. Dahmane N, Ruiz i Altaba A. (1999) Sonic hedgehog regulates the growth and patterning of the cerebellum. *Development* **126**, 3089–3100.
46. Wallace V. A. (1999) Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. *Curr. Biol*. **9**, 445–448.
47. Lee A., Kessler J. D., Read T. A., et al. (2005) Isolation of neural stem cells from the postnatal cerebellum. *Nat. Neurosci*. **8,** 723–729.
48. Dahmane N., Sanchez P., Gitton Y., et al. (2001) The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis. *Development* **128**, 5201–5212.
49. Berman D. M., Karhadkar S. S., Hallahan A. R., et al. (2002) Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* **297**, 1559–1561.
50. Romer J. T., Kimura H., Magdaleno S., et al. (2004) Suppression of the Shh pathway using a small molecule inhibitor eliminates medulloblastoma in Ptc1(+/–)p53(–/–)mice. *Cancer Cell* **6**, 229–240.
51. Sanchez P. and Ruiz i Altaba A. (2005) In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. *Mech. Dev*. **122**, 223–230.
52. Thompson M. C., Fuller C., Hogg T. L.,et al. (2006) Genomics identifies medulloblastoma

subgroups that are enriched for specific genetic alterations. *J. Clin. Oncol.* **24**, 1924–1931.
53. Svard J., Heby-Henricson K., Persson-Lek M., et al. (2006) Genetic elimination of Suppressor of fused reveals an essential repressor function in the mammalian Hedgehog signaling pathway. *Dev. Cell*. **10**, 187–197.
54. Goodrich L. V., Milenkovic L., Higgins K. M., and Scott M. P. (1997) Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109–1113.
55. Hahn H., Wojnowski L., Zimmer A. M., Hall J., Miller G., and Zimmer A. (1998) Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. *Nat. Med.* **4**, 619–622.
56. Oliver T. G., Read T. A., Kessler J. D., et al. (2005) Loss of patched and disruption of granule cell development in a pre-neoplastic stage of medulloblastoma. *Development* **132**, 2425–2439
57. Pazzaglia S., Tanori M., Mancuso M., et al. (2006) Two-hit model for progression of medulloblastoma preneoplasia in Patched heterozygous mice. *Oncogene* **25,** 5575–5580.
58. Gallo R., Zazzeroni F., Alesse E., et al. (2002) REN: a novel, developmentally regulated gene that promotes neural cell differentiation. *J. Cell Biol*. **158**, 731–740.
59. Argenti B., Gallo R., Di Marcotullio L., et al. (2005) Hedgehog antagonist REN(KCTD11) regulates proliferation and apoptosis of developing granule cell progenitors. *J. Neurosci*. 25, 8338–8346.
60. Ferretti E., Di Marcotullio L., Gessi M., et al. (2006) Alternative splicing of the ErbB-4 cytoplasmic domain and its regulation by Hedgehog signaling identify distinct medulloblastoma subsets. *Oncogene,* July 31 (epub ahead of print)
61. Tidcombe H., Jackson-Fisher A., Mathers K., Stern D. F., Gassmann M., and Golding J. P. (2003) Neural and mammary gland defects in ErbB4 knockout mice genetically rescued from embryonic lethality. *Proc. Natl. Acad. Sci. USA*. **100**, 8281–8286.
62. Rieff H. I., Raetzman L. T., Sapp D. W., Yeh H. H., Siegel R. E., Corfas G. (1999) Neuregulin induces GABA(A) receptor subunit expression and neurite outgrowth in cerebellar granule cells. *J. Neurosci.* **19,** 10,757–10,766.
63. Gilbertson R. .J., Clifford S. C., MacMeekin W., et al. (1998) Expression of the ErbB-neuregulin signaling network during human cerebellar development: implications for the biology of medulloblastoma. *Cancer Res*. **58,** 3932–3941.
64. Kainulainen V., Sundvall M, Maatta J. A., Santiestevan E., Klagsbrun M., and Elenius K. (2000) A natural ErbB4 isoform that does not activate phosphoinositide 3-kinase mediates proliferation but not survival or chemotaxis. *J. Biol. Chem*. **275,** 8641–8649.
65. Elenius K., Choi C. J., Paul S., Santiestevan E., Nishi E., and Klagsbrun M. (1999) Characterization of a naturally occurring ErbB4 isoform that does not bind or activate phosphatidyl inositol 3-kinase. *Oncogene*. **18**, 2607–2615.